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Drought response and resilience of water and carbon relations in mature European beech and Norway spruce

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I. Summary

Temperate forests face a major challenge with the ongoing climate change, especially in the last decade with exceptionally warm and dry years (e.g. 2015 and 2018/19). The ramifications of a drier climate on natural and planted forest stands, e.g. crown/stand die-back, have been observed all over Europe, but also worldwide. Trees' reactions to drought are manifold and differ among species. More isohydric species tend to close their stomata early under drought in order to minimize the water loss, potentially at the cost of limited carbon gain. While more anisohydric species keep their stomata open under drought to maximize carbon gain, but with a higher water loss as the consequence. Isohydric species are assumed to be prone to carbon starvation, a lack of carbon for maintenance processes, while anisohydric species are more endangered by hydraulic failure, a collapse of the water-conducting system, under drought conditions. Many studies of single drought events on juvenile trees have set the framework for our understanding of drought-induced reactions, acclimation patterns and tree mortality. Studies on mature trees under experimental drought are scarce due to the high effort and expense, but it is unclear whether findings in juvenile trees can be extrapolated directly to mature trees and natural forest stands. Additionally, the ramifications of repeated drought cycles, e.g. the two consecutive natural drought years 2018 and 2019 in Central Europe, on trees are widely unknown and long-term acclimation processes in forests stands are markedly understudied.

In the Kranzberg Roof (KROOF) experiment, a mature mixed forest stand composed of the more anisohydric European beech (*Fagus sylvatica* L.) and the more isohydric Norway spruce (*Picea abies* (L.) Karst.) was artificially drought stressed for five consecutive growing seasons (2014 to 2018). Therefore, a novel roof construction was installed on six plots beneath the canopy, to exclude any summer throughfall (throughfall exclusion = TE). Roofs were comprised of permanently closed parts and moveable shutters, which remained open for most of the time and only closed automatically during rainfall. The roofs withheld about 70% of the annual precipitation from the TE plots, while the control plots without roofs (CO) received the natural amount of precipitation. After five years of drought treatment, the TE plots were re-watered in summer 2019, to study the recovery and resilience of the trees from drought. A watering system composed of soaker hoses distributed over the whole plot area was used to bring the TE plots to a similar soil water content as the CO plots. Within two days the TE plots were watered with about 13000 L of water. During the whole experiment, biotic interactions with insect pests were excluded by spraying an insecticide. Different parameters were measured

to assess the implications of drought stress on the carbon and water relations of beech and spruce. The level of drought stress was quantified via the leaf water potential (pre-dawn and midday) and the plant available water in the soil. Non-structural carbohydrate (NSC) reserves were measured each winter in order to determine any occurrence of carbon starvation among different tree organs (e.g. root, stem and branches). With the help of a $^{13}CO_2$ labeling, the phloem transport velocity and mean residence time of newly fixed carbohydrates were examined for beech in two consecutive years. During the re-watering, the osmotic adjustment in leaves and the sap flow density were measured additionally and the resilience was calculated in comparison to CO trees for most of the mentioned parameters. The overall main research objectives of the single chapters were:

1. To address the realization and efficacy of the applied roof and watering system in the KROOF experiment.
2. To determine the level of carbon starvation and the restrictions of the phloem transport under repeated summer drought.
3. To quantify the resilience and time needed for recovery after repeated summer drought of the physiological water relations.

With the onset of drought strong impairments have been observed. Stem diameter increment decreased by 30%/70% and fine root abundance decreased by about 60%/70% in TE beech/spruce. Lower growth was accompanied by a reduction of the whole tree NSC pool by almost 50% in TE beech and spruce, while the NSC concentration remained stable even under repeated drought. However, no signs of carbon starvation were found in either tree species or any tree organ. Additionally, a slower phloem transport velocity in beech of about 50% in TE compared to CO and a higher mean residence time of newly assimilated carbohydrates by about 50% was found. The direct dependency of the phloem transport on the actual water status of the tree, i.e. strong correlation between pre-dawn water potential and phloem transport velocity, emphasized the phloem transport to be a critical bottleneck in the plant carbon-water-relations. Under drought, the carbon transport velocity in trees was decreased, which increased the threat of local carbon starvation (e.g. in fine roots far away from the source organ), as under drought additional carbon sinks arise. One additional sink, for example, are xylem embolism repair mechanisms, which are assumed to consume NSCs to refill the xylem embolisms with water. A lowered phloem transport plus a higher NSC demand could lead to a negative feedback loop for both resources, which increases the water and NSC scarcity in single tissues. Another additional sink for NSCs under drought is osmoregulation in different tree organs. NSCs used as osmolytes are not available for other processes (e.g. respiration), but are an important tool in the drought acclimation of plants. Both species showed signs of increased osmoregulation under drought, with lower osmotic potentials in leaves by about 0.9 MPa in beech and 0.8 MPa in spruce. Such direct interactions between the water and carbon relations are very critical mechanisms under drought and should be studied further in the context of tree mortality.

Upon the watering, both species overall displayed a very high resilience and recovered almost completely within the next growing season in their physiological water relations (e.g. osmotic adjustments reversed, increase in pre-dawn water potential, etc.), but often beech was faster in its recovery compared to spruce. The sap flow density in the outermost xylem tissue was strongly reduced under the drought treatment in both species by about 50% and 40% for beech and spruce, respectively in 2019. However, TE beech/spruce recovered within the next growing season to a level similar to CO, with TE beeches even surpassing the CO trees. However, the overall water consumption of TE spruce is probably still strongly reduced, because the lateral sap flow profile or sapwood area in TE spruce is supposedly reduced compared to CO spruce in 2020. This is most likely connected to a reduced leaf area in spruce with very short lateral shots and smaller needles. This is also supported by the higher water availability in the soil under TE spruce trees in the last years of the drought treatment. Therefore, the morphological acclimations/restrictions (e.g. leaf area, root abundance) did most likely not recover in the next growing season, unlike the physiological acclimations. Additionally, only minor differences were found during the drought and re-watering between the trees growing in mono or mixed competition situations. However, TE beech growing in mixture might have benefitted from the neighborhood with spruce in the later years of drought, due to the lower water consumption of spruce.

Beech and spruce survived five consecutive years of repeated summer drought and showing no signs of carbon starvation or hydraulic failure. However, both species were strongly impaired in their physiological functionality during the drought years, which potentially lowered the defense capacity and under natural conditions would have highly endangered spruce for biotic attacks (e.g. by bark beetles). Additionally, both species showed a high resilience after drought stress release and their physiological functionality recovered within the next growing season almost completely. More field manipulation studies in mature trees are needed to determine the resistance and resilience to drought of temperate forests under the ongoing climate change, focusing on the close interactions of the water and carbon relations, e.g. phloem transport, xylem embolism repair mechanisms and osmoregulation.

II. Kurzfassung

Die Wälder der gemäßigten Breiten stehen durch den fortschreitenden Klimawandel vor einer großen Herausforderung. Insbesondere im letzten Jahrzehnt traten außerordentlich warme und trockene Jahre auf (z. B. 2015 und 2018/19). Die Auswirkungen von Dürreperioden auf Wald- und Forstbestände (z.B. das Absterben einzelner Kronen bis ganze Bestände) wurden in ganz Europa und auch global beobachtet. Die Reaktionen der Bäume auf Trockenheit sind sehr vielfältig und artspezifisch. Eher isohydrische Arten neigen dazu ihre Stomata bei Trockenheit frühzeitig zu schließen, um den Wasserverlust auf Kosten eines geringen Kohlenstoffgewinns zu minimieren. Eher anisohydrische Arten dagegen, halten ihre Stomata auch bei Trockenheit für einen maximalen Kohlenstoffgewinn offen, was jedoch mit einem hohen Wasserverlust einhergeht. Es wird angenommen, dass isohydrische Arten daher bei Trockenstress anfällig für „Carbon Starvation“ sind. Das bedeutet, dass ein Mangel an freien Kohlenhydraten herrscht, der für Erhaltungsprozesse, wie z.B. der Atmung notwendig ist. Andererseits sind anisohydrische Arten unter Trockenheit eher durch „Hydraulic Failure“, einem Zusammenbruch des wasserleitenden Xylemsystems, bedroht. Die Theorien über trockenheitsbedingte Baumsterblichkeit und deren Reaktions- bzw. Akklimatisierungsmuster stammen fast ausschließlich von Studien an juvenilen Bäumen nach einmaliger Trockenheit. Experimentelle Studien an ausgewachsenen Bäumen sind aufgrund des hohen zeitlichen und finanziellen Aufwands eher selten. Dabei es ist unklar, ob die Erkenntnisse, die an jungen Bäumen gewonnen wurden, direkt auf ausgewachsene Bäume und natürliche Waldbestände übertragen werden können. Darüber hinaus sind die Auswirkungen wiederholter (oder anhaltender) Trockenheit, wie es z. B. in den beiden aufeinanderfolgenden natürlichen Trockenjahren 2018 und 2019 in Europa der Fall war, sowie langfristige Akklimatisierungsprozesse in Waldbeständen weitgehend unerforscht.

Im Kranzberg-Roof (KROOF) Experiment wurde ein adulter Mischwaldbestand, bestehend aus der eher anisohydrischen Rotbuche (*Fagus sylvatica* L.) und der eher isohydrischen Gemeinen Fichte (*Picea abies* (L.) Karst.) über fünf aufeinanderfolgende Vegetationsperioden (2014 bis 2018) künstlich trockengestresst. Dazu wurden in sechs Versuchsflächen (Plots) neuartige Dachkonstruktionen installiert, um jeglichen Sommerniederschlag (TE) auszuschließen. Die Dächer bestehen aus dauerhaft geschlossenen Teilen (um jeden einzelnen Baum) und beweglichen Rollläden, welche sich bei Regen automatisch schließen. Sie hielten etwa 70% des Jahresniederschlags von den TE-Plots ab, während die Kontrollplots ohne Dach (CO) die natürliche Niederschlagsmenge erhielten. Nachdem die Bäume auf diese Weise fünf Jahre lang

Trockenstress ausgesetzt waren, wurden die TE Plots im Sommer 2019 bewässert. Dadurch kann die Erholung und Resilienz der Bäume nach wiederholter Trockenheit bestimmt werden. Um den Bodenwassergehalt der TE Plots an den der CO-Plots anzugleichen, wurde ein Bewässerungssystem bestehend aus Perlschläuchen, welche über die gesamten Plotflächen ausgelegt waren, verwendet. Die TE-Plots wurden dabei innerhalb von zwei Tagen vorsichtig mit ca. 13000 L Wasser pro Plot bewässert. Während des gesamten Experiments wurden biotische Interaktionen mit Schadinsekten mit Hilfe eines Insektizids ausgeschlossen. Die Auswirkungen des Trockenstresses auf den Kohlenstoff- und Wasserhaushalt von Buche und Fichte wurden mittels verschiedener physiologischer Parameter bestimmt. Dabei wurden jeweils Bäume in intraspezifischer (mono) und interspezifischer (mix) Konkurrenzsituationen untersucht: Das Ausmaß der Trockenheit wurde über das Blattwasserpotential (pre-dawn und minimum) und das pflanzenverfügbare Wasser im Boden quantifiziert. Im Winter wurden jeweils die nicht-strukturell gebundenen Kohlenhydratreserven (NSC) gemessen, um ein eventuelles Auftreten von „Carbon Starvation“ in verschiedenen Baumorganen (z.B. Wurzel, Stamm und Äste) zu untersuchen. Ein $^{13}CO_2$-Markierungsexperiment diente dazu die Phloemtransportgeschwindigkeit und die mittlere Verweilzeit von neu fixierten Kohlenhydraten bei der Buche in zwei aufeinanderfolgenden Jahren zu untersuchen. Während der Wiederbewässerung wurde außerdem die osmotische Anpassung in Blättern und Saftflussdichte im Xylem gemessen und die Resilienz im Verhältnis zu CO-Bäumen für die meisten der genannten Parameter berechnet. Die Hauptforschungsfragen der einzelnen Kapitel hierbei waren:

1. Die Realisierung und anschließende Evaluierung des eingesetzten Dach- und Bewässerungssystems im KROOF-Experiment, sowie dessen Wirkungsgrad.
2. Die Bestimmung des Ausmaßes von „Carbon Starvation“ und die Einschränkungen des Phloemtransports nach wiederholter Sommertrockenheit.
3. Die Quantifizierung der Resilienz und Dauer der Erholung der Wasserhaushaltsphysiologie nach wiederholter Sommertrockenheit.

Mit Beginn der Trockenheit wurde ein starker Rückgang des Stammwachstums bei den TE-Bäumen beobachtet. Der Durchmesserzuwachs verringerte sich bei der Buche um 30% und bei der Fichte um 70%, und die Feinwurzelabundanz sank bei der Buche um etwa 60% und bei der Fichte um 70%. Das verminderte Wachstum ging mit einer starken Reduzierung des gesamten NSC-Pools der TE Buche und Fichte um fast 50% einher, während jedoch die NSC-Konzentration auch unter wiederholter Trockenheit stabil blieb. Daher wurden keine Anzeichen für „Carbon Starvation“, weder in Buche oder Fichte noch in irgendeinem Baumorgan gefunden. Die Phloemtransportgeschwindigkeit der TE Buchen verlangsamte sich im Vergleich zu CO Buchen um etwa 50%. Außerdem wurde eine um etwa 50% höhere mittlere Verweilzeit von neu assimilierten Kohlenhydraten in Blättern festgestellt. Die direkte Abhängigkeit des Phloemtransports vom aktuellen Wasserstatus des Baumes (Korrelation zwischen dem pre-dawn Wasserpotential und der Phloemtransportgeschwindigkeit), hebt den Phloemtransport als kritischen Engpass im Wasser-Kohlenstoff-Haushalt der Pflanze hervor. Unter Trockenheit ist die Kohlenstoffallokation in Bäumen verringert, was die Gefahr einer lokalen „Carbon Starvation“ (z.B. in Feinwurzeln weit entfernt von der primären Kohlenstoffquelle) erhöht, da zusätzliche Kohlenstoffsenken entstehen. Zusätzliche Senken stellen z.B. Emboliereparaturmechanismen da, von denen angenommen wird, dass sie NSCs verbrauchen, um auftretende Embolien im Xylem wieder mit Wasser zu füllen. Ein verminderter Phloemtransport plus ein höherer NSC Bedarf könnte zu einer negativen Rückkopplungsschleife für beide Ressourcen führen, welche den Wasser- und NSC-Mangel in einzelnen Geweben erhöht. Eine weitere Senke für NSCs unter Trockenheit ist die Osmoregulation in verschiedenen Baumorganen. NSCs, welche als Osmolyte verwendet werden, stehen höchstwahrscheinlich nicht für andere Prozesse, wie z. B. der Atmung zur Verfügung, sind aber für die Akklimatisierung der Pflanzen an die Trockenheit essentiell. Beide Arten zeigten Anzeichen für eine erhöhte Osmoregulation unter Trockenheit, welche sich jeweils mit einem um etwa 0.9 MPa niedrigeren osmotischen Potential in den Blättern der Buche und um etwa 0.8 MPa in denen der Fichte ausprägt. Solche direkten Interaktionen zwischen dem Wasser- und Kohlenstoffhaushalt sind essenzielle Mechanismen, die bei Trockenheit auftreten und sollten im Zusammenhang mit der trockenheitsbedingten Baumsterblichkeit weiter erforscht werden.

Nach der Bewässerung zeigten beide Arten insgesamt eine sehr hohe Resilienz in ihrer Physiologie und erholten sich innerhalb der nächsten Vegetationsperiode fast vollständig bis hin zu dem Niveau der CO Bäume (sichtbar z.B. durch Aufhebung der osmotischen Anpassungen, Anstieg des pre-dawn Wasserpotentials, etc.). Dabei erholte sich die Buche oftmals schneller als die Fichte. Die Saftflussdichte in den äußersten Jahrringen war während der Trockenheit bei beiden Arten stark reduziert, konkret um etwa 50% bei Buche und 40% bei Fichte, erreichte jedoch innerhalb der nächsten Vegetationsperiode ein ähnliches Niveau wie das der CO Bäume. Der Gesamtwasserverbrauch der TE Fichten war nach der Wiederbewässerung jedoch wahrscheinlich trotzdem stark reduziert, da die Bodenfeuchte unter TE Fichte deutlich höher war als unter CO Fichte. Dies hängt höchstwahrscheinlich mit einer reduzierten Blattfläche der TE Fichten zusammen und wird vermutlich durch reduzierte Splintholzfläche oder Profil in der Xylemflussdichte der TE Fichten in 2020 ausgelöst. Im Gegensatz zu den physiologischen Akklimatisierungen wurden die morphologischen Akklimatisierungen bzw. Einschränkungen (z.B. Blattfläche und Wurzelanzahl) daher in der nächsten Vegetationsperiode höchstwahrscheinlich nicht aufgehoben. Dies gilt insbesondere für die Fichte. Darüber hinaus wurden nur kleine Unterschiede während der Trockenheit und der Wiederbewässerung zwischen den Bäumen, die in Mono- oder Mischsituationen wuchsen, gefunden. Das deutet darauf hin, dass die Physiologie des Wasser- und Kohlenstoffhaushalts bei ausgewachsenen Bäumen kaum von der Identität der benachbarten Baumarten beeinflusst wird. Allerdings profitiert vermutlich die in Mischkultur wachsende TE Buche, aufgrund des geringeren Wasserverbrauchs der Fichte, von deren Nachbarschaft. Buche und Fichte überlebten fünf aufeinanderfolgende Jahre mit wiederholter Sommertrockenheit, wobei sie eine relativ hohe Resistenz gegen Trockenstress zeigten und keine Anzeichen von „Carbon Sstarvation“ oder „Hydraulic Failure“ aufwiesen. Allerdings waren beide Baumarten während der Trockenperiode in ihrer Physiologie stark beeinträchtigt. Unter natürlichen Bedingungen hätte das möglicherweise die Verteidigungsfähigkeit herabgesetzt und die Fichte für biotische Angriffe (z.B. durch Borkenkäfer) stark gefährdet. Darüber hinaus zeigten beide Arten nach Beendigung des Trockenstresses ein hohes Resilienzpotenzial und ihre Physiologie erholte sich innerhalb der nächsten Vegetationsperiode vollständig. Die Experimente zeigen, dass weitere Feldmanipulationsstudien an adulten Bäumen notwendig sind, um die Resistenz und Widerstandsfähigkeit von Wäldern der gemäßigten Breiten gegenüber Trockenheit zu bestimmen, vor allem in Hinblick auf den anhaltenden Klimawandel. Dabei sollten die engen Wechselwirkungen des Wasser- und Kohlenstoffhaushalts (z.B. Phloemtransport, Xylemreparaturmechanismen oder Osmoregulation) im Vordergrund stehen.

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IV. List of Abbreviations

BHD	breast height diameter of trees (at ~ 1.3 m)
C	carbon
CO	control plots/trees in the KROOF experiment
DACH	Germany, Austria and Switzerland
Day_{FR}	day after the watering when resilience reached 1
DOY	day of year
EER	evaporative enrichment of the leaf water
F_{label}	fraction of the labeled water
FWU	foliar water uptake
iSWD	integrated soil water deficit
KROOF	Kranzberg roof experiment
MRT	mean residence time
NSC	non-structural carbohydrates
PAW	plant available water in the soil
π_O	osmotic potential of the leaf at full turgor
$\Psi_{50/88}$	water potential at 50/88 loss of conductivity
$\Psi_{min/PD}$	minimum/pre-dawn water potential
Ψ_{TLP}	water potential at turgor loss point
PWP	permanent wilting point
SWC	soil water content
RCP	relative concentration pathway
$Resilience_0$	resilience value before the watering
$Resilience_{FR}$	resilience value at the day of full resilience
SPAC	soil-plant-atmosphere continuum

TE	throughfall-exclusion plots/trees in the KROOF experiment
u_{daily}	daily xylem sap flow density
WD_{plot}	water deficit of the TE plots compared to CO
WD_{soil}	water deficit of the soil
$WD_{toplayer}$	water deficit of the litter layer
WUD	water uptake depth
VPD	vapor pressure deficit
V_{phloem}	phloem transport velocity
V_R	resilience velocity

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VII. List of Publications

VII.I Included in this thesis

Grams, T.E.E., **Hesse, B.D.**, Gebhardt, T., Weikl, F., Rötzer, T., Kovacs, B., Hikino, K., Hafner, B.D., Brunn, M., Bauerle, T.L., Häberle, K.-H., Pretzsch, H., Pritsch, K., 2021. The Kroof experiment: realization and efficacy of a recurrent drought experiment plus recovery in a beech/spruce forest. Ecosphere 12.

Hesse, B.D., Goisser, M., Hartmann, H., Grams, T.E.E., 2019. Repeated summer drought delays sugar export from the leaf and impairs phloem transport in mature beech. Tree Physiol. 39, 192–200.

Hesse, B.D., Hartmann, H., Rötzer, T., Landhäusser, S.M., Goisser, M., Weikl, F., Pritsch, K., Grams, T.E.E.E., 2021. Mature beech and spruce trees under drought – Higher C investment in reproduction at the expense of whole-tree NSC stores. Environ. Exp. Bot. 191.

Hesse, B.D., Gebhardt, T., Hafner, B.D., Hikino, K., Reitsam, A., Häberle, K-H., Grams, T.E.E., Back to Routine? – Recovery Time and Resilience of the Water-Relations of Mature Beech and Spruce after Recurrent Summer Drought. Submitted to Global Change Biology in September 2021

VII.II Additional publications

Baumgarten, M., **Hesse, B.D.**, Augustaitiene, I., Marozas, V., Mozgeris, G., Bycenkiene, S., Mordas, G., Pivoras, A., Pivoras, G., Juonyte, D., Ulevicius, V., Augustaitis, A., Matyssek, R., 2019. Responses of species-specific sap flux, transpiration and water use efficiency of pine, spruce and birch trees to temporarily moderate dry periods in mixed forests at a dry and wet forest site in the hemi-boreal zone. J. Agric. Meteorol. 75, 13–29.

Hafner, B.D., **Hesse, B.D.**, Bauerle, T.L., Grams, T.E.E., 2020. Water potential gradient, root conduit size and root xylem hydraulic conductivity determine the extent of hydraulic redistribution in temperate trees. Funct. Ecol. 1–14.

Hafner, B.D., **Hesse, B.D.**, Grams, T.E.E., 2020. Friendly neighbours: Hydraulic redistribution accounts for one quarter of water used by neighbouring drought stressed tree saplings. Plant Cell Environ. 1–14.

Hesse, B.D., Hafner, B.D., Grams, T.E.E., 2019. Reverse conductivity for water transport and related anatomy in fine roots of six temperate tree species – a potential limitation for hydraulic redistribution. J. Plant Hydraul. 6.

Singh, A., Mayer, V.E., Zytynska, S.E., **Hesse, B.**, Weisser, W.W., 2021. The Efficiency of Plant Defense : Aphid Pest Pressure Does Not Alter Production of Food Rewards by Okra Plants in Ant Presence. Front. Plant Sci. 12, 1–13.

Tomasella, M., Beikircher, B., Häberle, K.-H., **Hesse, B.**, Kallenbach, C., Matyssek, R., Mayr, S., 2018. Acclimation of branch and leaf hydraulics in adult Fagus sylvatica and Picea abies in a forest through-fall exclusion experiment. Tree Physiol. 1–14.

Tomasella, M., Häberle, K.-H., Nardini, A., **Hesse, B.**, Machlet, A., Matyssek, R., 2017. Post-drought hydraulic recovery is accompanied by non-structural carbohydrate depletion in the stem wood of Norway spruce saplings. Sci. Rep. 7, 14308.

Tomasella, M., Nardini, A., **Hesse, B.D.**, Machlet, A., Matyssek, R., Häberle, K.-H., 2018. Close to the edge: effects of repeated severe drought on stem hydraulics and non-structural carbohydrates in European beech saplings. Tree Physiol. 1–12.

1. Introduction

1.1 Central European forests under climate change

The environment, respectively ecosystems, shaped species development and composition since the first emerge of life (Philippi et al. 1998, Beaman et al. 2016). Even under relatively stable ecosystem conditions species have to continuously adapt/acclimate to a certain extent. However, as soon as the system would be subjected to swifter changes, such as climate change or disturbances, e.g. natural catastrophes, species would be forced to adapt/acclimate quickly or go extinct in the long run (Lytle 2001, Crispo et al. 2010, Hoffmann and Sgró 2011, Banks et al. 2013). Under slow changes, e.g. natural climatic changes over centuries/millennia, species have the chance to either adapt to the new situation or migrate to more favorable locations (McCarty 2001, Iverson et al. 2004, Feeley et al. 2012, Morecroft et al. 2019). But with the ongoing climate change since the 20th century, accelerated by anthropogenic influences, to a warmer and drier climate with a higher frequency of drought periods in general, ecosystems not accustomed to drought or heat periods are subjected to a major challenge (Fuhrer et al. 2006, Seidenkrantz et al. 2009, Dai 2013a, IPCC 2014, Hansen and Stone 2016). Natural drought years have been occurring very frequently in the last decades (Briffa et al. 2009), especially in the 21st century (e.g. 2003, 2015, 2018 and 2019, Leuzinger et al. 2005, Hartmann, Moura, et al. 2018, Schuldt et al. 2020), with harsh consequences for our ecosystems (e.g. partial dieback (Baker et al. 2008, Allen et al. 2010, McDowell et al. 2020)). Climate research according to the last IPCC reports (e.g. IPCC 2014, 2019, Intergovernmental Panel on Climate Change) proclaimed an increase of about 0.9 °C in temperature until 2012 compared to the late 19th century. Additionally, modeled scenarios predict even further shifts to a warmer and drier climate (e.g. Mokhov and Eliseev 2012, Nazarenko et al. 2015). One of the most important climate change scenarios are the representative concentration pathways (RCP, IPCC 2014), which include next to meteorological and ecological aspects also socio-economic and political factors in the model. The RCP scenarios are therefore the result of a collaboration between many different fields in natural, social and economic sciences and integrate several different models (van Vuuren et al. 2011 and citations within). Within the RCP scenarios, four different versions can be distinguished for the possible climate in 2100, a best-case scenario (RCP 2.6), two moderate scenarios (RCP 4.5/6) and a worst-case scenario (RCP 8.5). The values 2.6, 4.5/6 and 8.5 refer to the radiative forcing (in W m^{-2}) in the year 2100 and can be translated to an atmospheric CO_2 equivalent and temperature increase in the year 2100 (Table 1).

Table 1: Details for the single representative concentration pathways (RCP) scenarios in 2100 according to IPCC (2014)

	Radiative forcing [W m^{-2}]	**CO_2 equivalent [ppm]**	**Temperature increase to present [°C]**
RCP 2.6	2.6	~ 490	~ 1.0
RCP 4.5	4.5	~ 650	~ 1.8
RCP 6	6	~ 850	~ 2.2
RCP 8.5	8.5	~ 1370	~ 3.7

Best case scenarios (RCP 2.6) with an increase of about 1 °C by 2100 compared to the late 20th century are presumable too optimistic, according to the newest modeling approaches (Raftery et al. 2017). Under present circumstances and partial success of the currently planned climate policy (e.g. CO_2 emission reductions (Breidenich et al. 1998, O'Neill and Oppenheimer 2002)), a moderate scenario (RCP 4.5/6) or even worst-case scenario (RCP 8.5) are more likely to happen. In either of these two scenarios, ecosystems would not only face increased temperatures and lower precipitation but additionally highly frequent extreme events, e.g. storms or flooding (Millán 2014). Also higher anthropogenic pressure, due to a higher recourse demand (Eggers et al. 2008, Elshkaki et al. 2018) or need for arable land (Smith and Myers 2018, Kopittke et al. 2019) as a consequence of an increasing global population (KC and Lutz 2017, Vollset et al. 2020), would threaten natural ecosystems. Especially immovable species with a long generation time/low generation change, such as trees and forest ecosystems, would be critically threatened under these scenarios (Allen et al. 2010, Hartmann, Schuldt, et al. 2018, Dauphin et al. 2020).

One of these ecosystems are temperate and boreal forests, which cover about one-third of the northern hemisphere, e.g. in Central Europe, North America, Russia and China (Goodale et al. 2002). The species composition of these ecosystems was mostly defined by the last ice age (Svenning and Skov 2007), but additionally by silviculture and historical human interventions (e.g. heavy logging/clearing (Ganz 2004, Dambrine et al. 2007)). Two major key species of Central European forests of today are European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst). In many parts of Central Europe, beech, a deciduous broadleaf tree species, would be the dominating species of forest ecosystems, as the peak of natural succession (currently dominant on ~16% of the forest area in Germany, BWI3 2014). This is due to its high plasticity and therefore wide range of habitat requirements (Ellenberg and Leuschner 2010, Cavin et al. 2013, Forster et al. 2019). The natural distribution of beech ranges from the Atlantic Ocean to the Black sea and Sicilia to the south of Scandinavia (Forster et al. 2019). While for Norway spruce, an evergreen conifer, the natural distribution is limited to boreal or (sub)alpine regions. However, it is the dominating tree species in many managed/planted monoculture forests, occupying 26% of the forest area in Germany (BWI3 2014). These stands are often located beyond spruce's natural range, due to its importance in silviculture and wood processing industry (Spiecker 2000, LWF 2014, Forster et al. 2019). A more detailed characterization of the two species can be found in chapter 2.1 later on. However, within the last decades, forest management intensifies the conversion of monoculture Norway spruce stands into mixed stands (Bravo-Oviedo et al. 2014, del Río et al. 2016) with beech and spruce being a common species couple (Knoke et al. 2008, Pretzsch and Schütze 2009, Pretzsch et al. 2010a).

1.2 Reaction patterns of trees' water and carbon relations under drought

Both species follow rather contrary water status regulation strategies under drought stress (Hochberg et al. 2018, Ratzmann et al. 2019), with beech being a more anisohydric (water-spending) species and spruce being a more isohydric (water-saving) species (Lyr et al. 1992, Hartmann et al. 2013, Pretzsch et al. 2014, Burkhardt and Pariyar 2016). On the one hand, anisohydric plants keep their stomata open even under drought conditions in order to keep carbon assimilation on a high level, but at the cost of high transpiration/water loss. On the other hand, isohydric plants close their stomata earlier under drought conditions in order to minimize transpiration and water loss, but at the cost of lower carbon assimilation (Hochberg et al. 2018, Ratzmann et al. 2019). In theory, these opposing strategies are adaptions to different kinds of drought. Anisohydric species are better adapted to long-term but mild drought periods and isohydric species to short-term but severe drought events (McDowell et al. 2008). In accordance with these two different strategies different theories about tree survival and death in relationship to the water and carbon relations were developed: hydraulic failure and carbon starvation (McDowell 2011, Anderegg et al. 2012, Sevanto et al. 2014).

Under hydraulic failure, the water conducting system would fail, due to emerging embolisms under high pressure e.g. very low xylem water potentials (Choat et al. 2012, Urli et al. 2013). According to literature the critical point in angiosperms is when 88% of the conductive system are embolized (Ψ_{88}, for beech: ~3.5 MPa (Barigah et al. 2013, Urli et al. 2013)) and in conifers when 50% are embolized (Ψ_{50}, for spruce: ~3.5 MPa), which could cause die back of the crown or even tree death (Brodribb and Cochard 2009, Bouche et al. 2014). Under well-watered conditions, the degree of xylem embolisms is close to zero but increases with a sigmoidal shape under decreasing water potentials, so-called vulnerability curves (Cai and Tyree 2010, Cochard et al. 2013). Due to this sigmoidal shape, small decreases in water potential would cause a huge increase in xylem embolisms. Therefore, pressure safety margins within the xylem, such as the difference between Ψ_{50}/ Ψ_{88} and the minimum water potential trees experienced, and between single tree compartments (e.g. leaf – twig) are important for tree survival (Johnson et al. 2012). According to the hydraulic segmentation theory, where leaves act as a safeguard to protect proximal organs, for example, trees could sacrifice certain organs in order to protect the remaining parts (Pivovaroff et al. 2014, Johnson et al. 2016). The range of these safety margins and their acclimative potential are important for forest resistance to hydraulic failure (Zwieniecki and Secchi 2014, Tomasella, Beikircher, et al. 2018).

Carbon starvation would occur when the tree is running into a long-term negative carbon balance. This would happen under very low/no carbon assimilation and depleted storages, which are not sufficient to cover maintenance costs for metabolic processes, such as respiration or cellular turgor (Hartmann and Trumbore 2016). Such carbon storages, so-called non-structural carbohydrates (NSCs), are mostly comprised of simple sugars (glucose, fructose, sucrose, etc.) and starch as a first more complex storage molecule (Hartmann and Trumbore 2016). These NSCs are used as an energy supply for the primary and secondary metabolism but can also be stored in different parts of the tree for later usage. Therefore, NSCs play a crucial role in trees' carbon relations. The understanding of their regulation and distribution patterns under drought is crucial for the knowledge about potential carbon starvation in mature trees. However, trees have many carbon sinks (Hartmann and Trumbore 2016), with different demands, depending on the current stage of the tree (e.g. ontogenetic stage) or climatic conditions, such as growth, defense via secondary metabolites, osmoregulation or export via root exudates to mycorrhiza or soil microbes. The amount of needed carbon is also different among organs and therefore the carbon allocation and remobilization plays an important role as well (Martínez-Vilalta et al. 2016, Furze et al. 2019, Schiestl-Aalto et al. 2019), especially under long-term and/or repeated severe drought events (Adams et al. 2013, Trugman et al. 2018). On this account, the ability to mobilize stored carbon (e.g. from starch (Sauter and van Cleve 1994, Sala et al. 2010)) and to re-allocate sugars seem to be crucial. Therefore phloem transport represents an important process in the carbon starvation scenario (Sevanto 2014 and citations within).

According to the carbon starvation and hydraulic failure theories, more anisohydric beech would be more prone to hydraulic failure in general, while more isohydric spruce would be more prone to carbon starvation under repeated severe drought (McDowell et al. 2008). Therefore, beech is assumed to keep its stomata (partially) open even under severe and repeated drought, while spruce would close its stomata very early already in the first drought period. However, both process-complexes are not independent of each other but often show a high degree of interaction (McDowell 2011, Adams et al. 2017). On the one hand, the phloem is taking water from the xylem, in order to maintain a low viscosity in the phloem sap (Hölttä et al. 2009). On the other hand, a high degree of carbohydrates is needed within the xylem tissue for the repair of xylem embolisms (Savi et al. 2016, Tomasella et al. 2017). Additionally, a high degree of NSCs is needed to maintain turgor pressure and leaf function (Bartlett et al. 2014). Under drought conditions, when both resources (water and carbon) are limited, this would lead to feedback loops between the water and carbon relations (McDowell 2011) further limiting plant fitness and defense capacity (Schlyter et al. 2006, Wiley et al. 2016, Huang, Kautz, et al. 2019). This might therefore increase susceptibility to biotic infestations with ongoing climate change (Logan et al. 2003, Ramsfield et al. 2016, Pureswaran et al. 2018, Linnakoski et al. 2019).

To understand the physiological processes in mature trees following water limitation due to drought is crucial to grasp forest die-back patterns during and after drought periods to develop counteracting strategies. For both species, beech and spruce, impairments due to drought and heat periods have been reported in mature (Brinkmann et al. 2016, Schuldt et al. 2020) and juvenile trees in the last years (Ruehr et al. 2019 and citations within). Yet reasons for tree mortality are still widely unknown (Adams et al. 2017, Choat et al. 2018, Anderegg et al. 2019). Dry periods with increased temperature have been reported worldwide in the last decades (Fuhrer et al. 2006, Briffa et al. 2009, Dai 2013b) and are often connected with forest mortality events (Allen et al. 2010, Schuldt et al. 2020). A better understanding of such events and their implications for single tree species is needed to reduce the risk of depletion of carbon storage in forests (Goodale et al. 2002, Pan et al. 2011). This depletion would create follow up problems, such as decreasing ecosystem services, e.g. a loss of biodiversity, site protection or recreational area (Paoletti et al. 2010, Seidl et al. 2016, Albrich et al. 2018) and increased economic uncertainties (Kirilenko and Sedjo 2007, Coomes et al. 2014).

1.3 Resilience after drought in natural forest ecosystems

Implications of drought stress on trees are a well-studied field compared to the recovery period and different suggestions for improved performances of forests have been made in the last decades (e.g. Chaves et al. 2003, Rennenberg et al. 2006, McDowell 2011). Such improvements include genotyping of species, in order to use more adapted provenances for new plantations (Howe et al. 2003, Gehring et al. 2017, Polle et al. 2019), mixing tree species in forest stands (Pretzsch and Schütze 2009, Pretzsch et al. 2010b, Toïgo et al. 2015) or to increase physiological plasticity by epigenetic analysis/breeding (Sow et al. 2018, Amaral et al. 2020). However, the survival of trees is not only defined by the drought period but also by the recovery period (Ruehr et al. 2019). Many studies so far focused only on the drought part, but nature is an ongoing cycle of drought and recovery periods, with survival sometimes being resolved not during the drought itself, but also in the failure to recover (Berdanier and Clark 2016, Trugman et al. 2018). Therefore, it is important to not only study the effects of abiotic stress but also to include the recovery period. Not only the quantity (how many survived) but also the quality of survival, in terms of resilience and recovery time, should be determined.

The term resilience is used in many different fields and therefore often following a very broad definition (Gessler et al. 2020 and citations within). Ecological resilience was originally defined by Holling (1973) as the persistence of a system against disturbances, without “losing” its original structure. Several aspects were added later on to the term “ecological resilience”, such as resistance, panarchy (influences between different ecosystem levels) or adaptive capacity (e.g. Gunderson 2000, Walker et al. 2004). This thesis follows in general the definition from Lloret et al. (2011) for resilience, as the capacity to reach pre-stress levels after a stress event.

$$Resilience = \frac{Post\ Drought\ Performance}{Pre\ Drought\ Performance} \quad (Formula\ 1)$$

The resilience (Formula 1) describes the relative level of a parameter after stress release in comparison to pre-stress/control values (measured on unstressed trees (CO), set to the value of 1 or 100%, Figure 1). A high resilience is given when post-stress values are very close to pre-drought or control values (resilience close to 1) and a low resilience when post-stress values stabilize at a lower level than pre-drought or control (resilience << 1). Additionally, the recovery time is defined as the amount of time that is needed for a parameter upon drought release to stabilize again. For details on calculations used in this thesis see chapter 2.5.

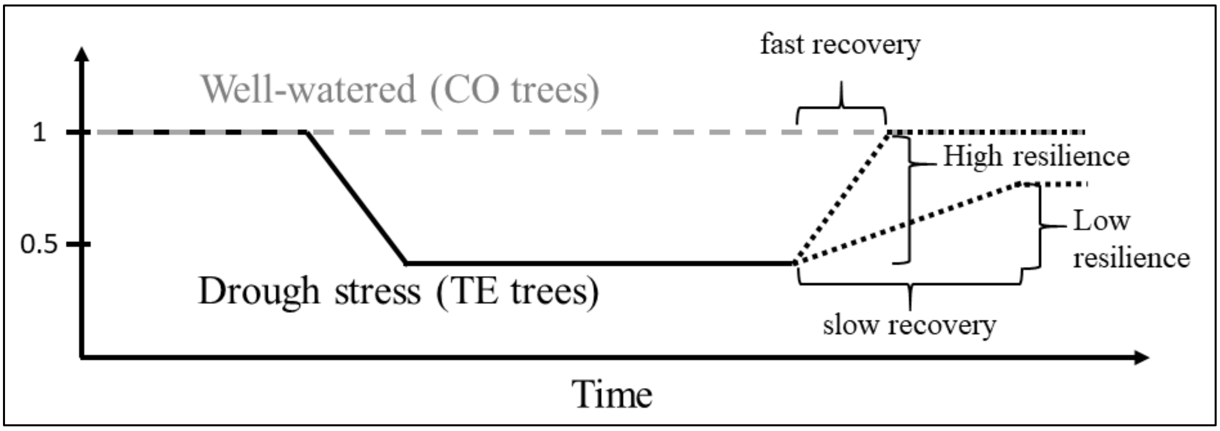


Figure 1: Example scheme for resilience and recovery time (from Article V)

For experiments with drought and recovery cycles, additionally to the resilience/recovery, the resistance (Formula 2) of forest stands to drought can be calculated.

$$Resistance = \frac{Drought\ Performance}{Pre\ Drought\ Performance} \quad (Formula\ 2)$$

Via the resistance and resilience, favorable mixtures/treatments (e.g. different genotypes, epigenetic breeding) might be identified and could support foresters with their decisions for future plantings.

Recent studies are suggesting that the recovery is not only species dependent, but also different among different parameters (Ruehr et al. 2019 and citations within). In these studies, the water potential and similar physiological parameters were found to recover within hours or several days after drought stress release. Structural damage/changes, including loss of conductivity in the xylem, took longer periods, up to several months or years, to recover completely (Ruehr et al. 2019 and citations within). However, such observations are still scarce in mature trees but are needed to determine the resilience potential among natural forest stands. Especially, the differences in the recovery processes between angiosperms vs. gymnosperms and anisohydric vs. isohydric species are widely unknown in general (Yin and Bauerle 2017).

1.4 Experiments in juvenile vs mature trees

In ecology and tree physiology, fundamental research is often conducted in (potted) seedlings or saplings and therefore theories mostly emerge from such experiments (Carpenter 1998, Schindler 1998, Calisi and Bentley 2009). This research is an important part of science and is needed to understand single processes and responses in plants (Gibson et al. 1999, Wilson 2009, Clobert et al. 2018, Halbritter et al. 2020). However, it is debatable how applicable these findings are for mature trees, as with ongoing ontogeny and ecosystem interactions reaction patterns often change (Robinson and Wareing 1969, Cavender-Bares and Bazzaz 2000, Vitasse 2013). While nowadays we are able to recreate climatic scenarios and growth conditions very accurately in greenhouse/climate chamber experiments, these are still restricted in many aspects and not a perfect copy of the complex natural situation. Therefore, it is not possible to extrapolate all findings from experiments with potted saplings to mature forest stands. This is due - among others - to restrictions in belowground growth (Coleman et al. 1996, Chen and Lieth 2019)), missing association with mycorrhiza fungi and soil microbes (Audet and Charest 2010), belowground interaction between trees (e.g. facilitative effects such as hydraulic redistribution (Hafner 2020)) or stand growing conditions (e.g. stand density (O'Connell and Kelty 1994, Landhäusser and Lieffers 2001)). That is why experiments in mature trees are needed to understand the implications of manipulated growing conditions (e.g. drought, heat or salinity) to forest stands and to investigate to what extent findings in juvenile trees can be extrapolated (Hanson 2000, Englund and Cooper 2003).

However, experiments in natural forest stands are not only connected with a high amount of work or financial costs (Asbjornsen et al. 2018) but also need a highly sophisticated experimental design (Dutilleul 1993, Legendre et al. 2004, Underwood 2009) and statistical evaluation (Zuur et al. 2009, Dingemanse and Dochtermann 2013, Harrison et al. 2018). The uncontrollability of nature must be included and to a certain extent manipulated, in order to generate reliable results. The basis for this thesis is a throughfall exclusion (TE) experiment in a mature forest stand. Summer precipitation was held off by a roof infrastructure in order to apply repeated drought stress over several years (for details see 2.1.1). Additionally, after the drought experiment, a re-watering was conducted on the same experimental site to investigate the recovery of the former stressed trees (for details see 2.1.2).

1.5 Objectives of this thesis

This thesis focuses on the understanding of tree reaction patterns and mechanisms in response to repeated drought stress and a subsequent recovery period in a mature forest stand. Within the first part, the efficacy of a long-term throughfall exclusion field experiment plus following watering was assessed in cooperation with all involved groups (Article I). The second part of this thesis focused on the carbon relations of mature trees under repeated drought. Therefore, the effects of drought on carbon allocation and transport were quantified (Article II and III). To follow up the drought restrictions, the third part of this thesis examined the recovery period after repeated drought. The focus was set on the water distribution/status within the soil and trees and quantifying the resilience of mature trees after drought release (Article IV and V).

The first part of this doctoral thesis was to test and demonstrate the efficacy of the throughfall exclusion and re-watering in the Kranzberg Roof experiment (KROOF). Therefore, physiological parameters were correlated with environmental data to detect the effects of the installed infrastructure (e.g. roofs) on growth conditions, quantify the drought intensity, introduce the irrigation system and demonstrate the successful re-watering (Article I).

As studies addressing hydraulic failure have already been conducted at the experimental site (Tomasella 2018), the second part of this thesis focused on the carbon relations of mature trees under repeated drought. One approach focused on the phloem transport velocity and hypothesized that under repeated drought the phloem transport would strongly reduce (Article II). The basic aims of this study were to quantify the restrictions of the phloem transport in the canopy and to assess the residence time of freshly assimilated carbohydrates within the leaf. For this purpose, a $^{13}CO_2$ labeling of branches was conducted in two repeated summers (for details see chapter 2.2.2). In a second study, the carbon allocation of winter reserves and the pool size of stored NSCs in single organs were determined (Article III). For this reason, trees were sampled in early winter in three consecutive years to analyze their NSC concentration and to calculate the whole NSC pools by relating it to the biomass (for details see chapter 2.3). This study hypothesized that NSC concentrations are reduced due to repeated drought and that NSC pools are reduced especially in sink organs (e.g. roots), with the isohydric spruce being stronger affected than the anisohydric beech.

The third part of this thesis focused on the recovery of trees upon rewatering after five years of repeated summer drought. The first study of part three focused on the distribution of added water within the soil and different tree organs (Article IV) after the experimental drought. For this reason, three control (CO) and three throughfall exclusion (TE) plots were watered with deuterium enriched water ($^{2}H_2O$, for details see chapter 2.2.1). The hypotheses for this study addressed any potential shifts in mean water uptake depth in CO and TE for both species and turnover times of water pools in different tree organs were assessed. In this process, shallow rooting spruce was expected to have access to the newly added water earlier than the deeper rooting beech trees. In a second study the resilience and recovery time of the water relations of TE in comparison to CO trees were assessed (Article V). Therefore, several parameters related to the water status of the trees were measured before, during and after the watering event (for details see chapter 2.4) and their resilience and recovery time were calculated (for details see chapter 2.5). The hypotheses for this study focused on the difference between the resilience of the different parameters in the TE treatment. Quick recovery to full resilience, within several hours, was expected in the water potential for both species. For the sap flow density, no complete recovery/resilience even in the next growing season was assumed, due to the huge amount of lost fine roots during the repeated drought. Finally, the reversal of osmoregulation was supposed to be faster in anisohydric beech compared to isohydric spruce.

2. Material and Methods

Descriptions of the single methods used for the different experiments can be found in the respective articles. Here, a more detailed summary of the main methods applied by the author is given.

2.1 Experimental site and species

The Kranzberg Roof (KROOF) experimental site is located near Freising in south-east Germany (coordinates: 11°39’42”E, 48°25’12”N, 490 m) in the Kranzberg forest. Long-term climate data for the experimental site was obtained from the nearby climate station Dürnast of the “Deutscher Wetterdienst” (DWD) via the online Climate Data Center (CDC, DWD 2021), which is about 2.7 km away from the forest stand. From 1981-2010, mean annual precipitation was about 790 mm and mean temperature about 8.6 °C (Figure 2).

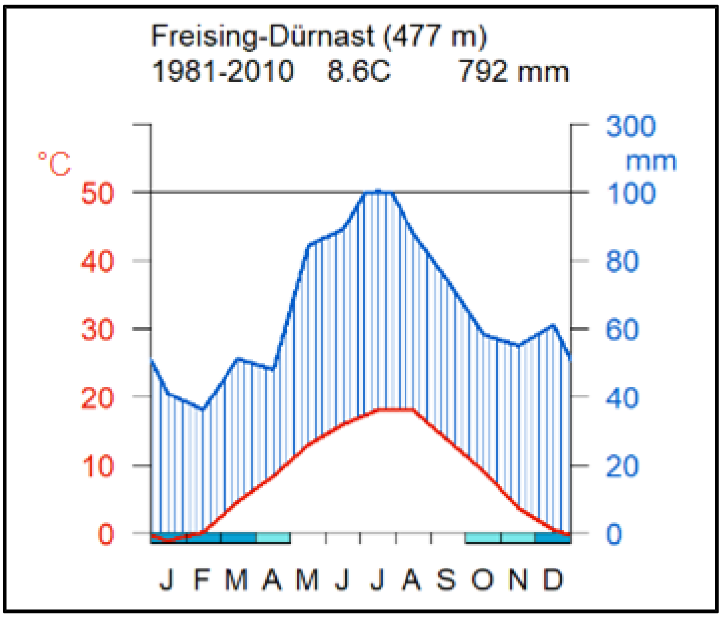


Figure 2: Climate diagram (temperature = red and precipitation = blue) for Freising-Dürnast with data from 1981-2010 after Walter and Lieth (1967) *as the long-term proxy for the experimental site. Data were obtained from the DWD climate station Dürnast. Blue colors on the x-axis symbolize potential (light blue) and definite (dark blue) freeze periods.*

The soil on the experimental site was classified as a luvisol type originating from loess over Tertiary sediments (Zech et al. 2014) and has hence a high nutrient and water supply (Zech et al. 2014), with an estimated water holding capacity of about 22 to 28 vol.-% in the loess layer (Göttlein et al. 2012). The loess layer is on average about 60 cm deep and supposedly composed of silt with a medium clay content (Ut3), followed by layers of silty (Lu) and sandy loams (Ls3) (Göttlein et al. 2012). The lower, loamy soil layers are very dense and difficult to penetrate, so most of the root growth happened within the first meter of the soil (Häberle et al. 2012). As the experimental site is located on a little hill, ground water level was supposed to be about 8 m below the surface and therefore unreachable for the trees. The experimental site is a fenced area of about 0.5 ha forest stand, dominated by European beech and Norway spruce (planted 1931 ± 4 AD and 1951 ± 2 AD, respectively, Table 2) intermixed with a few single *Pinus sylvestris* L., *Larix decidua* Mill. and *Quercus rubra* L. trees.

Table 2: Details of beech and spruce trees on the experimental site before the start of the KROOF experiment

	European Beech	**Norway spruce**
Planted in	1931 ± 4 AD	1951 ± 2 AD
Mean BHD [cm]	28.9 ± 3.3	34.3 ± 2.2
Mean height [m]	26.1 ± 0.6	29.0 ± 0.7
Drought stress strategy	anisohydric	isohydric
Leaf characteristics	deciduous broadleaf	evergreen conifer
Root system	heart-shaped	shallow with sinker roots

European beech, a deciduous broadleaf angiosperm species with a heart-shaped rooting system (Table 2), is naturally spread over all of Europe (Figure 3), ranging from south Italy to south Sweden and from northern Spain to the Black Sea. At the eastern distribution limit, the closely related *Fagus orientalis* (Lipsky) can be found, which is sometimes considered to be a subspecies of *Fagus sylvatica* (Müller et al. 2019). Its ecological potential is in general very high (3.8 out of 5 according to Otto (1994)), but the drought tolerance and resistance to biotic pests is only medium (3 out of 5). However, European beech is assumed to be the peak of natural succession in many parts of European forests. This is due to its high shade tolerance and plasticity in many physiological parameters in combination with low habitat requirements (e.g. nutrient availability, Ellenberg and Leuschner 2010, Cavin et al. 2013, Forster et al. 2019). Predictions about the future distribution of beech under climate change are diverse (Geßler et al. 2007), but it seems likely that beech remains one of the dominant species within Central Europe (Kramer et al. 2010, Forster et al. 2019).

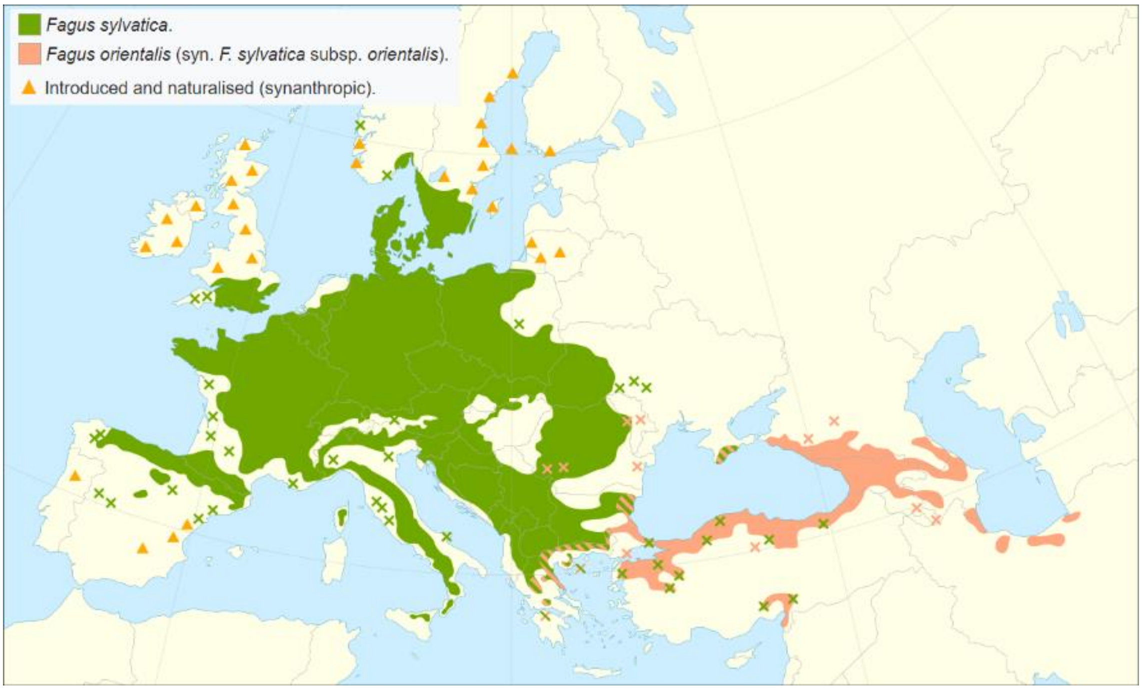


Figure 3: Distribution of European beech (Fagus sylvatica, green) Oriental beech (Fagus orientalis, salmon) in Europe (after Caudullo et al. (2017)*)*

Norway spruce, an evergreen conifer, is naturally distributed in (sub)alpine regions, Scandinavia and from the Czech Republic in the west to the Ural Mountains in the east (Figure 4). Additionally, it was introduced by forestry within Central Europe (e.g. France, Germany) and Great Britain (Figure 4) and is still one of the most important species for silviculture and timber industry (Spiecker 2000, LWF 2014, Forster et al. 2019). According to Otto (1994), its ecological potential is on a medium level (3.1 out of 5), with medium drought tolerance (3 out of 5). Its stability against windthrow is very low (1 out of 5), which is related to the plate-shaped root system just beneath the soil surface with only a few sinker roots (Puhe 2003). Additionally, its resistance to biotic pests is very low (1 out of 5), which could increase its overall susceptibility when combined with abiotic stresses (Netherer et al. 2015, 2019). With the ongoing climate change, the distribution of spruce is supposed to decrease drastically within the non-natural (introduced) areas but also the natural distribution could be shifted further north (Falk and Hempelmann 2013, Forster et al. 2019).

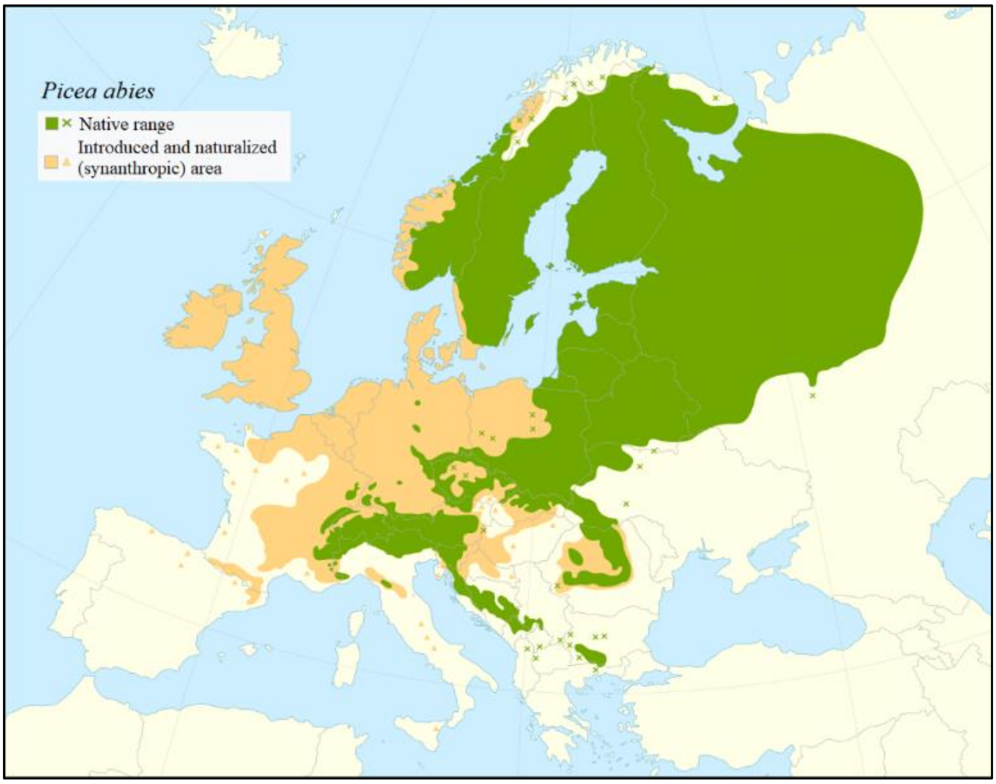


Figure 4: Natural (green) and introduced (orange) distribution of Picea abies (Norway spruce) in Europe (after (Caudullo et al. 2017)*)*

The understory of the experimental site is mainly comprised of juvenile beech, spruce and *Abies alba* Mill. trees with a moss (e.g. *Hylocomium splendens* (Hedw.) Schimp.) and *Rubus* spec. layer (e.g. raspberry or blackberry). An additional asset of the experimental site, a remnant of the SFB607 (project B4) ozone fumigation experiment (Häberle et al. 1998), are several scaffolding towers with climate stations below and above the canopy. These continuously measured the air temperature, relative humidity, global solar radiation and rainfall (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany). Additionally, a canopy crane was installed on the experimental site already in 2001 allowing for access of the major part of the canopy from the experimental plots.

2.1.1 Throughfall exclusion experiment (KROOF I) from 2014 to 2018

The KROOF phase I focused on the reactions of mature beeches and spruces to repeated summer drought. The experiment started in 2014 and lasted until 2018. All five experimental years showed an elevated temperature compared to the long-term average (1981-2010: 8.6 °C) of 3.4 ± 0.6 °C, with especially warm years 2015 and 2018 (Table 3). In four out of five years (2014-2017) a reduced annual precipitation was found by -73 ± 33 mm compared to the long-term mean (1981-2010: 792 mm). Only in 2018, the precipitation was similar to the long-term average (Table 3). Overall, 2015 was a particularly warm and dry year, while 2018 was very warm but not necessarily dry.

Table 3: Difference of the annual temperature and precipitation to the long-term mean of KROOF I

	2014	**2015**	**2016**	**2017**	**2018**
Δ Temperature [°C]	+ 2.7	+ 3.8	+ 3.1	+ 3.1	+ 4.2
Δ Precipitation [mm]	- 61	- 113	- 82	- 37	+ 11

The KROOF experiment is comprised of 12 plots, with 6 control (CO) and 6 throughfall-exclusion (TE) plots (Figure 3). From all plots, the canopy of 4 CO and 4 TE plots is accessible via the canopy crane. Each plot was trenched to a depth of 1m in 2010 and a thick plastic tarp was installed in the trench in order to interrupt any lateral water flow or root growth. Until the start of the KROOF experiment in 2014, trees were able to recover from the root trenching (Pretzsch et al. 2016). Drought stress was applied via roof constructions (Umwelt-Geräte-Technik GmbH, Münchnberg, Germany), excluding all summer precipitation from the 6 TE plots (Figure 3). Roofs were composed of an aluminum skeleton with movable and stationary (permanent closed) roof parts. The stationary parts of the roof (about 40% of the roof area), made of see-through acrylic glass, were fixed around each tree with additional perforation hoses around the stem to negate any stemflow down to the soil. The movable roof parts were composed of 20 to 30 rolling shutters, which could be opened and closed individually. Shutters closed and re-opened automatically only during and 1 hour after rain events, respectively, controlled by rain sensors (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany) on top of the scaffolding towers. The movable parts were installed in order to minimize changes in the microclimate and litter fall patterns. The construction of the roofs was completed within 2013.

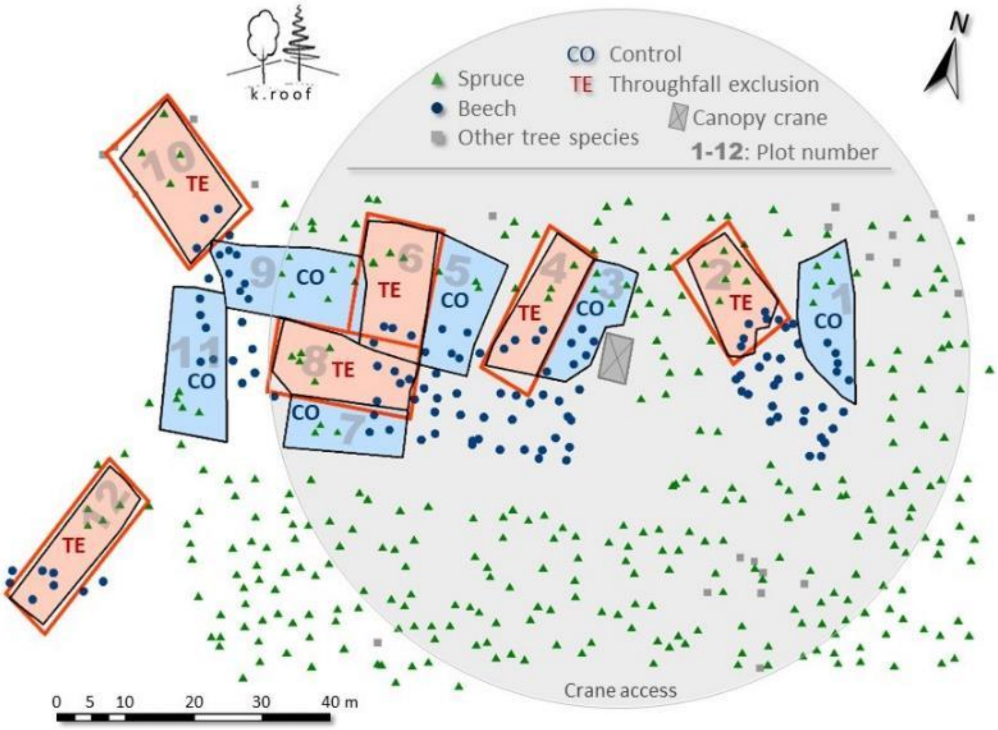


Figure 5: Map of the KROOF experiment with the control (CO, blue) and throughfall-exclusion (TE, red) plots. The gray box gives the position of the canopy crane and the grey circle shows the radius of the crane. Beech trees are symbolized by blue circles, spruce trees by green triangles and other species by grey squares. (From Article I)

Each plot was comprised of 3 to 7 beech and spruce trees at the opposing sides of each plot (intraspecific competition) and an interspecific competition zone in the middle (Figure 3). These three zones allowed to test for interspecific (beech vs spruce) and also intraspecific interaction effects (beech vs beech and spruce vs spruce) occurring during the applied drought periods (Goisser et al. 2016). In each plot two “focus trees” of each species were selected and studied intensely during the experiment, one of the two trees was standing in the monospecific and the other one in the interspecific zone. To control for any spatial effects occurring along the experimental site, a paired design was chosen with a CO plot always neighboring a TE plot (Figure 3). Plot size was on average 144.2 ± 26.4 m^2 and tree characteristics were very similar at the start of the experiment (see small SD in table 2 for tree height and diameter). The roofs withheld about 69 ± 7% of the annual precipitation on the TE plots.

2.1.2 Drought stress release/recovery experiment (KROOF II) from 2019 and 2020

The Focus for the second phase of the KROOF experiment was on the recovery upon watering after repeated summer drought. Watering was conducted in 3 campaigns (2 TE plots in parallel per campaign) in June/July 2019. While 2019 and 2020 were not as warm as the period from

2014 to 2018, temperature difference to the long-term average was only 2.0 ± 0.7 °C (Table 4), both years (especially 2019) showed a reduced amount of precipitation of -71 ± 57 mm compared to the long-term mean (Table 4).

Table 4: Difference of the annual temperature and precipitation to the long-term mean of KROOF II

	2019	**2020**
Δ Temperature [°C]	+ 2.5	+ 1.5
Δ Precipitation [mm]	- 111	- 30

The watering system was designed in order to accomplish a homogenous watering of whole plots. The watering system for each plot was composed of a garden hose with T-connectors every 15 cm, at the long site of the plot, connected to soaker hoses (CS Perlschlauch Premium, CS Bewässerungssyteme, Reichelsheim, Germany). These were laid out in parallel on the short side of the plot every 15 cm (soaker hoses were sealed at the other end with a cable tie, Figure 4). The accumulated length of all soaker hoses on each plot was 1111 ± 97 m.

Figure 6: Example of the watering system on one CO plot (left) and schematic set-up of the watering system on plot 4 (right). (Altered from Article 1)

The amount of flow within the system was controlled by a valve and electronic water meter (Wassermengenzähler, GARDENA Manufacturing GmbH, Ulm, Germany). As the infiltration rate on the TE plots was very low (0.25 ± 0.07 mm h^{-1}), water was applied at a very low rate (~ 2 L m^{-2} h^{-1}) for TE plots. In order to label the CO plots with deuterated water and to match the treatment of watering and mimic any immediate effects (e.g. soil temperature, nutrient availability or oxygen concentration), the control plots were watered with a small amount of water (15 L m^{-2} or 2034.5 ± 537.3 L per plot). The amount of water added to the TE plots was calculated via the soil water content (SWC in vol.-%, for details see chapter 2.4.1) and aimed at raising the water content to the level of the CO plots. Therefore, the soil water deficit per area (WD_{soil} in L m^{-2}) to a depth of 70 cm of the TE plots was calculated:

$$WD_{soil} = (SWC_{CO} - SWC_{TE}) * soil\ volume \qquad Formula\ 3$$

The absorptive capacity of the litter layer ($WD_{toplayer}$ of 14.40 ± 3.62 L m^{-2}) was tested in the lab by weighing dried and re-saturated litter samples taken from TE plots. To gain the absolute water deficit (WD_{plot} in L), the sum of WD_{soil} and $WD_{toplayer}$ was multiplied with the area plus the amount added to the CO plots.

$$WD_{plot} = WD_{soil} * area + WD_{toplayer} * area + (15\,L\,m^{-2} * area) \qquad Formula\ 4$$

On average 12849 ± 2801 L (90 L m^{-2}) of water were added to each TE plot. Watering started on June 25th, July 4th and July 10th 2019 for each campaign respectively at 4 a.m. and lasted on the CO plots for about 7 h and about 40 h on TE plots. Direct effects of the watering were measured on D-6/-1 (six/one day(s) prior to the watering), D0 (day of the watering) and D1/2/4/7/15/22/56 after the watering for each campaign. Fifteen days after the watering roofs of the TE plots were opened permanently and both treatments received the same amount of rainfall from thereon.

2.2 Stable isotope labeling and measurements

2.2.1 Labeling and measurement of ^2H_2O

Labeling of the forest soil water was performed with deuterium (^{2}H) enriched water in 2019. The concentration of ^{2}H in water samples will be expressed with the delta notation (δ^2H in ‰) relative to the Vienna Standard Mean Ocean Water (VSMOW) as calculated by formula 5.

$$\delta^2H = \left(\frac{\frac{^2H}{^1H}\ sample}{\frac{^2H}{^1H}\ VSMOW} \right) * 1000 \qquad Formula\ 5$$

During the watering 3 TE and 3 CO plots were labeled with ^{2}H enriched water. The water used for labeling CO plots had a signature of 1468.36 ± 44.47 ‰ ^{2}H, mixed in 1000 L tanks with 99.9% deuterated water (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and for TE plots

289.30 ± 2.48 ‰ 2H, mixed in a 60’000 L water pillow with 99.9% deuterated water. After the labeling, the distribution of ^{2}H in the soil and trees was assessed (for details see chapter 2.4.6).

To measure the ^{2}H concentration in the water of soil or tree samples, the water was extracted via cryogenic vacuum extraction for two hours (West et al. 2006, Hafner et al. 2017). The extracted water was analyzed with an isotope-ratio-mass-spectrometer (IRMS, Isoprime 100, Elementar, Langenselbold, Germany) coupled to a multiflow system (222 XL Liquid Handler, Gilson, Middleton, USA) against two monitoring lab standards (heavy: ~ 130 ‰ and light: ~ -180 ‰).

Additionally, in-situ measurements of the ^{2}H concentrations of the xylem sapwood were performed with the xylem water isotope probe (XWIP, Volkmann et al. 2016). For this technique, gas-permeable probes were installed into the xylem sapwood at breast height and connected to a nitrogen gas supply and a cavity ring-down spectrometer (CRDS, L2120-i, Picarro, Santa Clara, CA, USA). For details about the full set-up and measurement procedure see Volkmann et al. (2016). Each probe was measured daily in the course of the watering against two monitoring standards (light standard: -74.22 ± 1.42 ‰ and heavy standard: 198.34 ± 2.87 ‰).

To calculate the fraction of labeled water (F_{label}, Formula 6) and the turnover of water pools in the soil and tree organs (for details see chapter 2.4.6), a two-endmember mixing model was used (e.g. IsoError, Phillips et al. 2005, Hafner et al. 2020).

$$F_{label} = \left(1 - \frac{\delta^2 H_{target} - \delta^2 H_{source}}{\delta^2 H_{targetprelabeling} - \delta^2 H_{source}}\right) * 100 \qquad Formula\ 6$$

With:

F_{label} = fraction of labeled water in the target tissue [%]

$\delta^2 H_{target}$ = signature of the tissue after labeling [‰]

$\delta^2 H_{source}$ = signature of the labeled source water [‰]

$\delta^2 H_{targetprelabeling}$ = signature of the tissue before labeling [‰]

2.2.2 Labeling and measurement of $^{13}CO_2$

Labeling of beech branches was conducted with 99% $^{13}CO_2$ gas (Campro scientific GmbH, Berlin, Germany) in two consecutive years (2105 and 2016). The signature of ^{13}C in tree samples will be expressed with the delta notation ($\delta^{13}C$ in ‰) relative to the Vienna Pee Dee Belemnite (VPDB) as calculated by formula 7.

$$\delta^{13}C = \left(\frac{\frac{^{13}C}{^{12}C}\ sample}{\frac{^{13}C}{^{12}C}\ VPDB} \right) * 1000 \quad Formula\ 7$$

During the labeling, one branch per tree was covered with a transparent plastic back (ceramic-coated PET/PE mix of ~115 µm thickness, Long Life for Art, Eichstetten, Germany) and sealed with tape and Terostat (Henkel AG & Co. KGaA, Düsseldorf, Germany). Bags were inflated to a volume of ~ 45 L and a volume of 150-300 ml $^{13}CO_2$ gas was added to the bag, depending on the branch size. Leaf samples were taken before and several times after labeling, microwaved for 30 s, oven-dried (64°C for two days) and ball milled (Retsch® MM200, Haan, Germany) to a fine powder. The $\delta^{13}C$ signature of the samples was measured with an isotope-ratio-mass-spectrometer coupled with an element analyzer (Isoprime, Elementar, Hanau, Germany).

Additionally, the ^{13}C signature of the branch CO_2 efflux was measured in situ with a Delta Ray Isotope Ratio Infrared Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) in two darkened chambers per tree before and after the labeling. Chambers were installed close to the stem (proximal) and close to the bag (distal) on each branch. Via the occurrence of the ^{13}C signal in the respective chambers and the distance between the two chambers, the sugar transport velocity of the branch phloem was calculated (for details see chapter 2.3.2).

2.3 Carbon relations assessment

2.3.1 Mean residence time of recent photoassimilates in leaves

From the $\delta^{13}C$ values in the leaves upon the labeling, the mean residence time (MRT in h) of recently fixed photoassimilates was calculated. Values were normalized over the maximal measured value and pre-labeling values for each tree individually (Formula 8).

$$\delta^{13}C_{norm} = \frac{\delta^{13}C_{tx} - \delta^{13}C_{t0}}{\delta^{13}C_{\max(t)}} \qquad (Formula\ 8)$$

With:

$\delta^{13}C_{norm}$ = normalized $\delta^{13}C$ values at timepoint x

$\delta^{13}C_{tx}$ = $\delta^{13}C$ value at timepoint x

$\delta^{13}C_{t0}$ = $\delta^{13}C$ value at timepoint 0 (pre-labeling)

$\delta^{13}C_{max(t)}$ = maximal $\delta^{13}C$ value during the whole experiment

The normalized $\delta^{13}C$ were plotted against the time (t in h) after labeling and an exponential curve was fitted (Formula 9).

$$\delta^{13}C_{norm} = e^{k*t} \qquad (Formula\ 9)$$

The MRT was estimated as the inverse of *k*.

2.3.2 Phloem transport velocity

The distance between the stem proximal and distal chamber used to assess the $\delta^{13}C$ signal of branch respiration was 1.3 ± 0.2 m. All trees showed a steady $\delta^{13}C$ in their branch respiration before the labeling and a strong increase soon after the labeling. For each chamber, a sigmoidal curve with a steady value before the labeling as the lower limit and a steady value after the labeling as the upper limit was fitted (Formula 10).

$$\delta^{13}C = d + \frac{a-d}{1+\left(e^{\frac{t-c}{b}}\right)} \qquad Formula\ 10$$

With:

a = lower limit

b = spreading of the sigmoid

c = inflection point

d = upper limit

t = time in h

Two different approaches were used to calculate the arrival of the signal, first via the inflection point (V_{ip} in cm h^{-1}) and second via the point of the biggest change in slope (first zero of the third derivative, V_{cos} in cm h^{-1}). The time difference between the arrival of the signal in the stem proximal and distal chamber was divided by the distance between the two chambers to acquire phloem transport velocity for each tree individually. Both velocity calculations showed very similar results and correlated strongly positive ($\rho = 0.778$, $P < 0.001$) and therefore the mean of both was used as the phloem transport velocity (V_{phloem} in cm h^{-1}).

2.3.3 Non-structural carbohydrate concentrations and pools

Samplings of NSCs were conducted in November 2013 (pre drought) and 2014 plus 2015 (one/two years of experimental summer drought) during early winter dormancy. For each tree fine roots (diameter smaller than 2 mm), coarse roots (average diameter about 5 cm), stem phloem/xylem (at BHD) and twig phloem/xylem (2-3 years old) were sampled each year. Additionally, in spruce 1-year old needles were sampled. All samples were stored on ice, microwaved for 30 s, oven-dried at 64°C for two days and ball-milled to a fine powder (Retsch® MM200, Haan, Germany). For each sample, the concentration of soluble carbohydrates (glucose, fructose and sucrose) and starch was measured with a High performance liquid chromatography with pulsed amperometric detection on a Dionex® ICS 3000 ion chromatography system equipped with an autosampler (Raessler et al. 2010). The analyzed substances, i.e. glucose, fructose, sucrose and starch account for 80% of the mobile carbon pool in beech and spruce (Hoch et al. 2003). Sample processing followed the protocol of Hartmann et al. (2013) for sugars and of Landhäusser et al. (2018) for starch and was conducted by collaboration partners from the Max-Planck Institute in Jena.

Biomass and growth were modeled for the NSC pool calculations with the process-based growth model BALANCE (Grote and Pretzsch 2002) for the years 2013 to 2015 for each measured tree individually, by collaboration partners from the chair for Forest Growth and Yield Science at the TU Munich. The model BALANCE includes several abiotic factors (e.g. weather, soil conditions or CO_2 concentrations) as well as biotic factors (e.g. stand structure and mixing, competition for recourses) and simulates biomass in dependence of carbon, nitrogen, water and light availability (Fontes et al. 2011). BALANCE has been validated against empirical studies several times regarding the carbon and water relations (e.g. Rötzer et al. 2010, 2012, 2013, Rötzer, Biber, et al. 2017, Rötzer, Häberle, et al. 2017). To simulate the growth of the TE trees, precipitation in the model was reduced in accordance with the treatment (i.e. no rainfall during the growing season).

To derive the NSC pools (for soluble carbohydrates and starch individually) of the single tree organs, the biomass was multiplied with the respective NSC concentration. Additionally, for the stem and twig pool sizes the contribution of the phloem (annulus area) and xylem (circle area) was considered by estimating the individual fractions of the overall stem/twig biomass (Formula 11).

$$\begin{aligned} NSC_{pool} &= concentration_{phloem} * biomass_{phloem} * proportion_{phloem} \\ &\quad + concentration_{xylem} * biomass_{xylem} * proportion_{xylem} \qquad Formula\ 11 \end{aligned}$$

2.4 Water relations parameters

2.4.1 Plant available water in the soil

To assess the soil water status of the plots, the soil water content (SWC in vol.-%) was measured in four different depths (0-7 cm, 10-30 cm, 30-50 cm and 50 -70 cm) and at three positions within each plot (Mono Beech – Mix Zone – Mono Spruce, Goisser et al. 2016) via Time Domain Reflectometry (TDR, TDR100 & 200, Campbell Scientific, Logan, CT, USA). From the SWC the plant available water in the soil (PAW in vol.-%) was derived, by subtracting the soil water content of the permanent wilting point (PWP in vol.-%). The PWP was estimated from the SWC of late summer 2015 when SWC reached a plateau for several weeks and did not further decrease. Therefore, the plants were no longer able to take up the remaining water from the soil and this level was defined as the PWP.

To estimate the drought stress dose over a growing season, the integrated soil water deficit (iSWD in $\mathrm{cm_{(H2O)}^{3}\ cm_{(soil)}^{-3}}$) was calculated. The maximum measured of each TDR sensor was considered as the saturated state of the soil and the integral was calculated for the desired period. A non-linear model was fitted for each TDR sensor individually and the integral was calculated for the same period as for the saturated state. The difference between these two integrals was then defined as the iSWD for the defined period.

2.4.2 Pre-dawn and minimum leaf water potential

The water status of the trees was assessed via leaf water potential measurements with a scholander pressure chamber (mod. 1505D, PMS Instrument Co., Albany, OR, USA). The leaf water potential was measured on short twigs taken in the canopy, stored in humidified, sealed plastic bags and measured within 20 min. Minimum leaf water potential (Ψ_{min} in MPa) was measured between 1-3 p.m. and pre-dawn leaf water potential (Ψ_{PD} in MPa) before sunrise between 2-4 a.m. on several days during the growing season.

2.4.3 Leaf turgor loss point and osmotic potential from pressure-volume curves

Water potential isotherms, also known as pressure-volume curves (PV-curves, von Willert et al. 1995) were measured to assess the water potential at turgor loss point (Ψ_{TLP} in MPa) and the osmotic potential at full turgor (π_O in MPa). This was done by periodically measuring the water potential and the weight of the twigs, while slowly letting them dry out. For measurements, small branches were harvested in the late afternoon and rehydrated overnight with distilled water to a water potential of -0.1 MPa or higher. Calculations for the Ψ_{TLP} and π_O were done with the spreadsheet tool of Sack and Pasquet-Kok (2010) and in accordance with Tomasella et al. (2018).

From the measured differences between CO and TE and the increase in the osmotic potential of leaves upon watering (see Article V) the amount of (remobilized) osmolytes was calculated for one example beech and spruce TE tree. With the mean leaf area of TE beech (95 m^2) and spruce (70 m^2) on the experimental site (personal communication Benjamin Hafner and Kyohsuke Hikino) and the specific leaf area (SLA in m^2 kg^{-1}), the leaf mass was calculated (Table 5). In combination with the leaf water content (LWC in %, see Article IV) of 2019 the overall leaf water mass per tree (LWM in L) was then estimated (Formula 12).

$$LWM = \frac{LA}{SLA} * LWC \qquad Formula\ 12$$

From the difference in the osmotic potential of D-6 between CO and TE and between TE on D-6 to D22 ($\Delta\pi_O$ in MPa) during the watering (see Article V) and a derivation of the van‘t Hoff formula (Formula 13) the change in concentration of the leaf water solution under drought and after the watering was calculated for TE beech and spruce.

$$\pi = c * R * T \qquad Formula\ 13$$

With π = osmotic pressure in Pa, c = concentration of the solution in mol m^{-3}, R = ideal gas constant (8.134 J K^{-1} mol^{-1}) and T = temperature in K (here 293.15 K). The leaf water solution concentration (c_{LW} = c from Formula 15 in mol L^{-1}) was then multiplied with the LWM to gain the molar osmolytes equivalent (OE in mol), which were remobilized after the watering until D22. By multiplying them with the molar mass of sucrose (M= 342 g mol^{-1}), pinitol (M= 194 g mol^{-1}) and prolin (M= 115 g mol^{-1}), which are important osmolytes in beech and spruce (Peuke et al. 2002, Schiop et al. 2017), the total amount osmolytes ($AO_{sucrose/pinitol/prolin}$ in g) was calculated.

Table 5: Amount of osmolytes (AO in g), needed for osmoregulation under drought (CO vs TE) and re-mobilized until D56 (Recovery$_{D56}$) upon drought release in leaves of mature beech and spruce in 2019.

	Beech		**Spruce**	
	CO vs TE	**Recovery$_{D22}$**	**CO vs TE**	**Recovery$_{D22}$**
LA (m^2)	95		70	
SLA (m^2 kg^{-1})	8.8 ± 2.2		4.0 ± 0.3	
LWC (%)	50.6 ± 6.3		51.8 ± 8.3	
LWM (L)	5.8 ± 1.6		9.1 ± 0.7	
$\Delta\pi_O$ (MPa)	0.83 ± 0.15	0.47 ± 0.18	0.77 ± 0.13	0.44 ± 0.19
c_{LW} (mol L^{-1})	0.35 ± 0.06	0.20 ± 0.07	0.32 ± 0.06	0.19 ± 0.08
OE (mol)	2.02 ± 0.37	1.14 ± 0.31	2.93 ± 0.50	1.67 ± 0.13
$AO_{Sucrose}$ (g)	690 ± 128	390 ± 108	1001 ± 171	573 ± 45
$AO_{Pinitol}$ (g)	392 ± 76	222 ± 61	568 ± 97	324 ± 25
AO_{Prolin} (g)	232 ± 43	131 ± 36	337 ± 58	192 ± 15

2.4.4 Xylem sap flow density at BHD

The xylem sap flow density per area sapwood was measured in the outermost part of the sapwood (0-2 cm) every 10 minutes with the Granier heat dissipation system (Granier 1987) at breast height. On each measured tree, two sensors were installed (north and south side) and both sensors were averaged to gain the mean sap flow density per day (u_{daily} in L dm^{-2} d^{-1}). Calculations and corrections for the sap flow density data followed the suggestions of Lundblad and Lindroth (2002).

2.4.5 Mean water uptake depth

The mean water uptake depth (WUD in cm) of both species and treatments was assessed via the ^2H profile in the soil water and the ^2H signature of the xylem sapwood water after 5 years of summer drought. To get the ^2H profile of the soil, cores to a depth of 70 cm were taken (Pürckhauer soil sampler (diameter: 2cm and length: 100cm)) and divided into 10 cm segments (e.g. 0-10 cm, 10-20 cm, etc.). Of each segment a soil sample was taken into an exetainer vial, the water was extracted and measured for its ^2H signature (for details see 2.2.1). An exponential regression was fitted for the ^2H signature against their respective depth. By applying the ^2H signature of the sapwood water to the exponential regression formula the WUD was estimated per tree.

2.4.6 Water distribution upon watering via isotope mixing models

To follow the distribution of labeled water upon the watering, soil and different tree organs (stem xylem and leaves) were sampled into exetainer vials regularly before and after the watering event. ^{2}H signatures of the soil and leaves were measured via IRMS and for the stem xylem water via CRDS (for details see chapter 2.2.1). The fraction of labeled water in each organ was then calculated via the mixing models described in chapter 2.2.1. For the soil samples, one endmember was the signature of the labeling water and the second endmember was the ^{2}H signature of the soil directly before the watering. For the stem xylem water and the leaf water, one endmember was determined as the soil signature of the respective WUD and the other endmember as the values measured before the start of the watering. Additionally, for the leaves, the evaporative enrichment (EER in ‰) of the leaf water was considered (Farquhar and Cernusak 2005). Therefore, the ^{2}H signature of twig water (sampled before and after the watering) was subtracted from the leaf water ^{2}H signature of the same sampling day (Formula 12).

$$EER = \delta^{2}H_{Leaf} - \delta^{2}H_{Twig} \qquad (Formula\ 12)$$

EER did not change during the period of the watering for both species.

2.5 Resilience calculations

As stated in chapter 1.3, definition and calculation for resilience followed in general Lloret et al. (2011). Resilience, recovery time and resilience velocity were calculated for Ψ_{PD}, Ψ_{TLP}, π_{O}, PAW, u_{daily} for 2019 (first recovery year) and 2020 (second recovery year). Resilience was calculated for each TE tree individually at several timepoints in comparison to the mean of CO trees (Formula 13).

$$Resilience_{i} = \frac{TE_{i,x}}{Mean\ (CO_{i})} \qquad (Formula\ 13)$$

With

$Resilience_{i}$: degree of resilience at timepoint i after stress release

$TE_{i,x}$: value of TE at timepoint i for tree number x

Mean (CO_{i}): mean value of all CO trees at timepoint i

This was done due to the lack of pre-stress values for most measured parameters. However, the initial situation of the water relations before the start of the experiment in 2013 was compared between TE and CO. Changes in CO trees over the years were assumed to be solely owed to climate and the aging of trees. The comparison of PAW of 2013 between CO and TE plots showed no differences. Therefore, can the initial situation concerning the water relations of CO and TE plots be considered as equal and the water consumption before the drought was very similar between CO and TE plots.

The recovery time was defined as the period a tree needed to reach a new stable level after stress release or a resilience value of 1. Therefore, the DOY at the start of the experiment was subtracted from the DOY when either requirement was met. From the resilience and the recovery period, the resilience velocity (V_R in days^{-1}) was calculated (Formula 2). When the mean value of the resilience reached 1, the time until full resilience (Day_{FR} in days) was determined. These values were then multiplied with the resilience value of day_{FR} to account for any deviation from 1 (e.g. overcompensation).

$$V_R = \frac{1 - Resilience_{\ 0}}{Day_{FR}} * Resilience_{\ FR} \qquad (Formula\ 14)$$

With $Resilience_0$ = resilience value before the watering, Day_{FR} = day after the watering when resilience reached 1 or a stable level and $Resilience_{FR}$ = resilience value at the day of full resilience, to account for any deviances from 1 (e.g. by overcompensation).

2.6 General statistical methods

Within this chapter general statistical concepts are explained that were used for most experiments, for details on the statistical approaches refer to the individual articles.

Evaluations of data were performed with R (R Development Core Team 2008) in RStudio (RStudio Team 2015). Whenever necessary (e.g. statistical comparison of means) the homogeneity of variances was tested beforehand (e.g. Levene test). For all fitted models, the normality of residuals was tested (Q-Q-plot or Shapiro test). Whenever homogeneity of variances or the normality of residuals was not given, data were transformed to meet requirements or non-parametric tests were used.

Most data were analyzed with linear mixed effect models (Zuur et al. 2009). In most cases, the species (beech vs. spruce), treatment (CO vs. TE), competition (Mono vs. Mix) and their interactions were used as fixed factors. As a random factor mostly the tree number nested in the plot identity was used to account for any spatial effects or repeated measurements (e.g. for the comparisons of different years).

3. Abstracts and contributions to the single publications

3.1 Article I: The Kroof experiment – realization and efficacy of a recurrent drought experiment plus recovery in a beech/spruce forest

Grams TEE*, **Hesse BD***, Gebhardt T, Weikl F, Rötzer T, Kovacs B, Hikino K, Hafner BD, Brunn M, Bauerle T, Häberle K-H, Pretzsch H, Pritsch K (2021) The Kroof experiment: realization and efficacy of a recurrent drought experiment plus recovery in a beech/spruce forest. Ecosphere 12.

Forest ecosystems play a central role in global water and carbon cycles, yet the impact of global climate change, in particular drought, on trees and forests is poorly understood. Therefore, there is an urgent need for forest-scale experiments in improving our understanding of trees' responses to extreme drought events and subsequent recovery under field conditions.

Here, we present the design and efficacy of a novel throughfall-exclusion experiment with retractable roofs in a mature forest allowing for flexible drought and recovery periods. A total of 12 plots (144 ± 26 m^2 on average) with 3-7 European beech and Norway spruce trees each were established by root trenching to a depth of one meter, four years prior to the experiment. Subsequent installation of roofs ($n = 6$) allowed for the removal of throughfall precipitation and almost a complete non-availability of soil water in the upper 70 cm during five subsequent growing seasons, i.e. 2014-2018. This reduction in available soil water resulted in pre-dawn leaf water potentials down to -1.8 MPa in mature trees. Stem diameter growth decreased by 30% in beech and 70% in spruce, and fine root abundance was reduced by 57% in beech and 73% in spruce compared to controls. After only one growing season, the mycorrhizal community composition changed in response to drought. Careful watering of hydrophobic forest soils in early summer of 2019 resulted in recovered pre-dawn leaf water potentials of drought stressed trees within one week. Recovery of stem diameter growth, however, did not occur within the same growing season and remained reduced by 33% in beech and 69% in spruce compared to controls.

The implemented throughfall-exclusion system imposed recurrent seasonal drought events on a mature beech/spruce forest with high efficacy. Shifts in community composition of mycorrhizae in parallel to tree growth decline, advocate for a more holistic view on forest scale drought and watering experiments, particularly in light of more frequently predicted drought events in the future. The perennial nature of mature trees and their subsequent slow recovery

from drought, i.e. over multiple growing seasons, argues for more long-term experiments that span several years.

Contributions: I finalized the experimental design, collected/processed the samples, analyzed/interpreted the data and drafted the final manuscript. Thorsten Grams originally designed the study, interpreted the data and drafted a first version of the manuscript. Timo Gebhardt, Fabian Weikl, Ben Kovacs, Kyohsuke Hikino, Benjamin Hafner and Melanie Brunn helped with the sampling and analyzing the data. Thomas Rötzer, Taryn Bauerle, Karl-Heinz Häberle, Hans Pretzsch and Karin Pritsch originally designed the study and helped interpreting the data. All co-authors revised the manuscript and gave final approval before publication. About 50% of the overall work was done by me.

3.2 Article II: Repeated summer drought delays sugar export from the leaf and impairs phloem transport in mature beech

Hesse BD, Goisser M, Hartmann H, Grams TEE (2019) Repeated summer drought delays sugar export from the leaf and impairs phloem transport in mature beech. Tree Physiol 39:192–200.

Phloem sustains maintenance and growth processes through transport of sugars from source to sink organs. Under low water availability, tree functioning is impaired, i.e., growth/photosynthesis decline and phloem transport may be hindered. In a 3-year throughfall exclusion (TE) experiment on mature European beech (Fagus sylvatica L.), we conducted $^{13}CO_2$ branch labeling to investigate translocation of recently fixed photoassimilates under experimental drought over 2 years (2015 and 2016). We hypothesized (H1) that mean residence time of photoassimilates in leaves (MRT) increases, whereas (H2) phloem transport velocity (V_{phloem}) decreases under drought. Transport of carbohydrates in the phloem was assessed via $\delta^{13}C$ of CO_2 efflux measured at two branch positions following $^{13}CO_2$ labeling. Pre-dawn water potential (Ψ_{PD}) and time-integrated soil water deficit (iSWD) were used to quantify drought stress. The MRT increased by 46% from 32.1 ± 5.4 h in control (CO) to 46.9 ± 12.3 h in TE trees, supporting H1, and positively correlated ($P < 0.001$) with iSWD. Confirming H2, V_{phloem} in 2016 decreased by 47% from 20.7 ± 5.8 cm h–1 in CO to 11.0 ± 2.9 cm h–1 in TE trees and positively correlated with Ψ_{PD} ($P = 0.001$). We suggest that the positive correlation between MRT and iSWD is a result of the accumulation of osmolytes maintaining cell turgor in the leaves under longer drought periods. Furthermore, we propose that the positive correlation between V_{phloem} and Ψ_{PD} is due to a lower water uptake of phloem conduits from surrounding tissues under increasing drought leading to a higher phloem sap viscosity and lower V_{phloem}.

The two mechanisms increasing MRT and reducing V_{phloem} respond differently to low water availability and impair trees' carbon translocation under drought.

Contributions: I finalized the experimental design, collected/processed the samples, analyzed/interpreted the data and drafted the manuscript. Michael Goisser originally designed the study and helped during sampling. Henrik Hartmann processed the NSC samples. Thorsten Grams originally designed the study, helped with data collection and supported me in data interpretation. All co-authors revised the manuscript and gave final approval before publication. About 75% of the work was done by me.

3.3 Article III: Two years of experimental summer drought cut down NSC pools, but not NSC tissue concentrations and reproductive investment in a mixed beech/spruce forest.

Hesse, B.D., Hartmann, H., Rötzer, T., Landhäusser, S.M., Goisser, M., Weikl, F., Pritsch, K., Grams, T.E.E.E., 2021. Mature beech and spruce trees under drought – Higher C investment in reproduction at the expense of whole-tree NSC stores. Environ. Exp. Bot. 191.

Under drought, potential plant death from depletion of carbon (C) stores, referred to as carbon starvation, is thought to result from negative carbon balance during (partial) stomatal closure. As evidence for C starvation is rare for mature trees, we investigated the C dynamics in mature beech and spruce under drought, focusing on non-structural carbohydrates (NSC) as an indicator of their C balance. Trees were exposed to complete exclusion of precipitation throughfall over two consecutive growing seasons. We assessed NSC concentrations during the early dormant season in leaves, twigs, stem phloem/xylem, coarse and fine roots. Up-scaling to whole-tree NSC pools was achieved using a process-based single-tree model ("BALANCE"), estimating tree biomass increment. While there were distinct differences in NSC concentration among different tissues in both beech and spruce (root < stem < twig < leaves and xylem < phloem), drought did not affect NSC concentrations. However, compared to controls, the whole-tree NSC pool size significantly decreased under drought in both beech (42 %) and spruce (36 %), in parallel to a significant growth decline of overall 52 % and 57 %, respectively. Nevertheless, drought-stressed beech and spruce invested almost twice as much C in reproductive structures relative to total C investment (i.e. 6.0 ± 3.3 and 52.3 ± 8.71 %) compared to control trees (3.1 ± 1.8 and 29.2 ± 7.8 %). This highlights the high priority of C investment into reproduction relative to growth under drought. Given that NSC concentrations are maintained even under severe drought over two growing seasons, NSC pool sizes appear to be a better proxy to assess whole-tree's carbon status in mature trees. Overall, trees maintained NSC availability, avoiding carbon starvation, by downregulating a major C sink (i.e. growth) while upholding reproduction.

Contributions: I finalized the experimental design, collected/processed the samples, analyzed/interpreted the data and drafted the manuscript. Michael Goisser originally designed the study and helped during sampling. Henrik Hartmann and Simon Landhäusser processed and measured all NSC samples. Thomas Rötzer computed the biomass data with the model BALANCE. Fabian Weikl and Karin Pritsch helped in processing/measuring the samples. Thorsten Grams originally designed the study and supported me in data interpretation. All co-authors revised the manuscript and gave final approval before publication. About 75% of the work was done by me.

3.4 Article IV: Location vs allocation – the fate of newly available water under recovery after repeated drought in a mature stand of beech and spruce

Hesse BD, Hafner BD, Gebhardt T, Seeger S, Hikino K, Stempfle E, Seiler R, Häberle KH, Weiler M, Grams TEE (2021) Location vs allocation – the fate of newly available water under recovery after repeated drought in a mature stand of beech and spruce. Draft

Upon drought stress release, the uptake and distribution of water within the (dehydrated) plant tissues would be one of the first processes to happen. In tall plants (e.g. trees) this can pose a major challenge as the water transport along the soil-plant continuum might be disrupted at several points (e.g. soil root contact, xylem embolisms). Here we deuterium-labelled the newly available water in a stand of mature European beech and Norway spruce in control (CO) and repeatedly droughted (i.e. throughfall-exclusion, TE) plots. We assessed the deuterium concentration in the soil, stem xylem and leaf water pools, before and after the drought stress release to calculate the turnover times of single water pools. Irrespective of the previous drought, the labeling was distributed within 24 hours in the soil and quickly refilled the TE soil to a similar level as CO. However, trees showed a significant delay in the water arrival on TE compared to CO plots, with spruce (on day 7) being more delayed than beech (on day 4), for both the xylem and leaf water. A similar pattern was found for the turnover of water pools in the studied tissues, which happened quickly in CO, but was slower for both species in previously drought stressed trees. One reason for this delay might be the loss of vital fine roots during the drought period. Especially in spruce the loss of fine roots was tremendous, as most fine roots are close to the soil surface and therefore exposed to drought conditions very early. Another reason might be the difference in usage of the internal water storage, which is supposedly more important in the isohydric spruce than in the anisohydric beech. The internal water storage and its daily/seasonal contribution to transpiration is still a black box but could help in understanding trees' reactions to drought (release).

Contributions: I finalized the experimental design, collected/processed the samples, analyzed/interpreted the data and drafted the manuscript. Timo Gebhardt, Kyohsuke Hikino, Eva Stempfle and Regina Seiler helped in processing/measuring the samples. Benjamin Hafner helped to interpret the data. Stefan Seeger and Markus Weiler set up the online measurements for the stem water isotopic signature and helped to interpret the data. Thorsten Grams provided support for the study design and data interpretation. All co-authors will revise the manuscript before publication. About 80% of the work was done by me.

3.5 Article V: Back to Routine? – Recovery Time and Resilience of the Water-Relations of Mature Beech and Spruce after Recurrent Summer Drought

Hesse BD, Gebhardt T, Hafner BD, Hikino K, Reitsam A, Häberle KH, Grams TEE (2021) Back to Routine? – Recovery Time and Resilience of the Water-Relations of Mature Beech and Spruce after Recurrent Summer Drought. Submitted to Global Change Biology in September 2021.

The ability to acclimate to a changing environment defines the potential of survival for every species. Acclimation processes include the ability to endure and recover from abiotic stress events (e.g. drought), which challenges especially immobile and long-living species, such as trees. The ability and extent of stress resilience depend on site conditions, severity and duration of the stress, but may also be species dependent. European beech (Fagus sylvatica) and Norway spruce (Picea abies), two economically and ecologically important tree species in European temperate forests, are threatened by increasing drought frequency. We tested the resilience and recovery time of the water relations in mature beech and spruce upon stress release after five years of recurrent summer droughts in a throughfall-exclusion experiment in southern Germany. We measured pre-dawn leaf water potential, sap flow density and leaf osmoregulation under drought and two years after drought release. Both species were severely impaired by drought with an average decrease of 33% in water related parameters. Nevertheless, both species displayed high resilience, reaching values close to control trees during two years after drought release. Beech reached full resilience in water potential and sap flow density earlier than spruce and even exceeded sap flow density of control trees in the second year of recovery. Differences found in recovery between beech and spruce appear to be related to their "opposing" drought stress mitigation strategies, with beech being more anisohydric and spruce more isohydric. Additionally, species-specific recovery times to full resilience were found for different physiological parameters, with water potential recovering faster (hours to days) than osmoregulation (weeks) and the sap flow density (months-years). With the anticipated increased frequency of drought events under ongoing climate change, short recovery times will be beneficial for plant fitness, with faster acclimating species benefiting at the expense of slower acclimating species.

Contributions: I finalized the experimental design, collected/processed the samples, analyzed/interpreted the data and drafted the manuscript. Timo Gebhardt processed the xylem sap flow data and helped to interpret the data. Kyohsuke Hikino and Anna Reitsam helped in processing/measuring the samples. Benjamin Hafner and Karl-Heinz Häberle helped to interpret the data. Thorsten Grams provided support for the study design and data interpretation. All co-authors will revise the manuscript before publication. About 85% of the work was done by me.

4. General discussion

In the general discussion, the results of all experiments are brought together and further evaluated. The detailed discussions about the single experiments can be found in the respective articles in the appendix.

4.1 Tree survival in experimental and natural drought events in mature forest stands

Experimental approaches, such as KROOF, and observations of natural drought events are two essential tools to understand the effects of drought stress on tree physiology. However, both approaches have benefits as well as disadvantages. Observations of natural drought events, for example, cannot be planned ahead but happen spontaneously, e.g. during long-term studies, and are often a combination of several abiotic (drought and heat) and biotic stresses (e.g. insect calamities). Nevertheless, drought induced tree mortality has been observed in the last decades very frequently worldwide and was supposedly connected to hydraulic failure of the xylem system (Allen et al. 2010, Klein 2015, Schuldt et al. 2020). One example of a recent natural drought year was 2018 in Central Europe, e.g. across Austria, Germany and Switzerland (DACH region). As in many natural drought events, also 2018 was accompanied by a severe heatwave (Hartmann, Moura, et al. 2018, Schuldt et al. 2020) with an increased temperature by on average 3.3 °C compared to the long-term mean (Schuldt et al. 2020). In most cases high temperatures lead to an increase in the vapor pressure deficit (VPD), which is a very important factor to define the plants performance (Grossiord et al. 2020). Initially, a higher VPD would increase the evapotranspiration of whole ecosystems (Breshears et al. 2013). The increase of water evaporating from the soil would decrease the plant available water and could therefore increase the drought stress intensity for plants. The transpiration by plants would initially be increased under high VPD, but plants sooner (isohydric behavior) or later (anisohydric behavior) would close their stomata, in order to counteract the high water loss (Breshears et al. 2013, Grossiord et al. 2017). With closed stomata, the cooling effect of transpiration is lost for the leaves and the leaf temperature could increase strongly (Gates 1968, Monteiro et al. 2016), which could lead to a leaf/canopy dieback (Perez and Feeley 2020). Under experimental drought, e.g. in field experiments with mature trees, this situation mustn't occur, as the air temperature cannot be controlled. But the increasing temperatures in leaves allow, up to a certain degree, to disentangle the effects of drought and heat induced impairments of the tree physiology. Additionally, it would create an ideal setting to test for the influence of foliar water uptake (FWU), to mitigate the drought stress impact (Berry et al. 2019). The magnitude of FWU seems to be very species dependent and also related to the level of isohydry (Schreel et al. 2019). However, FWU is only supposed to happen under moderate drought stress and might cease with ongoing leaf dehydration (Limm et al. 2009). While it has been demonstrated, that FUW can benefit the leaves/branches it remains unclear if FUW can be distributed also to the roots in mature trees (Schreel and Steppe 2019). Nevertheless, experiments with seedlings showed a release of FWU into the soil, indicating a complete reversal of the water flow along the SPAC (Berry et al. 2019).

Such different framework conditions might also explain the relatively low mortality rates of the experimental drought approach in KROOF over five years (Pretzsch et al. 2020) compared to high mortality in the natural drought in 2018 (Schuldt et al. 2020). One important driver for tree mortality, i.e. insect calamities, had been partially excluded in the KROOF experiment by spraying an insecticide. This was done to protect the very susceptible spruce from bark beetle infestations (Synek et al. 2020). However, in the KROOF experiment with its harsh drought treatment (see Article I), other restrictions, such as heat or increased VPD, have not been included constantly artificially. So, while trees experiencing the natural drought of 2018 were confronted with multiple stress (drought, heat, high VPD, etc.) in a short time, trees in the KROOF experiment only had to deal with mostly one stressor (drought). The reason why the natural drought of 2018 didn't have such a strong effect on the KROOF experiment is due to the relatively mild weather conditions at the experimental site. While the temperatures were significantly higher than the long-term average by about 4°C, the precipitation was very similar to the long-term average (+11 mm). So such effects as FWU might have helped the trees of the KROOF experiment to survive the heat and drought, while in places with fewer precipitation trees succumb to the drought/heat (Schuldt et al. 2020). Additionally, trees in the KROOF experiment must have developed some acclimations over time in order to mitigate the drought, which can be seen in the stabilization/recovery of stem growth already during the repeated drought years (Pretzsch et al. 2020).

4.2 Water/carbon related physiology under repeated drought – differences between intra- and interspecific competition?

Impairments of forests under drought is a well-studied field and it has often been shown that tree performance decreases under stress. However, studies also suggested that mixed species forests are superior in dealing with abiotic stress to monocultures, regarding growth and stability (Pretzsch 2013, Metz et al. 2016, Hafner 2020). According to the stress-gradient-hypothesis, facilitative interactions between individuals are more frequent under more stressful conditions (e.g. Holmgren and Scheffer 2010, Michalet et al. 2014). Such facilitative effects, e.g. hydraulic redistribution (Hafner 2020), are likely to be higher in interspecific interactions (Pretzsch et al. 2013, del Río et al. 2014), as different complementary niches are used by different species (e.g. rooting depth, Bello et al. 2019). Such facilitative interactions are related to phenological/morphological differences between species, which are often complementary among interacting species (e.g. stand structure diversity, Rothe and Binkley 2001, Forrester and Bauhus 2016), but the physiological implications are still widely unknown for mature forest stands. In the KROOF experiment, beech and spruce growing in interspecific competition showed a higher growth increment than trees growing in intraspecific competition (Pretzsch et al. 2020). However, the intensity of drought stress was the same for both competition situations, as indicated by the pre-dawn water potential (Figure 7).

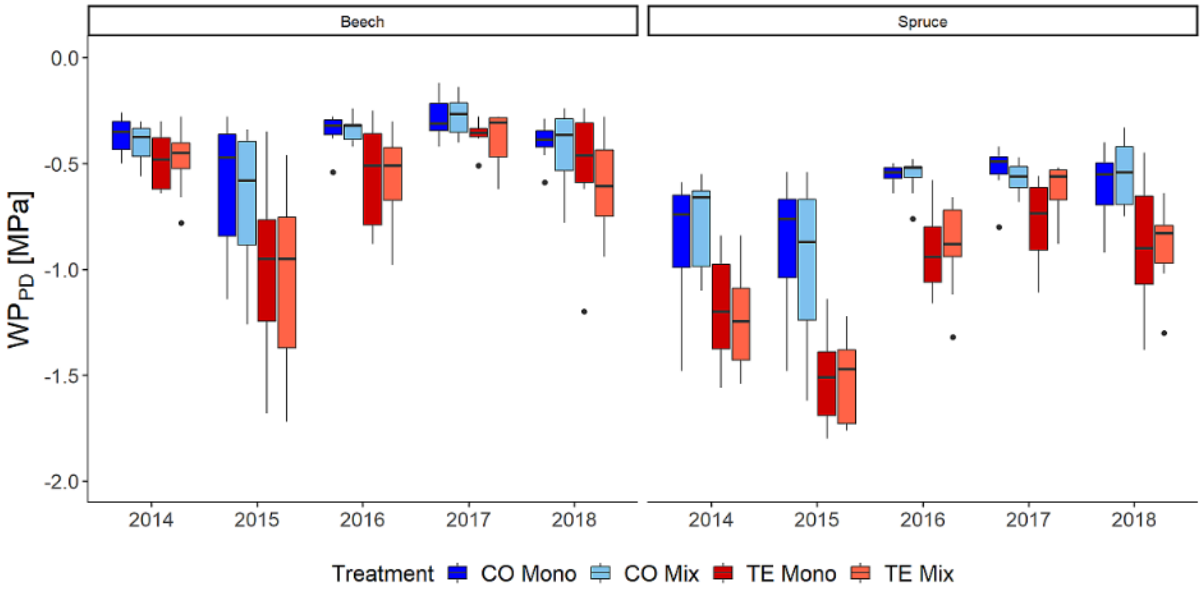


Figure 7: Pre-dawn water potential of beech and spruce in the KROOF experiment from 2014 to 2018. Dark blue = control mono, light blue = control mix, dark red = drought stress mono and light red = drought stress mix (data redrawn from article I). The only differences that occurred were between CO vs TE but not between competitions (intraspecific= mono vs interspecific = mix).

Both species and competition situations displayed similar behavior in their water relations (water potential and osmoregulation) and no significant differences in physiology have been found between the intra- and interspecific competition situation. However, the plant available water to 70 cm depth in the soil displayed a different pattern. The CO trees seemed to have consumed a similar amount of water in all years (similar annual courses of the blue lines, Figure 8). However, in the TE interspecific zone, the water consumption of drought stressed trees became gradually lower from 2016 till 2018 (proximity between blue and red line in mix, Figure 8). This would indicate a lower water consumption of the trees in the interspecific competition, while in intraspecific competition, especially in beech, trees spend all available water (shown by a stable plateau close to 0 from about DOY 180 on, Figure 8). Studies focusing on drought and the interaction between soil moisture and water use found similar results with drought stressed trees decreasing their sensitivity of transpiration to VPD (Grossiord et al. 2018 and citations within).

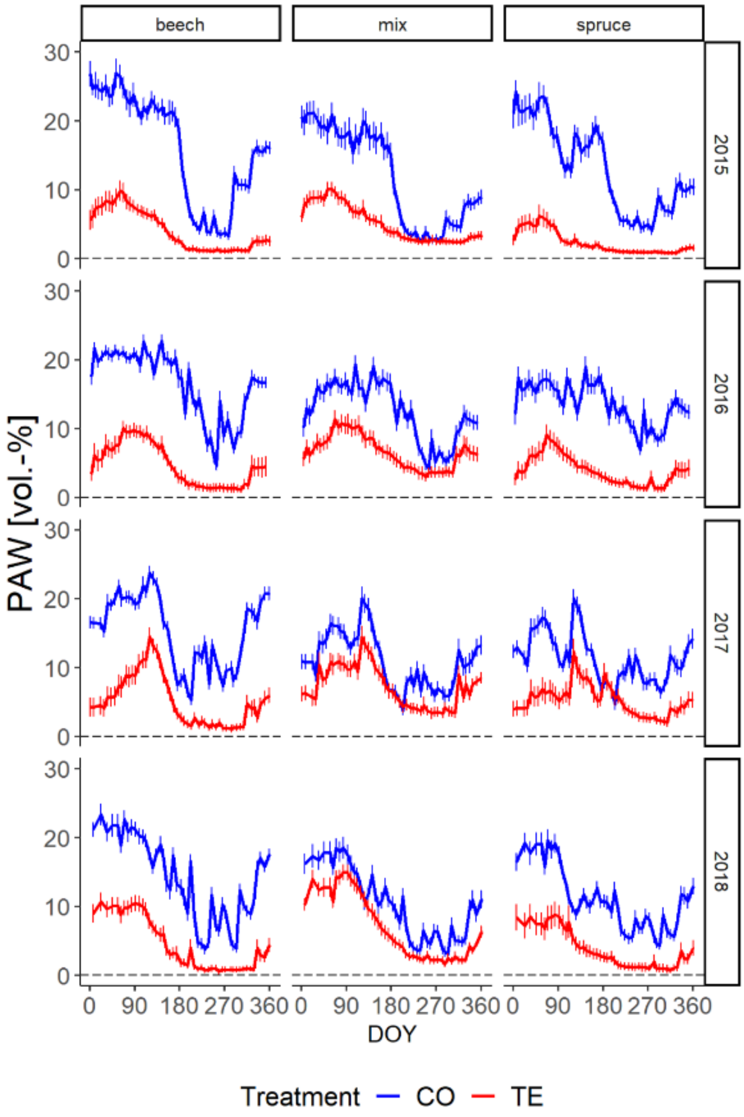


Figure 8: Plant available water (PAW in vol.-%) from 0 to 70 cm depth for CO (blue) and TE (red) in the intraspecific beech, interspecific (mix) and intraspecific spruce competition zone for 2015 to 2018. Data are redrawn from article I.

Additionally, in 2019 before the watering the xylem sap flow density of drought stressed trees were not significantly different between the interspecific and the intraspecific competition for both species (Figure 9). However, for both species, the xylem sap flow density was significantly reduced compared to CO trees (see Article V). The reduction of the xylem sap flow density suggests partially closed stomata in TE trees due to the drought treatment (Stöhr and Lösch 2004, Köcher et al. 2009a), which were higher in spruce than in beech. The differences in the water consumption since 2016, shown here by the PAW, indicate that in the same time less water is consumed in the interspecific competition, as well as in the intraspecific spruce competition zone compared to the intraspecific beech competition zone. Together with very similar xylem sap flow density between the two competitions for both species, this would indicate lower overall water consumption of the spruce trees from 2016 on. This lower water consumption is probably linked to a reduction of the total leaf area in spruce on the TE plots (personal observation). The reduction of the transpiring area would also fit to the heavy loss of fine roots and mycorrhizae association, found on the same experimental site under drought (Nickel et al. 2018, Zwetsloot and Bauerle 2021, see Article I), which might have reduced the water uptake capacity of the trees.

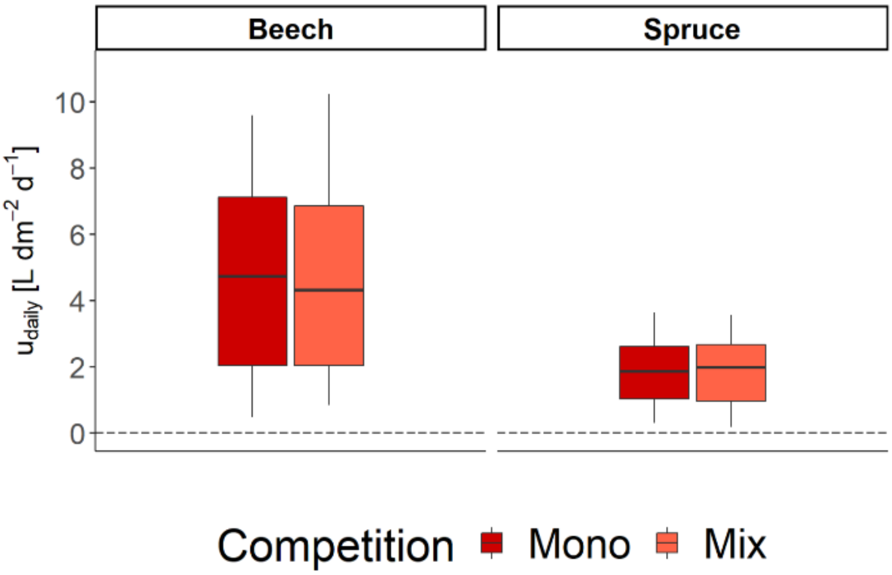


Figure 9: Xylem sap flow density of 2019 before the watering of TE beech and spruce. Dark red = intraspecific competition (mono) and light red = interspecific competition (mix). No differences were found between competitions for both species. Data are redrawn from article V.

Despite the reduction of the C uptake under drought for both species (Zwetsloot and Bauerle 2021), estimated here by the reduction of xylem sap flow density, no differences were found in the NSC concentrations among different tree organs from twigs/branches over the stem down to the roots (Figure 10). However, combined with the recently published differences of stem growth increments between intra- and interspecific competition (Pretzsch et al. 2020), the overall NSC stores might have differed between the two competitions. As the NSC stores are a combination of the NSC concentrations (Figure 10) and the overall biomass for each tree organ, over the whole drought period the aboveground NSC stores were supposedly higher in spruce trees growing in interspecific competition compared to the intraspecific competition. As the phloem transport velocity seems to be highly correlated with the pre-dawn leaf water potential (see Article 2, Hikino et al. in prep.), no differences can be expected between competitions (data not shown) as the pre-dawn water potential was very similar in intra- and interspecific competition (Figure 7).

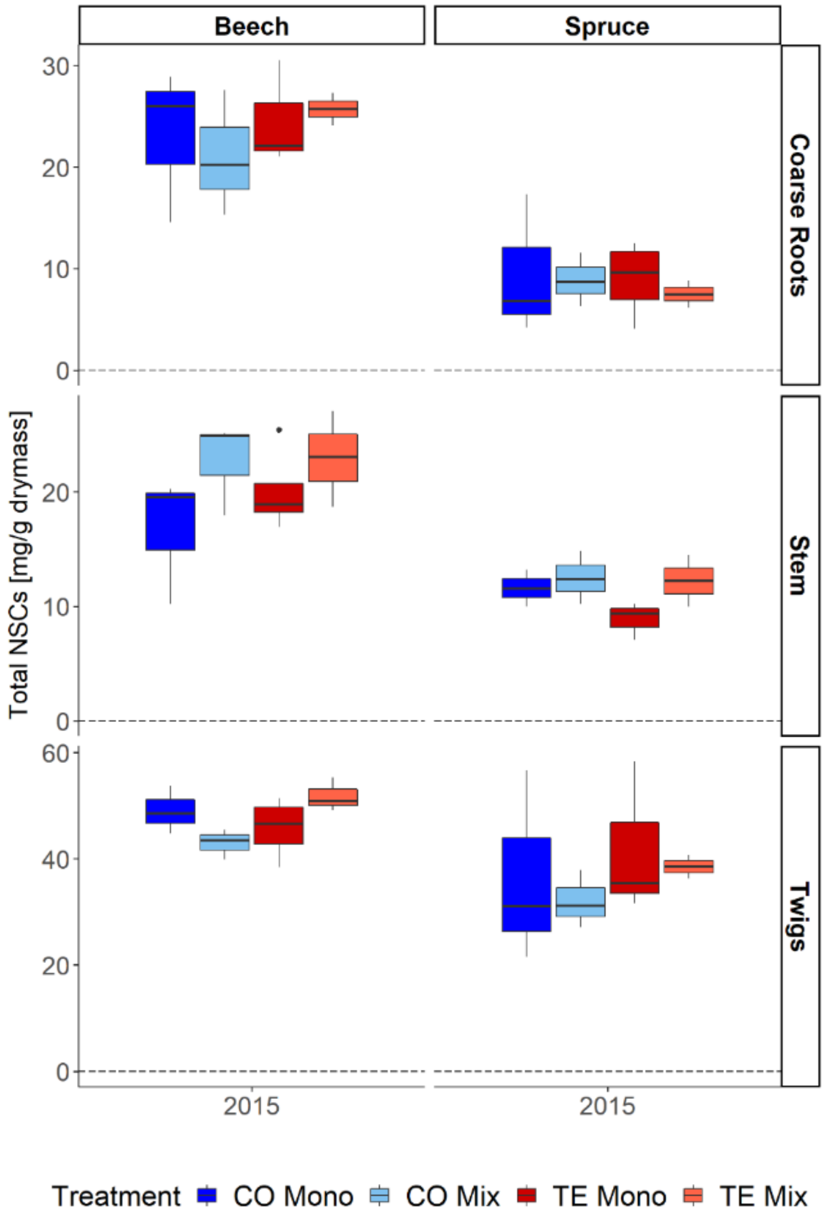


Figure 10: NSC concentrations in coarse roots, stem (weighted mean of xylem and phloem tissue) and twigs (weighted mean of xylem and phloem tissue) for beech and spruce in 2015. Dark blue = control mono, light blue = control mix, dark red = drought stress mono and light red = drought stress mix (data redrawn from article III). Note the different scales for each tree organ.

Despite the differences in growth increment between intra- and interspecific competition, physiological measurements did not show clear differences between the two competition situations. However, morphological changes/reductions have been found in the fine root/mycorrhiza abundance (Nickel et al. 2018, Zwetsloot and Bauerle 2021, see Article I) and supposedly in the leaf area for spruce. In the interspecific competition the reduction of the fine root abundance/production was less strong only for spruce but not for beech (Zwetsloot and Bauerle 2021), which could be connected to higher facilitative effects such as hydraulic redistribution (Hafner 2020) or plant communication via mycorrhiza (Gorzelak et al. 2015) in mixed forest stands under drought. Additionally, as spruce is known to preserve its roots under drought (Nikolova et al. 2020), the higher PAW probably elongated the life span of the fine roots in the interspecific competition zone for spruce. Therefore, probably slightly less C was needed to build new fine roots, while in the intraspecific competition zone such benefit did not occur for spruce. Perhaps the leaf area, due to the higher PAW, was also less strong reduced in the interspecific competition in comparison to the intraspecific competition for spruce. Within the first and second years of drought both species were strongly impaired in their physiology, which was also reflected in the reduced stem growth increment (Pretzsch et al. 2020). Therefore, during the first but particularly during the second drought year, the morphology (e.g. fine root abundance or leaf area) was strongly reduce in spruce (see Article I), especially in the intraspecific competition zone (Zwetsloot and Bauerle 2021). These morphological changes probably enabled the trees to maintain a reduced but stable level of water and carbon related physiology. The plasticity of physiological vs. morphological acclimations are still under debate, especially for belowground parameters (Grams and Andersen 2007), but several studies indicate that a change of morphology could help to maintain physiology under long-term abiotic stress in plants (Grassi and Bagnaresi 2001, Delagrange et al. 2004, Gao et al. 2017, Augustine and Reinhardt 2019). Understanding to what extent the morphological plasticity is species dependent or influenced by the neighboring tree species could help to prepare forest stands for the ongoing climate change. However, the drought stress seemed to not only reduce the physiology but, as a first consequence, to also reduce the fine root abundance in both species. The damage of the water up-taking system might have increased the general C demand belowground for increased maintenance, repair and re-growth processes.

4.3 Local carbon starvation – a crucial bottleneck in the trees' water-carbon relations under drought?

The carbon balance was strongly impaired by the repeated drought stress, represented by strongly reduced NSC storages (see Article III), reduced C transport (see Article II) and growth increment (Pretzsch et al. 2020, Zwetsloot and Bauerle 2021) for both species. However, no overall signs of carbon starvation (see Article III) and hydraulic failure (Tomasella 2018) have been found, still, some individuals succumbed due to the drought treatment (Pretzsch et al. 2020). While neither carbon starvation nor hydraulic failure was responsible for tree death alone, most likely a combination of both could trigger tree death (McDowell 2011). Such a combined cause for tree death would likely be linked to mechanisms that are a close conjunction between water and carbon related physiology. The reduced NSC pools and the slower C transport (see Article II and III) could critically endanger C sinks which are further away from the primary C source, i.e. leaves, and cause local carbon starvation. One of these aforementioned C sinks could be fine roots of mature trees. Beech and spruce are known to differently cope with fine root loss due to drought stress. While beech would preferentially shed endangered fine roots and build new ones, spruce would try to maintain its fine roots, e.g. via suberization (Nikolova et al. 2020). In the KROOF experiment, both species suffered heavy losses of fine roots due to the repeated drought treatment (Zwetsloot and Bauerle 2021, see Article I). A significant amount of carbohydrates and water would be needed for regrowth/maintenance of fine roots and other drought-imposed acclimations/countermeasures. Such acclimations/countermeasures include for example the repair of xylem embolisms, defense against pests or osmoregulation.

As an important direct interaction between the carbon and water relations, the repair of xylem embolisms (Klein et al. 2018) is directly linked to the occurrence of hydraulic failure (Sevanto et al. 2014). Different species have displayed different capacities for xylem embolism repair, but in most cases a decrease in the NSC concentration was observed, indicating the repair mechanism to be a C demanding process (Savi et al. 2016, Nardini et al. 2017, Trifilò et al. 2019). Under drought stress, this would add another maintenance C sink next to respiration and deficient xylem embolism repair could potentially facilitate hydraulic failure. As root xylem seems to be more vulnerable to cavitation, the demand for repair in the root tissue would be increased compared to stem tissue (Kavanagh et al. 1999, McElrone et al. 2004, Maherali et al. 2006). This would add to the local carbon starvation scenario, as trees either need more C for the repair of embolisms or a higher turnover rate of fine roots if they abandon cavitated roots instead of repairing them (Nikolova et al. 2020). However, xylem embolism repair capacity seems to be species dependent (Brodersen and McElrone 2013) and further depends on the cellular anatomy (e.g. amount of parenchyma cells, Kiorapostolou et al. 2019). Therefore, the C demand will probably strongly vary between species or even genotypes. Studies in juvenile beech and spruce revealed, that only spruce seems to be able to repair xylem embolisms but not beech (Tomasella et al. 2017, Tomasella, Nardini, et al. 2018).

Plant defense mechanisms more likely going to be triggered under drought stress than well-watered conditions, as “weakened” plants are considered more vulnerable to biotic attacks (Anderegg et al. 2015). Plant defense compounds, mostly secondary metabolites, can either accumulate under drought stress (induced response) or always be present (constitutive response), which depends on species, age and drought intensity (Jamieson et al. 2012, Holopainen et al. 2018, Huang, Kautz, et al. 2019). Under the growing threat of bark beetle infestations a functional defense is important, especially for spruce (Netherer et al. 2019). For example in 2015, several spruce trees of the TE plots were attacked by bark beetle and later succumbed, while no CO spruce trees were attacked (see Article I). In juvenile spruce trees, defense seems to be maintained at the cost of growth and even NSC storages under C limitation (Huang, Hammerbacher, et al. 2019), but it remains unclear if mature spruce trees would react similarly.

Another important C sink, especially under drought stress, is the osmoregulation of plant tissues (Hartmann and Trumbore 2016), which was found for both species under the TE treatment, with a high activity in leaves (Tomasella et al. 2018, see Article V). The osmoregulatory acclimations remained stable even during five years of summer drought (similar results of Tomasella et al. 2018 and article V), which is indicating a high priority for osmoregulation under drought in mature trees. For the osmotic acclimation of leaves in beech and spruce in 2019 respectively about 690 ± 128 g and 1001 ± 171 g of sucrose have been needed (Table 5). Taking the different assimilations rates for CO_2 of the two species under drought (personal communication Kyohsuke Hikino) into account, the effort, needed to generate this level of osmoregulation, would be very different between the two species. For beech, about 10 days of assimilated sucrose are enough to generate the osmotic acclimations in the leaves, while for spruce about 46 days are needed. As osmoregulation is not only occurring in leaves but most in living plant tissues (Morgan 1984), the total amount of sugars needed for the osmoregulation in trees are probably significantly higher. Additionally, this raises the question whether the whole NSC pools can be seen as metabolically available (Prescott et al. 2020, Hartmann et al. 2021), because sugars/sugar alcohols used for osmoregulation are unlikely to be re-mobilized or respired, so they should probably not be considered as “available” for as long as the drought continues.

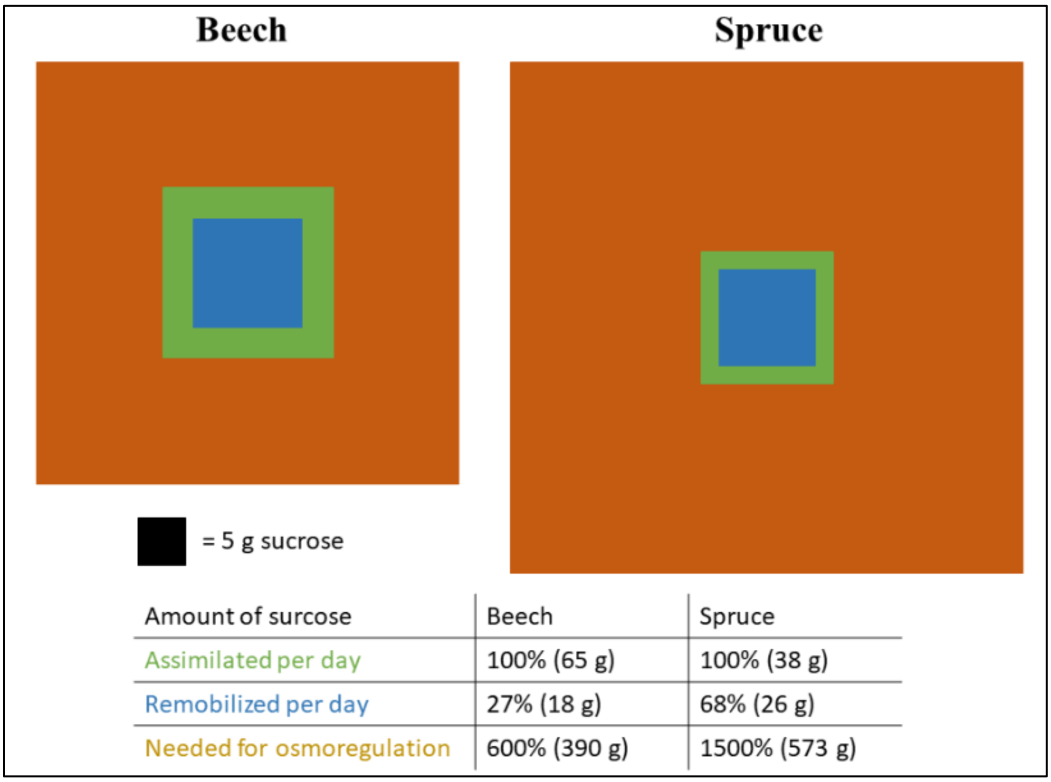
The increased need for C to maintain the hydraulic system of trees coupled with a decreased C transport velocity could facilitate a negative C balance in single tree organs, e.g. fine roots. Such local carbon starvation might be a crucial bottleneck of plant physiological functionality under drought and could even be facilitated in negative feedback loops (McDowell 2011). In mature trees, however, the C demand of single mechanisms, e.g. osmoregulation or NSC dependency of embolism repair, is mostly unclear, as well as to what extent stored C can be utilized (Hartmann and Trumbore 2016). Therefore, it is crucial to further study these close interactions of the water and carbon balance and to disentangle to what extent local carbon starvation might occur and how it influences hydraulic failure. The uncertainty for the possibility to extrapolate findings in juvenile trees to mature trees (Ryan and Asao 2014, Ruehr et al. 2019) regarding the C balance is strongly connected to the phloem transport and the longer transport ways with a high exchange between different tissues, as carbon and water balance are directly linked in the phloem transport. Under repeated drought, such local carbon starvation scenarios might have harsher consequences for spruce than for beech due to the different strategies of fine root management (Nikolova et al. 2020). Beech generally tends to build new fine roots to replace the loss of it and therefore local carbon starvation coupled with a loss of fine roots would not cause additional costs. Yet for spruce, with the proposed maintenance of fine roots under drought, which is most likely connected with a high C demand, local carbon starvation and the possible loss of fine roots would be very costly. Such loss of fine roots would additionally impair the tree during a potential recovery phase.

4.4 Resilience of mature beech and spruce after repeated summer drought – species or parameter dependent?

Both species experienced summer drought for five consecutive years (see Article I), but the level of drought stress seemed to be slightly harsher in spruce. The lowest pre-dawn water potential of spruce per tree was -1.68 ± 0.14 MPa, representing high drought stress (Kurjak et al. 2012), especially within the first two years of drought. In beech trees the lowest pre-dawn water potential per tree was -1.45 ± 0.25 MPa, representing moderate to high drought stress (Leuschner 2020, Walthert et al. 2021). However, both species were strongly impaired in their physiology (see Article I, II, III, Tomasella 2018) and the above- (Pretzsch et al. 2020)/ belowground growth (Zwetsloot and Bauerle 2021), with spruce being stronger affected than beech in some parameters. Nevertheless, both species recovered their physiology within hours to months (see Article V) and took up/distributed the newly added water within several days (see Article IV).

While the quality of recovery was very high in all measured physiological parameters, shown by full resilience, the time needed for recovery was very parameter dependent (see Article V). Differences between the two species during the recovery are addressed in the respective articles (see Article V). Parameters solely dependent on physiological or biochemical reactions, such as pre-dawn leaf water potential and leaf hydraulics (e.g. osmotic potential) recovered quickest, even after repeated drought. This was in accordance with data published from other experiments, which were mostly conducted in juvenile plants (Pflug et al. 2018, Ruehr et al.

2019). The reversal of the osmotic acclimation at the leaf level (see Article V) until D22 released continuously about 390 ± 108 g of sucrose in beech and 573 ± 45 g in spruce (Table 5). Next to photosynthesis, this would potentially act as a second C source in plants that recover from drought stress. Remobilized osmolytes make up about 27 % of the daily fixed sucrose in beech and 68 % in spruce, which gives the TE trees up to 1.7 times the amount of sucrose in the first three weeks of recovery compared to the CO trees (Figure 11). This might facilitate recovery/reparation and re-growth processes. The combination of increased phloem transport (Hikino et al. in prep.) and the re-mobilization of osmolytes upon drought release could help the trees to counteract some of the impairments imposed by drought (e.g. by enhanced root growth or embolism repair).



Amount of surcose	Beech	Spruce
Assimilated per day	100% (65 g)	100% (38 g)
Remobilized per day	27% (18 g)	68% (26 g)
Needed for osmoregulation	600% (390 g)	1500% (573 g)

Figure 11: Comparison between daily assimilated sucrose (green), remobilized sucrose from osmolytes (blue) and the total amount of remobilized osmolytes until D22 upon watering (orange) in 2019 for beech and spruce. Data was redrawn from Article V.

Another important aspect of the tree-water-relations is the hydration level of different tree tissues. Especially isohydric species depend on the internal stem water storage (Hartmann et al. 2021), which can contribute up to 50% to the transpired water (Waring et al. 1979, Hao et al. 2013). The internal water storage is likely to be emptied under drought and would get refilled upon drought release (Betsch et al. 2011). Therefore, especially in mature trees, such internal water storage could be very important for a stable physiological level (Zweifel et al. 2001, Vieira et al. 2013). Upon the drought release, the turnover of the stem water pool was very high in both species and up to 80 % originated from the newly added water in TE trees compared to 20-40 % in CO trees (see Article IV). This would indicate that at least the sapwood and probably the phloem/bark tissues were partially re-hydrated after the drought release. This emptying and refilling of internal stem water storages could be very important for mature trees as up to 50% of transpired water can originate from internal storages (Waring et al. 1979, Hao et al. 2013) and could be tested with point dendrometers via the changes in stem radius (Zweifel et al. 2001, Vieira et al. 2013). To what extent the internal water storage refilling is NSCs demanding, like the xylem embolism repair is still not fully evaluated, but some studies suggest that both processes are similar (Trifilò et al. 2019, Tomasella et al. 2020).

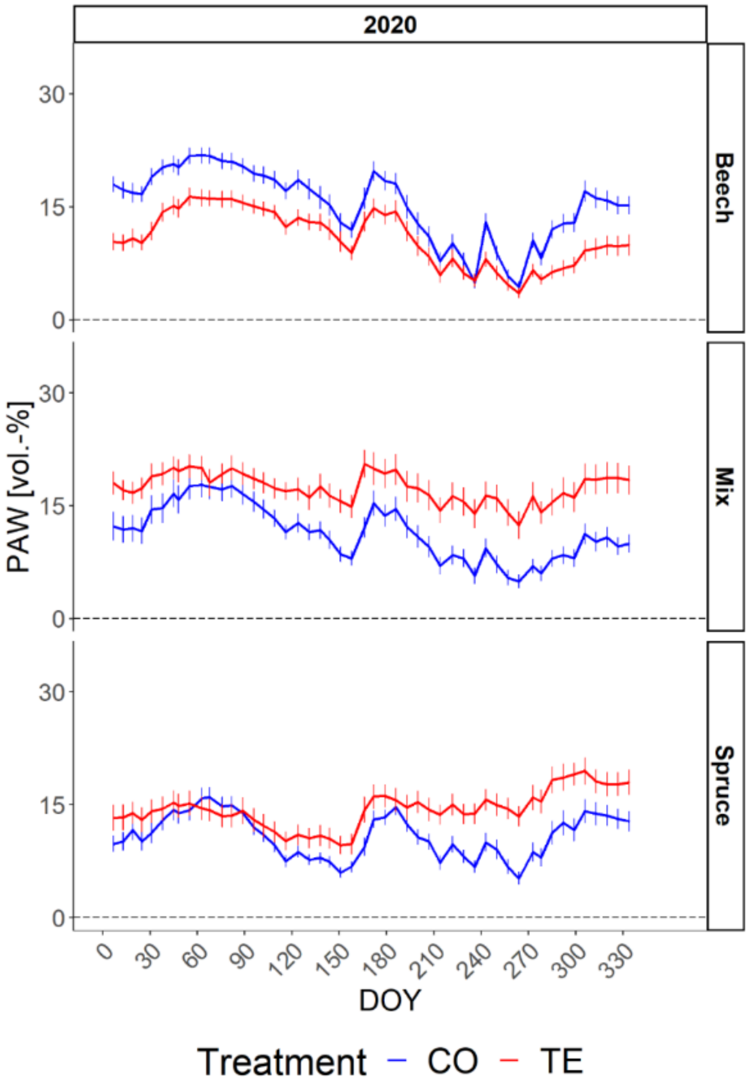


Figure 12: Plant available water in the soil down to 70 cm depth in the interspecific (Mix) and intraspecific zones (Beech and Spruce) in 2020 for CO (blue) and TE (red) plots.

While the water related physiology recovered (i.e. pre-dawn water potential and osmotic potential) also the xylem sap flow density recovered to the level of the CO trees (see Article

V). Therefore, supposedly also the stomatal conductance recovered (Stöhr and Lösch 2004, Köcher et al. 2009b) and the CO_2 uptake was no longer decreased. Despite the recovery of the xylem sap flow density, the overall water consumption of the TE plots were reduced, especially in the interspecific and spruce intraspecific competition zone, indicated by the PAW (Figure 12). The PAW down to 70 cm was significantly higher in the mixed and spruce zone than on the corresponding CO plots, but very similar for the beech zone between both treatments (Figure 12). This would support the idea of a reduced leaf area in spruce, as less water is consumed despite the physiological recovery. However, the recovery of xylem sap flow density in TE trees up to the level of CO combined with a lower water consumption in TE than CO could indicate a change in the lateral xylem sap flow profile (Delzon et al. 2004) or a decreased sap wood area (Sellin 1996) due to the reduced growth increment of spruce under drought (Pretzsch et al. 2020). The still reduced leaf area of spruce is probably connected to the evergreen strategy of spruce. On average spruce trees shed their needles after five years (Muukkonen and Lehtonen 2004). As a reduced leaf growth probably happened for several years during the drought treatment, it will take several years for a full recovery of the leaf area in spruce (Ruehr et al. 2019). This lower water consumption of spruce on the TE plots could benefit beech in the interspecific competition zone during and after the watering.

The full recovery of the physiology is antithetic to the still reduced/impaired morphology, e.g. the reduced leaf area of spruce and the possibly still reduced abundance of fine roots in both species. The slower recovery of morphological acclimations/damages is in accordance with studies on juvenile trees and the framework of Ruehr et al. (2019). As the recovery is likely to take several years in mature trees, such morphological acclimations are likely one of the last measures to be deployed but probably needed to maintain a stable level of physiology. One open question that remains is whether beech also recovers its morphology quicker than spruce (Figure 13). This could be possible due to the higher CO_2 uptake in beech compared to spruce and additionally due to the reduced leaf area of spruce.

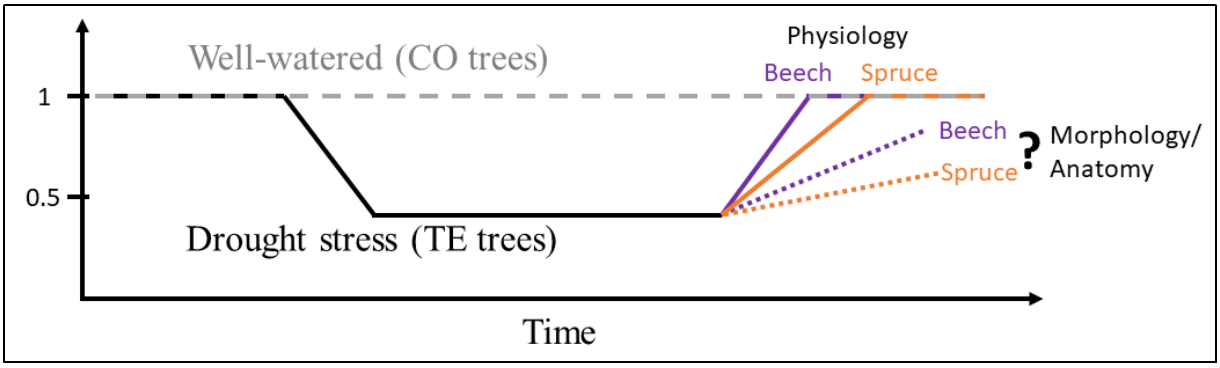


Figure 13: Resilience behavior of beech (purple) and spruce (orange) for physiological (solid lines) and morphological (dotted lines) parameters upon drought stress release

5. Conclusion and outlook

Both species survived five years of repeated summer drought. However, the water and carbon relations were strongly impaired (see Article I, II, III, Tomasella, Beikircher, et al. 2018), especially in the first two/three years of treatment, as well as the growth increment (Pretzsch et al. 2020, Zwetsloot and Bauerle 2021). In many parameters spruce was stronger affected by the repeated drought than beech, but different acclimations were found in both trees, such as shifts of vulnerability curves of branches and leaves or enhanced osmoregulation in leaves (see Article V, Tomasella, Beikircher, et al. 2018). Since the second or third drought year morphological acclimations/damages have been found in beech and especially spruce, probably in order to maintain their physiological functionality. Overall both species showed a rather high degree of drought resistance, resulting in rather low mortality rates (Pretzsch et al. 2020) compared to naturally occurring drought events (Schuldt et al. 2020). Therefore, the overall vulnerability of spruce and beech to drought alone seems to be significantly lower than to combined stresses in natural droughts (Grossiord et al. 2017, 2020), especially for spruce and biotic pests (e.g. bark beetles). In the physiology no differences have been found between the intraspecific and interspecific competition, despite the differences founded for the stem growth increment between the competition zones (Pretzsch et al. 2020). So, drought mitigation in mixture seems to be more driven by niche complementation and thereby related facilitative effects in forests (Rothe and Binkley 2001, Forrester and Bauhus 2016), e.g. different rooting depths coupled with hydraulic redistribution (Hafner 2020).

Despite the generally low mortality rate in the KROOF experiment, the drought treatment significantly increased the mortality, especially in spruce (Pretzsch et al. 2020). However, no signs of hydraulic failure (Tomasella 2018) or carbon starvation (see Article III) have been found in either species. Yet the possibility of local carbon starvation in fine roots, due to reduced phloem transport velocity (see Article II), reduced NSC storages (see Article III) and increased C demand for maintenance (e.g. osmoregulation, defense or xylem embolism repair) remains. Therefore, close interactions of the water-carbon relations, especially osmoregulation under drought, could be crucial bottlenecks in tree physiology. Estimating the amount of C needed to maintain the aforementioned mechanisms could be substantial in understanding tree mortality under drought periods.

Upon drought stress release both species displayed full resilience in their water related physiology (see Article V) and the rehydration of plant tissues (see Article IV). On the contrary, the recovery of morphological parameters were probably not completed even one year after the watering. Since beech was recovering faster than spruce in most measured physiological parameters, this could also apply for the restoration of morphological parameters. The assumed reduction of the leaf area of spruce and the so caused lower overall CO_2 uptake, could further slowdown the recovery in spruce compared to beech. One question that arises is, whether these differences between beech and spruce are solely species dependent or more related to the level of isohydry or foliage type. As the frequency and intensity of drought events are supposed to increase due to the ongoing climate change, the ability to recover quickly will be essential for tree fitness and/or survival. Faster reacting species would thereby benefit at the expense of slower reacting species. Additionally, species that would need to apply morphological acclimations to a high degree might have an additional disadvantage during the recovery period. To assess the drought resistance and resilience potential of temperate tree species, more studies on mature trees and different mixtures are needed. To determine the physiological and morphological plasticity under (repeated) stress for more species will be crucial to understand the mortality/survival pattern in forest ecosystems under natural occurring droughts.

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8. Appendix

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- Article II (published) + permission for reproduction
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- Article IV (draft)
- Article V (submitted)

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The Kroof experiment: realization and efficacy of a recurrent drought experiment plus recovery in a beech/spruce forest

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Abstract. Forest ecosystems play a central role in global water and carbon cycles, yet the impact of global climate change, in particular drought, on trees and forests is poorly understood. Therefore, there is an urgent need for forest-scale experiments in improving our understanding of trees' responses to extreme drought events and subsequent recovery under field conditions. Here, we present the design and efficacy of a novel throughfall exclusion experiment with retractable roofs in a mature forest allowing for flexible drought and recovery periods. A total of 12 plots (144 ± 26 m^2 on average) with 3–7 European beech and Norway spruce trees each were established by root trenching to a depth of one meter, four years prior to the experiment. Subsequent installation of roofs ($n = 6$) allowed for the removal of throughfall precipitation and almost a complete non-availability of soil water in the upper 70 cm during five subsequent growing seasons, that is, 2014–2018. This reduction in available soil water resulted in pre-dawn leaf water potentials down to −1.8 MPa in mature trees. Stem diameter growth decreased by 30% in beech and 70% in spruce, and fine root abundance was reduced by 57% in beech and 73% in spruce compared with controls. After only one growing season, the mycorrhizal community composition changed in response to drought. Careful watering of hydrophobic forest soils in early summer of 2019 resulted in recovered pre-dawn leaf water potentials of drought-stressed trees within one week. Recovery of stem diameter growth, however, did not occur within the same growing season and remained reduced by 33% in beech and 69% in spruce compared with controls. The implemented throughfall exclusion system imposed recurrent seasonal drought events on a mature beech/spruce forest with high efficacy. Shifts in community composition of mycorrhizae in parallel to tree growth decline advocate for a more holistic view on forest-scale drought and watering experiments, particularly in light of more frequently predicted drought events in future. The perennial nature of mature trees and their subsequent slow recovery from drought, that is, over multiple growing seasons, argues for more long-term experiments that span several years.

Key words: Central European forest; growth increment; soil water content; throughfall exclusion; watering.

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INTRODUCTION

While controlled experiments in greenhouses or phytotrons are helpful in elucidating mechanistic reactions and responses in plants, ecosystem-level field manipulation studies contribute to the understanding of these responses in complex environmental networks. Naturally, both kinds of studies are needed to grasp a clearer understanding of nature (Gibson et al. 1999, Wilson 2009). However, there remains a preference for laboratory and greenhouse experiments, especially in the field of ecology and forestry, due to simplified experimental setups. Nonetheless, experiments on natural and mature stands are needed, to test whether extrapolations of controlled greenhouse or phytotron experiments hold across scales (Englund and Cooper 2003) and under manipulation of environmental conditions in the field (e.g., amount of available water, Hanson 2000). Additional uncertainty in our rapidly changing climate requires current ecological investigations to include spatial and temporal analyses of longer drought and heat periods (IPCC 2014). While climatic shifts have occurred for millions of years, the rapid acceleration since the 1850s as a result of the industrial revolution has caused unprecedented shifts (Seidenkrantz et al. 2009). While every ecosystem will be influenced by a changing climate, long-lived systems such as forests are particularly susceptible (Allen et al. 2010, Hartmann et al. 2018). To address questions on the extent that temperate forests will be affected by a changing climate, experiments should consider a range of forest ages and species arrangements, from saplings/seedlings to mature forest stands (e.g., Lola da Costa et al. 2010, Pangle et al. 2012). For many years, foresters have strived to introduce mixed tree species assemblages in central Europe to promote stand stability under unfavorable environmental conditions (Pretzsch et al. 2010). One of the favored mixtures includes Norway spruce (*Picea abies* Karst. (L.)), the most important economical species for forestry in central Europe in the last centuries (Spiecker 2000, LWF 2014) and European beech (*Fagus sylvatica* (L.)), a peak successional species in temperate European forests (Cavin et al. 2013). These two species represent diverse hydrologic anatomy and physiology. Spruce is an evergreen gymnosperm, with tracheids, few stem parenchyma cells, needles, and a mostly shallow-rooting system, while beech is a deciduous angiosperm with xylem vessel elements, higher proportions of xylem parenchyma, broadleaves, and a heart-rooting system, with coarse roots spreading horizontally and vertically from the rootstock with the peak of vertical fine root distribution below that of spruce (Ellenberg and Leuschner 2010). Spruce is hypothesized to be more isohydric, closing its stomata early during drought to minimize water loss (Lyr et al. 1992, Hartmann et al. 2013), while beech maintains open stomata for longer periods of time representing a more anisohydric strategy (Pretzsch et al. 2013). Both hydraulic strategies have advantages and disadvantages. Isohydric plants are more likely to be susceptible to carbon starvation under long-term drought (McDowell and Sevanto 2010), while anisohydric plants are more likely to suffer from hydraulic failure, especially under short extreme drought periods (McDowell et al. 2008). The aim of the first phase of the Kroof project (Kranzberg roof project) was to investigate the effects of recurrent summer droughts on a stand of mature beech and spruce, in monoculture and mixed plantings. For this reason, novel precipitation exclusion roofs were constructed over 6 of 12 total plots, in order to exclude any summer precipitation (from April to November, ~70% of annual precipitation; Appendix S1: Table S1) on the throughfall exclusion plots (TE, drought treatment). Subsequent to the drought in 2014–2018 (Kroof I), the experiment continued with a controlled irrigation during early summer 2019 (Kroof II). This was done in order to examine the reaction of the deflected system when drought stress is released (Ruehr et al. 2019).

The aim of this publication was to detail the design of the throughfall exclusion infrastructure and the system for watering of the hydrophobic soil. Moreover, we focus on the system performance during drought and recovery in view of soil moisture and respective effects on mature beech and spruce trees, demonstrating the efficacy of the employed systems.

MATERIAL AND METHODS

Experimental site and design

The Kroof experiment is located in the Kranzberg forest near Munich in the south-east of

Germany (coordinates 11°39′42″ E, 48°25′12″ N). The average precipitation (1971–2000) was 750–800 mm/a (with 460–500 mm during the growing season), and mean air temperature was 7.8°C (13.8°C during growing season from May to October). The experimental site is approximately 0.5 ha and 490 m above sea level. The site has luvisol soil that originated from loess over Tertiary sediments resulting in high, nutrient and water provision (Pretzsch et al. 2014). The theoretical water-holding capacity for plant available water was estimated to be between 22 and 28 vol.-% (volumetric content of water per volume soil, e.g., dm^3 water/dm^3 soil) in the loess layer (Göttlein et al. 2012). The loess layer is between 50 and 70 cm thick and mostly composed of silt with a medium clay content (Ut3). Below, layers of silty (Lu) and sandy loams (Ls3, Göttlein et al. 2012) follow, respectively. Because these layers were very dense and difficult for roots to penetrate, most of the roots grow within the first meter of the soil (Häberle et al. 2012). The experimental site consists primarily of Norway spruce and European beech trees that were planted in 1951 ± 2 AD and 1931 ± 4 AD, respectively (Pretzsch et al. 2014).

The experimental setup is comprised of 12 plots that contain clusters of 3–7 beech and spruce trees at the opposing sides of the plot (Fig. 1 and Table 1). This design allowed us to test for mixing effects, with monospecific (intraspecific competition) zones at the ends of each plot and an interspecific mixing zone in the middle of the plots (Goisser et al. 2016). In each plot, two beech and two spruce trees were selected as focus trees that were intensively studied with one tree of each species representing monospecific interactions and the other one in the center of the plot representing interspecific interactions (i.e., beech/spruce mixture). Additionally, a pair design was used to control for small scale variations in, for example, soil characteristics, by pairing each control (CO, untreated plot) plot with a throughfall exclusion (TE) plot (Fig. 1). Overall, the plots were similar in size (144.2 ± 26.4 m^2) and tree characteristics (Table 1). For example, only small differences existed between CO and TE for the tree diameter at breast height (DBH; spruce 35.8 ± 7.8 in CO, 34.7 ± 9.9 cm in TE; beech 29.3 ± 9.6 cm in CO, 28.4 ± 9.4 cm in TE). A canopy crane was installed at the Kroof experimental site in 2001 (45 m height, crane jib of 50 m (see black circle in Fig. 1)) allowing access to the canopy of eight plots (4 control [CO] and 4 throughfall exclusion [TE]). In 2015, all spruce trees of plot 2 and 10 were infested with bark beetles and consequently removed. Therefore, since the spring of 2016 stems of all spruce trees were sprayed with an insecticide (Karate Forst flüssig [λ-cyhalothrin agent], Syngenta Agro GmbH, Maintal, Germany) to minimize future biotic infestations.

Kroof Phase I—throughfall exclusion

In 2010, the 12 experimental plots were trenched to 1 m soil depth, where the layer of sandy/silty loam extremely minimizes deeper root growth. The trenching was completed 4 yr before the start of the drought experiment in 2014, to give trees enough time to recover, in particular of root surface area (Pretzsch et al. 2016). Overall, stem diameter growth in the present drought experiment was similar to growth decline under natural drought conditions of beech and spruce trees in the same forest (Pretzsch et al. 2020). However, to further minimize trenching effects, we chose focus trees as far away from the trench as possible. In order to avoid root re-growth outside of the plots and most importantly to disrupt lateral water flow across plots, a thick plastic tarp was installed in the trench. In early summer 2019, tarps were partially re-excavated, confirming the intact, unperforated status of the inserted tarp. In addition, we tested for the possibility of tree water uptake from outside the trenched plots (e.g., by root growth below 1 m soil depth underneath the trench) by applying deuterated water (^2H_2O) to deep soil layers outside of the plots as recommended by Asbjornsen (2018). Methodological details and results are presented in the supplemental files. There were no indications that beech trees growing inside a TE plot took up labeled water from outside the plot (Appendix S1: Fig. S1). Likewise, beech trees outside of the plot did not or minimally took up labeled water (see Appendix S1: Fig. S1). Therefore, in both beech and shallow-rooting spruce, the water uptake from 1 m soil depth was minimal at the experimental site (Appendix S1: *Material and Methods* and Fig. S1).

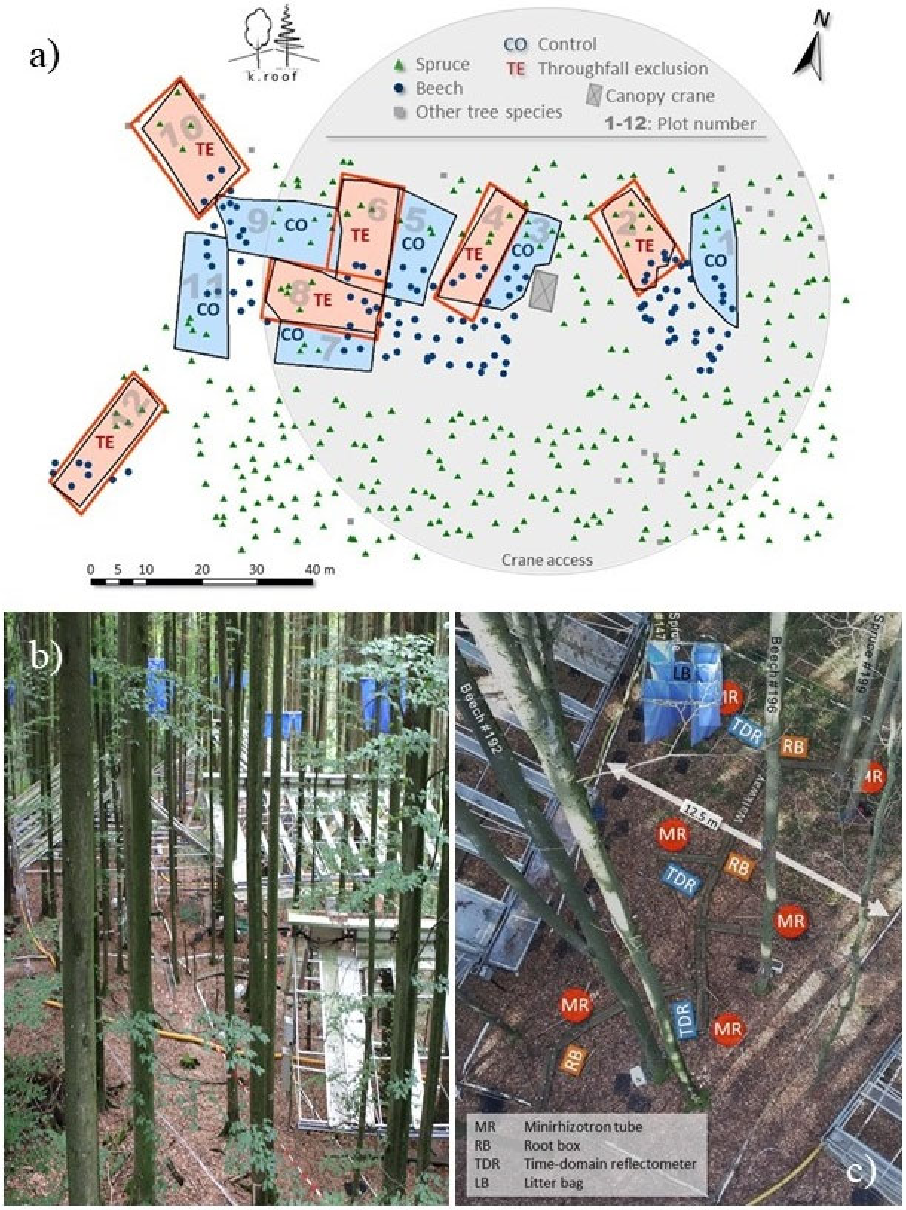

Fig. 1. Map of Kranzberg (top, a), view of the roofs from the crane (bottom left, b) and overview of installed structures per plot (bottom right, c).

To manipulate the precipitation throughfall on the TE plots, a novel roof construction was developed and built in summer 2013 on six of the twelve plots (Umwelt-Geräte-Technik GmbH, Münchberg, Germany). During the construction process, the forest floor was covered with wooden pallets (120 × 80 × 14 cm), to avoid compaction of the soil from construction work. Forest floor vegetation cover was absent due to the high density of the tree crowns. To further minimize soil compaction, each plot was only accessible by elevated wooden walkways (Fig. 1c) and movable perforated crate pads (30 × 40 cm). The roofs were fabricated from light penetrating acrylic sheets secured by an aluminum skeleton. Each roof extended ~40 cm beyond the footprint

Table 1. Plot characteristics with plot size (Area), number of trees on the plot (*N*), mean height of trees per plot (*h*), and diameter at breast height (DBH, mean ± 1 SD). Plots 1, 3, 5, 7, 9 and 11 are control plots (CO), whereas plots 2, 4, 6, 8, 10 and 12 are throughfall exclusion plots (TE).

		Spruce			Beech		
Plot	Area (m^2)	*N*	*h* (m)	DBH (cm)	*N*	*h* (m)	DBH (cm)
1	131.8	5	32.0	34.5 ± 3.2	5	27.8	27.6 ± 9.0
3	109.8	4	32.3	36.2 ± 6.6	6	26.5	25.9 ± 10.7
5	142.1	3	32.2	35.6 ± 6.8	5	27.8	27.2 ± 6.2
7	111.3	3	32.5	37.2 ± 6.7	3	30.5	36.1 ± 10.8
9	199.0	7	32.3	37.7 ± 12.9	7	27.5	27.5 ± 7.3
11	164.3	5	31.6	33.8 ± 10.7	5	28.6	31.2 ± 13.7
CO Mean ± SD	**143.1 ± 31.2**	**4.5 ± 1.4**	**32.1**	**35.8 ± 7.8**	**5.2 ± 1.2**	**28.2**	**29.3 ± 9.6**
2	115.2	(6)	32.7	37.8 ± 6.5	5	26.2	23.6 ± 7.9
4	127.6	3	32.3	36.4 ± 8.9	4	27.4	27.2 ± 11.9
6	161.7	3	31.6	34.4 ± 10.5	4	28.9	33.6 ± 16.5
8	156.2	5	32.1	35.4 ± 7.4	5	27.0	25.0 ± 5.0
10	174.3	(4)	31.3	34.6 ± 14.1	3	29.2	31.6 ± 9.9
12	137.1	4	30.3	29.7 ± 12.1	4	27.9	27.4 ± 6.0
TE Mean ± SD	**145.4 ± 20.5**	**4.2 ± 1.1**	**31.7**	**34.7 ± 9.9**	**4.2 ± 0.7**	**27.7**	**28.2 ± 9.4**

of the drought plots to completely prevent throughfall onto each TE plot (red areas in Fig. 1a). Each roof was comprised of a suit (20–30) of rolling shutters (Appendix S1: Fig. S2) that remained furled throughout the year but close automatically when rainfall started. The shutters were operated in response to three rain sensors (Umwelt-Geräte-Technik GmbH, Münchberg, Germany), which signaled the roofs to close during, and re-open 1 h after the end of a rainfall event. The rolling shutters were installed to minimize the effects of the roofs on microclimate and nutrient input through litter fall and account for ~60% of each roof area. The stationary portions of the roof were secured around individual trees and were permanently closed (Appendix S1: Fig. S2). Stemflow was negated via perforation hoses encircling each tree stem above the roof, and the collected water was directed via hoses to the roof gutters. All rain that was intercepted by the roof was transported off of the experimental site via plastic hoses attached to gutters at each side of the roofs (Appendix S1: Fig. S2). Phase I (drought phase) of the Kroof experiment started in March 2014 and continued until November 2018. During this phase, the roofs were automatically closed during rainfall in the growing season (Appendix S1: Table S1) and on average withheld 69 ± 7% of the annual rainfall (Appendix S1: Table S1).

Meteorological station

We logged the climate above the experimental site canopy at every 10 min with a weather station (Umwelt-Geräte-Technik GmbH, Münchberg, Germany). Tracked climate parameters included air temperature (T in °C), relative humidity (RH in %, used to calculate vapor pressure deficit [VPD in hPa]), global radiation (GR in W/m^2), and rainfall (RF in mm). The climatic conditions during the experiment (2014 till 2019) are shown in Fig. 2, with the daily average of air temperature above the canopy (mean during experiment 12.1° ± 7.8°C), VPD (mean 3.7 ± 3.8 hPa), global radiation (mean of 151.5 ± 96.8 W/m^2), and monthly sum of rainfall (61.1 ± 32.0 mm). Additionally, we recorded the air temperature 1 m above the forest floor and soil temperature (10 cm depth) in summer 2014 on the CO and TE plots in the middle of each plot for several months, to monitor for potential temperature effects of the throughfall exclusion roofs.

Assessment of drought stress in trees and soil

To assess the level of drought stress intensity on the trees, pre-dawn (WP_{PD} in MPa) and midday (WP_M in MPa) leaf water potentials of the four focus trees per plot were regularly measured with a Scholander pressure chamber (mod. 1505D, PMS Instrument, Albany, Oregon, USA).

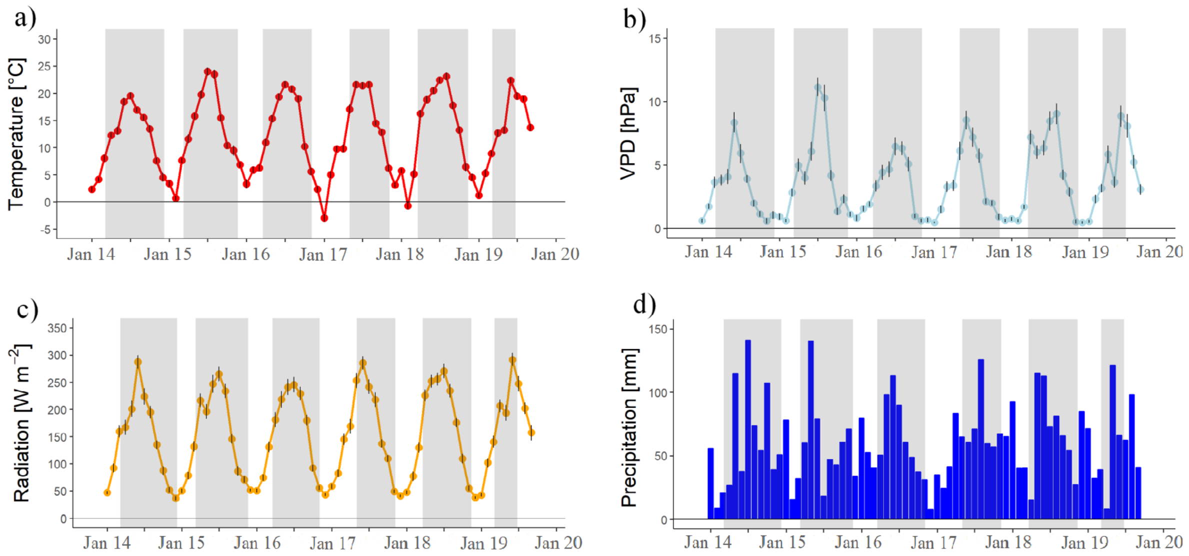

Fig. 2. Meteorological data (mean ± 1 SD) from 2014 to 2019 (Kroof I and II): monthly means of temperature (a), vapor pressure deficit (b) and global radiation (c) and monthly sum of precipitation (d), with gray areas showing the time of roofs activated.

To determine the drought stress in the soil, we assessed soil water content (SWC in vol.-%) with custom-built time domain reflectometry sensors (TDR, sensor length: 20 cm) attached to either a TDR100 or a TDR200 (Campbell Scientific, Logan, Utah, USA). On each plot, TDR sensors were installed (Fig. 1) in the beech and spruce monospecific zones (opposing plot sides) and in the plot center (mixed zone). At each zone, sensors were installed at four depths, 0–7 cm, 10–30 cm, 30–50 cm, and 50–70 cm soil depths, resulting in six replicates for each depth, position (spruce, beech, and mix), and treatment (144 sensors in total). Measurements were carried out weekly. Experimental data revealed that every soil layer has a different threshold for non-plant available water (permanent wilting point, PWP). At the PWP, adhesion of water to soil pores is too strong for plant roots to take up the water (Kirkham 2014). The PWP for the soil layers in Kranzberg forest ranges from 7.4 ± 3.6 vol.-% in the top layer (0–7 cm depth), 13.5 ± 2.0 vol.-% in 10 to 30 cm, 19.1 ± 5.7 vol.-% in 30 to 50 cm, and up to 25.8 ± 2.4 vol.-% in the deepest layer of 50 to 70 cm depth (dashed horizontal lines in Fig. 3). Data for the PWP were obtained in the summer of the natural drought year 2015 on TE plots, as SWC reached a plateau (reflecting that no more water was taken up by roots) and remained stable for several weeks.

Band and automatic dendrometers

To assess the growth increment of the 48 focus trees, automatic point dendrometers (Deslauriers et al. 2003; DR-type, Ecomatik, Dachau, Germany) were installed to measure stem growth at breast height (1.3 m) and at 50% tree height. To minimize the swelling and shrinking effect of the bark, the spruces' outermost bark was removed. The dendrometers were fixed in NE direction and consisted of aluminum frames which were fixed with screws on the tree stem. The root diameter increment was measured by using circumference dendrometers (DC2 type, Ecomatik, Dachau, Germany), which were fixed on one course root near the stem. Slide rings helped to diminish the friction between the wire cable and the tree bark. All measurements were based on precision linear variable transducers which were placed directly in contact with the bark. Data were recorded every 10 min.

Minirhizotron tubes

To track root dynamics, six acrylic minirhizotron tubes were installed in 2010 on each plot, two in each monospecific species zone, and two

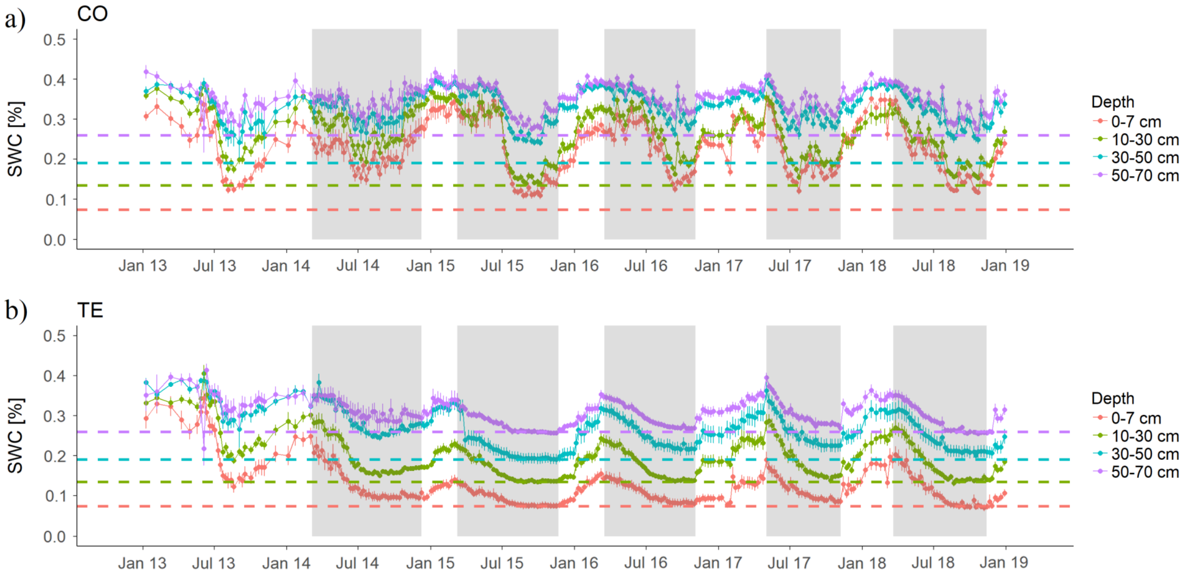

Fig. 3. Volumetric soil water content (SWC, mean ± 1 SD) from 2014 to 2018 on CO (a) and TE plots (b) in four different depths (red 0–7 cm, green 10–30 cm, blue 30–50 cm, and purple 50–70 cm) with gray areas showing the time of roofs activated and horizontal dashed lines (same color code for depths) representing the threshold of water un-available to plants (permanent wilting point).

in the mixed species zone (Fig. 1b). Tubes were 70 cm long and 6 cm in outer diameter and installed 60° to the vertical to reach a maximum viewing depth of 50 cm. Forty-four images (width, 18 mm; height, 15 mm) along each tube were imaged biweekly during the growing season (May–August) and once monthly (September–April) with a laparoscopic camera (BTC-100X, Bartz Technology, Carpinteria, California, USA). Images were analyzed for absorptive fine root production (birth and death), calculated as the mid-point between disappearance and death (for details, see Zwetsloot et al. 2019).

Ectomycorrhizal community composition and functionality

Root, ectomycorrhiza, and soil samplings for analyzing ectomycorrhizal composition and enzyme activity profiles have been described in detail along with morphological-, DNA-, and enzyme activity-based analyses in Nickel et al. (2018). Briefly, 4 cm diameter soil cores were sampled from three zones (monospecific beech and spruce zones and mix zone in plot center) in each plot at the end of the growing seasons from 2013 until 2019. Soil cores were each separated into an upper (combined the $O_f + A_h$ horizons) and lower section (A_lB_v). The root material from these 96 subsamples per year was used to determine fine root vitality and to morphotype mycorrhizae. Per subsample, extracellular enzyme activities (six hydrolytic enzymes, and laccase) of 21 representative ectomycorrhizal tips per subsample were measured and fungal internal transcribed spacer ribosomal DNA sequences (ITS) of individual tips determined, where necessary for identification. DNA of the remaining fine roots was extracted per subsample, fugal ITS amplified, sequenced via Illumina MiSeq v3 600 high-throughput sequencing cycles kit (Illumina, San Diego, California, USA), and processed via the PIPITS v.1.3.6 analysis pipeline (Gweon et al. 2015) to phylotype tables of each subsample's fungal community composition.

Water repellency after 5 yr of drought

The hydrophobicity of the soil in summer 2019 was tested by the water drop penetration time (WDPT in s). The repellency of the soil was determined by placing drops of deionized water on the soil surface and measuring the time until complete penetration, which is based on the phenomenon that soil does not wet spontaneously when water is applied to the surface (Chenu et al. 2000, Leelamanie et al. 2008). To run this test, we used mineral soil collected with a root auger after separating the organic layer. The upper 1-cm section of the mineral soil was used to determine the WDPT of shallow soil. Since the wettability of this soil layer was decisive for the effectiveness of watering, we focused on the characteristic of this layer. However, we also tested the water repellency in deeper (10 cm depth) soil. The soil was dried, sieved <2 mm, and properly prestled. Five drops of deionized water were placed on the soil surface with a pipette at 1 cm distance from the soil. The time until complete penetration was measured by a stopwatch. Repellency was categorized into WDPT $\leq$ 1 s, non-repellent; 1–60 s, slightly repellent; 60–600 s, strongly repellent; 600–3600 s, severely repellent; and $\geq$3600 s, extremely repellent (Leelamanie et al. 2008). The WDPT increased under drought (*P* value treatment:species = 0.0372) in the shallow soil for beech (CO 84 ± 139 s and TE 3368 ± 3390 s) and spruce (CO 1315 ± 1546 s and TE 3267 ± 3222 s), while the drought impact on the wettability was less strong at 10 cm depth (beech CO 168 ± 328 s and beech TE 714 ± 1030 s; spruce CO 716 ± 656 s and spruce TE 409 ± 728 s). Independent of treatment and species, the water repellency decreased with soil depth (Table 2).

Kroof Phase II—watering and recovery

In phase II of the Kroof experiment, the trees were watered to assess their recovery after five years of repeated summer drought. To accomplish simultaneous watering of an entire plot, a watering system composed of soaker hoses (CS Perlschlauch Premium, CS Bewässerungssysteme, Reichelsheim, Germany) and garden hoses was designed (Appendix S1: Fig. S3). A garden hose with T-connectors every 20 cm was installed along the length of each plot. From each T-connector, a soaker hose ran the entire width of the plot and was sealed at the end with a cable tie (mean length of all soaker hoses per plot 1111 ± 97 m; Appendix S1: Fig. S3). To pair the watering treatment of the drought plots to the control plots, we aimed to bring the TE plots to the same SWC level as CO plots. In parallel, we watered the CO plots to a minor extent, that is, 15 L/m^2 of water (approximately 2034.5 ± 537.3 L, depending on plot size). This was done to minimize differences between CO and TE plots introduced by the watering, such as changes in soil temperature or direct availability of nutrients and to bring the upper soil layer (upper 10 cm) to full saturation also on the CO plots. Therefore, for each of the 12 TDR probes installed at different depths, we calculated the difference in SWC between the TE plot and its neighboring CO plot. Relative SWC values (in vol.-%) were transformed into the amount of water deficit per square meter to a depth of 70 cm (WD_{soil} in L/m^2).

$$\text{WD}_{\text{soil}} = (\text{SWC}_{\text{CO}} - \text{SWC}_{\text{TE}}) \times \text{soil volume} \quad (1)$$

WD_{soil} was multiplied with the individual plot size to gain the total amount of water, which was necessary to irrigate the TE plots to the desired level of the CO plots. Additional water was added to account for the absorption capacity of the litter layer ($\text{WD}_{\text{toplayer}}$ of 14.40 ± 3.62 L/m^2). As a last step, we added the amount of water applied to the CO plots to determine the total amount of water to be irrigated per plot (WD_{plot} in L).

$$\text{WD}_{\text{plot}} = \text{WD}_{\text{soil}} \times \text{area} + \text{WD}_{\text{toplayer}} \times \text{area} + \left(15\,\text{L/m}^2 \times \text{area}\right) \quad (2)$$

On average, we added 12,849 ± 2801 L (this amount corresponds to a precipitation event of ~90 L/m^2) to the TE plots (Table 3). We controlled the amount of water added to each plot with an electronic water meter (Wassermengenzähler, GARDENA Manufacturing GmbH, Ulm, Germany).

Due to the high hydrophobicity of the topsoil layers on the TE plots (infiltration rate of 0.25 ± 0.07 mm/h and water drop penetration time of 3317 ± 2864 s in the shallow soil for beech and spruce combined), the watering was initially applied at a slow rate (2 $\text{L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) for TE that amounted to about 7 and 40 h of watering for the CO and TE plot, respectively. The irrigation took place in three separate campaigns (2 CO and 2 TE plots in each campaign) to allow time to follow single tree responses and to distribute the number of labor-intensive measurements over a longer period. Watering dates were

Table 2. Water drop penetration time (WDPT) of shallow soil (depth = 0 cm) and deep soil (depth = 10 cm below the organic layer) and its repellency categories for beech and spruce under the different treatments.

			Shallow soil		Deep soil		
Treatment	Plot	Species	WDPT ± SE (s)	Repellency category	WDPT ± SE (s) - deep	Repellency category	*n*
CO	3	Beech	40 ± 10	Slightly	2 ± 1	Slightly	5
	5		2 ± 0	Slightly	1 ± 0	Non-repellent	5
	7		291 ± 98	Strongly	659 ± 657	Severely	5
	9		2 ± 0	Slightly	9 ± 8	Slightly	5
	3	Spruce	912 ± 150	Severely	100 ± 17	Strongly	5
	5		742 ± 154	Severely	807 ± 806	Severely	5
	7		3564 ± 1294	Severely	360 ± 359	Strongly	5
	9		40 ± 12	Slightly	1596 ± 1595	Severely	5
TE	4	Beech	21 ± 3	Slightly	1 ± 0	Non-repellent	5
	6		4717 ± 1268	Extremely	2219 ± 1823	Severely	5
	8		1247 ± 339	Severely	102 ± 90	Strongly	5
	10		7485 ± 1956	Extremely	532 ± 530	Strongly	5
	4	Spruce	4030 ± 305	Extremely	1500 ± 565	Severely	5
	6		7478 ± 2084	Extremely	60 ± 58	Slightly	5
	8		304 ± 73	Strongly	71 ± 24	Strongly	5
	10		1254 ± 362	Severely	3 ± 0	Slightly	5

Note: Standard errors represent the variability of one sample.

Table 3. Watering details for each plot with the calculated water deficit (Calculated $\mathrm{WD}_{\mathrm{plotl}}$) and the actual amount of water added to each plot (actual added water).

Plot	Calculated $\mathrm{WD}_{\mathrm{plot}}$ [L]	Actual added water [L]
1	1977	1977
3	1647	1647
5	2132	1928
7	1670	1405
9	2985	2985
11	2465	2465
Mean ± SD	**2146 ± 467**	**2035 ± 537**
2	7978	7978
4	14596	14703
6	16977	16977
8	13691	13691
10	11499	11499
12	11964	12248
Mean ± SD	**12784 ± 2802**	**12849.3 ± 2802**

25 June, 4 July, and 10 July 2019. Watering was initiated at 4 am during each watering event (D 0). During the watering phase, we assessed the same parameters as during phase I (SWC, $\mathrm{WP}_{\mathrm{PD}}$, growth increment, etc.), starting on day 6 before the watering (D-6). On day 15 (D 15), we took a set of samples and then opened the roofs permanently (Appendix S1: Table S1), so that both CO and TE plots received the same amount of rainfall from thereon.

Xylogenesis

For detailed analysis of stem growth response to watering, we utilized a stem micro core (2 mm diameter, 20 mm length, Rossi et al. 2006). Sampling was conducted on days − 1, 2, 7, 15, 21, 60, and 120 after watering. We embedded the core in superglue (UHU Alleskleber Kraft, UHU GmbH KG, Bühl/Baden, Germany), cut 20 µm thick longitudinal sections with a microtome (Sledge Microtome G.S.L. 1, Schenkung Dapples, Zürich, Switzerland), and examined the sections with a light microscope (Olympus BX41TF, Olympus Austria, Vienna, Austria). For each sample, the number of tracheid cells of the current growth ring was counted in five rows and averaged. We avoided counting vessels, to permit comparison between the cellular growth of spruce and beech and to gain a more homogenous result for beech trees. For beech samples, when encountering a vessel, the row of counting was moved parallel and continued then. The number of cells was divided by the days since onset of the growing season (average temperature constantly above 5°C = 1st of April in 2019) to calculate the average cellular growth rate per day (CGR in N_{cells}/d).

Statistical concepts

In general, physiological data were analyzed with mixed-effect models, due to their ability to test for fixed (factorial and continuous variables and interactions) and random effects. With random effects, we controlled for repeated measurements (e.g., measurements on the same tree in different campaigns) and non-independent datapoints (e.g., trees that grow on the same plot). In most cases, we used the tree individual nested in the plot identity as a random effect to control for both limitations. To validate the correctness of our models, we tested the data for homogeneity of variances (when needed, e.g., fixed factor is a factorial variable) beforehand, for example, with Levene's test. We tested for normality of residuals with the Shapiro-Wilk test and additionally graphically with a quantil–quantil plot (Q–Q plot). To test for differences between single interaction groups (e.g., between CO beech, TE beech, CO spruce, and TE spruce), we used a post hoc test, estimated marginal means with Tukey correction. Two replicates of each tree species per plot were selected as focus trees ($n = 8$), for crown sampling from the crane. All other measurements had 12 replicates per species and treatment for each measured parameter. For the focus trees, this resulted in 12 trees per species nested in 6 plots per treatment and within the crane radius in 8 trees per species nested in 4 plots per treatment.

Data shown here were analyzed for statistical differences using R (version: 3.6, R Development Core Team 2008) in RStudio (version 1.2.1335, R Studio Team 2015). For differences in WP_{PD} vs. SWC, CGR, WDPT, and radial increment of phase I, a linear mixed-effect model (lme function) was calculated, using the year, species, and/or the treatment as fixed and the tree individual nested in the plot as a random effect (package nlme, version 3.1-137). For phase II the species, treatment and timepoint in relation to the watering were used as a fixed effect. If the mixed-effect model showed significant effects, we ran a post hoc test with the emmeans function with Tukey correction (package emmeans, version 1.3.1). Data were plotted with the boxplot (package graphics, version 3.5.2) or ggplot function (package ggplot2, version 3.1.0) and are given in text and tables as means ± 1 SD.

For the ectomycorrhizal community analysis, morphotyping community abundance data were log-transformed, aggregated per plot and year, and used to calculate a Bray-Curtis dissimilarity matrix for each year. From these matrices, principle coordinate analyses (PCA) were calculated to show the overall divergence between ectomycorrhizal communities of each plot.

RESULTS

Kroof Phase I—repeated summer drought 2014–2018

Effects of the roofs on air and soil temperature.— No statistical difference was found between the air temperature on CO and TE plots. Therefore, roofs did not affect subcanopy air temperature (Table 4), with an overall average air temperature of 11.9° ± 2.8°C in September 2014 and 10.7° ± 3.0°C in October 2014 (Table 4). Soil temperature was slightly, however not significantly, higher in the CO plots (average of 14.1°–11.5°C from August to October 2014) than in the TE plots (average of 13.5°–11.1°C from August to October 2014).

Ectomycorrhizal community under drought.—The principal coordinate analysis of dissimilarities before throughfall exclusion showed that spatial proximity of ectomycorrhizal community samples explained most differences/similarities between the neighboring plots (e.g., plot 1 and 2 or plot 3 and 4; Fig. 4a). After the first year of summer drought, ectomycorrhizal communities of CO plots and TE plots were already clearly separated by treatment along the first principal coordinate and drought became a strong explanatory factor when comparing the samples at the plot level (Fig. 4b).

Water budget.—After establishment of the throughfall exclusion experiment in 2013, the SWC in the TE plots dropped below the CO plot levels every summer. The soil moisture in the shallowest soil layers, 0–7 cm soil depth, was above 15 vol.-% SWC in the CO plots, but only about 8–10 vol.-% in the corresponding TE plots during the summer months (Fig. 3). A similar pattern was found for the other three soil depths as well, where the TE values were consistently about 10 vol.-% lower than the CO (Fig. 3a). The only exception from this pattern was the natural

Table 4. Mean (± 1 SD) of soil and air temperature (in °C) in 2014.

	Position and Treatment			
	Air temperature		Soil temperature	
Month	CO	TE	CO	TE
Aug	n/a	n/a	14.12 ± 1.51	13.51 ± 1.22
Sep	11.93 ± 2.79	11.93 ± 2.78	12.39 ± 1.31	11.94 ± 1.31
Oct	10.64 ± 2.98	10.65 ± 3.00	11.53 ± 1.43	11.10 ± 1.47

Note: No statistical differences were found between CO and TE plots. n/a = not assessed.

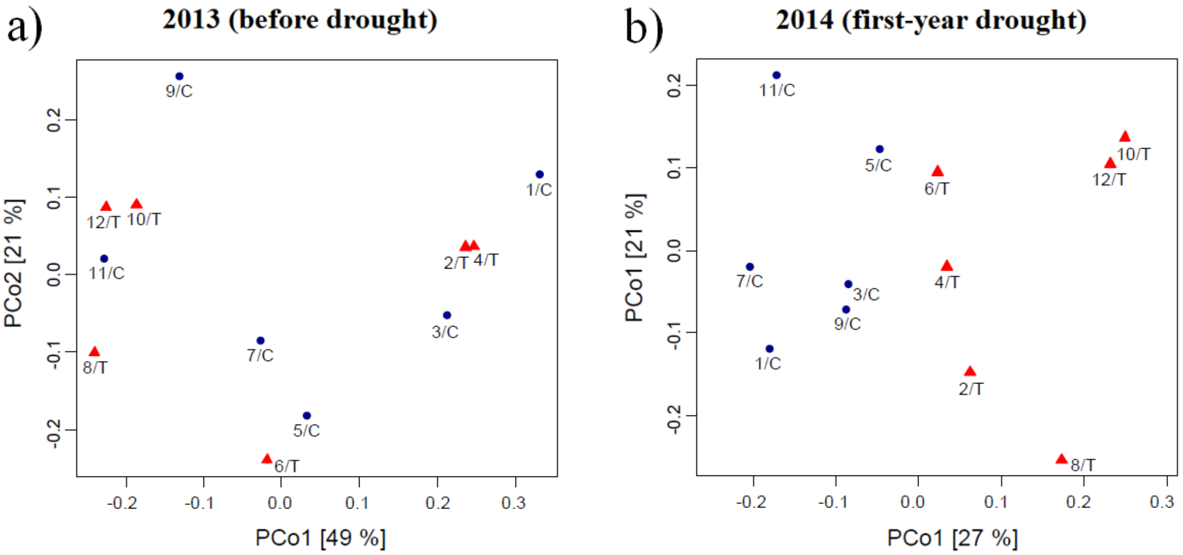

Fig. 4. Principal coordinate analyses showing the ectomycorrhizal (ECM) community before (a, autumn 2013) and at the end of the first throughfall exclusion period (b, autumn 2014). Red triangles represent plots assigned for throughfall exclusion and blue circles represent plots assigned for untreated control; distances represent differences in ECM community composition based on Bray-Curtis dissimilarities gained from log-transformed abundance data of ECM morphotypes.

drought year of 2015 with low annual and summer precipitation and high VPD (Fig. 2, Appendix S1: Table S1), which resulted in similar SWC values of 10 and 15 vol.-% in the 0–7 and 10–30 cm soil depths in the CO and TE plots, respectively. In every year expect for 2014, the SWC in all four depths was close to the PWP in TE plots, reflecting that within the first 70 cm of soil there was limited plant accessible water (distance between curve and horizontal dashed lines in Fig. 3b). In the CO plots, 2015 was the only year where soil water availability approached the PWP for several weeks.

The decline in SWC (mean of all 4 depths) was correlated with the WP_{PD} for both species (Fig. 5). In particular in beech and spruce, SWC and WP_{PD} of CO trees were similar over all years (around 23 ± 3 vol.-%, −0.37 ± 0.09 MPa and 23 ± 3 vol.-%, −0.64 ± 0.17 MPa, beech and spruce, respectively), except for the natural drought year of 2015 (21 ± 3 vol.-%, −0.86 ± 0.20 MPa and 21 ± 3 vol.-%, −1.08 ± 0.16 MPa, respectively; Fig. 5). Both SWC and WP_{PD} were significantly (P value for treatment for spruce 0.0001 and beech 0.0002) lower in TE plots with values of 18 ± 3 vol.-%, −0.66 ± 0.18 MPa and 18 ± 2 vol.-%, −0.98 ± 0.31 MPa for beech and spruce, respectively, even in the drought year 2015 (17 ± 2 vol.-%, −1.30 ± 0.24 MPa and 16 ± 3 vol.-%, −1.63 ± 0.14 MPa, respectively).

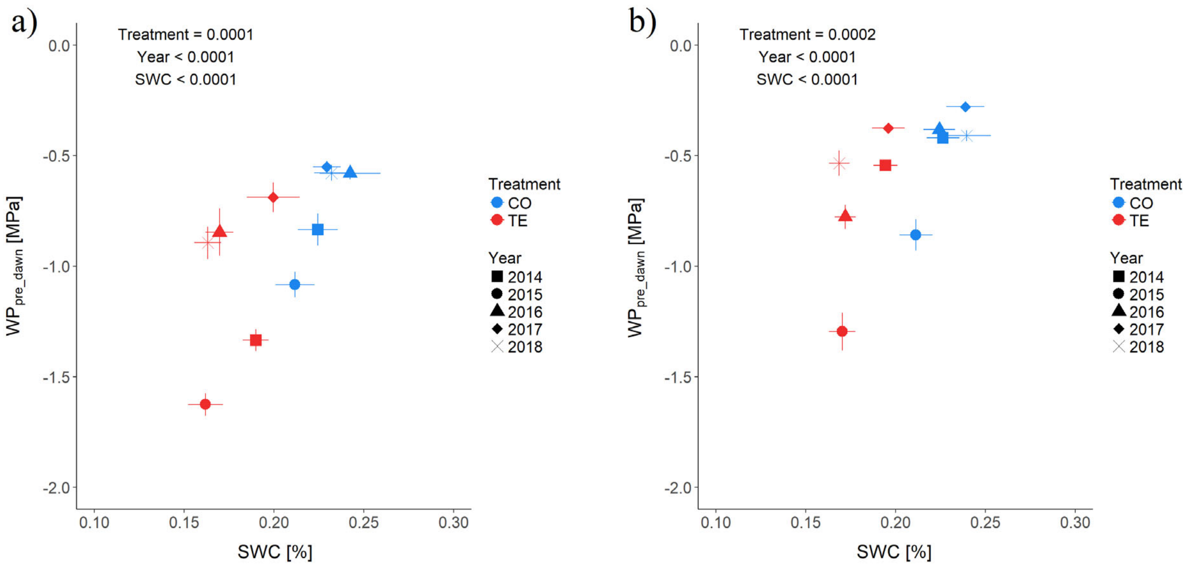

Fig. 5. WP$_{PD}$ of summer of Phase I vs. SWC of spruce (a) and beech (b, mean ± 1 SD, with treatment CO, blue; TE, red; year 2014, rectangles; 2015, circles; 2016, triangles; 2017, diamonds; 2018, saltires). For both species the treatment, year and the SWC had a significant influence on the WP$_{PD}$.

Effects of drought on above- and belowground growth.—For both species, radial stem diameter growth at DBH was reduced by drought. For spruce, a significant 70% reduction in radial growth was recorded for TE in all years (only for non-bark beetle infested trees; Fig. 6a) compared to CO with a rather stable growth of about 2000 μm each year. In 2015, ten of the TE spruce trees became heavily infested by bark beetles and subsequently died. The presence of bark beetles was verified by sawdust and small holes along the stem that were minor in neighboring spruce trees. Spruce trees with smallest stem diameter growth in the previous year, that is, 2014, were infested by bark beetles in 2015 (red boxplot in Fig. 6a). Beech also reduced its stem diameter growth under drought, although not significantly. Nevertheless, from 2015 to 2017, beech growing under drought conditions had an approximately 30% reduction in growth compared with CO trees, which grew on average 1400 μm each year (Fig. 6b).

In 2013, before the start of the experiment, similar numbers of fine root tips were present across both drought and control plots for beech (mean for both treatments: 12.79 ± 8.85 × 100/m^2 window) and spruce (mean for both treatments: 4.59 ± 4.19 × 100/m^2 window). With the onset of drought, a similar pattern as in aboveground growth was found for fine root tip production. In 2014, only one year after the drought treatment was initiated (Fig. 6d), the number of fine root tips was decreased by approximately 29% in beech TE plots (29.71 ± 26.21 × 100/m^2 window) compared with CO (41.92 ± 33.86 × 100/m^2 window), and in 2015, the number of root tips was reduced by 85% in TE (2.19 ± 2.50 × 100/m^2 window) vs. CO (14.98 ± 7.86 × 100/m^2 window); however, TE was not significantly different from CO (p value for treatment: 0.1774). Spruce (Fig. 6 c) reduced the number of root tips by 56% in the first year after drought (2014; 8.00 ± 4.98 × 100/m^2 window) compared with CO (18.27 ± 12.12 × 100/m^2 window), and in 2015, the root production in drought plots (1.09 ± 1.18 × 100/m^2 window) was reduced by 90% compared with CO (11.36 ± 8.08 × 100/m^2 window). Both treatment (P value 0.0500) and year (P value 0.0189) and their interaction (P value 0.0135) had significant effects on the amount of fine root tips for spruce. Therefore, we assume that interannual variability in climate and precipitation influenced belowground growth to the same extent as drought periods (Fig. 6).

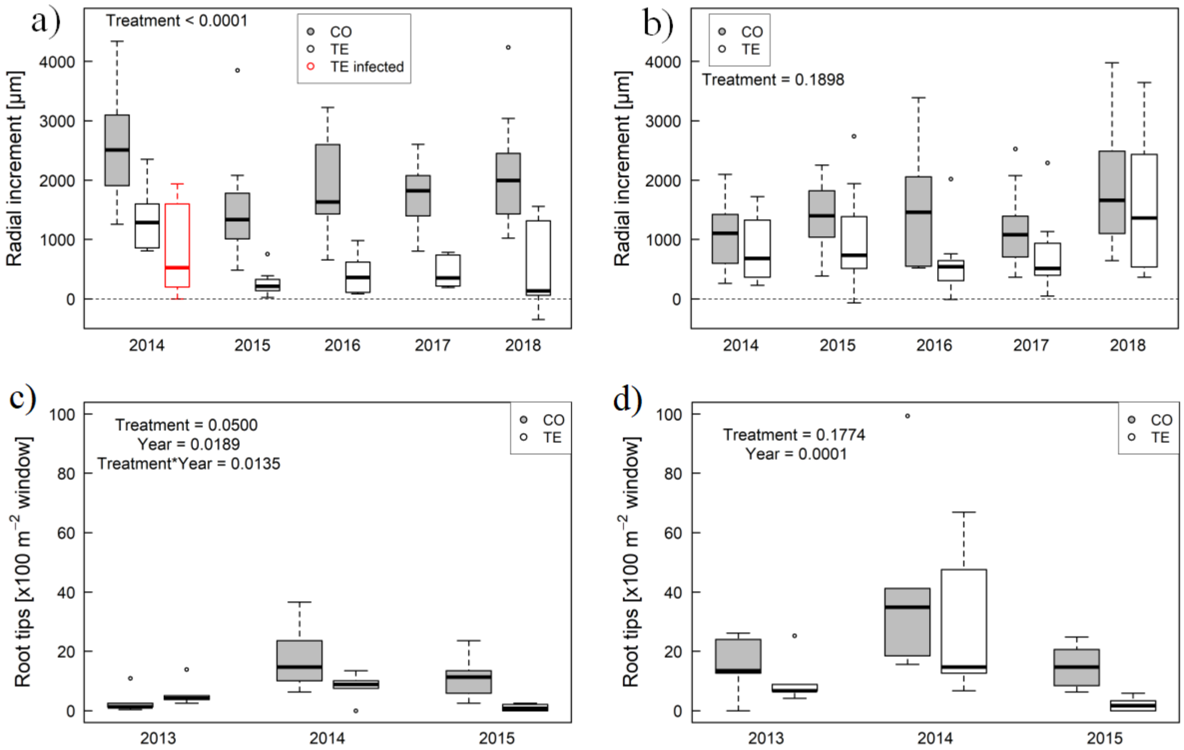

Fig. 6. Radial growth increment of spruce (a, treatment showed a significant influence) and beech (b, no influence of the treatment) and number of root tips for spruce (c, treatment and year showed a significant influence) and beech (d, only the year showed a significant influence, with CO, gray; TE [not infested], white; and TE bark beetle infested, red).

Kroof Phase II—recovery

Water budget.—In 2019 for CO trees of both species, average SWC across all four soil depths was similar during all timepoints, that is, days −6, 7, and 15 (overall mean of 24.5 ± 2.5 vol.-%). Correspondingly, WP_{PD} of CO trees was similar over all timepoints (Fig. 7a,b), with a WP_{PD} of −0.48 ± 0.11 and −0.63 ± 0.08 MPa for beech and spruce, respectively. Before watering and irrespective of tree species, TE (18.54 ± 2.28 vol.-%) plots had significantly lower soil moisture than CO (26.57 ± 1.82 vol.-%). Pre-dawn leaf water potential indicated mild leaf drought stress −0.79 ± 0.07 and −0.93 ± 0.07 MPa in beech and spruce, respectively. Seven and 15 d after watering, differences between CO and TE in both SWC and WP_{PD} disappeared for both species at levels similar to CO (close proximity of blue and red data points for d7 and d15 in Fig. 7a,b).

Xylogenesis.—The average cellular growth rate (CGR) of new tracheids in beech CO trees (0.61 ± 0.07 cells/d) was 2.4 times higher than in spruce (0.25 ± 0.04 cells/d; Fig. 8). Over the course of the watering, the CGR of CO beech and spruce remained fairly constant (ranging from 0.67 to 0.46 cells/d and 0.19 to 0.30 cells/d) with a small decline toward the end of the growing season (November). In TE trees before watering, the CGR was 35% lower for beech (0.39 ± 0.06 cells/d) and 69% lower for spruce (0.08 ± 0.01 cells/DOY) compared with CO trees. The rate slightly increased upon watering in TE beech (maximum of 0.47 ± 0.34 cells/d) but remained lower than in CO trees (Fig. 8), while for spruce, CGR did not change. For TE trees, there was a slight decrease in the CGR toward the end of the growing season (Fig. 8).

DISCUSSION

We aimed to bring mature trees to the edge of their survival, opening the opportunity to study

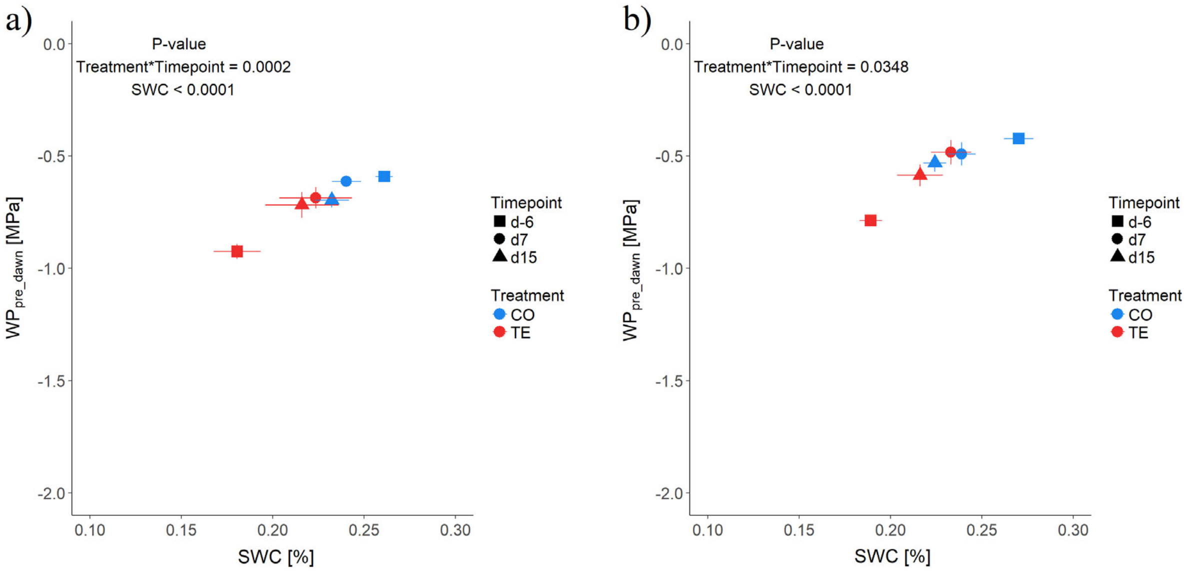

Fig. 7. Pre-dawn water potential vs SWC of spruce (a) and beech (b) in 2019 during the watering phase (mean ± 1 SD, for both species, the interaction of treatment and timepoint was significant as well as the SWC, with treatment CO, blue; TE, red; day after watering −6, circles; 7, triangles; 15, diamonds).

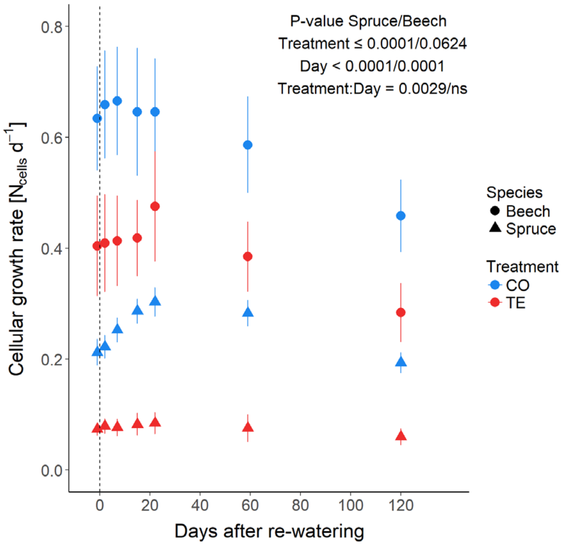

Fig. 8. Cellular growth rate (mean ± 1 SD) of the growing season in 2019 during the watering phase for spruce (triangles) and beech (circles, with treatment CO, blue; TE, red). For spruce, a significant influence of the treatment and day, as well as their interaction was found, while for beech, only the day showed a significant influence, and for the treatment, only a trend was found.

tree acclimation under severe drought. To this end, we chose two economically and ecologically important tree species in central Europe, that is, European beech and Norway spruce, with contrasting hydraulic strategies in response to drought; in the case of beech a more anisohydric and in the case of spruce a more isohydric stomatal drought response (Lyr et al. 1992, Ellenberg and Leuschner 2010).

Kroof represents an extended novel thoughfall exclusion experiment with retractable roof infrastructure that resulted in 69% annual reductions in throughfall precipitation reaching the soil. A strong decline in SWC and pre-dawn leaf water potential attests to the efficacy of the experimental setup in reducing soil water availability. In particular, shallow-rooting spruce trees were negatively affected during recurring severe summer droughts and were unable to fully recover stem growth (i.e., meristematic activity at DBH) upon watering. After five years of summer drought, careful watering of the highly hydrophobic soil (Table 2) allowed for a detailed analysis of tree drought recovery across two Central European tree species. While both species significantly increased their WP$_{PD}$ within only a few days, spruce growth was detrimentally affected by recurring severe summer droughts and beech was also unable to fully recover growth during the first season despite a return of water to the soil.

Design of the throughfall exclusion system with retractable roofs

The initial establishment for the experimental infrastructure costs approximately 0.65 M € including crane, retractable roofs, and commissioned external construction processes. Our throughfall exclusion design resulted in regular needs for maintenance, for example, weekly cleaning and occasional repair of movable parts caused by falling branches (material costs of approximately 15,000 € per year). Overall failure of the throughfall exclusion roofs was <1% of time, respectively, area. However, having retractable roofs, that only closed during rain events and otherwise stay unfurled, minimized micrometeorological effects below the roofs such as increases in air temperature (cf. Table 4), contrasting with static designs and in more open ecosystems (e.g., Pangle et al. 2012). Nevertheless, soil temperature during the growing season decreased by 0.4°C (non-significantly) compared with control plots. Non-retractable parts such as permanent rain shelters, for example, covering engines or around tree stems, slightly reduced winter snow cover and irradiance reaching the forest floor and thus may have caused the reduced soil temperatures to some extent (Gundersen et al. 1998, Kreyling et al. 2012).

An easier to maintain alternative to retractable roofs would have been to cover plots permanently, either completely (Bredemeier et al. 1998, Gundersen et al. 1998) or partially, as done in several recent experiments using troughs (Pangle et al. 2012, Ward et al. 2015, Lu et al. 2017). However, in these experimental approaches, nutrient input on drought plots would likely be affected as litter fall is completely or partially intercepted, an interaction that is less prominent using retractable roofs allowing for largely unchanged litter fall (annual visual observation on site) reaching the forest floor. In addition, sprinkler systems are often used to distribute irrigation water either underneath roofed control plots (Gundersen et al. 1995) or for (subsequent) watering experiments (Bredemeier et al. 1998, Gherardi and Sala 2013). This approach, however, changes the spatial distribution of soil moisture that is naturally heterogeneous through, for example, positions of crowns and stemflow (Breshears et al. 1997, Bellot and Escarre 1998). Similarly, systems partially removing precipitation by troughs are introducing unnatural distribution of soil moisture and thus may alter spatial abundance of roots and microorganism, an impact less prominent with systems employing retractable roofs. The strongest advantage of the system presented here, however, may be its flexibility in controlling the drought treatment. For instance, it can be used to adjust the percentage of throughfall exclusion during the growing season or to impose drought spells of different durations throughout a season.

Another important prerequisite for the drought experiment was to avoid root growth outside of plots (Asbjornsen 2018). To this end, we trenched all plots to a soil depth of about 1 m four years before the start of the drought treatment. Upon trenching, none of the trees displayed any visible negative symptoms or were compromised in a subsequent storm event. Nevertheless, stem diameter growth was initially minimally negatively affected, and beech in particular was effected by initial trenching, but recovered after two growing seasons (Pretzsch et al. 2016). Moreover, even after five subsequent years of drought, trenching remained effective as there was no indication of roots penetrating the inserted plastic tarp or taking up water from outside of the plots. Overall, we highly recommend trenching of both control and experimental drought plots, but negative growth responses should be carefully addressed and enough time should be given for tree recovery before the start of the drought treatment.

Because the Kranzberg forest is a planted site, species distribution is not random, and therefore, plot design was easily delineated into multiple groups of beech trees within a spruce forest. However, we assume that our system can be translated to natural forest stands, with some slight modifications. Trenching and if possible, a pairwise design (pairing one CO plot with a TE plot in close proximity) is highly recommended and most likely obtainable in natural forests. Plot size might be varied, depending on the stand density, individual tree size, and the number of species. Roof size could also be varied to a certain extent, as seen in our experiment in which roofs cover areas from about 100 to 200 m^2. Even larger areas should be feasible. Another important requisite for our experimental design is a power supply on site, and this might also be feasible with generators, as the roofs have a low energy consumption.

Efficacy of the throughfall exclusion

Recurrent exclusion of precipitation throughfall including stemflow during the growing season was effective in reducing SWC even in a soil with rather high water-holding capacity as the one in the present study (Göttlein et al. 2012). In the summers 2014–2018, available soil water was used up down to 70 cm almost every year, supporting the high efficacy of the throughfall exclusion system. We did not aim to simulate future drought scenarios (e.g., a 50% reduction of throughfall precipitation); nevertheless, minimum SWC and leaf water potentials reached during the experiment were similar to values reached during recent naturally occurring droughts in central Europe, for example, in 2015 and 2018 (Fuchs et al. 2020) with means of leaf water potential lower than −1.5 MPa causing critical hydraulic problems in Central European trees (Schuldt et al. 2020). Correspondingly, the low soil moisture availability severely affected tree growth, in particular leading to an 80% reduction in stem diameter expansion in spruce and a reduced production of absorptive root tips (Fig. 6a, c, see also Gaul et al. 2008). These severe curtailments in growth suggest that spruce was at the edge of its survival. In fact, 10 spruce trees died due to bark beetle infestation in the second year of the drought treatment (i.e., 2015), a year with very high abundance of bark beetles in the surrounding forest (Krüger 2016). Interestingly, only spruce trees under throughfall exclusion were successfully infested and subsequently died. As shown in other publications, trees weakened by abiotic stress (e.g., drought) are more vulnerable to biotic attacks and often succumb to a combination of abiotic and biotic stress (McDowell 2011, Netherer et al. 2015). Already in the year preceding their death, those trees had lower stem diameter increments than surviving spruces on throughfall exclusion plots. Despite active measures to prevent further loss of spruce to bark beetle through the use of insecticide from 2016 on, two more spruce trees died—from drought as no visible symptoms of biotic interactions were observed. Overall, spruce seemed to be more vulnerable to re-occurring drought and was limited in above- and belowground growth with lower pre-dawn water potentials than beech. This might be linked to the different stomatal closure strategies (isohydric vs. anisohydric) and the ability of beech to root somewhat deeper than spruce (Zwetsloot et al. 2019, Pretzsch et al. 2020).

Experimentally induced drought already impacted the mycorrhizal community composition in the first year. Before drought was induced, the mycorrhizal community composition was most similar among neighboring plots, supporting the use of a pairwise plot design of one control and one TE plot (Fig 1a). After only one year of summer drought, ectomycorrhizal community samples were clearly grouped into their respective treatment category (Fig. 4b, cf. Nickel et al. 2018). The observed change in mycorrhizal community composition might be an adaptive change in high relevance and reflects the holistic nature that trees have with their associated microorganisms, that is, the holobiont (Vandenkoornhuyse et al. 2015).

Overall, the complete exclusion of throughfall, including stemflow, for 190–257 d during the growing seasons of 2014–2018 evoked strong drought responses in a mature beech/spruce forest with 70- to 90-yr-old trees. For comparisons with other naturally occurring or experimental drought, one should keep in mind the high water-holding capacity of the soil in this experiment. Moreover in the present experiment, precipitation did wet the trees' canopies potentially mitigating drought through leaf or stem water uptake (Katz et al. 1989, Burgess and Dawson 2004, Breshears et al. 2008). During rain events in fact, we occasionally observed increased leaf water potentials of trees growing in the TE plots (data not shown); however, these increases were short-lived. Therefore, we believe aboveground water uptake did not interfere with our long-term drought stress treatment. Additionally, throughfall exclusion introduced a somewhat artificial situation during rainy days when evaporative demand was essentially zero, but soil water potentials were low. This situation, however, would be ideal to test the capacities of aboveground water uptake and reverse flow of water from the canopy toward roots and soils (Prieto et al. 2012).

Recovery upon watering

After five recurrent summer droughts, forest soils on TE plots were hydrophobic with nearly five times higher repellency per plot (Table 2) and low water infiltration rates of about 0.25 ± 0.07 L/h. Thus, for the watering experiment, careful application of irrigation water was mandatory to avoid surface runoff (Olorunfemi et al. 2014). Therefore, our method of water application (soaker hoses) initially used an irrigation rate of about 1 mm/h at the beginning. In all TE plots, a total of 90 L/m^2 was applied within 36 h that resulted in an average increase of 20% in the SWC on the TE plots within one week and successfully increased the SWC in TE plots to levels similar to controls SWC (Fig. 7). We therefore conclude that the hydrophobicity of the soil was overcome and the applied water was distributed consistently within and between plots through our watering experiment. In both species, TE tree leaf water potential recovered to a similar level as controls with one week of watering. In contrast, stem diameter growth at DBH, in particular in spruce, did not recover within the first growing season upon watering similar to findings of Montwé et al. (2014). Before watering in late June/early July, xylem tissue cellular growth rates were about double as fast in control compared with TE trees. Drought trees did not recover their cell growth in response to water. This slow recovery reflects the severity of the previously applied drought stress (Ruehr et al. 2019); however, long-term recovery within the next growing seasons is to be expected (Montwé et al. 2014).

CONCLUSIONS

Responses of mature forests to future climate scenarios are critically understudied. The presented throughfall exclusion system provides high efficacy in imposing drought on mature trees and forest ecosystems. The design with retractable roofs allows for high flexibility in duration and intensity during the drought experiment. Trenching of experimental plots to restrict root growth outside of drought plots and giving trees sufficient time for recovery upon trenching is highly recommended. High responsiveness of soil microorganisms to drought, for example, mycorrhizae, advocates for holistic forest-scale studies. Tree species differ not only in drought susceptibility but also in recovery potential. Thus, recovery responses should not be neglected, in particular as frequency of droughts is expected to increase. During long-term drought experiments, highly hydrophobic forest soils need carefully adapted watering with irrigation intensity not exceeding the uptake rate of the soil to avoid runoff. Subsequent responses of mature trees to watering may be slower than expected from experiments with juvenile trees and should be studied for several growing seasons, in particular after intense, long-term droughts.

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Research paper

Repeated summer drought delays sugar export from the leaf and impairs phloem transport in mature beech

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Phloem sustains maintenance and growth processes through transport of sugars from source to sink organs. Under low water availability, tree functioning is impaired, i.e., growth/photosynthesis decline and phloem transport may be hindered. In a 3-year throughfall exclusion (TE) experiment on mature European beech (*Fagus sylvatica* L.) we conducted $^{13}CO_2$ branch labeling to investigate translocation of recently fixed photoassimilates under experimental drought over 2 years (2015 and 2016). We hypothesized (H1) that mean residence time of photoassimilates in leaves (MRT) increases, whereas (H2) phloem transport velocity (V_{phloem}) decreases under drought. Transport of carbohydrates in the phloem was assessed via $\delta^{13}C$ of CO_2 efflux measured at two branch positions following $^{13}CO_2$ labeling. Pre-dawn water potential (Ψ_{PD}) and time-integrated soil water deficit (iSWD) were used to quantify drought stress. The MRT increased by 46% from 32.1 ± 5.4 h in control (CO) to 46.9 ± 12.3 h in TE trees, supporting H1, and positively correlated ($P < 0.001$) with iSWD. Confirming H2, V_{phloem} in 2016 decreased by 47% from 20.7 ± 5.8 cm h^{-1} in CO to 11.0 ± 2.9 cm h^{-1} in TE trees and positively correlated with Ψ_{PD} ($P = 0.001$). We suggest that the positive correlation between MRT and iSWD is a result of the accumulation of osmolytes maintaining cell turgor in the leaves under longer drought periods. Furthermore, we propose that the positive correlation between V_{phloem} and Ψ_{PD} is due to a lower water uptake of phloem conduits from surrounding tissues under increasing drought leading to a higher phloem sap viscosity and lower V_{phloem}. The two mechanisms increasing MRT and reducing V_{phloem} respond differently to low water availability and impair trees' carbon translocation under drought.

Keywords: drought stress dose, *Fagus sylvatica*, mean residence time, phloem transport velocity, stable carbon isotope (^{13}C) labeling, water potential.

Introduction

In times of ongoing climate change, forests face several challenges to survival (Dale et al. 2001, Allen et al. 2010, Lindner et al. 2010, Choat et al. 2012) such as extended periods of severe droughts (IPCC 2007). Especially for temperate and boreal forests, warmer climate could be critical for tree survival (Anderegg et al. 2012) as observed in Central Europe during 2003 and 2015 (Ciais et al. 2005, Leuzinger et al. 2005, Hartmann et al. 2018b). Recent debates on drought-induced tree mortality have focused on two interacting abiotic mechanisms, carbon starvation and hydraulic failure (McDowell et al. 2008, Sevanto et al. 2014). According to the carbon starvation hypothesis, declining levels of carbohydrates in plant tissues impede metabolism and defense (McDowell 2011), as the uptake of carbon decreases under drought with plants closing their stomata to minimize water loss (Flexas and Medrano 2002). Moreover, not all carbohydrates may be available for metabolism during drought, as sugars are used for several different purposes (e.g., respiration processes) or impaired phloem transport may result in parts of a plant suffering from low carbohydrate levels (McDowell and Sevanto 2010, Hartmann et al. 2013). So when the replenishment with sugars falters, sink organs are forced to use up their stores to maintain functionality and once stores are used up these organs suffer from carbon starvation. In fact, drought can significantly delay phloem transport from source leaves to the soil (Ruehr et al. 2009, Zang et al. 2014), either by reducing phloem velocity, e.g., through increased phloem sap viscosity (Epron et al. 2015) or source–sink relationship of carbohydrates, i.e., phloem loading and unloading processes (Ryan and Asao 2014, Sevanto 2014, Woodruff 2014). Hydraulic failure results from cavitation in xylem vessels and the formation of embolisms (Cochard et al. 2002, Choat et al. 2012, Barigah et al. 2013, Sevanto et al. 2014), and eventually leads to a loss of xylem conductivity and a shortage in water supply (Delzon et al. 2010, Urli et al. 2013). Recently, both mechanisms, i.e., carbon starvation and hydraulic failure, were shown to co-occur (Adams et al. 2017) and are likely interrelated via the potential role of carbohydrates, in particular sugars, in osmoregulation (Savi et al. 2016) and embolism repair (Bucci et al. 2003, Savi et al. 2016). Sugar and water transport in plants is therefore not decoupled but strongly intertwined, for example, water required for sugar transport in the phloem originates from the xylem and thus changes in the xylem water potential will have a direct effect on phloem transport properties (Hölttä et al. 2009). The mean residence time (MRT) is the average time that recent photoassimilates stay in assimilative organs (e.g., leaves), before they are transported away via the phloem (Epron et al. 2012). Therefore, MRT can be used as a proxy for how effectively new sugars are loaded into the phloem (Zang et al. 2014). Additionally, the MRT of photoassimilates in leaves increases with accumulative drought, i.e., the drought stress dose, during the vegetation period in young potted beech trees (Zang et al. 2014, Blessing et al. 2015). The impairment of phloem transport under drought, especially the connection between MRT and phloem transport velocity (V_{phloem}) is not well understood but may be related to an increase in viscosity of the phloem sap (Sevanto 2014) with high sugar concentrations, increasing resistance in the phloem sieve elements (Hölttä et al. 2006). Moreover, sieve elements produced at low water potential during drought could restrict phloem sap transport, as they are supposedly smaller and thinner in diameter (Woodruff 2014).

Studies on sugar transport in the phloem under drought were mostly performed with potted saplings (Ruehr et al. 2009, Zang et al. 2014, Blessing et al. 2015) or young trees in the field (Epron et al. 2015). To our knowledge, no studies about phloem transport under drought stress have been done on mature trees in a natural stand. Recently emerged criticism about the extrapolation of carbon relations from seedlings studies to the level of mature trees raised the call for studies on mature forest stands (Hartmann et al. 2018a). We took advantage of a long-term drought stress experiment in a forest stand with canopy access via crane in south eastern Germany and investigated the effect of repeated summer drought on the transport of recently fixed photoassimilates in branches of mature European beech (*Fagus sylvatica* L.), the dominant species of temperate European forests (Cavin et al. 2013). We hypothesized that drought (i) increases the MRT of recent photoassimilates in leaves of mature beech and (ii) reduces the transport velocity in branch phloem. In a large-scale 3-year throughfall exclusion experiment on mature beech (Pretzsch et al. 2014, Tomasella et al. 2017) we applied $^{13}CO_2$ labeling of individual branches and continuously measured CO_2 efflux and its $\delta^{13}C$ signature at two positions along the branch to calculate phloem transport velocity (Dannoura et al. 2011, Epron et al. 2012). Mean residence time of photoassimilates in leaves was assessed by measuring leaf non-structural carbohydrates (NSC) concentration and $\delta^{13}C$ at a high temporal resolution. Both parameters we tested for their dependency on pre-dawn water potential and drought stress dose, accumulated over the vegetation period.

Materials and methods

Plant material and experimental design

The experiment was part of the Kranzberg Forest Roof Experiment (KROOF) in SE Germany near Freising. The experimental site is a mixed stand of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* Karst.) planted around 1931 AD and 1951 AD, respectively. The KROOF project started in 2013 and is composed of 12 plots (Pretzsch et al. 2014), trenched to one-meter depth in 2010, with three to seven beech and spruce trees within each plot (Pretzsch et al. 2016). Half of the plots, i.e., the throughfall exclusion plots (TE), were covered with a unique rainfall-activated roof construction, which closes automatically only during rain periods during the vegetation period to minimize any roofing effect, like changes in air temperature. For eight of these plots (four control (CO) and four TE) the canopy was accessible via scaffolding and a canopy crane, allowing repeated leaf sampling and measurements.

Data presented here were produced in 2015 (August 21 to September 24) and 2016 (July 19 to September 5). Thirteen sun-exposed branches of each five CO and TE trees (i.e., three trees with two branches) accessible by canopy crane were selected for similar foliage mass and diameter (see Results). Several weeks before the experiment, we installed cuvettes for sampling of branch CO_2 efflux, hereafter referred to as 'respiration'. Environmental data were collected continuously at a climate station at 27 m height right above the canopy of the experimental site.

$^{13}CO_2$ labeling of branches

Labeling was done during morning hours between 07:50 and 10:50 MEZ for 2.5 ± 0.34 h. During labeling daily average temperatures (23.7 ± 2.7 °C), relative humidity (65.3 ± 6.2%) and max.

photosynthetic photon flux density (1405 ± 130 μmol photons s^{-1} m^{-2}) were similar.

One branch per beech tree and measurement campaign was covered with a transparent plastic bag (ceramic-coated PET/PE mix of ~115 μm thickness, Long Life for Art, Eichstetten, Germany), which was sealed with tape and Terostat (Henkel AG & Co. KgaA, Düsseldorf, Germany) and pumped up to a volume of 45 l with ambient air. Depending on leaf biomass, each branch was exposed to a known volume of 150–300 ml of 99 atom% $^{13}CO_2$ gas (Campro scientific GmbH, Berlin, Germany) and the air inside the bag was mixed via a fan. The CO_2 concentration inside the labeling bag was 5302 ± 1110 ppm (~91.54 atom% ^{13}C) right after label injection. At the end of labeling, CO_2 concentration was reduced to 87 ± 20 ppm (~10.61 atom% ^{13}C), confirming label uptake. During labeling air temperature inside the labeling bags was 26.6 ± 5.6 °C. For all three parameters, temperature, CO_2 and ^{13}C concentration, no differences were found between CO and TE.

Mean residence time of recently fixed carbohydrates in leaves

Five leaves, distributed along the branch, were sampled before, directly after and eight times during three days following labeling. Samples were immediately put on ice (0 °C), microwaved for 30 s within 1 h and eventually oven-dried at 64 °C for 2 days, before being ball milled (Retsch® MM200, Haan, Germany) and analyzed with an isotope-ratio-mass-spectrometer coupled with an element analyzer (Isoprime, Elementar, Hanau, Germany) for its ^{13}C content. For each branch, the measured values were normalized over the maximal measured value per branch and an exponential curve was fitted:

$$\delta^{13}\mathrm{C} = e^{k*t}$$

The mean residence time (MRT in h) was then estimated as the invers of *k*.

Phloem transport velocity

Two darkened chambers were installed on the labeled branches, one close (proximal) to the stem and one close to the foliated part of the branch (distal). The distances between the two chambers were 1.30 ± 0.20 m. During and after labeling, the ^{13}C signature of the branch CO_2 respiration of each cuvette was measured with a Delta Ray Isotope Ratio Infrared Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Trees showed a steady $\delta^{13}C$ value before labeling and a strong increase a few hours after labeling (see Figure 1). The sigmoidal part of the curve (steady value before labeling as the lower limit, the increase after labeling and the maximum as the upper limit) for each chamber was fitted with the following function:

$$\delta^{13}\mathrm{C} = d + \frac{a-d}{1+\left(e^{\frac{t-c}{b}}\right)}$$

with '*a*' being the lower limit, '*b*' the spreading of the sigmoid, '*c*' the inflection point and 'd' the upper limit. For the calculation of the phloem transport velocity (V_{phloem}, cm h^{-1}), two different approaches were used, (i) via the inflection point (V_{ip}, cm h^{-1}) and (ii) over the point of the biggest change in slope (first zero of the third derivative of the function, V_{cos}, cm h^{-1}). The time difference (Δt in h) between the two cuvettes was divided by the distance between the proximal and distal branch chamber to derive the phloem transport velocity. The velocities calculated by the two approaches correlated well ($\rho = 0.778$, $P < 0.001$) (Figure 2) and the mean of both was used as V_{phloem}.

Leaf water potential and integrated soil water saturation deficit

Two to five days before each labeling, predawn water potential of sun-exposed leaves (Ψ_{PD} in MPa) was measured with a Scholander pressure bomb (mod. 1505D, PMS Instrument Co., Albany, OR, USA). For calculating the time-integrated soil water deficit (iSWD in day $\mathrm{cm}_{(H2O)}{}^{3}$ $\mathrm{cm}_{(soil)}{}^{-3}$), weekly Time Domain Reflectometry (TDR) (TDR100, Campbell Scientific, Logan, CT, USA) measurements were used (probes buried at three positions per plot at each four depths, i.e., 0–7 cm, 10–30 cm, 30–50 cm and 50–70 cm; for details see Goisser et al. 2016). The maximum soil water saturation measured in the year before the start of throughfall exclusion in 2014 was considered as the water saturated value under natural field conditions for each sensor (black solid line in Figure 3). The integral of this saturated value over time during the growing seasons (from bud break to the labeling point) in 2015 and 2016 was calculated (hatched area in Figure 3a). A non-linear model was fitted for each TDR probe over the same time period (gray solid line in Figure 3b and c), and the integral was calculated (gray area in Figure 3b). The iSWD was then calculated as the difference between the two integrals (black area in Figure 3) for each TDR probe as a time-integrated deficit in soil water, indicating the drought stress dose.

Assessment of NSC

We assessed leaf concentrations of soluble carbohydrates (SC_{leaf} in mg g^{-1} dry weight, i.e., sum of individually assessed water soluble glucose, fructose and sucrose and starch ($\mathrm{Starch}_{\mathrm{leaf}}$ in mg g^{-1} dry weight) from the same samples used for MRT measurements (see Table S1 available as Supplementary Data at *Tree Physiology* Online). Samples were milled with a ball mill (Retsch® MM200) to fine powder and 10 mg of the samples were added to 1 ml distilled water. The mixture was vortexed, incubated for 10 min at 65 °C in a thermomixer (1050 rpm) and then centrifuged for 15 min at 2300*g*. The supernatant was removed with a pipette, stored on ice and the procedure was repeated twice. The

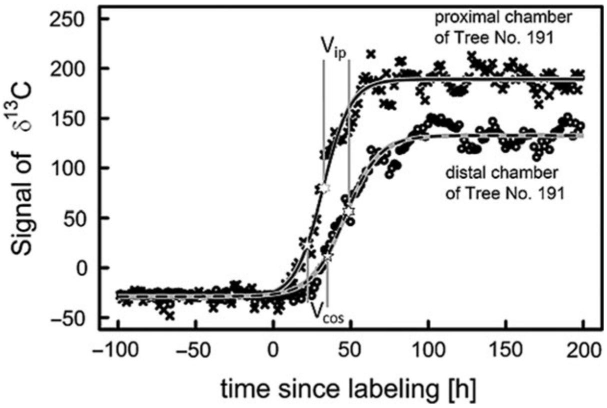

Figure 1. Example of $\delta^{13}C$ of branch respiration of tree 191 (solid line represents sigmoidal curve of the proximal chamber, dotted line represents sigmoidal curve for the distal chamber, five pointed stars show the point of greatest change in slope giving V_{cos} and six pointed stars show the inflection point giving V_{ip}).

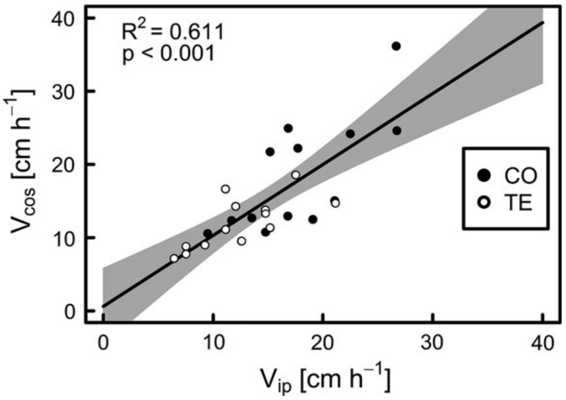

Figure 2. Correlation between V_{cos} (phloem transport velocity calculated by the biggest change of slope in the first part of the sigmoidal curves) and V_{ip} (phloem transport velocity calculated by the inflection point of the curves) for phloem transport velocity calculations (solid line shows the prediction of the linear model, gray area shows the 95% confidence interval, $n = 13$ for CO and TE).

supernatants were pooled and stored frozen at −20 °C for later measurement (Raessler et al. 2010).

Starch digestion was performed with an incubation of the remaining pellet, for 2 h at 85 °C, using α-amylase from *Bacillus licheniformis* (Sigma cat. no. A4551). After centrifuging at 13,000*g* for 1 min, the resulting soluble glucans contained in the supernatant were then hydrolyzed into glucose at 55 °C for 2 h using amyloglucosidase from *Aspergillus niger* (Sigma cat. no. ROAMYGLL) (see Landhaeusser et al. submitted).

Sugar and starch extracts were diluted (1:20 and 1:55, respectively) before measurement with high pressure liquid chromatography pulsed amperometric detection on a Dionex® ICS 3000 ion chromatography system equipped with an autosampler (Raessler et al. 2010). Starch concentrations were computed as the glucose concentration in the hydrolyzed extract multiplied by a conversion factor of 0.9 (Sullivan 1935).

Statistical analyses

Data were analyzed for statistical differences using R (version 3.1.2, R Development Core Team 2008) in RStudio (version 0.97.332, RStudio Team 2015). The decay curves for the MRT and the sigmoidal curves for the branch respiration data were done with the 'nls' function (package: stats, version: 3.1.2) and with the functions shown in the respective paragraphs. Every model was tested for normality of the residuals (Shapiro test) and data was tested for homogeneity of variances (Levene test) beforehand. For differences in Ψ_{PD}, iSWD and V_{phloem} a linear mixed effect model was calculated, using the year (2015 and 2016) and the treatment (CO and TE) as fixed and the tree individual as a random effect (package: nlme, version: 3.1-118). In none of our measured parameters the factor month (August 2016 and July 2016) showed any influence, therefore we excluded it form the analyses. If the mixed effect model showed significant effects, we did a post-hoc test with the 'lsmeans' function with Tukey correction (package: lsmeans, version: 2.20-23). Data were plotted with the boxplot function (package: graphics, version: 3.1.2, showing the first, second (i.e., median, solid line within the box) and third quartile as a box, the whiskers indicate the 1.5 interquartile range and empty dots showing outliers) and the 'plotFit' function (package: investr, version: 1.4). Data in text are given as the mean ± 1 SD.

Results

Branch characteristics

The year had no influence on any of the branch characteristics parameter and we therefore excluded it from the analyses. At the stem proximal position branch diameter was somewhat larger (CO: 22.7 ± 4.0 mm and TE: 22.3 ± 2.2 mm, $P = 0.80$) than at the distal position (CO: 17.0 ± 2.5 mm and TE: 17.8 ± 2.2 mm, $P = 0.31$) (Table 1). No differences between CO and TE trees were found in total leaf dry mass (77.9 ± 24.6 and 74.3 ± 19.3 g, respectively; $P = 0.86$). Likewise, concentration of soluble carbohydrates (71.1 ± 11.4 mg g^{-1} and 78.6 ± 11.6 mg g^{-1}, $P = 0.16$) and starch (10.8 ± 5.1 mg g^{-1} and 9.9 ± 5.2 mg g^{-1}, $P = 0.90$) in the leaves of CO and TE trees, respectively, did not show any significant difference (Table 1).

Drought treatment

We found no differences in the Ψ_{PD} during our first experiment in 2015 (CO: –0.92 ± 0.19 MPa and TE: –0.93 ± 0.22 MPa, Table 2), because a natural drought reduced Ψ_{PD} of CO trees in late summer. However, iSWD was significantly lower for the CO trees (62.7 ± 8.9 d $\mathrm{cm_{(H_2O)}}^3\ \mathrm{cm_{(soil)}}^{-3}$) compared with the TE trees (121.1 ± 6.2 d $\mathrm{cm_{(H_2O)}}^3\ \mathrm{cm_{(soil)}}^{-3}$, Table 2) as drought started much earlier during the year under TE. Ψ_{PD} in TE trees

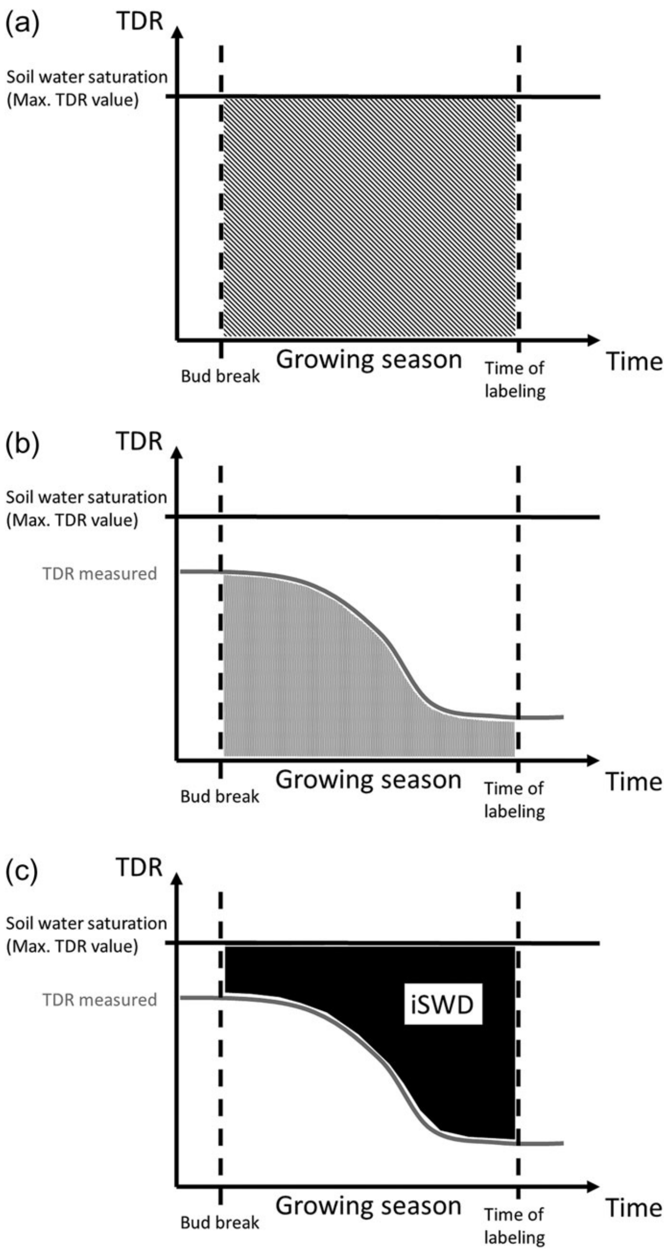

Figure 3. Schematic figure for the calculation of the time integrated soil water deficit (iSWD). (a) Value for saturated soil (hatched area) given by the maximum assessed TDR value before the start of TE in 2014 (black solid line), (b) value of available water in the soil (gray shaded area) given by the assessed TDR values during the growing season for each sensor (gray solid line shows a model curve for the decline of TDR over the growing season) and (c) iSWD results from the difference between the saturated and available water in the soil (black shaded area); dotted black lines mark the growing season (from bud break until the time point of labeling).

was below −1 MPa from beginning of July on, while drought in CO trees started about 1 month later (data not shown). In 2016, both Ψ_{PD} and iSWD were significantly different, with CO trees (−0.37 ± 0.07 MPa) showing higher pre-dawn water potentials compared with TE trees (−0.73 ± 0.23 MPa, Table 2) and lower values for the iSWD (CO: 52.2 ± 13.6 d $\text{cm}_{(\text{H2O})}{}^{3}\ \text{cm}_{(\text{soil})}{}^{-3}$ and TE: 84.3 ± 19.2 d $\text{cm}_{(\text{H2O})}{}^{3}\ \text{cm}_{(\text{soil})}{}^{-3}$, Table 2).

Mean residence time of photoassimilates in leaves

The different time-points of measurement campaigns did not affect the MRT of photoassimilates in leaves and thus were excluded from further statistical analyses. On average for both years photoassimilates stayed for 32.1 ± 5.4 h and 46.9 ± 12.3 h in the leaves of CO and TE trees, respectively ($P < 0.01$, Figure 4). We found this pattern for both years, despite no difference in Ψ_{PD} between CO and TE trees in 2015 (Table 2).

Phloem transport velocity

In 2015, we found no difference for V_{phloem} between CO (14.2 ± 2.8 cm h^{-1}) and TE (14.1 ± 3.6 cm h^{-1}, Figure 5). In the following year, V_{phloem} was significantly increased and almost twice as high in CO trees (20.7 ± 5.8 cm h^{-1}) compared with TE trees (11.0 ± 2.9 cm h^{-1}).

Relation of plant water status with MRT of recently fixed carbohydrates in leaves and V_{phloem} of branches

We related MRT of recently fixed carbohydrates in leaves and V_{phloem} of branches with both Ψ_{PD}, representing the current water status of the tree (indicating drought stress at the time of measurement) and iSWD, indicating accumulative drought stress over the vegetation period (Figure 6). The MRT of leaves correlated positively with iSWD, whereas not relation was found for Ψ_{PD} (Figure 6a and b). Conversely, V_{phloem} in branches correlated positively with Ψ_{PD}, but not with iSWD (Figure 6c and d). This indicates MRT of recently fixed carbohydrates in leaves to be not dependent on the current water status of a plant, but to increase with the time-integrated drought stress dose during the vegetation period. Conversely, V_{phloem} in branches was negatively affected by decreasing water status of the tree (Ψ_{PD}) but not by the drought stress dose.

Discussion

In our study the seasonal cumulative effect of drought (iSWD, Figure 6b) increased the MRT of photoassimilates in leaves but independent from current drought levels, i.e., Ψ_{PD}, as seen in the experiment of 2015 (Figure 6a). By contrast, low Ψ_{PD} limits sugar transport velocity in branch phloem (Figure 6c) independent of the seasonal cumulative effects of drought as seen in the experiment of 2016 (Figure 6d).

Mean residence time of recently fixed carbohydrates increases in leaves during drought

Carbohydrates have diverse functions in plants, especially under stress conditions (e.g., energy source, energy transport, osmotic adjustments, storage, etc., Hartmann and Trumbore 2016). Under drought, sugars are used for osmotic adjustment of the mesophyll cells to maintain functionality and structural integrity (Sala et al. 2010). In fact, osmotic adjustment in the drought stressed trees of our study has been reported before (Tomasella et al. 2017) and a consistent tendency was found in the present

Table 1. Proximal ($D_{proximal}$) and distal (D_{distal}) branch diameter, leaf dry mass (LDM), soluble carbohydrates (SC_{leaf}) and starch ($Starch_{leaf}$) concentration of leaves for the experiment in 2015 and 2016 combined. Values are given as the mean ± 1 SD (n.s. means no significant differences between CO and TE).

	$D_{proximal}$ (mm)		D_{distal} (mm)		LDM (g)		SC_{leaf} (mg g^{-1})		$Starch_{leaf}$ (mg g^{-1})	
CO	22.7 ± 4.0	n.s.	17.0 ± 2.5	n.s.	77.9 ± 24.6	n.s.	71.1 ± 11.4	n.s.	10.8 ± 5.1	n.s.
TE	22.3 ± 2.2		17.8 ± 2.2		74.3 ± 19.3		78.6 ± 11.6		9.9 ± 5.2	

Table 2. Predawn water potential (Ψ_{PD}) and time-integrated soil water deficit (iSWD) for the experiment in 2015 and 2016. Values are given as the mean ± 1 SD. (Different letters indicate differences between treatments and years within the parameter.)

	Ψ_{PD} (MPa)		iSWD (d $cm_{(H2O)}^{3}$ $cm_{(soil)}^{-3}$)	
	2015	2016	2015	2016
CO	−0.92 ± 0.19 a	−0.37 ± 0.07 b	62.7 ± 8.9 AB	52.2 ± 13.6 B
TE	−0.93 ± 0.22 a	−0.73 ± 0.23 a	121.1 ± 6.2 C	84.3 ± 19.2 A

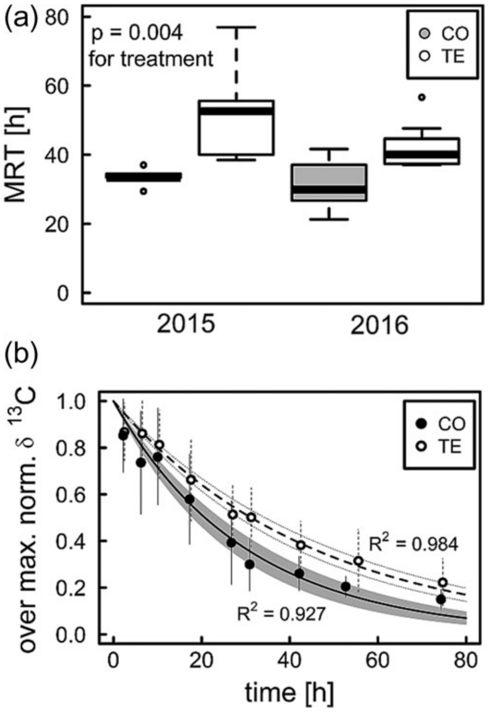

Figure 4. (a) Mean residence time (MRT) of photoassimilates in CO (gray) and TE (white) leaves (tested with lme with post-hoc test lsmeans with Tukey corrections) and (b) fall-off curve of $\delta^{13}C$ in bulk tissue of leaves of CO (black dots, solid line shows the prediction of the non-linear model, gray band shows the 95% confidence interval) and TE trees (open dots, dashed line shows the prediction of the non-linear exponential model, dotted lines shows the 95% confidence interval, all values shown here are corrected by subtracting the natural abundance from the actual measured value).

study (Table 1). Thus, one reason for an increase in the MRT could be that newly fixed carbon is not directly exported from the leaf but undergoes mixing with 'older' carbon as it is used for the osmotic adjustment in leaves (Keel et al. 2007, Nio et al. 2011). Given that the MRT is dependent on iSWD (Figure 6b), the latter may be a good indicator for accumulation of osmolytes (e.g., sugars) in drought-stressed plants. It appears unlikely that the increase in MRT is influenced by the phloem anatomy (e.g., phloem conduit size), as a few days after rewetting of potted saplings, MRT in beech leaves recovers to the level of control plants (Zang et al. 2014). Moreover, MRT appears to be independent from the reduction of sugar transport in the phloem, as during 2015 transport velocity was not reduced in TE compared with CO trees, while MRT was still increased (Figures 4 and 5). Therefore, MRT seems to be rather determined by the turnover time of carbon or the carbon demand in leaves itself than the phloem loading capabilities or sink strength of other organs. Accordingly, the present findings on mature trees fit well with previous studies on juvenile and potted saplings (Ruehr et al. 2009, Zang et al. 2014, Blessing et al. 2015) as the mechanistic basis behind the increased carbon residence time appears to be on the leaf level rather than on the whole-tree level. Moreover, this would also explain why different aspects of drought, i.e., iSWD and Ψ_{PD}, affect MRT and V_{phloem} in different ways, respectively (Figure 6).

Reduction of phloem transport velocity (V_{phloem}) in the branch under drought

Whereas the increase in MRT was related to long-term effects of drought, and independent of the current water status of the tree, the opposite was found for V_{phloem}, which decreased with Ψ_{PD}. Ernst Münch postulated that long distance phloem transport is driven by osmotically generated pressure differences between source and sink organs (Münch 1930). However, phloem sieve tubes are not strictly isolated from but exchange water with surrounding cells (Phillips and Dungan 1993, Knoblauch and Peters 2010) and xylem elements (Nikinmaa et al. 2014). Moreover, under drought the concentration of osmotically active substances increase in neighboring cells of the phloem conduits (e.g., companion cells or parenchyma cells, Sala et al. 2010), further reducing the amount of available water in the phloem conduits (Phillips and Dungan 1993). Consequently, low water availability in the xylem (i.e., low Ψ_{PD}) will reduce phloem transport velocity, as the flux of water from neighboring cells into the phloem conduits is one of the main driving factors for phloem transport (Jensen et al. 2009). While this effect was not found under mild drought (Ψ_{PD} ~–0.29 to –0.38 MPa) in young eucalypt trees (*Eucalyptus grandis*, Epron et al. 2015), phloem transport velocity was limited at Ψ_{PD} below –0.5 MPa in mature beech (Figure 6c). If water availability of the phloem decreases, either by lower exchange capability (e.g., high concentration of osmolytes) or a shortage of water supply, the viscosity of the phloem sap is likely to increase (Epron et al. 2015). This would lower the transport velocity in the phloem, especially as the viscosity of sugar solutions increases exponentially with the sugar concentration in the solution (Morison 2002). As a result of their high water storage capacity (e.g., in the stem), mature trees are expected to be better buffered against drought effects on V_{phloem} than saplings. Nevertheless in the present KROOF experiment with two subsequent years of intense summer drought, V_{phloem} was significantly reduced by about 50% in mature beech trees. In juvenile trees, carbon translocation from leaves to soils has been demonstrated to be delayed by drought (Ruehr et al. 2009, Zang et al. 2014, Blessing et al. 2015), however direct effects on V_{phloem} have not been studied. In addition, phloem transport velocity may be reduced through impaired source–sink relations. In general, reduction in tree growth under drought is a well known phenomenon and has been documented for the study trees (Bréda et al. 2006, Rötzer et al. 2017). As plants use less carbon for growth under drought and additionally may mobilize their carbon stores (e.g., starch), reduced sink strength may add to the decrease of V_{phloem}, driven by the growth rate, size and number of sink organs (Marcelis 1996, Muller et al. 2011, Körner 2015). Under long-term drought, this may lead to severe problems in sink organs, such

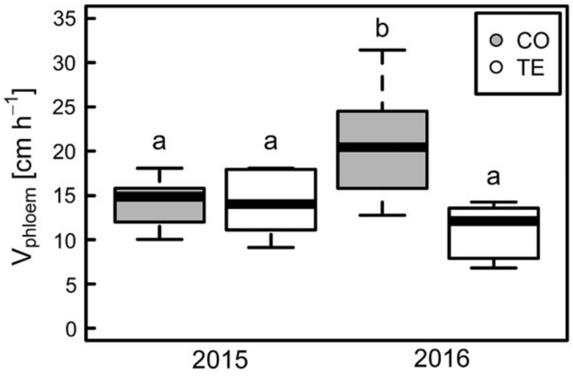

Figure 5. Boxplot of the phloem transport velocity (V_{phloem}) of CO (gray) and TE (white) trees in 2015 and 2016. Different letters indicate significant differences between treatments (P < 0.05, tested with lme with post-hoc test lsmeans with Tukey corrections, n = 13 for CO and TE).

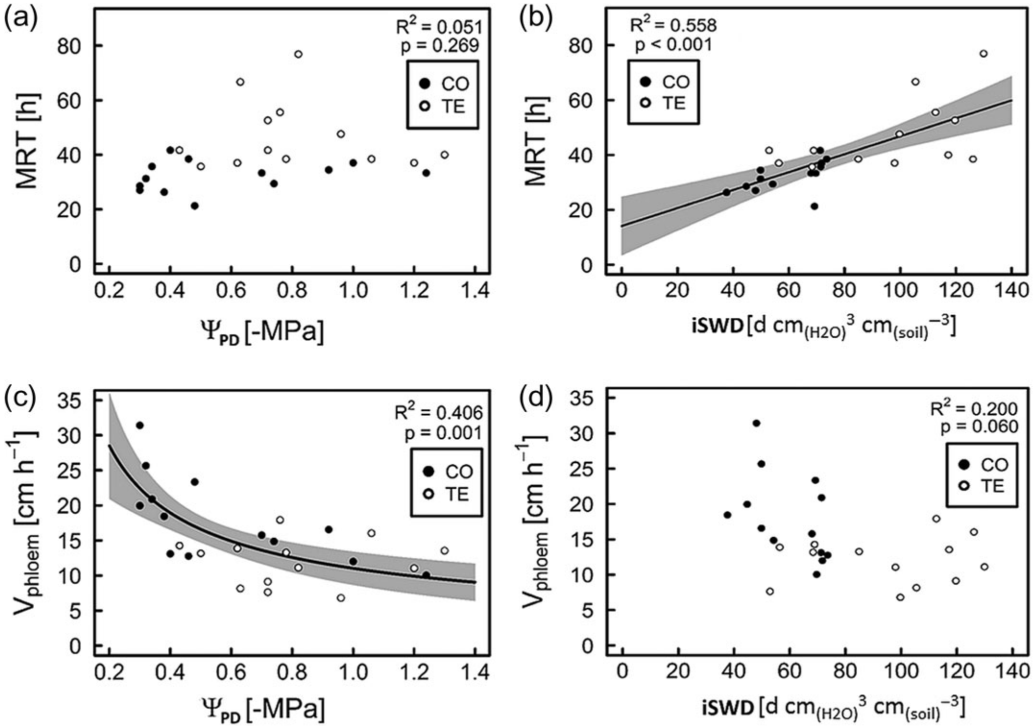

Figure 6. Correlation between the mean residence time of photoassimilates (MRT) in leaves and the predawn water potential (Ψ_{PD}) (a) and the soil water saturation deficit (iSWD) (b), and between the phloem transport velocity (V_{phloem}) and the predawn water potential (Ψ_{PD}) (c) and the soil water saturation deficit (iSWD) (d) (solid lines show the prediction of the linear (b) and power function (c) model, gray area shows the 95% confidence interval, n = 13 for CO and TE).

as roots. When storages are used up or can no longer be mobilized these parts of the tree may end up suffering from carbon starvation and eventually die off. Although trees can increase their osmotic potential in roots, similar to above ground organs, this seems to be species or even genotype dependent and is therefore rather difficult to predict (Brunner et al. 2015).

Conclusions

Carbon allocation within trees is a fundamental process for the regulation of growth and survival. Sugar export from leaves and subsequent phloem transport are key elements in this process, responding independently to drought. The increase in MRT of recently fixed carbohydrates in leaves is increased with long-term, time-integrated drought stress dose (i.e., iSWD), whereas V_{phloem} decreased with the current water status of the tree (i.e., Ψ_{PD}). With ongoing climate change, trees' carbon allocation will be challenged by both longer and more severe drought periods. As shown here for mature beech trees, increased MRT of recent photoassimilates in leaves together with decreased transport velocity along the phloem pathway may limit trees' capability to respond to drought. Additionally, this limitation fosters drought stress by restricting the carbon supply of sink tissues and defense metabolism, eventually increasing the risk of tree mortality under drought, e.g., if roots, one of the main sink organs for carbon, is no longer supplied with sufficient carbon.

Supplementary Data

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

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Conflict of interest

None declared.

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Dedication

We would like to dedicate this work to Prof. Dr Rainer Matyssek on the occasion of his retirement.

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Mature beech and spruce trees under drought – Higher C investment in reproduction at the expense of whole-tree NSC stores

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ABSTRACT

Under drought, potential plant death from depletion of carbon (C) stores, referred to as carbon starvation, is thought to result from negative carbon balance during (partial) stomatal closure. As evidence for C starvation is rare for mature trees, we investigated the C dynamics in mature beech and spruce under drought, focusing on non-structural carbohydrates (NSC) as an indicator of their C balance. Trees were exposed to complete exclusion of precipitation throughfall over two consecutive growing seasons. We assessed NSC concentrations during the early dormant season in leaves, twigs, stem phloem/xylem, coarse and fine roots. Up-scaling to whole-tree NSC pools was achieved using a process-based single-tree model ("BALANCE"), estimating tree biomass increment. While there were distinct differences in NSC concentration among different tissues in both beech and spruce (root < stem < twig < leaves and xylem < phloem), drought did not affect NSC concentrations. However, compared to controls, the whole-tree NSC pool size significantly decreased under drought in both beech (42 %) and spruce (36 %), in parallel to a significant growth decline of overall 52 % and 57 %, respectively. Nevertheless, drought-stressed beech and spruce invested almost twice as much C in reproductive structures relative to total C investment (i.e. 6.0 ± 3.3 and 52.3 ± 8.71 %) compared to control trees (3.1 ± 1.8 and 29.2 ± 7.8 %). This highlights the high priority of C investment into reproduction relative to growth under drought. Given that NSC concentrations are maintained even under severe drought over two growing seasons, NSC pool sizes appear to be a better proxy to assess whole-tree's carbon status in mature trees. Overall, trees maintained NSC availability, avoiding carbon starvation, by downregulating a major C sink (i.e. growth) while upholding reproduction.

1. Introduction

During drought, plants are facing several difficulties with the lack of water and decreased carbon (C) gain as severe consequences (McDowell, 2011). Under harsh drought conditions, these can lead to hydraulic failure and/or carbon starvation (Sevanto et al., 2014) and eventually kill the plant. The water conducting system will be disrupted as conductive elements, i.e. vessels and tracheids, begin to cavitate and water transport is limited, resulting eventually in hydraulic failure (Barigah et al., 2013; Urli et al., 2013). With less water being transported to the leaves, stomatal conductance is often reduced and therefore less C can be assimilated (Brodribb and McAdam, 2011). Plants can follow different strategies to deal with drought. Isohydric plants close their stomata early under drought to minimize water loss and avoid hydraulic failure at the risk of carbon starvation, while anisohydric plants keep their stomata open to maximize C gain, avoiding carbon starvation at a higher risk of hydraulic failure (McDowell et al., 2008). With ongoing climate change, drought periods and heat waves will occur more frequently (Burke et al., 2006; IPCC, 2014), and such stressful conditions will be a major challenge for ecosystems, especially for long-living and slow growing organisms such as trees (Hartmann et al., 2018). Consequences of such extreme events for Central European

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forests have been obvious upon extreme drought years in 2003, 2015 and 2018/19 (Asner et al., 2016; Büntgen et al., 2021; Schuldt et al., 2020) with severe decreases of photosynthesis and primary production (Ciais et al., 2005), as such the C balance of trees could be critically disturbed (Anderegg et al., 2012). During extended drought stress, C stores may become depleted and the plant could be limited in its anabolism (Palacio et al., 2014).

Carbon gained by photosynthesis is needed for many catabolic and anabolic processes. NSCs, consisting mainly of soluble sugars (e.g. sucrose) and starch are invested in five predominant sinks: storage, metabolism (e.g. growth), transport, osmoregulation and export (e.g. exudates, Hartmann and Trumbore, 2016). Under drought, these sinks will be affected in different ways. One of the first consequences is a decrease of the photosynthetic rate accompanied by a depletion of C stores (Maguire and Kobe, 2015). The export of metabolites, e.g. root exudates or volatile organic compounds, also changes under drought (Gessler et al., 2017; Rennenberg et al., 2006). In mature trees, transport of photoassimilates and water, which are highly connected, are strongly decreased under drought (Adams et al., 2017; Hesse et al., 2019; Sevanto, 2018) and therefore C sinks at a longer distance from the primary source (i.e. leaves) could be affected stronger under whole-tree C limitation (Landhäusser and Lieffers, 2012). Especially in mature trees with long transport distances, this could potentially lead to a C imbalance along the tree under drought, with fine roots (longest transport distance from the source organs) being affected stronger than e.g. branches (short transport distance, Ryan and Asao, 2014). So far, most experiments on C allocation at the whole plant level under drought were carried out on juvenile trees (e.g. Hagedorn et al., 2016; Chuste et al., 2020) or under short-term drought (Anderegg et al., 2012). In this paper, we focus on long-term drought effects (i.e. two subsequent growing seasons) on the primary metabolism, in particular NSC, and its consequences for growth and investment in reproduction in a mixed forest with mature trees of Norway spruce (*Picea abies* (L.) H Karst) and European beech (*Fagus sylvatica* L.). The two species follow a rather opposing strategy, when it comes to stomatal sensitivity and regulation under drought (Pretzsch et al., 2014). The degree of isohydry can be seen as continuum with two extreme ends (Hartmann et al., 2021; Hochberg et al., 2018). Strictly isohydric species close their stomata very early under drought stress and try to avoid a sudden drop in water potential, at the expense of a limited CO_2 uptake. Conversely, more anisohydric species keep their stomata open even during severe drought conditions to further assimilate CO_2 at the expense of continued water loss (Hochberg et al., 2018). Following the literature and own observations at the experimental site of this study (Pretzsch et al., 2014), mature spruce is generally behaving more isohydric under drought (Lyr et al., 1992; Oberhuber et al., 2015; Pashkovskiy et al., 2019), although some variability in provenances exist in seedlings (Jamnická et al., 2019). Conversely, mature beech follows a more anisohydric drought strategy (Leuschner, 2020; Magh et al., 2019). We assessed NSC concentrations of different tree organs and tissues during winter early dormancy. We took advantage of the KROOF experiment, a long-term drought experiment, with retractable roofs to simulate summer drought (Grams et al., 2021). By means of the process-based single-tree model BALANCE (Grote and Pretzsch, 2002; Rötzer et al., 2010), we estimated the growth increments of tree's organs allowing for an upscaling of NSC concentrations to estimate overall NSC pools to test the following hypothesis:

H1. NSC concentration is reduced by drought, with concentrations in sink organs being more affected than in source organs and with distance from source organs, i.e. leaves > branches/twigs > stems > roots.

H2. Total NSC pools, measured during dormancy, are reduced in drought stressed trees,

a with the more isohydric spruce being stronger affected than the more anisohydric beech and

b with sink organs being stronger affected than source organs, i.e. leaves > branches/twigs > stems > roots

due to decreases in NSC tissue concentration and growth.

H3. The amount of invested C in reproductive structures decreases under drought.

2. Material and methods

2.1. Plant material and experimental design

The experimental site, a mature mixed forest composed of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) planted around 1931 AD and 1951 AD, respectively, is part of the Kranzberg Forest Roof (KROOF) experiment in Southern Germany near Freising, Bavaria. In 2010, 12 plots were trenched to one-meter depth, allowing no further lateral water transport between the plot and outside soil (Pretzsch et al., 2016). Each plot contained between three to seven beech and spruce trees each (Grams et al., 2021). The KROOF experiment started in 2014 when six of the 12 plots were covered with a unique rainfall-activated retractable roof, which closed automatically for precipitation events during the growing season, to exclude any throughfall as well as stem run-off and retracted after the rain events passed, to minimize any other climatic effects. For further details on the experimental design, site (e.g. soil characteristics) and treatment description see Grams et al. (2021). Data presented in this publication were collected in 2013, 2014 and 2015 from two beech and spruce trees of eight plots accessible via canopy crane (four assigned to the throughfall exclusion (TE) plots and four control plots (CO)) to a total of eight TE and CO trees for each species.

The long-term average in temperature is 13.8 °C during the growing season (7.8 °C for whole year) and 750−800 mm annual precipitation. For 2013 and 2014, the precipitation was 640 mm (growing season: 444 mm) and 651 mm (growing season: 501 mm), a bit lower than the long-term average. At the same time, growing season temperature was slightly higher than average, i.e. 14.8 °C and 15.2 °C in 2013 and 2014, respectively. With only 495 mm (growing season: 330 mm) of precipitation and a growing season temperature of 17.2 °C, the year 2015 was extraordinarily warm and dry. For further details see Grams et al. (2021).

2.2. Drought stress assessment

To assess the effects of the throughfall exclusion on the trees' water status, pre-dawn leaf water potential (Ψ_{PD} in MPa) was measured with a Scholander pressure chamber (mod. 1505D, PMS Instrument CO., Albany, OR, USA) towards the end of the drought period in the middle of October of each year. Volumetric soil water content (SWC in vol.-%), was assessed weekly by Time Domain Reflectometry (TDR, TDR100, Campbell Scientific, Logan, CT, USA) in four different depths (0−7 cm, 10−30 cm, 30−50 cm and 50−70 cm) and at three positions within each plot (total of 144 TDR probes, for details see Goisser et al., 2016). Data were averaged over all four depths and three potions for each plot.

2.3. NSC sampling and concentration assessment

NSC sampling was done in early November 2013 to 2015 at the start of winter dormancy, in order to get the carbon status at the end of the vegetation period, hence one year before the onset and two consecutive year/s of experimentally induced summer drought. For each sample, the sum of soluble carbohydrates (SC, Table S1) and starch (Table S2) was assessed. Via the canopy crane, one-year old leaves of spruce (SC_L & $starch_L$) and branches/twigs of beech and spruce were sampled. For the branches/twigs (2-3 years old), the living bark (inner and outer bark; hereafter referred to as phloem, SC_{TP} & $starch_{TP}$) and xylem (SC_{TX} & $starch_{TX}$) were separated. With an increment borer and a cork borer, the xylem (SC_{SX} & $starch_{SX}$) and phloem (with the dead bark removed, SC_{SP} & $starch_{SP}$) of the stem were collected at 1.3 m above the ground, respectively. For NSC measurements in the xylem, the last 10 years of each increment core were used, combining the conductive and non-conductive portions of the xylem. Additionally, wood chippings of coarse roots (average diameter about 5 cm, SC_{CR} & $starch_{CR}$) were sampled with a driller and fine roots (with a diameter of less than 2 mm, SC_{FR} & $starch_{FR}$) of both species were directly sampled. All samples were stored on ice (0 °C) and microwaved for 30 s within 30 min after sampling, to avoid respiration and degradation losses of NSCs. In the lab, samples were dried for 72 h at 64 °C and ball milled to a fine powder (Retsch® MM200, Haan, Germany). We assessed the concentration of soluble sugars (glucose, fructose and sucrose) and starch, which account for at least 80 % of the mobile carbon pool in both species (Hoch et al., 2003). High performance liquid chromatography with pulsed amperometric detection on a Dionex® ICS 3000 ion chromatography system equipped with an autosampler was used to determine sugar/starch concentration (Raessler et al., 2010). Sample preparation for sugars followed the protocol in Hartmann et al. (2013) and for starch in Landhäusser et al. (2018).

2.4. Sampling of reproductive structures

In autumn 2015, a masting year for both species in southern Germany, reproductive structures (RS) of beech (complete beechnuts, fruits plus huskers) and spruce (cones) were sampled. For beech, a random column of 1 m^2 ground area per plot (often combining the canopy of a few beech individuals) was selected and the number of beechnuts of this area were counted ($N_{beechnut}$ in m^{-2}). Afterwards, these beechnuts were harvested and oven-dried at 64 °C and the dry mass of each beechnut was assessed ($DM_{beechnut}$, in g). In combination with the sum of the beech tree crown spread area per plot (average for CO plots: 149.1 ± 25.2 m^2 and TE plots: 147.9 ± 69.5 m^2), we then estimated the dry mass of beechnuts per plot ($DM_{beechnut\_plot}$ in kg). For spruce, five random branches from the main stem of 8 CO and 8 TE trees were selected and the number of cones were counted, afterwards the number of branches of the main stem was counted and the total number of cones was calculated (N_{cones}, unitless). Subsequently, the cones of the five branches were harvested, oven-dried at 64 °C and each cone was weighed (DM_{cones}, in g). To get the total dry mass of cones per tree ($DM_{cones\_per\_tree}$, in kg), we multiplied N_{cones} with DM_{cones} for each tree individually.

2.5. Modeling biomass increment with process-based model BALANCE

Biomass and growth of organs of individual trees were calculated using the eco-physiological growth model BALANCE (Grote and Pretzsch, 2002), which simulates the three-dimensional development of individual trees based on external environmental factors such as weather (e.g. temperature, precipitation, etc.), CO_2 concentration, soil conditions and water availability. Further on, the model describes the consequences of the individual competition for light, water and nutrients. As individual environmental conditions change with the tree development, the influence of stand structure, species mixture and management options are taken into account, too. Consequently, biomass increment is simulated in dependence of the carbon and nitrogen uptake for each segment on the basis of its energy supply and resource (e.g. water) availability (Fontes et al., 2011). Model outcomes are validated against empirical measurements of the carbon and water balance (including drought scenarios) several times (Rötzer et al., 2017b, 2013, 2012, 2010) and also for the above vs. below ground biomass ratio (Rötzer et al., 2009). For our study, BALANCE was used to generate the biomass of the fine roots (BM_{FR}), the coarse roots (BM_{CR}), the stem (BM_S), the branches/twigs (BM_T) and of the leaves (BM_L, for spruce only, Table S1). The simulation was based on meteorological data from 2013 to 2015 from the nearby weather stations in Freising with initial tree data and soil characteristics as described in Rötzer et al. (2017a, b). Precipitation of the drought plots was reduced according to the throughfall exclusion experiment, i.e., rainfall was set to zero for the period when the roofs were closed (Grams et al., 2021). To validate the model outcome, we compared the modeled biomass increment of stems in 2014 and 2015 (BI_{stem}, in kg) with measured data of band dendrometers (Dendrometer D1, METER, Munich, Germany) at 1.3 m above the ground (diameter increment D_{stem}, in cm) for both species and treatments (i.e. CO and TE).

2.6. NSC pool calculations

We scaled the concentration of SC and starch in each tissue with its modeled biomass (BM in kg, Table S3) to calculate the overall C pools ($SC_{pool\_CO/TE}$ and $Starch_{pool\_CO/TE}$, both in kg, except for spruce $Starch_{pool}$ in g). For stem and branches/twigs, we additionally estimated the proportion of xylem and phloem by measuring the stem circle area at DBH (assessed via measuring tape) and the annulus area for phloem (phloem thickness was assessed with a caliper on the samples taken with the cork borer). For beech, the proportion of the stem xylem was CO: 0.989 ± 0.002 and TE: 0.990 ± 0.003, and for spruce it was CO: 0.988 ± 0.003 and TE: 0.989 ± 0.002, respectively. We then applied the following formula to calculate the stem and branch/twig pool sizes:

$$SC/Starch_{pool} = concentration_{phloem}*biomass_{phloem}*proportion_{phloem} + concentration_{xylem}*biomass_{xylem}*proportion_{xylem}$$

Overall, the phloem was thicker in spruce compared to beech ($P < 0.001$). In spruce there was a significant difference between the phloem thickness, with CO trees having a thicker phloem (0.46 ± 0.05 cm) than TE trees (0.39 ± 0.06 cm) ($P < 0.05$), while in beech we did not find any statistical difference in phloem thickness ($P = 0.17$). However, in beech there appeared to be a similar trend with CO trees (0.35 ± 0.05 cm) having somewhat thicker phloem compared to TE trees (0.31 ± 0.05 cm). For beech, phloem thickness correlated strongly positive with the stem diameter regardless of treatment ($P < 0.05$, $R^2 = 0.31$), while in spruce, this correlation was only valid for TE trees ($P < 0.01$, $R^2 = 0.76$) and not for CO trees ($P = 0.91$, $R^2 = 0.00$).

2.7. Statistical analyses

Data were analyzed for statistical differences using R (version 3.6, R Development Core Team, 2008) in RStudio (version 1.2.1335, RStudio Team, 2015). Every model was tested for normality of the residuals (Shapiro test) and data was tested for homogeneity of variances (Levene test) beforehand. For differences in Ψ_{PD}, biomass increment, NSC concentrations and pools, a linear mixed effect model ('lme' function) was calculated using the species (beech and spruce), year (2013, 2014 and 2015) and the treatment (CO and TE) as fixed and the tree individual nested in the plot as a random effect (package: nlme, version: 3.1-137). If the mixed effect model showed significant effects, we performed a post-hoc test with the 'emmeans' function with Tukey correction (package: emmeans, version: 1.3.1). For data of cones and beechnuts, the same functions were used, but with the plot as the random effect. To validate the BALANCE-data we used a linear model (package: stats, version: 3.5.0) with the BI_{stem} as the dependent variable and the D_{stem} as the test variable. Data were plotted with the 'boxplot' function (package: graphics, version: 3.5.2, showing the first, second (i.e., median, solid line within the box) and third quartile as a box, the whiskers indicate the 1.5 interquartile range and empty dots showing outliers), the 'plot' function (package: graphics, version: 3.5.2) and the 'ggplot' function (package: ggplot2, version: 3.1.0). Data in text and tables are given as mean ± 1 SD.

Table 1
Pre-dawn water potential of beech and spruce at the end of the vegetation period 2013, 2014 and 2015 (Data are given as mean ± 1 SD, different letters indicate significant differences between groups (treatment*year)).

Ψ_{PD} [MPa]	Spruce	
	CO	TE
2013	−0.52 ± 0.06 a	−0.49 ± 0.05 a
2014	−0.78 ± 0.31 a	−1.33 ± 0.12 b
2015	−1.38 ± 0.22 b	−1.68 ± 0.13 c
Ψ_{PD} [MPa]	Beech	
	CO	TE
2013	−0.41 ± 0.10 ab	−0.40 ± 0.08 ab
2014	−0.30 ± 0.04 a	−0.62 ± 0.08 bc
2015	−0.76 ± 0.24 c	−1.14 ± 0.31 d

3. Results

3.1. Drought treatment effects on soil and tree water status

In 2013 prior to establishing the drought, leaf Ψ_{PD} was not different between trees in the treatment plots for both species (beech: -0.41 ± 0.09 MPa and spruce: -0.51 ± 0.06 MPa Table 1). Upon throughfall exclusion, TE trees of both species had significantly lower Ψ_{PD} than CO trees. In beech in 2014 and 2015, Ψ_{PD} was reduced to -0.62 ± 0.08 and -1.14 ± 0.31 MPa (Table 1) corresponding to reductions by 51 and 178 % compared to 2013, respectively. Ψ_{PD} in Spruce was reduction stronger to -1.33 ± 0.12 and -1.68 ± 0.13 MPa in 2014 and 2015 (Table 1), respectively (reductions by 160 and 229 % relative to 2013, respectively). However, during the extraordinary dry year of 2015 the CO trees of both species showed signs of drought stress in their water potential (beech: -0.76 ± 0.24 MPa and spruce: -1.38 ± 0.22 MPa), with values similar to those found in TE trees in 2014, for both species respectively (Table 1).

Before the onset of the drought treatment in 2013, soil water content

Table 2
Mean monthly volumetric soil water content (SWC) of control (CO) and throughfall-exclusion (TE) plots to a soil depth of 70 cm (from January 2013 to December 2015). Timespan of roof closure: from 6th of March to 12th of September in 2014 and 10th of March to 21 st of November in 2015. Data are given as the monthly mean ± 1 SE per treatment and year. Statistically significant differences between CO and TE are given between each month for each year separately with p-value > 0.05 = ns, <0.05 = *, <0.01 = ** and <0.001 = ***). For further details see Grams et al. (2021).

SWC [vol.-%]		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2013	CO	34.1 ± 1.2	38.4 ± 1.0	31.9 ± 0.7	34.2 ± 0.9	30.8 ± 1.1	32.5 ± 0.6	24.9 ± 0.6	18.6 ± 0.6	20.4 ± 0.7	23.5 ± 0.6	24.5 ± 1.2	28.7 ± 1.1
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	TE	32.7 ± 1.3	34.3 ± 1.4	32.7 ± 0.8	34.5 ± 1.0	31.9 ± 1.1	32.7 ± 0.7	25.3 ± 0.6	20.0 ± 0.7	21.0 ± 0.7	22.9 ± 0.6	23.1 ± 1.3	26.0 ± 1.2
2014	CO	30.6 ± 0.8	33.2 ± 1.0	27.8 ± 0.6	29.2 ± 0.5	29.0 ± 0.7	24.7 ± 0.6	22.1 ± 0.5	24.1 ± 0.6	24.7 ± 0.6	26.3 ± 0.7	30.6 ± 0.7	32.7 ± 0.6
		ns	ns	ns	ns	*	*	*	**	**	**	***	***
	TE	27.7 ± 1.0	29.1 ± 1.1	25.4 ± 0.7	27.8 ± 0.6	23.4 ± 0.7	19.5 ± 0.6	17.5 ± 0.5	16.5 ± 0.6	16.7 ± 0.5	17.0 ± 0.6	17.4 ± 0.6	17.6 ± 0.6
2015	CO	35.6 ± 0.5	35.0 ± 0.5	33.8 ± 0.5	34.2 ± 0.4	35.5 ± 0.5	34.6 ± 0.4	24.9 ± 0.5	20.4 ± 0.4	20.1 ± 0.5	22.5 ± 0.5	24.3 ± 0.5	27.7 ± 0.5
		***	***	**	***	***	***	**	ns	ns	*	**	**
	TE	20.1 ± 0.6	21.3 ± 0.6	21.3 ± 0.5	22.6 ± 0.5	20.6 ± 0.5	19.0 ± 0.4	17.5 ± 0.5	16.8 ± 0.4	16.7 ± 0.4	16.7 ± 0.4	16.7 ± 0.4	17.8 ± 0.4

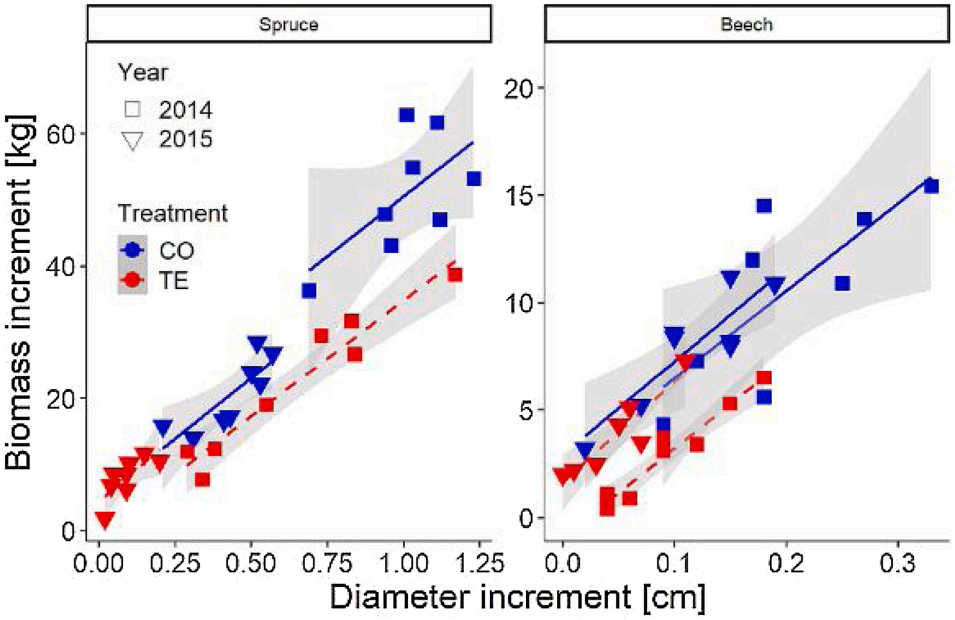

Fig. 1. Measured dendrometer increment at 1.3 m height vs modeled stem biomass increment versus; with triangles showing the values for spruce and circles for beech and in red for throughfall exclusion (TE) and blue for control (CO). Red and blue lines show Pearson correlations for each group of beech and spruce for CO (solid lines) and TE (dashed lines). Gray areas show the 95 % confidence interval for each group. Please note the different scaling of the beech and spruce graph. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Modeled biomass increment (BI) by the model BALANCE of spruce (top) and beech (bottom) for 2014 and 2015. Data are given as mean ± 1 SD, p-values: ns > 0.1, • < 0.1, * < 0.05, ** < 0.01 and *** < 0.001.

Spruce	2014			2015		
BI [kg]	CO		TE	CO		TE
Fine root	1.4 ± 0.4	•	0.9 ± 0.2	1.3 ± 0.2	ns	0.9 ± 0.2
Coarse root	7.0 ± 3.1	ns	7.5 ± 3.4	6.2 ± 2.0	*	2.8 ± 1.4
Stem	50.9 ± 9.1	***	22.2 ± 11.1	20.6 ± 5.5	*	8.0 ± 3.1
Branch/ Twig	2.6 ± 0.7	*	1.9 ± 0.7	2.7 ± 0.4	*	1.9 ± 0.4
Leaf	4.7 ± 1.9	ns	3.3 ± 1.3	3.9 ± 1.5	ns	2.4 ± 1.1

Beech	2014			2015		
BI [kg]	CO		TE	CO		TE
Fine root	1.4 ± 0.2	*	0.7 ± 0.1	1.4 ± 0.4	•	0.8 ± 0.2
Coarse root	3.3 ± 1.8	ns	2.1 ± 1.2	4.5 ± 1.7	*	2.2 ± 1.2
Stem	10.5 ± 4.3	**	3.1 ± 2.2	8.0 ± 2.7	**	3.4 ± 2.2
Branch/Twig	2.6 ± 0.2	**	1.2 ± 0.7	2.6 ± 0.6	*	1.6 ± 1.0

(SWC) to a soil depth of 70 cm was very similar between CO and TE plots (mean difference between CO and TE averaged over all depths: 0.1 ± 1.8 vol.-%, p-value > 0.05, Table 2). During the growing seasons of 2014 and 2015, SWC was generally lower on TE compared to CO plots. In 2014, the mean difference between CO and TE was 6.0 ± 4.0 vol.-% (p-value = 0.023) and in 2015 9.5 ± 4.5 vol.-% (p-value = 0.002, Table 2). During winter, when roofs were permanently open, SWC on all plots was partially recharged by precipitation. In August and September 2015, the SWC on CO plots decreased due to very warm and dry conditions and reached values similar to TE plots.

3.2. Modelled biomass increments using BALANCE

We found strong overall positive linear correlations (Pearson) between the measured stem increment at 1.3 m above ground level and modeled stem biomass increment (Fig. 1), with strong positive correlations for each group (Pearson correlation: P-value/Pearson coefficient of CO spruce 2014: 0.090/0.64 & 2015: 0.011/0.83, TE spruce 2014: <0.001/0.97 & 2015: 0.027/0.76, CO beech 2014: 0.028/0.76 & 2015: 0.003/0.89 and TE beech 2014: <0.001/0.97 & 2015: 0.003/0.92, Fig. 1). Slopes and intercepts were not significantly different between treatments for beech and spruce within the single years, suggesting that our model appears to generate realistic results, with the TE trees showing significantly decreased growth for almost all tissues compared to CO trees. Overall, spruce showed a higher growth than beech, especially of stems and coarse roots (Table 3). Modelled biomass of spruce fine root increment (BI_{FR}) was 34 % lower for both drought years in TE compared to CO trees but not significantly different (p-value 2014: < 0.1 and 2015: > 0.1), and significantly lower by 46 % in TE beech compared to CO trees (p-value 2014: < 0.05 and 2015: <0.1 0.1). Coarse root growth increment (BI_{CR}) was significantly different between the two treatments for both species only in 2015 (p-value spruce: <0.05 and beech: <0.05), with 2-times higher growth increment in CO than TE trees (Table 3).Stem diameter growth of both species was significantly different between CO and TE in both years, with a growth reduction in TE by more than 50 % (p-value spruce 2014: <0.001 & 2015: <0.05 and beech 2014: <0.01 & 2015: <0.01, Table 3). For branch/twig tissue, growth reduction was also about 30–40 % in TE trees compared to CO trees and significant different between treatments for both species and years (p-value spruce 2014: <0.05 & 2015: <0.05 and beech 2014: <0.01 & 2015: <0.05, Table 3). For leaves of spruce, there were no significant differences in both years, nevertheless reductions of 30 and 39 % under TE compared to CO were estimated in 2014 and 2015, respectively (Table 3).

3.3. Biomass investment in reproductive structures during a masting year (2015)

In the masting year, TE trees of spruce produced about three times more cones per tree than CO trees (N_{cones}, CO: 235 ± 66 and TE: 775 ± 349, Fig. 2), while the dry mass per cone (DM_{cone}) was on average about three times smaller in TE than in CO trees (TE: 22.0 ± 6.5 and CO: 59.0 ± 17.2 g, respectively, Fig. 2). Thus, the overall invested biomass in cones per tree ($DM_{cones\_per\_tree}$) was not different between CO (13.4 ± 4.8 kg) and TE (15.7 ± 5.6 kg, Fig. 2). In beech, neither the amount of beechnuts per ground area ($N_{beechnut}$, CO: 21.0 ± 1.6 m^{-2} and TE: 19.3 ± 1.8 m^{-2}) nor the mass per beechnut ($DM_{beechnut}$, CO: 0.73 ± 0.21 g and TE: 0.82 ± 0.28 g) differed between the treatments. Therefore, also the total dry mass of fruits per area ($DM_{beechnut\_plot}$, CO: 2.27 ± 0.66 kg and TE: 2.31 ± 1.32 kg) was very similar in CO and TE (Fig. 2). Accordingly, the invested biomass of both tree species into reproductive structures was not affected by drought although the number of spruce cones was three time higher in TE compared to controls.

3.4. Biomass investment (BI) in structural sinks vs. reproductive structures in 2015

For the masting year 2015, we compared the investment of biomass (BI) into the sink tissues (sum of fine/coarse root, stem and branch/twig tissues, BI_{sum} in kg) and reproductive structures (cone-mass and whole beechnut-mass ($DM_{beechnut\_plot}$ divided by the number of trees on each plot)) per tree. For beech, the BI_{sum} of TE trees (8.4 ± 3.4 kg) was about half of CO trees (17.5 ± 4.8 kg, P-value < 0.01), while the beechnut-drymass did not change (CO: 0.5 ± 0.3 kg and TE: 0.5 ± 0.3 kg, Fig. 3a). The relative biomass investment was calculated by dividing the weight of single tissues by the overall sum of all tissues (including reproductive structures). Therefore, in beech the ratio of reproductive structures compared to structural tissues (e.g. reproduction/(roots + stem + branches)) was 3 ± 1 % and 7 ± 3 % (P-value < 0.01), for CO and TE, respectively (Fig. 3b). For spruce, the BI_{sum} was reduced by 57 % (Fig. 3a) in TE (13.6 ± 3.1 kg) compared to CO (31.5 ± 5.6 kg, P-value $<$ 0.0001), but the cone-drymass was very similar between CO (13.4 ± 4.8 kg) and TE (15.7 ± 5.7 kg, Fig. 3a). Thus, the relative ratio of the investment in reproductive structures compared to other tree tissues combined (e.g. reproduction/(roots + stem + branches)) in spruce was 44 ± 7 % and 115 ± 42 % (P-value < 0.001), for CO and TE, respectively. Most of the difference in structural tissues between CO and TE was caused by a reduced stem and coarse root growth (Fig. 3a). However, the relative biomass investment of structural tissues was only significantly reduced in the stem (p-value = 0.0020) and coarse roots (p-value = 0.0475) of spruce and tendentially in stem of beech (p-value = 0.0774, Fig. 3b).

3.5. NSC concentrations and NSC pools

For both species, there was no significant influence of drought on the concentration of SCs during the dormant season (P = 0.45 for beech and 0.23 for spruce) detected and thus all data points are close to the 1:1 line in Fig. 4a and c. However, concentrations were different among plant tissues. Structural tissues with low metabolic activity, i.e. stem xylem (SX) and coarse roots (CR), showed the lowest concentrations (mean of SX and CR: 22.0 ± 6.1 mg g^{-1} for beech and 7.4 ± 5.0 mg g^{-1} for spruce). Conversely, recently built, highly active and/or transport tissues, i.e. fine roots (FR), stem phloem (SP), branch/twig phloem/xylem (TP/TX) and spruce leaves (L), showed higher concentrations (mean of FR, SP, TP, TX and L: 40.2 ± 8.9 mg g^{-1} for beech and 50.0 ± 11.0 mg g^{-1} for spruce, Fig. 4a, c). Thus, differences in NSC concentrations between structural and active tissues (excluding spruce needles) were more pronounced in beech that in spruce.

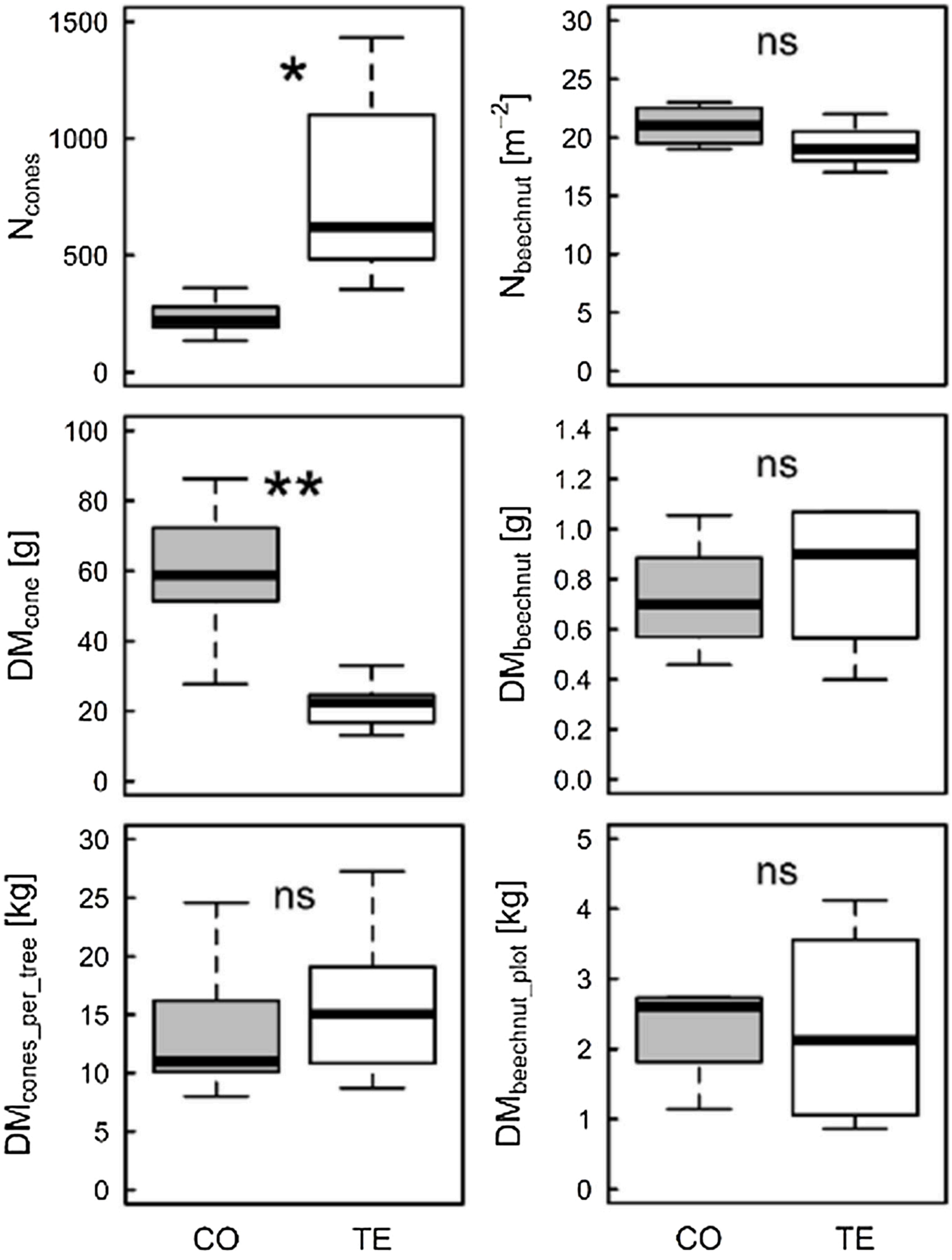

Fig. 2. Number (top), dry mass of single (middle) and accumulated mass (bottom) of reproductive structures in spruce per tree (left) and beech per plot (right; p-values: ns > 0.05, * < 0.05 and ** < 0.01).

In comparison to the SC, starch concentrations of the tissues were on average 10 and 6 times lower for spruce and beech, respectively. Similar to SC, starch concentrations did not differ between CO and TE for both species ($P = 0.408$, Fig. 4b, d). Starch concentrations of beech were highest in stem and branch/twig phloem (all years and both treatments averaged: 8.58 ± 3.48 and 11.49 ± 2.69 mg g^{-1}, respectively), lowest in the stem xylem and coarse roots (all years and both treatments averaged: 1.50 ± 0.68 and 1.62 ± 0.90 mg g^{-1}, respectively) and in between for the branch/twig xylem and fine roots (all years and both treatments averaged: 4.08 ± 1.39 mg g^{-1} and 7.30 ± 2.56 mg g^{-1}, Fig. 4d). A similar pattern was found for spruce, with high values in leaves (average over all years and both treatments: 7.76 ± 1.64 mg g^{-1}) and somewhat lower levels in stem and branch/twig phloem as well as branch/twig xylem (averaged over all years and both treatments: 4.08 ± 2.55, 4.25 ± 1.77 and 5.31 ± 2.77 mg g^{-1}, respectively, Fig. 4b). Except from the above, fine root tissue showed the highest values in spruce (averaged over all years and both treatments: 12.15 ± 4.7 mg g^{-1}). Much lower concentrations were found in the coarse roots and stem xylem (averaged over all years and both treatments: 0.59 ± 0.30 and 0.76 ± 0.71 mg g^{-1}, respectively.

In 2013, the SC pools of tissues were similar in CO and TE trees for both species (green symbols in Fig. 5a, c), but in 2014 and 2015 SC pools decreased significantly in TE, except for fine roots and leaves in spruce (Fig. 5a, c, please note logarithmic scaling of axes). Overall, drought significantly reduced the overall SC pools (for beech: $P < 0.001$ and spruce: $P < 0.0001$), with TE trees showing smaller pool sizes in 2014 and 2015 for almost all tissues (for beech, averaged for all tissues: -49 ± 8 % and spruce, averaged for all tissues (expect leaves): -45 ± 23 %). Concentration of SCs were not affected by the treatment but varied between tissues, while the SC pools were strongly reduced by in the TE treatment. However, the natural drought year of 2015 did not seem to influence the pool size of CO trees (no differences within CO between the

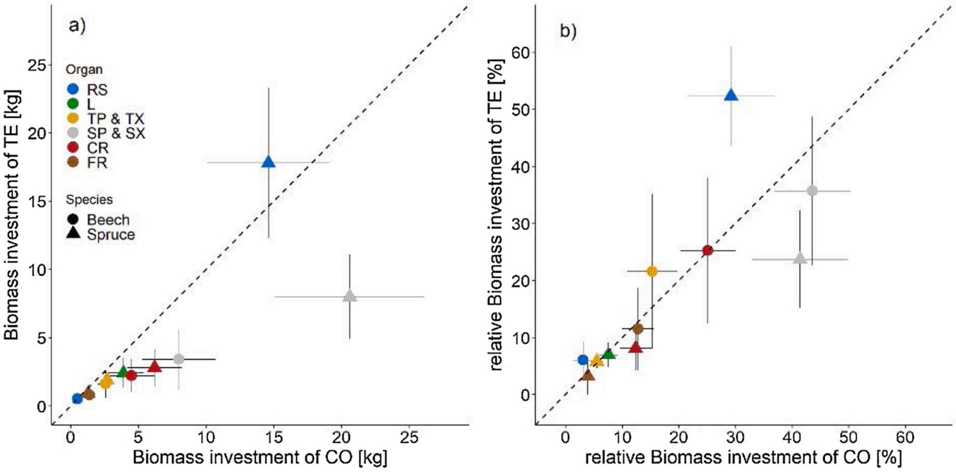

Fig. 3. Total (a, data from Table 3) and relative modeled biomass investment (b) in the masting year 2015. (RS = beechnut or cones (blue), leaves = L (green), branches/twigs = TP & TX (beige), stem = SP & SX (gray), coarse roots = CR (red) and fine roots = FR (brown). Data are given as the mean ± 1SD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

years for both species).

For the starch pools of beech, the influence of drought was significant ($P < 0.05$, Fig. 5d). For 2014 and 2015, a strong decrease in the starch pool size of TE beech compared to CO was found for CR (by 53 %), stem tissue (by 45 %) and branch/twig tissue (by 58 %), except for fine roots which showed an increase (by 47 %, Fig. 5d). For spruce, this signal was not as clear as for beech and no significant reduction was found (Fig. 5b). Stem tissue and needle were not much affected by drought in their starch pool size and the variation in branch/twig tissue and coarse/fine roots over the different years was high (Fig. 5b).

Whole-tree pool sizes of NSCs in beech trees were 11.1 ± 4.7 and 7.0 ± 1.9 kg under CO and TE, respectively (values averaged over 2014 and 2015, Fig. 6a). Under drought, NSC pools sizes were significantly reduced by 40 % in 2014 (P-value < 0.05) and 42 % in 2015 (P-value <0.01, Fig. 6a) compared to controls. In spruce averaged over 2014 and 2015, whole-tree pool sizes of NSCs were smaller compared to beech with 7.4 ± 1.7 and 4.8 ± 0.7 kg under CO and TE, respectively. Reductions in spruce under drought were less strong compared to beech trees with 23 % in 2014 (P-value <0.01) and 36 % in 2015 (P-value <0.001, Fig. 6a). Irrespective of the drought treatment, the relative proportion of the single tissues in the NSC pool size were remarkably constant in beech with CR = 4.1 ± 0.8 %, FR = 3.1 ± 1.8 %, SX/SP = 90.7 ± 1.4 and TX/TP = 2.1 ± 0.5 % (averaged over all years and treatments, Fig. 6b). Conversely, in spruce, the relative proportion of the CR was strongly reduced under drought in both years (by 75 %, P-value < 0.0001) and strongly increased in leaves (by 55 %, P-value < 0.0001, Fig. 6b). Additionally, in 2015 the TE spruce trees showed an increase in the fine root proportion by 33 % (P-value <0.1) compared to previous years and a decrease in the stem proportion by 24 %, P-value < 0.05, Fig. 6b).

4. Discussion

Comparisons with data from mature trees in other studies for the same NSC components indicate that the trees from this study had rather low NSC levels (specially starch) (e.g. Hoch et al., 2003). This difference, could be related to site conditions and stand structure, such as high stand density with ongoing self-thinning (Gruber et al., 2012; Tsamir et al., 2019). However, the values measured during this experiment were still in the range of other studies (Oberhuber et al., 2011; Schiestl-Aalto et al., 2019) and similar to values from the same stand sampled in 2003 (Nikolova et al., 2020). The low values presented here could reflect the seasonal variation of NSC concentrations with lowest levels in early winter (Furze et al., 2019; Martínez-Vilalta et al., 2016) and the parallel hydrolization of starch to soluble sugars (Ramirez, 2017).

In response to through-fall exclusion during the growing seasons, both species showed signs of moderate to harsh drought stress in 2014 and 2015 with Ψ_{PD} as low as -1.8 MPa, while in 2013, before the start of the experiment, no differences were found between the treatments. This should be accompanied by a reduction in stomatal conductance and assimilation of CO_2 and water potential regulation at the leaf level (Hochberg et al., 2018). This reduction/regulation was stronger and earlier in spruce than in beech, reflecting the more isohydric strategy of spruce under drought compared to beech (Hesse et al., under preparation).

4.1. NSC concentration vs. NSC pools

Two subsequent years of summer drought did not significantly decrease the NSC concentrations of various tissues in both species, indicating the possibility for trees to maintain them at a steady level. The meta-analysis of He et al. (2020) revealed, that only under severe drought saplings would show a decrease in their NSC concentrations. In addition, mature trees are supposedly less strong affected than seedlings (Zhang et al., 2020). As drought did not reduce the NSC concentrations in any tissue in both species, we rejected our first hypothesis that sink organs further away from the source organs will be stronger affected by drought in their NSC concentrations. Nevertheless, we confirm the expected differences in NSC concentrations between tissues, with source and transport (e.g. phloem) structures showing higher values than predominant sink tissues, such as stem growth, which was strongly reduced under drought in the experimental trees (Table 3, Pretzsch et al., 2020). Even in C sinks with a transport distance of more than 25 m from the C source, e.g. from leaves to fine roots (Fig. 4) NSC concentrations remained stable, which is well in accordance with studies on juvenile trees (Hagedorn et al., 2016; Hartmann et al., 2013; Muller et al., 2011).

NSCs are needed for many other sinks next to growth and maintenance, such as defense compounds, e.g. secondary metabolites, and osmoregulation (Chaves et al., 2003; Kozlowski, 1992). NSC investments into osmoregulation, repair processes or defense are unavailable for maintenance processes, but play an important role in acclimation processes of trees to abiotic and biotic stresses. An important sink under drought is the osmoregulation of cells, mostly accompanied by the incorporation of sugars or amino acids as osmotic substances (Jamnická et al., 2019; Sevanto et al., 2014). As reported before (Tomasella et al., 2018), in the second drought summer, i.e. 2015, both beech and spruce showed osmotic adjustments at the leaf level, resulting in a decrease of the turgor loss point by 0.5 MPa, accompanied

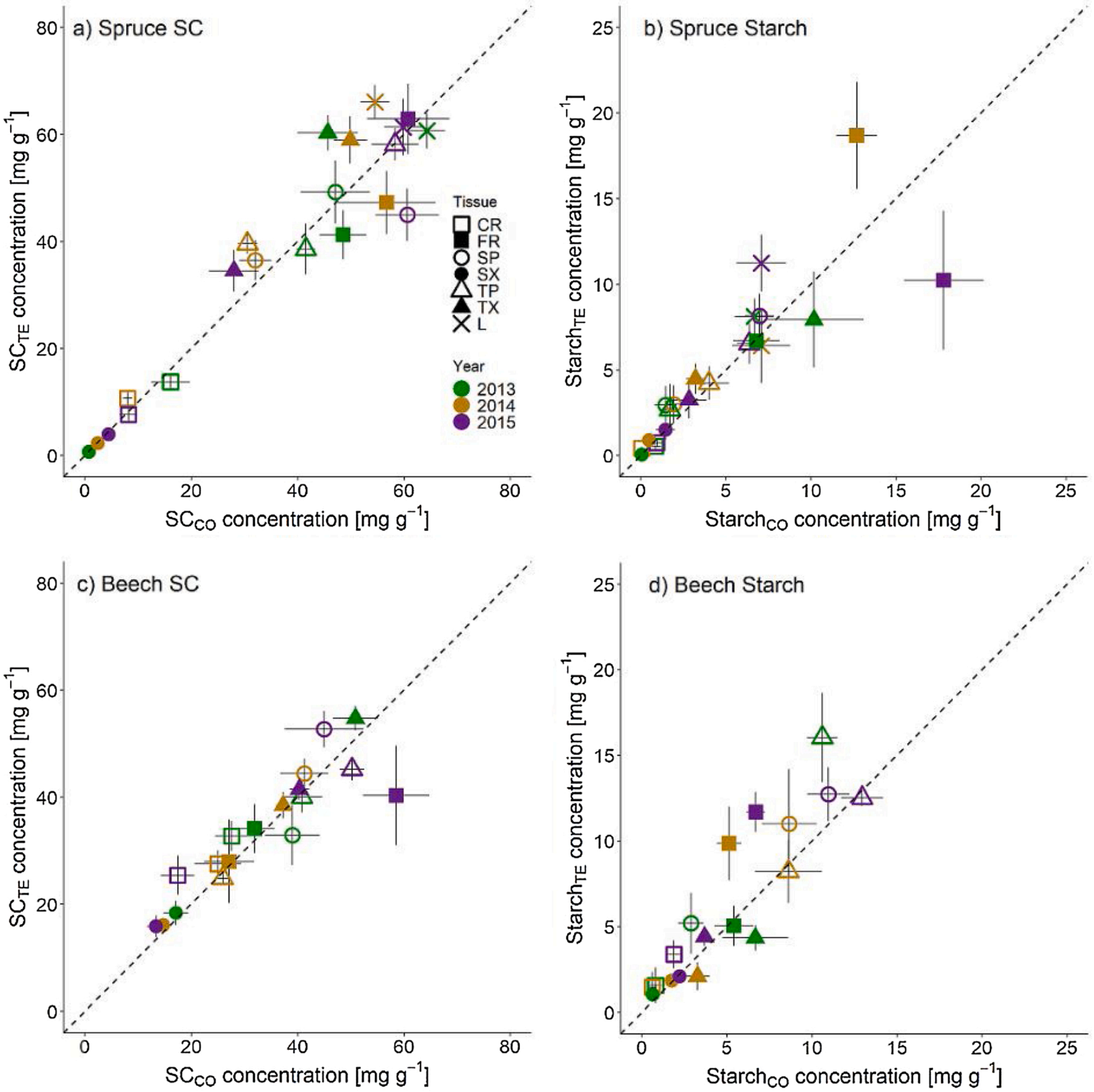

Fig. 4. Mean soluble carbohydrates (SC, left) and starch (right) concentrations of spruce (top) and beech (bottom) in different tissues. X-axis is giving the values of control (CO) trees and the Y-axis of throughfall-exclusion (TE = drought stressed) trees. Coarse root = CR (open square), fine root = FR (closed square), stem phloem = SP (open circle), stem xylem = SX (closed circle), branch/twig phloem = TP (open triangle), branch/twig xylem = TX (closed triangle) and leaves = L (cross). Years are given by colors: 2013 (before drought in green), 2014 in beige and 2015 in purple. Data are given as mean ± 1 SE, dashed line = 1 to 1 line, with drought treatment: $P > 0.05$, tissue: $P < 0.0001$ and year: $P < 0.0001$ for both species). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

by a similar decrease of the osmotic potential at full turgor. This change is based on osmoregulation (Bartlett et al., 2012) another C sink the drought-stressed trees had to cope with. However, the increased C demand for osmoregulation under drought and the stable level of NSC concentrations in all tree organs, raises the questions whether all measured NSCs can be seen as “metabolically available” carbohydrates (e.g. for transport or respiration (Prescott et al., 2020). Further studies are needed to separate the “stored or unavailable” amount of NSCs from the “metabolically available” NSCs in different tree organs, as this could prove to be very different between well-watered and drought-stressed plants. Nevertheless, it seems reasonable that trees maintain a certain concentration of NSCs even under drought, yet not much is known about the minimum required to maintain functionality, especially not for mature trees (Adams et al., 2013).

Looking at the NSC pool sizes, all growth sinks of the tree (but especially coarse roots) showed a decrease in the total amount of NSCs under drought. Despite the stable concentration of NSCs in almost all organs, the overall carbon pool of tree individuals was reduced by 42 % in beech and 36 % in spruce after two subsequent summer droughts. Winter NSC pools were reduced under drought in both species, generally consistent with our second hypotheses. However, beech and spruce were affected to a similar extent, which is against the second part of that hypotheses assuming more isohydric spruce to be stronger affected by drought than more anisohydric beech.

However, starvation thresholds, i.e. lethal NSC concentrations or pools, are widely unknown for mature trees or long lasting drought periods and many experiments showed NSC remnants in dead tissues and for juvenile trees (Weber et al., 2018; Wiley et al., 2019, 2017). Although NSC pools are significantly reduced in drought-stressed trees of both species after two subsequent summer droughts, they can be estimated sufficient for supporting respiratory processes of trees for at least two years. Assuming that whole-tree respiration in μmol CO_2 s^{-1} is equal to 0.8 times the whole-plant mass in kg and that 1 g of NSC is needed for 6 g of respired CO_2 (Mori et al., 2010), drought stressed

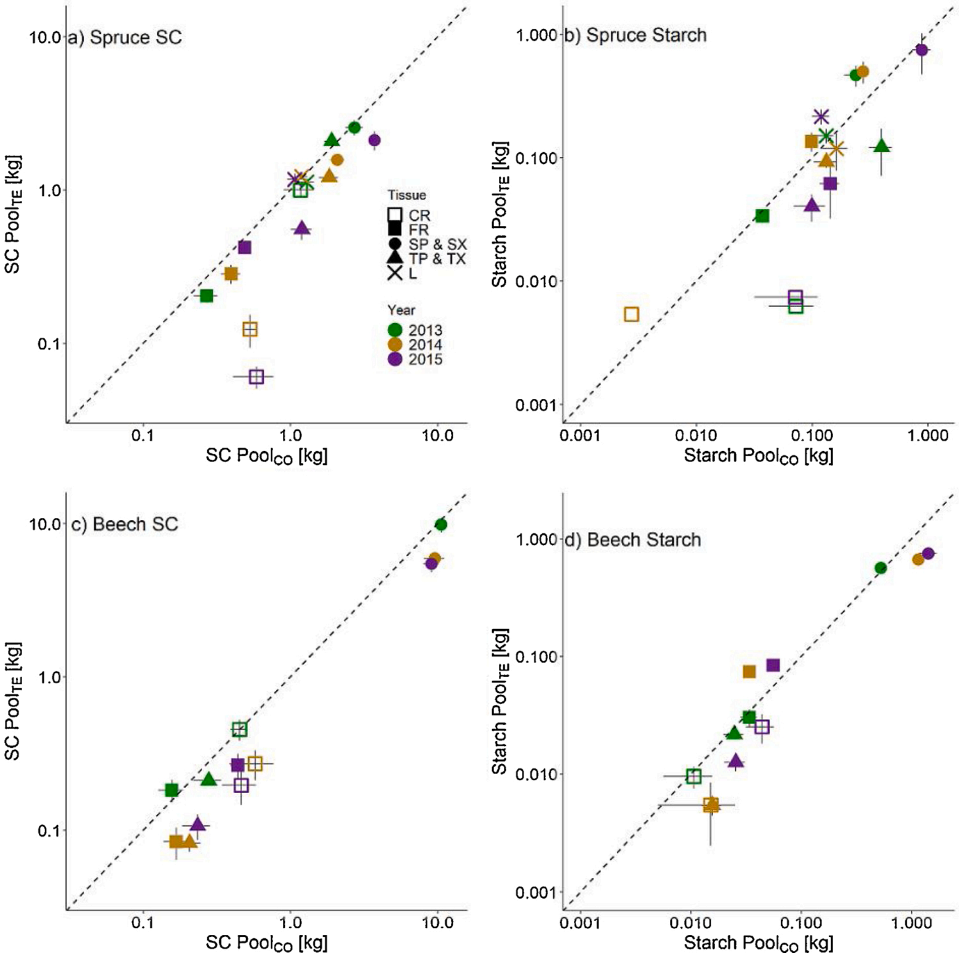

Fig. 5. Mean soluble carbohydrates pool (SC, left) and starch pool (right) sizes of spruce (top) and beech (bottom) in different tissues. Coarse root = CR (open square), fine root = FR (closed square), stem phloem and xylem = SP & SX (closed circle), branch/twig phloem and xylem = TP & TX (closed triangle) and leaves = L (cross) and years (2013 (green) = before drought, 2014 (beige) = 1st year drought and 2015 (purple) = 2nd year drought) with the x-axis (in log-scale) showing the values of control (CO) trees and the y-axis (in log scale) of throughfall-exclusion (TE = drought stressed) trees. (Data are given as mean ± 1 SE, dashed line = 1 to 1 line, with treatment: $P < 0.01$, tissue: $P < 0.0001$ and year: $P < 0.0001$ for both species). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

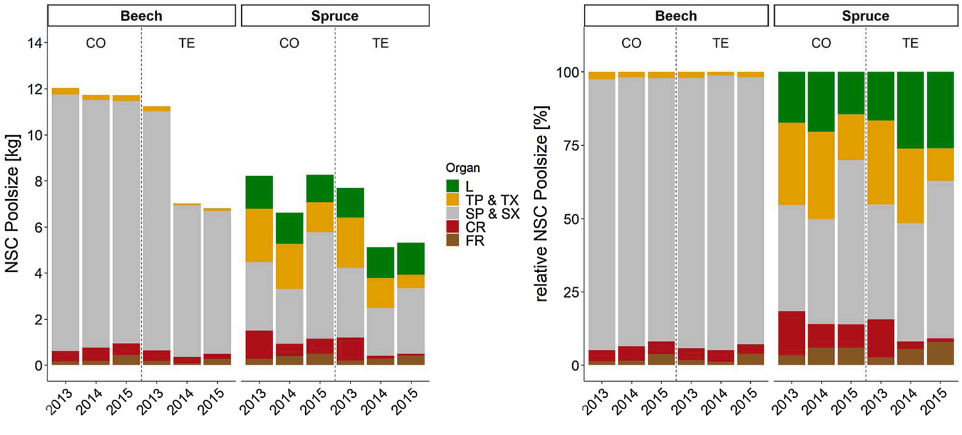

Fig. 6. Total (a) and relative (b) NSC pool size of beech and spruce for different tissues (leaves = L (green), sum of branch/twig phloem and xylem = TP & TX (beige), sum of stem phloem and xylem = SP & SX (gray), coarse roots = CR (red) and fine roots = FR (brown) over three years, i.e. 2013 (before onset of drought treatment) and in 2014 and 2015 in control (CO) and drought stressed (TE) trees. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

spruce and beech could survive for almost 2 and 4 years, respectively, if the whole NSC pools could be accessed and used for maintenance respiration to keep trees alive. While this is certainly not possible, as NSCs are needed for many other mechanisms (see above/below), it demonstrates that, neither the more anisohydric beech nor the more isohydric spruce seems therefore to be limited in their overall C availability under drought (Hoch, 2015; Körner, 2003; Leuschner, 2020). Trees keep a rather constant concentration of NSCs as a buffer by reducing selected C sinks under stress. This is also in accordance with Garcia-Forner et al. (2017), who reported that more isohydric plants are not necessarily more carbon limited than anisohydric ones.

The rather small reduction of NSC pool size in spruce fine roots (FR) might be explained by a somewhat overestimated spruce FR biomass in the model compared to the observed loss of vital fine roots under drought (Nickel et al., 2018; Zwetsloot and Bauerle, 2021). However, accumulation of NSCs in roots of drought-stressed trees has been reported previously for saplings (Hagedorn et al., 2016 and citations within) and is supported here for mature beech by the increased starch concentrations (Fig. 4d). The reduction of NSC pools in sink tissues, in particular of stem xylem (SX) and coarse roots (CR) over time, suggests that phloem transport is hindered under drought as previously reported in the same experiment (Hesse et al., 2019; Hikino et al., submitted). The impaired C transport to roots is also supported by the fact that NSC pools in spruce leaves and by that the replenishment of carbon is not affected by drought. Thus, capacity to transport carbon plays an important role in the carbon starvation scenario (Hartmann et al., 2013; Ivanov et al., 2019), as stems and coarse roots might empty their storages to maintain fine roots as the most important tissues for water uptake and exploitation of new water sources (Jackson et al., 2000). As the annual carbon allocation of the control trees was not affected by the natural drought in 2015, but as the phloem transport was reduced for the same experimental trees (measured in 2015 by Hesse et al., 2019 on the same experimental trees), short term droughts seem to be buffered by tree C stores when carbon transport decreases for a short time. However, overall both species still seem to be far from carbon starvation or even carbon limitation, even in the masting year 2015, as the remaining NSC pools can potentially sustain respiration for more than one whole year.

4.2. *Carbon investment in reproduction under drought*

While it has been shown that growth is one of the first C sinks to decrease under drought (Chuste et al., 2020; Muller et al., 2011), this is not clear for reproduction and might also differ among species. As the total C investment into reproductive structures in beech and spruce was unaffected even under recurrent summer drought with significant growth reductions, reproduction must be of high priority in the hierarchy of carbon sinks in mature trees (Fatichi et al., 2013; Lacointe, 2000). Accordingly, beech is known for showing signs of resource switching towards reproduction in dry masting years (Hacket-Pain et al., 2017 and citations within). Other C investments under stress, e.g. investments in defense (Huang et al., 2019a, b) and osmoregulation (Morgan, 1984) are – at least partially – quick responses to stress (Hartmann and Trumbore, 2016), and as they are partially reversible, they might not be too costly for the plant after stress release. Conversely, C investments in growth or reproduction is irreversible, but seems to be regulated very different in different mature tree species under drought. C demand for reproduction might be partially met by the green inflorescence tissue itself (Hoch et al., 2013; Landhäusser, 2011; Mund et al., 2020), or largely supported by leaves of the same branchlet as reported for e.g. beech (Hoch, 2005). Knowledge about the carbon autonomy of spruce cones is scarce, but cone biomass increment dependents largely on C assimilated in the canopy (Koppel et al., 1987). Under drought, spruce trees invested similar amounts of C in reproductive and structural tissues. This might reflect a strategy to distribute seeds by zoochory (Dobrovolný and Tesař, 2010) or anemochory (Dobbs, 1976) to locations with potentially more favorable microclimatic conditions or available water. In contrast to our third hypothesis, under drought C invested in reproductive structures was not reduced in either species. Apparently, reproduction has a high priority in mature beech and spruce even under severe summer drought.

5. Conclusions

Under two-years of experimentally induced, severe summer droughts with distinct growth decline, mature beech and spruce trees maintained a stable level of NSC concentrations across all studied tissues. This preserves trees' ability to react to their environment, e.g. defense against biotic attacks. Shorter drought events, as the natural summer drought of 2015, hardly affected the C allocation of beech and spruce, as the control trees were not affected in their NSC household compared to previous years. While C pools in all tissues, except leaves and fine roots of spruce, were reduced at least by about half after two consecutive drought years, both species, even the more isohydric spruce, were apparently not carbon limited. Trees were still able to maintain functionality by preserving rather constant NSC concentrations through reducing C demand for growth. With NSC pool size in leaves in spruce being rather unaffected by drought, C transport might be critical for carbon availability in non-green tissues in mature trees, as NSC pool sizes in branch/twig, stem and coarse roots were affected the most. Conversely, C investment in reproduction remained unaffected, partial supported by carbon autonomy of inflorescence structures and the proximity to C source tissue but clearly representing a high priority among C sinks of trees. Finally, a decrease in NSC concentration would only occur once the NSC pools run completely empty. Therefore, we suggest assessing not only NSC concentrations, but also the overall amount, i.e. pool sizes, of NSCs to assess the carbon status of mature trees under drought.

Author statement

Benjamin D. Hesse finalized the experimental design, collected/processed the samples, analyzed/interpreted the data and drafted the manuscript. Michael Goisser originally designed the study and helped during sampling. Thomas Rötzer modeled the biomass increment with the model BALANCE. Henrik Hartmann and Simon Landhäusser measured the NSC content of the samples. Fabian Weikl and Karin Pritsch helped in processing the samples. Thorsten Grams originally designed the study and supported me in data interpretation. All co-authors revised the manuscript and gave final approval before publication.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2021.104615.

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Dear Ladies and Gentlemen,
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Thank you in advance
Benjamin Hesse

Title

Location vs allocation – the fate of newly available water under recovery after repeated drought in a mature stand of beech and spruce

Running head

Water allocation upon watering after repeated drought in a mature stand of beech and spruce

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TBA

Abstract

Upon drought stress release, the uptake and distribution of water within the (dehydrated) plant tissues would be one of the first processes to happen. In tall plants (e.g. trees) this can pose a huge challenge as the water transport along the soil-plant continuum might be disrupted at several points (e.g. soil root contact, xylem embolisms). Here we labelled a stand of mature European beech and Norway spruce upon five years of experimental summer drought (throughfall-exclusion, TE) and under well-watered (control, CO) conditions with deuterated water. We then assessed the deuterium concentration and turnover time of the soil, stem xylem and leaf water pools, before and after the drought stress release. In both treatments the labeling was distributed within 24 hours in the soil and quickly refilled the TE soil to a similar level as CO. However, the trees showed a significant delay in the water uptake on TE compared to CO (day 1), with spruce (day 7) being more delayed than beech (day 4), for both the xylem and leaf water. A similar pattern was found for the turnover of water pools, which happened quickly in CO, but was delayed for both species in TE. We assumed that the reasons for this time delay might be related to a refilling of internal water storages, a decreased hydraulic conductivity (e.g. smaller conduits) or a loss of vital fine roots (especially in spruce as most fine roots are close to the soil surface and therefore exposed to drought conditions very early). Another reason might be the difference in stomatal control upon drought release (beech: hydraulic and spruce: hormonal), which would allow beech to react earlier than spruce. The internal water storage and its daily/seasonal contribution to transpiration is still a black box, but could help in understanding trees' reactions to drought (release).

Introduction

Drought stress is without doubt one of the most demanding challenges for our ecosystems. In course of ongoing climate change, with enhanced amount and duration of dry and hot periods (IPCC 2014), this challenge will become more and more a struggle for survival. Especially ecosystems that have "little" experience with drought and a long generation time will be threatened, as seen in the intense drought years of 2003 and 2015 (Leuzinger et al. 2005, Hartmann, Moura, et al. 2018). One of these ecosystems are forests of the temperate regions, as can be found in Central Europe (Allen et al. 2010, Hartmann, Schuldt, et al. 2018). Many studies have shown how vulnerable the dominant species of Central European forests, e.g. Norway spruce (*Picea abies* Karst.(L)) or European beech (*Fagus sylvatica* (L)), are to drought and heat stress (Milad et al. 2011 and citations within). However, many studies were performed on saplings/seedlings and might not fully grasp the consequences of drought for a natural forest stand (Englund and Cooper 2003, Niinemets 2010), as mature trees may have the ability to buffer the stress to a certain extent (Betsch et al. 2011), e.g. by stem water storage which can play an important role in temperate tree species (Köcher et al. 2013), but could probably run low after several heavy drought periods. Additionally, an important part of drought periods, the recovery (when water is available again), was not examined very thoroughly. Although very often damages of drought are only visible when plants are not able to fully recover, as recovery is a very demanding process in terms of water and carbon (Gallé et al. 2007), for repair (of e.g. the water transport system (Brodribb et al. 2010)) and regrowth processes (Trugman et al. 2018, Ruehr et al. 2019). To understand how "new" water gets distributed in the soil and trees after drought in a mature forest stand, we took advantage of the Kroof experiment (Kranzberg roof), which after 5 years of repeated summer drought (Grams et al. 2021),was watered in 2019. The Kroof experiment is focusing on two of the dominant tree species in Central European forests, the more isohydric (water saving) Norway spruce, the most important species for foresters in Germany (Spiecker 2000, Hartmann et al. 2013) and the more anisohydric (water spending) European beech, the dominant species in natural succession for Central Europe (Cavin et al. 2013, Pretzsch et al. 2013). Via deuterated water labeling (^2H_2O) we assessed the distribution of water in formerly stressed (TE) and untreated stands (CO). We focused on the soil, which had lost almost all the plant available water and was very hydrophobic (Grams et al. 2021) and is therefore supposed to be slowly refilled with water from the top downwards (Brinkmann et al. 2018). Additionally, we examined differences between spruce and beech under watering, whose root distributions are very different, with spruce having most of its roots in the shallow soil and beech rooting further down (Zwetsloot et al. 2019). As a next step we calculated the turnover of the different water pools with mixing models (Phillips et al. 2005), in order to see where most of the newly available water is needed/stored and how long it remains in the single compartments of the soil-plant(-atmosphere) continuum (SPC). We hypothesized that:

H 1. TE trees shift their mean water uptake depth due to the drought years to deeper soil layers, with beech rooting deeper than spruce.

H 2. The deuterium (δ^2H) signal on the CO plots is quickly distributed within the already moist soil while for the TE plots the dry soil gets refilled slowly from the top downwards.

H 3. Trees on the TE plots take up the newly available water as soon as possible and distribute it within the tree with shallower rooting spruce having access to irrigated water earlier than deeper rooting beech.

H 4. Water pools in the dry TE soil/trees have a very short turnover time compared to moist CO soil/trees.

Material and Methods

Experimental site and design

The Kroof experiment is located in Kranzberg Forest near Munich in south-eastern Germany (11°39’42”E, 48°25’12”N). The tree stand is composed of Norway spruce (*Picea abies* Karst.(L.)) and European beech (*Fagus sylvatica* (L.)), planted in 1951 ± 2 AD and 1931 ± 4 AD, respectively (Pretzsch et al. 2014). With a luvisol soil that originated from loess of tertiary sediments and an average precipitation of 750-800 mm a^{-1} the water supply for the site is very high (for details see Grams et al. 2021). For five years (2014 to 2018) the Kroof experiment “phase I” focused on the effects of repeated summer drought on Central European forests. Drought stress was generated via a throughfall-exclusion system (TE), which held of the summer precipitation from 6 experimental plots. For comparison, 6 untreated control plots (CO, Grams et al. 2021) were established next to the TE plots. In 2019 the Kroof experiment “phase II” started by watering the TE plots to the level of the CO plots.

Soil water content

To measure the soil water content (SWC in vol.-%) of the mineral soil beneath the litter layer time domain reflectometry sensors (TDR100 and TDR200, Campbell Scientific, Logan, USA) were installed. On each plot three TDR installations were made one each at the beech site, spruce site and in-between (mix) respectively (Goisser et al. 2016, Grams et al. 2021). For each installation four sensor were installed at four different depths to record the SWC down to 70 cm. The shallowest sensor records SWC at 0-7 cm depth, the second one at 10-30 cm, the third at 30-50 cm and the deepest at 50-70 cm, resulting in n = 6 for each depth, position and treatment. SWC was measured 9 and 1 days before (D-9 and D-1) and directly after the watering (D1, D2, D4 and D7). From there on weekly measurements were performed.

Labeling approach

In the course of the watering, 3 CO and 3 TE plots were labeled with deuterated water. For the application of the labeled water, a watering system composed of soaker hoses (CS Perlschlauch Premium, CS Bewässerungssyteme, Reichelsheim, Germany) and garden hoses was designed, to simulate natural rainfall. At the one side of each plot a garden hose with T-piece connectors every 20 cm was installed. From these T-pieces soaker hoses ran over the whole width of the plot and were sealed at the end with a cable tie (for details see Grams et al. 2021). CO plots were watered to apply the labeling and to match effects of saturated topsoil, temperature effects and nutrient availability (Grams et al. 2021). To match the treatment of watering for CO (n = 3 plots) and TE (n = 3 plots), we decided to add 15 mm (approx. 2034.5 ± 537.3 L) to each CO plot with a deuterium signature of 1468.36 ± 44.47 ‰. The labeled water for the CO plots was mixed with 99.9 % deuterated water (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) in 1000 l tanks. The amount of water added to each TE plot was calculated via the SWC data. We attempted to bring the TE plots to the same SWC level as the control. On average we added 12849.3 ± 2801.7 L (corresponds to ca. 90 mm) of water to the TE plots with a deuterium signature of 289.30 ± 2.48 ‰. The labeled water for the TE plots was mixed in a water pillow (volume of 60000 l) with 99.9 % deuterated water (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) We controlled the amount of water added to each plot with an electronic water meter (Wassermengenzähler, GARDENA Manufacturing GmbH, Ulm, Germany). The duration of the watering was about 7 h for the CO plots and 40 h for the TE plots. The watering was performed in three separate campaigns (1 CO and 1 TE plot per campaign). The watering of the plots started at 4 AM at day 0. For details on the calculations and watering see Grams et al. (2021).

Deuterium signature in single compartments of the soil-plant-continuum (SPC)

An overview on the samples taken for deuterium measurement can be found in the supplemental material (Tab. S1).

In leaf and xylem water

Leaf samples (δ^2H_{leaf}) were taken with a canopy crane from the sun exposed part of each tree crone. For each sample about 5 leaves of beech or 100 needles of one 1-year old shoots of spruce were sampled into airtight exetainer vials (Labco, Lampeter, UK) and stored at -20°C. We took samples before the watering (D-6 and D-1), immediately after the watering (D0, D1, D2, D4, D7) and for longterm observation on D15 and D22 (Tab. S1). Twig (δ^2H_{twig}) samples were only taken at the measurement campaigns on D-6 and D15 (Tab. S1). Twigs with a diameter of about 0.5 cm were freed of bark, cut in to approx. 1 cm long pieces and stored in exetainer vials.

For the measurement of the stem water isotopic signature (δ^2H_{stem}) we used the probes and measurement set-up described in Volkmann et al. (2016) on one CO/TE plot. To assess the δ^2H signature of the stem sapwood xylem water, the probes, consisting of a water-vapor permeable membrane installed at DBH inside the trunk xylem, were connected to a cavity ring-down spectrometer (CRDS, Picarro, Santa Clara, CA, USA). The probes were installed in one (for CO)/two (for TE) beech/spruce trees at DBH and in the mixed zone soil at 10 cm and 30 cm depth. Each probe was measured once a day (from D0 to D22, Tab. S1) before midday against two monitoring standards (light standard: -74.22 ± 1.42 ‰ and heavy standard: 198.34 ± 2.87 ‰).

In soil water

Samples of the mineral soil (δ^2H_{soil}) were taken with a Pürckhauer soil sampler (diameter: 2cm and length: 100cm) on the same days as leaf samples (Tab. S1). For each plot one core was taken at the beech, spruce and mixed zone respectively, down to 70 cm. Each core was then divided in 10 cm long sections (e.g. 0 to 10 cm, 10 to 20 cm, etc.) and one soil sample was taken from each section into exetainer vials. Right before sampling the surface exposed to air was removed to avoid any evaporative contamination of the soil water. After each sampling the whole core was freed of remaining soil, rinsed with tap water and dried.

Cryogenic extraction of water and isotopic analysis

All samples were stored at -20°C until cryogenic water extraction. Water was extracted by cryogenic vacuum distillation for 2 h (West et al. 2006, Hafner et al. 2017). We analyzed the extracted water for its δ^2H (against VSMOW standard) with an isotope-ratio-mass-spectrometer (IRMS) linked with a multiflow system (Isoprime, Elementar, Langenselbold, Germany, for details see Hafner et al. 2017) against two monitoring standards (heavy: 127.14 ‰ and light: -179.22 ‰). Before the extraction, right after extraction and after a cleaning, the exetainer vials were weighted for leaf water content calculations (LWC in %). We divided the amount of water in the sample (exetainer before extraction minus exetainer after extraction) by the fresh mass of the sample (exetainer before extraction minus the clean exetainer). Treatment (CO vs. TE) had no influence on the LWC for both species and also watering did not change LWC (Tab. 1)

Mixing models to calculate the turnover of water pools

To calculate the amount of labeled water in the samples a two-endmember mixing model was used (e.g. IsoError, Phillips et al. 2005, Hafner et al. 2020).

Leaf and xylem water

Using the δ^2H values from before the watering, we calculated the mean water uptake depth of beech and spruce on CO and TE plots. Therefore, we plotted the deuterium values of the soil water against the depth and calculated an exponential regression for each plot (mean R^2 = 0.7122, data not shown). Applying the deuterium value of the sapwood water into the regression formula, we estimated the mean water uptake depth (WUD in cm). In both species the mean WUD on the CO plots were close to 30 cm depth (Tab. 2), with beech being slightly shallower

(27.5 ± 11.4 cm) than spruce (33.5 ± 6.9 cm). On the TE plots the WUD was shifted upwards in both species (Tab. 2), with beech being again slightly shallower (10.9 ± 7.6 cm) than spruce (15.9 ± 4.3 cm). We decided to use the soil δ^2H values of 10-20 cm depth for the TE spruce trees and mean δ^2H value of 0-10 cm and 10-20 cm for TE beech trees, as the deuterium signature of the mean water uptake depth ($\delta^2\mathrm{H}_{\mathrm{water\_uptake}}$). For CO trees of both species a mixture of the 20-30 cm and 40-50 cm δ^2H values were calculated and used as the $\delta^2\mathrm{H}_{\mathrm{water\_uptake}}$:

$$\delta^2\mathrm{H}_{water\_uptake} = \frac{\delta^2\mathrm{H}_{20-30\mathrm{cm}} + (\frac{\delta^2\mathrm{H}_{20-30\mathrm{cm}} - \delta^2\mathrm{H}_{40-50\mathrm{cm}}}{2})}{2}$$

The deuterium value of the soil of the mean probable water uptake depth ($\delta^2\mathrm{H}_{\mathrm{water\_uptake}}$), were used as one endmember (deuterium mix-signature of the soil water taken up from the tree) in the mixing model for the twig water and the other was the deuterium signature of the twig water before the start of the watering ($\delta^2\mathrm{H}_{\mathrm{twig}}$ of D-6) to calculate the fraction of labeled water in the twigs (F_{twig} in %).

$$1 - F_{twig} = \frac{\delta^2\mathrm{H}_{twig} - \delta^2\mathrm{H}_{water\_uptake}}{\delta^2\mathrm{H}_{twig_{D}-6} - \delta^2\mathrm{H}_{water\_uptake}} * 100$$

For the leaf data we had to consider the evaporative enrichment (EER in ‰, Tab.2) of the leaf water (Farquhar and Cernusak 2005). Therefore, we calculated the difference between the leaf water and the twig water isotopic signature (on D-6 and D15) and subtracted the mean from each remaining measured leaf water.

$$ERR = \delta^2H_{Leaf} - \delta^2H_{Twig}$$

Evaporative enrichment was not different between D-6 and D15 for each combination (Tab. 1). We used the corrected values of D-1 leaf water as one endmember and the same soil water signature as in the mixing model for the twig water to calculate the fraction of labeled water in the leaves (F_{leaf} in %).

$$1 - F_{leaf} = \frac{(\delta^2\mathrm{H}_{leaf} - EER) - \delta^2\mathrm{H}_{water\_uptake}}{(\delta^2\mathrm{H}_{leafD-1} - ERR) - \delta^2\mathrm{H}_{water\_uptake}} * 100$$

Soil water

For the soil samples the values measured at D-1 and the tank water ($\delta^2\mathrm{H}_{\mathrm{tank}}$, for CO: 1468.36 ‰ and TE: 289.30 ‰) were used as endmembers. For each depth, position and day the fraction of labeled water (F_{soil} in %) was calculated.

$$1 - F_{soil} = \frac{\delta^2\mathrm{H}_{soil} - \delta^2\mathrm{H}_{tank}}{\delta^2\mathrm{H}_{soilD-1} - \delta^2\mathrm{H}_{tank}} * 100$$

Statistical analysis

For the statistical analyses, the isotopic data were displayed relative to the SLAP2 isotopic standard for ^{2}H (IAEA 2017). Additionally, we calculated the ^{2}H excess by subtracting the mean of D-6 and D-1 from the single timepoint and the relative enrichment after labeling. For each treatment, day and species, 6 trees on 3 plots were sampled, giving 6 replicates for each measurement point in trees and 3 replicates for soil samples. Data were analyzed for statistical differences using R (R Development Core Team 2008) in RStudio (RStudio Team 2015). Data were plotted with the 'ggplot' function (package: ggplot2, version: 3.1.0) or the boxplot function (package: graphics, version: 3.5.2). Data were tested for homogeneity of variances (Levene test) beforehand and the residuals of every model used were tested for normality (Shapiro test/Q-Q-Plot). For differences in deuterium signature of soil, stem, twig and leaf, a linear mixed effect model ('lme' function) was calculated, using the day, species and the treatment as fixed and the tree individual nested in the plot as a random effect (package: nlme, version: 3.1-137). If the mixed effect model showed significant effects, we did a post-hoc test with the 'emmeans' function with Tukey correction (package: emmeans, version: 1.3.1). Data are given in text and tables as the means ± 1SD.

Results

Changes in the soil water content upon watering

The water status of the soil before the watering on the CO plots was with 35 vol.-% in the deepest and 20 vol.-% in the shallowest layer very high (Fig. 1). The TE plots were up to 60% drier than the corresponding CO plot soil layers (Fig. 1). Especially the soil top-layer on TE was with less than 10 vol.-% exceptionally dry, but also the layers from 10-30 cm and 30-50 cm were with 17 vol.-% and 22 vol.-%, respectively, particularly dry. Only the deepest layer from 50-70 cm was relatively moist with 29 vol.-%. However, taking the permanent wilting point (PWP) into account, the plant available soil water on the TE plots was close to zero (distance between the dashed lines and curve in Fig. 1, data taken from Grams et al. 2021). So, before the start of the watering the TE plots only had 40 % to 80 % of the water content found on CO plots. In the CO plots, only a small peak was found on D0 and afterwards a slow decline in SWC was found, as expected with ongoing growing season. For the TE plots, a huge peak in the SWC was found from D0 lasting until D3 for all layers, but the weakest in 0-7 cm depth. From D3 on a small decline was found with the plants taking up the newly available water, until from D42 on a weak increase of SWC was found for all layers and from D63 on CO and TE plots displayed a very similar SWC (Fig. 1).

Tracing of "new" water along the SPC

Initial situation before watering

For the deuterium signature of the soil water the depth and treatment showed a significant influence, with a decrease of the deuterium signature with increasing depth and generally higher values in CO compared to TE (Fig. 2). δ^2H values of CO ranged from -53.3 ± 5.7 ‰ in the shallowest layer (0-10 cm) to -81.2 ± 7.8 ‰ in the deepest layer (60-70 cm), while TE was on average 10.8 ± 3.2 ‰ lower (top-layer: -66.7 ± 11.6 ‰ and deepest layer: -91.6 ± 3.9 ‰, Fig. 2).

Before the watering, no differences in the deuterium isotopic signature between CO vs. TE and beech vs. spruce were found in the twig and leaf water. Deuterium signature of the twig water on D-6 in beech was at -71.2 ± 8.1 ‰ (CO) and -65.2 ± 11.3 ‰ (TE), respectively and for spruce -75.5 ± 4.5 ‰ (CO) and -72.1 ± 6.0 ‰ (TE), respectively (Fig. 2). For the leaf water, no differences were found between values of D-6 and D-1, with beech trees showing slightly lower values (CO: -29.5 ± 3.5 ‰ and TE: -28.1 ± 7.5 ‰) than spruce (CO: -24.5 ± 5.8 ‰ and TE: -27.5 ± 5.4 ‰, Fig. 2).

Changes in the isotopic signature upon labeling/watering

Upon watering, soil deuterium signature changed drastically for CO and TE. On CO plots especially the three uppermost layers (0-30 cm) changed within the day of the watering by plus 180 to 350 ‰ (Fig. 3). The isotopic excess of the soil water in lower depths on CO changed less strong, for 40-50 cm on D0 about plus 45 ‰ and to a maximum of plus 120 ‰ on D4 to D7 and for 60-70 cm constantly to plus 45 ‰ from D0 onwards. While on TE plots a more homogenous distribution of the "new" water has been observed, soil water deuterium excess rose until D4 in all depths only to a maximum of plus 150 to 200 ‰ over all 5 depths (Fig. 3).

In the woody plant tissues, CO trees showed signs of deuterium enrichment earlier than TE trees, for beech and spruce. In both species already on D1 an enrichment of about 10 ‰ was found at DBH. Over the following two weeks the deuterium level in CO tress increased with an excess of maximum 55 ‰ in beech and 35 ‰ in spruce. Trees on the TE plots displayed a deuterium enrichment in the sapwood xylem water at DBH on D5 for beech and D7 for spruce (Fig. 4). The uptake of deuterium labeled water was significantly delayed in the TE tress, with spruce (maximum excess of 60 ‰) reacting slower than beech (maximum excess of 80 ‰). In the twig water a significant enrichment was found on D15 for beech and spruce, but no differences were found between CO and TE anymore (Tab. 1).

The dynamics in the leaf water signature were similar to the xylem signature. In CO trees already on D1 after the labeling a significant enrichment was found in the leaf water for both species (beech: 10 ‰ and spruce: 7 ‰, Fig. 5). In the TE trees this was significantly delayed for both species. For beech on D4 a significant enrichment in the leaf water was found (excess of 14 ‰) and for spruce only on D7 (excess of 13 ‰) after the watering. After 15 days both treatments showed a similar excess of deuterium in the leaf water, with values of plus 65 to 85 ‰ in beech and plus 35 to 55 ‰ in spruce (Fig. 5).

Allocation of newly up-taken water along the SPC

In the soil water of the control plots only a minor fraction consists of labeled water, but on a constant level. The three shallower layers consist of up to 20 % of "new" water, while the two deeper layers only rise to 8 % in the fraction of labeled water (Fig. 6). For the TE plots a major fraction of the water found after the labeling was enriched water. Up to 80 % was "new" water and labeling seemed to be distributed homogenous among the different depths after 7 days (Fig. 6).

A similar pattern was found in the stem sapwood xylem water, with CO trees only showing a very minor fraction of new water (average of 38 % in beech and 13 % in spruce over time) but more or less constant from D2 on (Fig. 6). In the TE trees the fraction of labeled water rose on average to 58 % in beech and 62 % in spruce from D7 on. The level in both species and treatments remained relatively constant over time until D15 (Fig. 6).

The fraction of labeled water in the leaves changed in a different manner. A constant rise was found over two weeks for both species and treatments (Fig. 6). For CO trees already on D1 a significant fraction of "new" water was found for beech (8 %) and spruce (7 %). Until D15 levels rose to 61 % in beech and 55 % in spruce in the CO treatment (Fig. 6). A similar delay of arrival of labeled water of 4 days for beech and 7 days for spruce in the TE trees as found for the sapwood xylem water was found in the leaf water as well. Levels of "new" water rose in beech to 9 % on D4 & 66 % on D15 and for spruce to 12 % on D7 & 30 % on D15 (Fig. 6).

Discussion

Effects of drought on the isotopic signature of soil and plant water

The difference between CO and TE in soil water isotopic signature seems counterintuitive at first. From literature, drought treatments are known to increase the isotopic signature of soil water (Dawson and Ehleringer 1998). As however in this special case only the summer precipitation was excluded, we additionally have to take the precipitation signature and its change over the season into account (Dansgaard 1964). According to Bowen et al. (2005) the deuterium signature of the winter precipitation (roofs open, Nov. till Feb.) for the KROOF experiments is about -94.3 ± 8.7 ‰, while the summer precipitation (roofs closed, Mar. till Oct.) is much higher: -51.8 ± 20.2 ‰ (annual mean: 65.9 ± 26.8 ‰). As TE plots only received the winter precipitation, the soil water deuterium signature must be lower than the signature of CO, although evaporative enrichment is counter acting to some extent.

With the difference in water uptake depth between CO and TE for both species, with TE plants on average using more water of the shallower layers than the CO trees, the very similar values in twig and leaf water deuterium signature seem plausible, as the deuterium signature on the CO plots is slightly higher in all measured depths. However, the shift in water uptake depth to shallower layers in the drought treatment was surprising at first sight and contradicting to our hypothesis H1 (TE trees shift their mean water uptake depth to deeper soil layers during the drought years). Nevertheless given the fact that over the whole depth only very few water was available, according to the SWC, might have forced the plants to grow roots closer to the surface, in order to get the winter precipitation as early as possible, especially as both species lost a significant amount of vital roots tips during the drought treatment (Nickel et al. 2018). Therefore, we reject H1 as both species moved their WUD to shallower layers, with beech being even shallower than spruce.

Location and allocation of "new" water under recovery in the soil-plant-continuum

Deuterated water added in the course of the watering was found in every part of the SPC for CO and TE. However, the timepoint of discovery and allocation was very different between treatments and species. In contradiction to H2 (deuterium signal is quickly distributed in CO and slowly refilling TE from the top on downwards), the deuterium signal was distributed within ~24 h in the soil of CO and TE plots. Additionally, the labeling of TE was very homogenous, as all depths show a very similar deuterium signature from the day of labeling on (for detailed discussion see next paragraph), while for CO the shallow soil layers showed a higher signature for the whole experimental time. This is in accordance with other studies, which showed that water of deeper soil layers is only replaced/mixed by heavy rainfall or snowmelt (Gazis and Feng 2004, Brinkmann et al. 2018). Therefore, we rejected H2 as in both treatments the "new" water was distributed within the whole plot in the same amount of time.

Within the trees the deuterium signature was found earlier in CO trees than in TE trees in both beech and spruce, i.e. on D1 after labeling. In the TE trees, labeled water was detected earlier in beech than in spruce in the stem sapwood xylem and the leaf water (by ~3 days), however 3 to 6 days delayed when compared to CO trees, in contradiction to H3 (trees on TE plots take up the water immediately when it is available in the soil and shallow rooting spruce has access to the added water before deeper rooting beech). For TE beech the leaf water showed signs of deuterated water on D4 and for spruce on D7, even though already on D0 the TE soil showed an enriched water signature. This delay could be related to refilling of water reservoirs within the coarse roots and stem xylem structures, as it is proposed that mature trees have a huge capacity to store water within "dead" tissues (Holbrook 1995, Čermák et al. 2007). During the drought periods these storages could have been emptied/reduced and were refilled in the course of the watering (Betsch et al. 2011). The difference between beech and spruce could be explained by their water usage strategy (more anisohydric beech vs. more isohydric spruce), as anisohydric plants are supposed to react faster than isohydric ones, as the stomatal response to release of drought stress in anisohydric plants is supposed to be under direct hydraulic and for isohydric plants under hormonal control (e.g. ABA, Brodribb and McAdam 2013, Tramontini et al. 2014). Another explanation could be impaired xylem water transport, as drought stressed trees tend to suffer from embolisms and a reduced hydraulic conductivity (Geßler et al. 2007), due to the formation of smaller vessels, which would mostly affect beech over spruce (Tomasella et al. 2017, Tomasella et al. 2018). The general very different hydraulic architecture (e.g. lower hydraulic conductivity) of conifers compared to angiosperms (Brodribb et al. 2005, Johnson et al. 2012) could also explain the time difference of 3 days in leaf deuterium signature enrichment between TE beech and TE spruce. Another reason could be the heavy loss of fine roots and ecto-mycorrhiza in TE spruce compared to TE beech on the experimental site (Nickel et al. 2018) or a loss of root-surface contact (North and Nobel 1997), and the consequently lower ability to take up water (Tschaplinski and Blake 1985). WUD did not seem to play an important role for the uptake of "newly" added water, as both species use water from similar soil layers. We therefore rejected H3 as CO trees took up the labeled water quicker than TE trees and beech trees reacted earlier than spruce on the TE plots.

The allocation of deuterated water after the watering was very different between CO and TE in general. This is surely linked to the amount of water added to each plot and the already present amount of water. The high fraction of deuterated water in the soil of the TE plots compared to the small fraction on CO is most likely linked to the very little amount of water present at the start of the watering on the TE plots. However, on CO a pattern emerged after which shallower layers contained more deuterated water than deeper soil layers. Therefore, we believe that water was first mixed within the uppermost layer and then slowly infiltrating further down, getting more diluted on the way (Gisi 1997, Huang et al. 2013). In TE, the pattern was very different, with high fluctuations, but in the end very similar fractions in all depths. This could happen when the deuterated water was directly transported into the different depths and not necessarily passing through them. As the soil on the experimental site was extremely hydrophobic (Grams et al. 2021), the infiltration for water was strongly reduced. As this hydrophobicity needed to be overcome before water could infiltrate the soil, which can take several days (Burch et al. 1989, Bauters et al. 2000), some part of the water might have taken different ways driven by gravity. One possibility would be cracks in the soil, which as a consequence of heavy drought naturally occur (Novàk et al. 2000), or root and animal pipes (Amelung et al. 2018), which have been found on the experimental sites on several occasions (personal observations). The isotopic fraction of the stem sapwood xylem water was quickly changed in CO trees. The newly added water was therefore not directly transported upward to the leaves, but mixture with the stored water in the stem must have occurred, studies for other species report up to 50 % of transpired water to originate from stem water storage (Waring et al. 1979, Hao et al. 2013). In the TE trees a similar pattern for the fraction of deuterated water in the sapwood was found, with an even strong rise (and a plateau afterwards only in beech) but with a time delay of several days in both species. This delay could mean that within several days/weeks the water storage of the trees was re-filled with the deuterated water, with beech again reacting faster (~7 days) than spruce (at least 15 days), before transporting water up to the leaves (Anderegg et al. 2012). Despite the time lag in TE the same pattern of the fraction of deuterated water was found in the leaves for both species. The fraction of labeled water increased continuously over the whole experimental time to the same level for CO and TE in beech and also almost in spruce respectively. This indicates that leaves have a high priority when it comes to the allocation of water and that leaves themselves have no to little capacity for storage of water (as values continuously increased and no plateau was found). Therefore, we partly accept H4, as TE trees and soil showed much higher fractions of deuterated water, except for leaves.

Conclusions

While the deuterated label was found in every compartment of the SPC, huge differences were found between CO and TE in the fraction of deuterated water in different tissues and TE trees reacted much slower than CO trees, with beech taking up water faster than spruce. While soil water content changed very quickly, the water content of leaves remained unchanged for both species. With stem sapwood xylem and soil water showing very similar patterns in the isotopic fractions upon labeling it is likely that also the xylem changed in water content after the watering, while the capacitance for water storage of leaves is very small or was not affected from the drought treatment. Therefore, it would be necessary to investigate the role of stem water storage capacitance and its role under well-watered and drought conditions, as it could buffer the effects of drought for mature trees.

Acknowledgements

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Figures

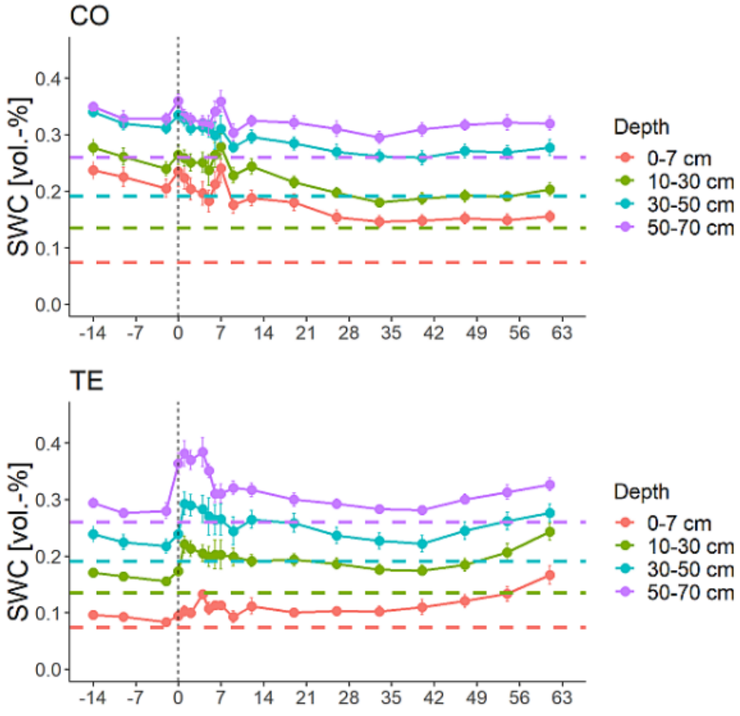

Figure 1: Soil water content (SWC in vol.-%) in CO and TE of different soil depths (0-7 cm = orange, 10-30 cm = green, 30-50 cm = blue and 50 to 70 cm = purple, dashed lines = PWP of different depths according to Grams et al. 2021)

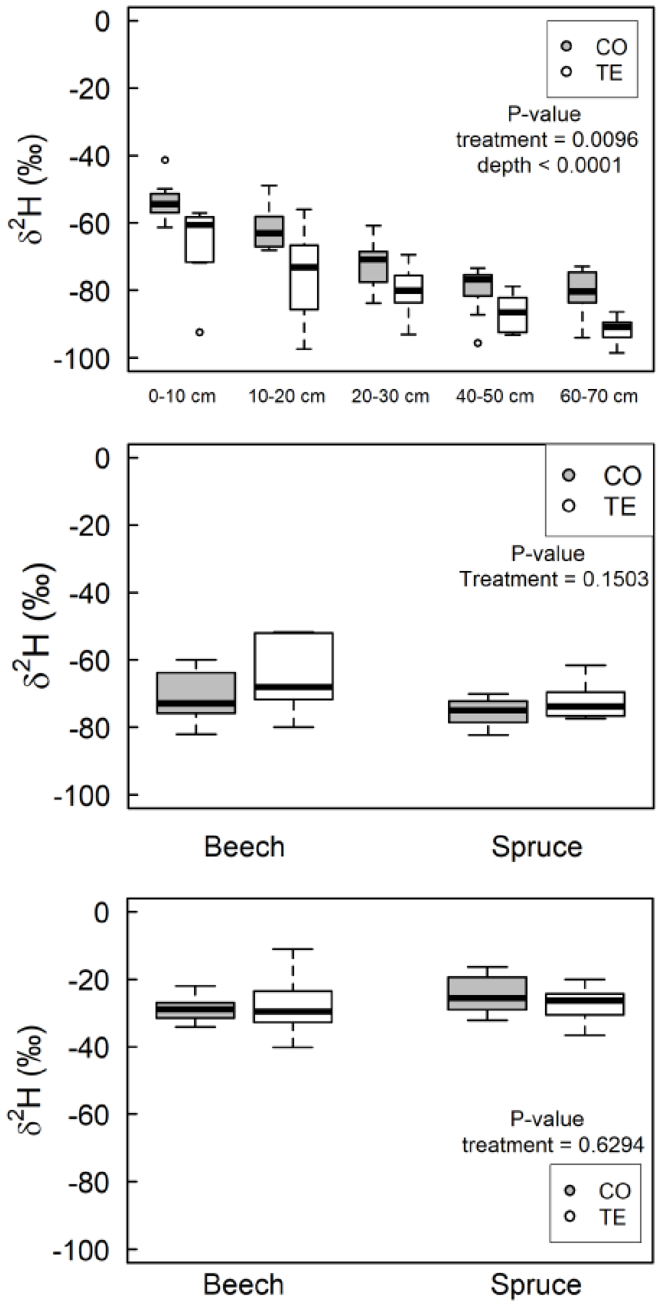

Figure 2: Initial deuterium signature (δ^2H in ‰) on D-6 of soil (top), twig (middle) and leaf water (bottom) for beech and spruce (CO = gray boxplots and TE = white boxplots)

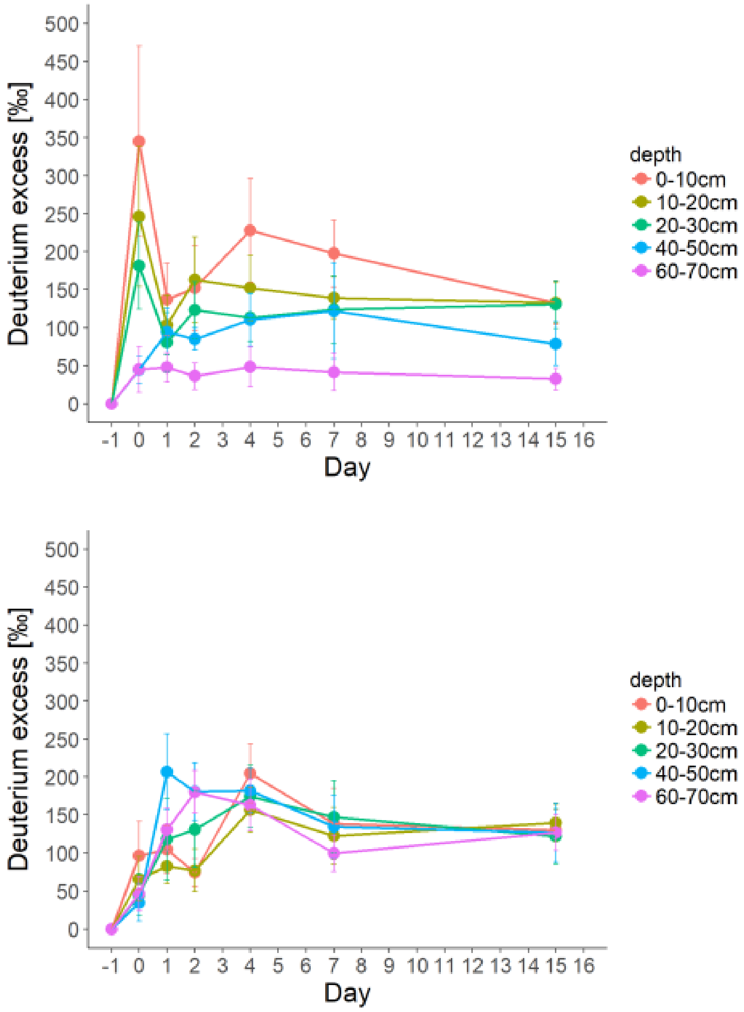

Figure 3: Soil water deuterium excess based on values of D-1 for CO (top) and TE (bottom) of different depths (0-7 cm = orange, 10-20 cm = ochre, 20-30 cm = green, 40-50 cm = blue and 60 to 70 cm = purple)

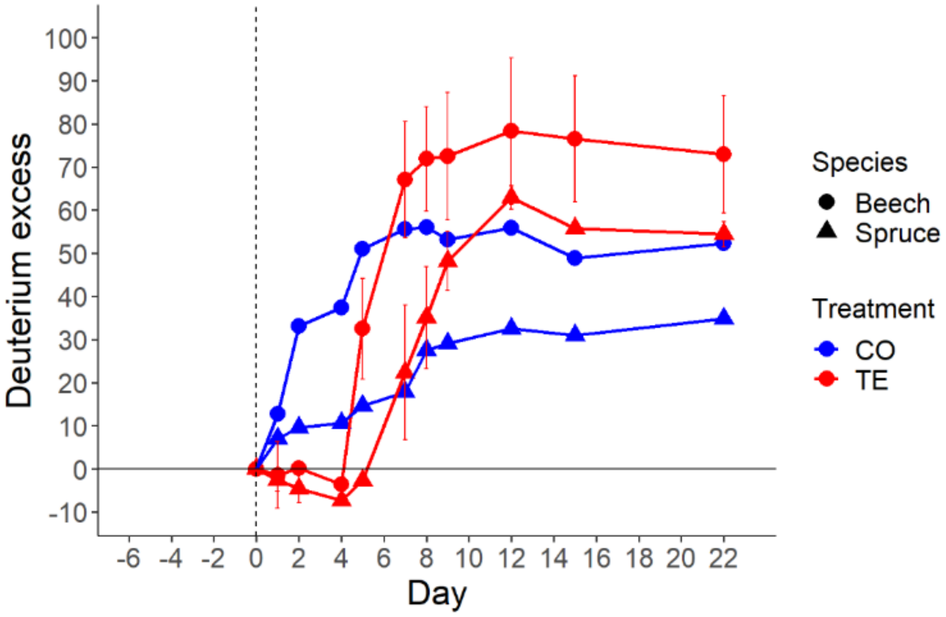

Figure 4: Deuterium excess of stem sapwood water for beech (circle) and spruce (triangle) and CO (blue) and TE (red). Significant increase in CO beech and spruce on D1, TE beech on D5 and TE spruce on D7. Vertical dashed line marks the watering on D0.

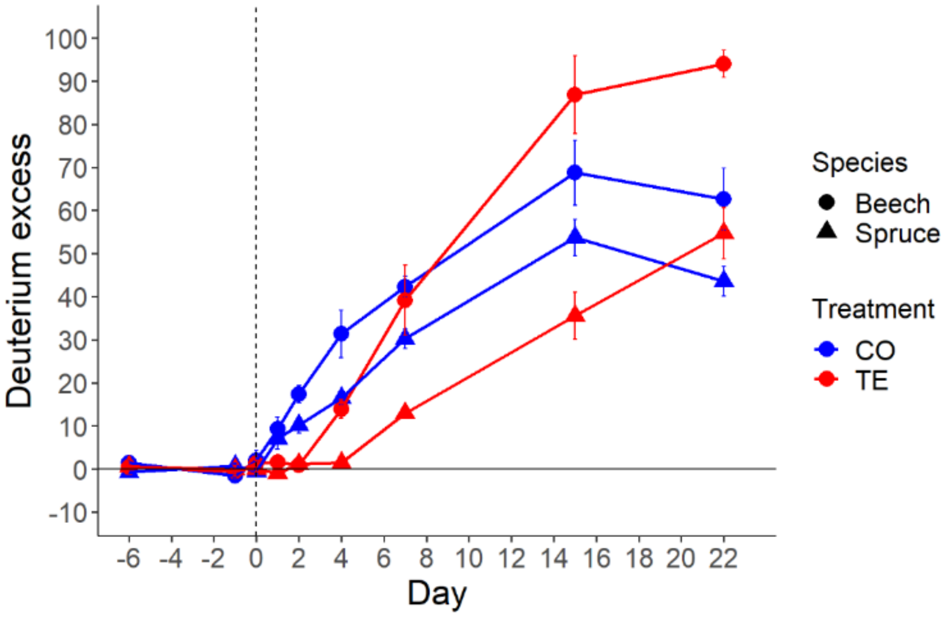

Figure 5: Deuterium excess of leaf water for beech (circle) and spruce (triangle) and CO (blue) and TE (red). Significant increase in CO beech and spruce on D1, TE beech on D4 and TE spruce on D7. Vertical dashed line marks the watering on D0.

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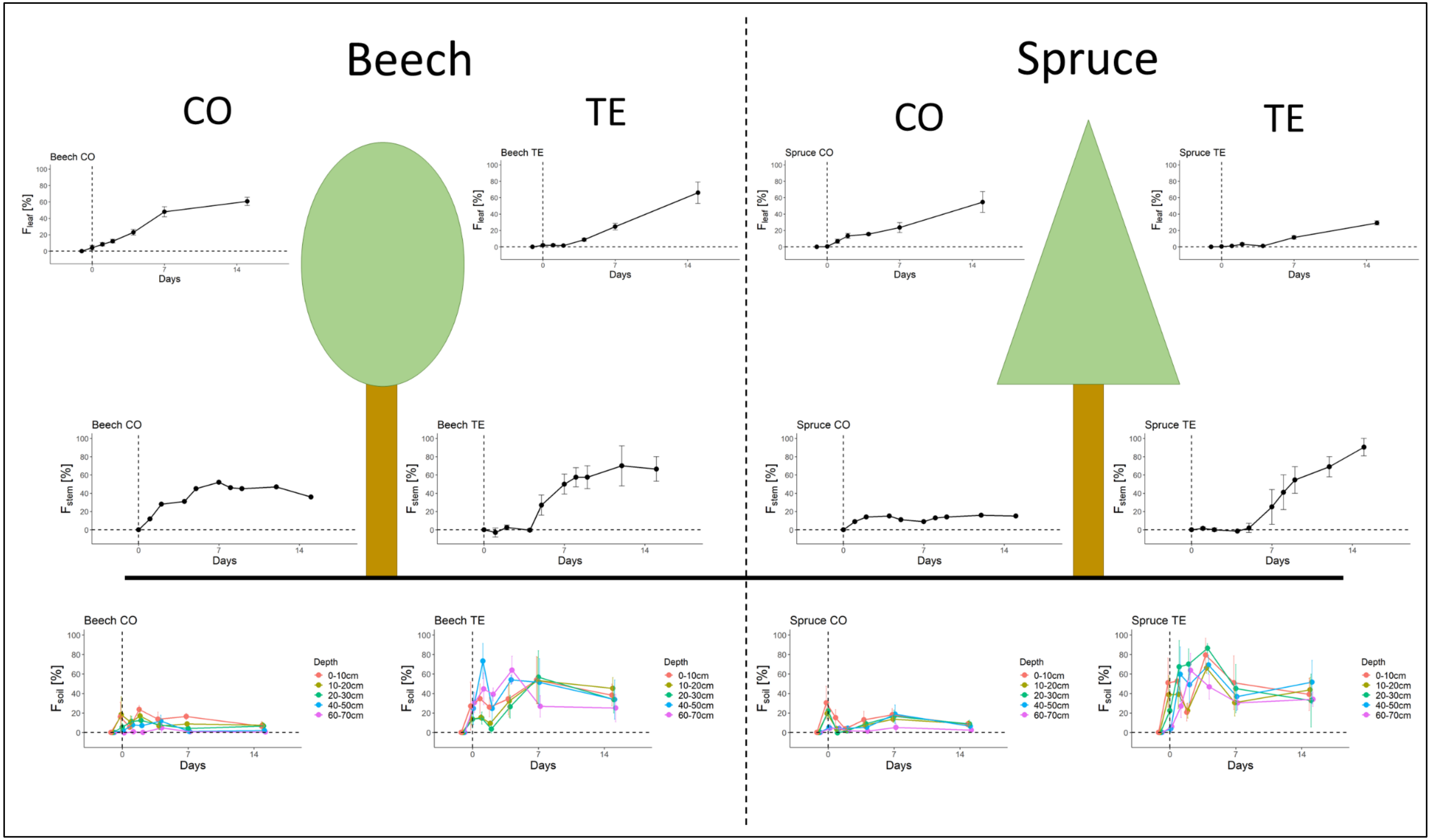

609 *Figure 6: Fractions of labeled water in the SPC*

Tables

Table 1: Leaf water content (LWC) and evaporative enrichment of leaf water (EER)

Species	**Beech**				**Spruce**			
Day	**D-6**		**D15**		**D-6**		**D15**	
Treatment	**CO**	**TE**	**CO**	**TE**	**CO**	**TE**	**CO**	**TE**
LWC [%]	52.7 ± 7.5	51.9 ± 7.1	50.1 ± 1.5	52.0 ± 0.9	51.8 ± 0.9	51.3 ± 13.1	51.1 ± 0.7	49.9 ± 1.4
EER leaf water [‰ ^{2}H]	43.9 ± 7.9	37.7 ± 6.9	46.1 ± 9.9	40.7 ± 5.8	50.3 ± 7.1	45.1 ± 5.9	42.3 ± 4.9	39.4 ± 4.4
δ^2H twig water [‰ ^{2}H]	-71.2 ± 8.1	-65.2 ± 11.3	-6.2 ± 24.2	20.6 ± 30.9	-75.5 ± 4.5	-72.1 ± 6.0	-6.4 ± 21.6	-31.6 ± 10.6

Table 2: Water uptake depth of beech and spruce

	Beech		**Spruce**	
Depth of water uptake [cm]	**CO**	**TE**	**CO**	**TE**
	27.5 ± 11.4	10.9 ± 7.6	33.5 ± 6.9	15.9 ± 4.3

Supplemental material

Table S 1: Sampling table for deuterium measurement in different tissues

Tissue	**D-6**	**D-1**	**D1**	**D2**	**D4**	**D7**	**D15**	**D22**
Soil	x	x	x	x	x	x	x	x
Stem		x	x	x	x	x	x	x
Twig	x						x	
Leaf	x	x	x	x	x	x	x	x

Title

Back to Routine? – Recovery Time and Resilience of the Water-Relations of Mature Beech and Spruce after Recurrent Summer Drought

Running head

Drought resilience of mature beech/spruce

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Keywords

Climate change, forest ecosystems, drought stress release, water potential, sap flow density, osmotic potential

Abstract

The ability to acclimate to a changing environment defines the potential of survival for every species. Acclimation processes include the ability to endure and recover from abiotic stress events (e.g. drought), which challenges especially immobile and long-living species, such as trees. The ability and extent of stress resilience depend on site conditions, severity and duration of the stress, but may also be species dependent. European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*), two economically and ecologically important tree species in European temperate forests, are threatened by increasing drought frequency. We tested the resilience and recovery time of the water relations in mature beech and spruce upon stress release after five years of recurrent summer droughts in a throughfall-exclusion experiment in southern Germany. We measured pre-dawn leaf water potential, sap flow density and leaf osmoregulation under drought and two years after drought release. Both species were severely impaired by drought with an average decrease of 33% in water related parameters. Nevertheless, both species displayed high resilience, reaching values close to control trees during two years after drought release. Beech reached full resilience in water potential and sap flow density earlier than spruce and even exceeded sap flow density of control trees in the second year of recovery. Differences found in recovery between beech and spruce appear to be related to their “opposing” drought stress mitigation strategies, with beech being more anisohydric and spruce more isohydric. Additionally, species-specific recovery times to full resilience were found for different physiological parameters, with water potential recovering faster (hours to days) than osmoregulation (weeks) and the sap flow density (months-years). With the anticipated increased frequency of drought events under ongoing climate change, short recovery times will be beneficial for plant fitness, with faster acclimating species benefiting at the expense of slower acclimating species.

Introduction

Forest ecosystems have undergone many changes during their evolution (Looy, Brugman, Dilcher, & Visscher, 1999; Spencer, Mapes, Bateman, Hilton, & Rothwell, 2015), overcoming different challenges, adapting to changing environmental conditions, interacting with many different species and ultimately better adapted genotypes survived (González-Martínez, Krutovsky, & Neale, 2006; Iwasa, Sato, Kakita, & Kubo, 1994). Nowadays natural and managed ecosystems face the challenge of rapid climate change (IPCC, 2014), largely caused and accelerated by humankind (Seidenkrantz, Kuijpers, & Schmith, 2009). As relatively slow-growing ecosystems, forests will be endangered to survive in the shape, e.g. species composition, as we have known them for past centuries due to the quickly advancing climate change (Allen et al., 2010; Hartmann et al., 2018). As any system deflected by stress, forests will have to adjust to the new situation or endure stress periods and regenerate afterward (Thompson, Mackey, McNulty, & Mosseler, 2009). The capacitance for resilience and recovery (*sensu* Lloret, Keeling, & Sala, 2011) of temperate forest ecosystems is one of the major questions in how climate change might affect forest ecosystems in the northern hemisphere (Ruehr, Grote, Mayr, & Arneth, 2019). Especially in Central Europe, species' composition might have to adjust drastically with ongoing climate change (Bakkenes, Alkemade, Ihle, Leemans, & Latour, 2002; Brodribb, Powers, Cochard, & Choat, 2020).

According to Ruehr et al. (2019), the recovery intensity and time of plants are largely depending on the studied parameter, but also on the experienced drought stress level, as it includes high energy demanding repair and regrowth processes for severely stressed plants. Many studies have shown that the water potential of formerly drought stressed plants increases almost immediately upon drought release (Ruehr et al., 2019 and citations within), while stomatal or leaf conductance, which is supposedly linked directly to the level of osmoregulation (Salmon et al., 2015), can be delayed by up to several weeks (Ruehr et al., 2019 and citations within). However, most of these studies were conducted on juvenile (potted) plants and information on mature forest stands is scarce. Basic physical principles (e.g. rehydration upon watering) should be similar across ontogenetic stages (e.g. Cermak et al., 1993), while properties such as the presence of heartwood, tree size or amount of living tissues may affect the hydrological recovery processes depending on tree maturity

The present study is focusing on two of today's most dominant tree species in Central Europe, i.e. European beech (*Fagus sylvatica* (L.)), which is assumed to be the peak of natural succession on many sites (Cavin, Mountford, Peterken, & Jump, 2013), and Norway spruce (*Picea abies* Karst.(L.)), most frequently used in silvicultural stands (LWF, 2014; Spiecker, 2000). While drought response of beech is supposed to be more anisohydric (Leuschner, 2020; Magh et al., 2019) and that of spruce more isohydric (Hartmann, Ziegler, Kolle, & Trumbore, 2013; Oberhuber, Hammerle, & Kofler, 2015), recent drought periods in 2013, 2015 and 2018/19 have uncovered high sensitivity of both species to drought and heat and sometimes a low potential for recovery afterward (Asner et al., 2016; Buras, Rammig, & Zang, 2019; Schuldt et al., 2020). The study was conducted as part of the Kroof (Kranzberg Forest roof) experiment, which applied severe drought stress to a mixed stand of mature beech and spruce trees for five consecutive years (2014 to 2018) via throughfall-exclusion (TE = drought stress treated trees, Grams et al., 2021). This resulted in a strong decrease in growth, by up to 70% decline in the annual stem diameter growth (Pretzsch et al., 2020) and reduced fine root production (Zwetsloot & Bauerle, 2021) already in the first drought years. Also, the carbon and water relations were strongly affected by drought. Indicating severe drought stress, the pre-dawn water potential was significantly decreased in both species under the TE compared to unstressed control trees (CO, Grams et al., 2021). The speed of carbon transport in the phloem was reduced by almost 50% and the mean residence time of newly assimilated sugars in leaves was almost doubled under drought (Hesse, Goisser, Hartmann, & Grams, 2019). Additionally, osmotic acclimations to drought have been found in the leaves of both species already in the second drought year (i.e. 2015) (Tomasella et al., 2018).

The focus of the present work is on the drought release of European beech and Norway spruce after five years of experimentally induced summer drought (Grams et al. 2021). To this end, throughfall exclusion plots of the Kroof experiment were watered to the soil water content of neighboring control plots. We assessed the water relations of the trees via pre-dawn leaf water potential, as a primary drought stress indicator, and xylem sap flow density, for morphological changes (e.g. fine root abundance or leaf area) (Gebhardt, Häberle, Matyssek, Schulz, & Ammer, 2014). Additionally, osmotic regulations (Bartlett, Scoffoni, & Sack, 2012), namely the turgor loss point and the osmotic potential in leaves, were monitored. As a more anisohydric species, beech is assumed to have a higher potential for osmotic adjustments (Hartmann, Link, & Schuldt, 2021) and to open stomata upon drought release faster than the more isohydric spruce (Brodribb & McAdam, 2013). We hypothesized that the pre-dawn water potential of both species will increase within hours after watering to the level of CO trees (H1). Additionally, for both species, we hypothesized that the daily sap flow density will not fully recover within one year due to the strong decrease in stem diameter growth and fine root biomass during drought (H2). Finally, we hypothesized that the reversal of osmoregulation, i.e. the decrease of turgor loss point and osmotic potential, occurs more rapidly in beech than in spruce, which is due to the more anisohydric strategy of beech (H3).

Material and Methods

Experimental site and design

The study was part of the Kroof experiment, based in Kranzberg Forest near Munich in south-eastern Germany (11°39’42”E, 48°25’12”N). The stand is composed of Norway spruce (*Picea* *abies* Karst.(L.)) and European beech (*Fagus sylvatica* (L.)), planted in 1951 ± 2 AD and 1931 ± 4 AD respectively (Pretzsch et al., 2014). With a luvisol soil that originated from loess of tertiary sediments and average precipitation of 750-800 mm a^{-1}, the water supply of the site is high (Grams et al. 2021). Twelve plots with over 100 trees altogether were established in 2010. In the years 2014 to 2018 the trees of 6 plots were exposed to recurrent summer droughts with pre-dawn water potentials as low as -1.8 MPa and strong annual basal area growth reduction by up to 45 % and 70 % in beech and spruce, respectively (Grams et al., 2021; Pretzsch et al., 2020). Drought stress was imposed via a throughfall-exclusion system (TE), which completely held off the summer precipitation and stem run-off on 6 plots (50 trees). These TE plots were compared to 6 untreated control plots (58 trees, CO, Grams et al. 2021). At the end of June 2019, a controlled drought release by watering of the TE plots to the level of soil water content of the CO plots was conducted. To this end, TE plots were slowly watered for c. 48 h with a rate of about 2 mm h^{-1} to a total amount of 90 mm water m^{-2} via a soaker-hose based watering system. In order to match the treatment of watering on the TE plots and its immediate effects on the soil (e.g. temperature, oxygen level) the CO plots were watered with 15 mm (for details see Grams et al., 2021). Three watering campaigns with two CO and two TE plots each were conducted in consecutive weeks at the end of June/beginning of July 2019 (for details see Grams et al. 2021). Climatic data, i.e. air temperature (in °C), vapor pressure deficit (VPD in kPa), photon flux density of the PAR spectrum (PPFD, µmol m^{-2} s^{-1}) and precipitation (in mm) were recorded by a climate station on the experimental site above the canopy.

Measurement parameters were taken six and one day(s) before the watering (D-6 and D-1) and repeatedly after the watering on D0 (day of watering), D1, D2, D4, D7, D15, D22, D56 and D365 (July 4th 2020 for all three campaigns). From D14 on the roofs were opened permanently and both treatments received the natural amount of precipitation from thereon. Eight CO trees of both species and 8 respectively 6 TE trees of beech and spruce were measured on four CO and three TE plots.

Plant available water in the soil

On each plot, time domain reflectometry sensors (length: 20 cm; TDR100 and TDR200, Campbell Scientific, Logan, USA) were installed under beech and spruce trees and in the mixing zone between species to measure the soil water content (SWC in vol.-%, Goisser et al., 2016; Grams et al., 2021). For each installation, sensors were buried at four depths down to 70 cm (0-7 cm, 10-30, 30-50 and 50-70 cm, n = 6 for each depth, position and treatment). The permanent-wilting points (PWP) of the experimental site were determined as a stable plateau with no further decrease of SWC and used to calculate the plant available water (PAW in vol.-%) in each soil layer (Grams et al., 2021). The field capacity (θ_{fc} in vol.-%) was determined for each soil depth as the highest PAW values measured during the whole experiment. The θ_{fc} was highest in the shallowest soil layer (0-7 cm: 27.6 ± 5.0 vol.-%) and gradually decreased in the deeper soil layers (10-30 cm: 22.7 ± 3.8 vol.-%, 30-50 cm: 20.9 ± 3.6 vol.-% and 50-70 cm: 15.7 ± 3.5 vol.-%). Finally, the relative extractable water (REW in %) was calculated for each measurement, by dividing the PAW by θ_{fc}.

Pre-dawn leaf water potential (Ψ_{PD})

To assess the drought intensity, pre-dawn leaf water potential (Ψ_{PD} in MPa) was assessed. A fully sun-exposed branch (c. 20 cm) of spruce (2 needle age classes) or beech was inserted in a scholander type pressure chamber (mod. 1505D, PMS Instrument Co., Albany, USA), and the pressure was slowly increased until water became visible at the cut surface (darken of the xylem area). Pre-dawn measurements were carried out at 3 to 4 AM, before sunrise.

Daily sap flow density at breast height (u_{daily})

Sap flow density per unit sapwood area was measured with Granier-type heat dissipation sensors (Granier, 1987) in 10 min intervals. On two trees per species and plot at breast height sap flow was measured in the outer xylem sapwood (0-2cm depth) with two sensors (north and south exposure). Data from both sensors were averaged and the mean sap flow density per day and tree was calculated (u_{daily} in L dm^{-2} d^{-1}). In 2019, sap flow density was measured from D-80 to D110 (growing season of 2019) and D362 to D392 (1st June to 31st July 2020).

Turgor loss point (Ψ_{TLP}) and osmotic potential (π_O)

Following Tomasella et al. (2018), pressure volume curves (PV-curves, or water potential isotherms) were assessed using a scholander type pressure chamber (mod. 1505D, PMS Instrument Co.). Fully sun-exposed branches (c. 20 cm) of spruce (2 needle age classes) and beech were cut in the afternoon and rehydrated overnight to a water potential > -0.02 MPa. Upon full saturation, the twigs were weight and their water potential was measured repeatedly. Calculations for the turgor loss point (Ψ_{TLP} in MPa) and osmotic potential (π_O in MPa) were carried out with the spreadsheet tool of Sack and Pasquet-Kok (2010). PV-curves were assessed on D-6, D2, D7, D22, D56 and D365.

Calculation of resilience, recovery time (RT) and resilience velocity (V_R)

Resilience was calculated according to (Lloret et al., 2011) as the relative proportion of TE values compared to the respective CO values, which were set as 1, for each measurement timepoint and species respectively (Fig. 1, Formula 1). The resilience of TE trees was determined by how close they reached the value of the control trees. Thus, if TE trees reached the same value as CO trees a resilience of 1, i.e. full resilience, was assumed (Fig. 1). Recovery time (RT, in days) was determined as the timespan upon drought release that was needed to reach full resilience or a stable level below 1 (Fig. 1).

$$Resilience_i = \frac{TE_{i,x}}{Mean\ (CO_i)} \quad Formula\ 1$$

With i = each individual timepoint and x = each individual tree. From the resilience and the recovery time, the resilience velocity (V_R in days^{-1}) was calculated (Formula 2). When the mean value of the resilience reached 1, the time until full resilience (day_{FR} in days) was determined and the result was multiplied with the resilience value at day_{FR} in order to account for any deviation from 1 (e.g. overcompensation).

$$V_R = \frac{1 - Resilience_{\ 0}}{Day_{FR}} * Resilience_{\ FR} \quad Formula\ 2$$

With $Resilience_0$ = resilience value before the watering, Day_{FR} = day after the watering when resilience reached ~1 or higher and $Resilience_{FR}$ = resilience value at day_{FR}.

Statistical analysis

Data were analyzed for statistical differences using R (version: 4.0.2, R Development Core Team 2008) in RStudio (version 1.3.1073, RStudio Team 2015). Data were tested for homogeneity of variances (Levene test) and the residuals of every model were tested for normality (Q-Q-Plot/Shapiro test). For differences in Ψ_{PD}, Ψ_{TLP}, π_O and u_{daily}, linear mixed effect models ('lme' function) were calculated, using the day/date (respective to the watering), species and the treatment as fixed and the tree individual nested in the plot as a random effect (package: nlme, version: 3.1-148). Whenever a fixed effect was not statistically significant (p-value > 0.05) it was excluded from further analysis. If the mixed effect model showed significant effects, we did a post-hoc test with the 'emmeans' function with Tukey correction (package: emmeans, version: 1.5). Data were plotted with the 'boxplot' function (package: graphics, version: 4.0.2) and 'ggplot' function (package: ggplot2, version: 3.3.2). Data are given in text and tables as the mean ± 1SD unless stated otherwise.

Results

Climate of 2019 and 2020

During the growing season (April to October) average values for air temperature of 16.8 ± 4.0 °C, VPD of 0.66 ± 0.25 kPa, PPFD during daytime of 609 ± 143 µmol m^{-2} s^{-1} and precipitation of 406 mm (Fig. 2 a-d) characterized 2019 as a relatively warm and dry year in comparison to the long-term annual climate (Grams et al. 2021). Mean climatic conditions during the main experimental period (June 2019 to September 2019, light gray area in Fig. 2 a-d) were 18.7 °C (daily maximum 28.8 °C /daily minimum 8.9 °C), 0.72 kPa VPD (daily maximum 2.11 kPa / daily minimum 0 kPa) and 620 µmol m^{-2} s^{-1} PPFD (daily maximum 1109 µmol m^{-2} s^{-1} / daily minimum 99 µmol m^{-2} s^{-1}) respectively, and summed precipitation amounted to 276 mm. In comparison to 2019, the growing season of 2020 was slightly milder with average values for air temperature of 15.5 ± 3.2 °C, VPD of 0.54 ± 0.17 kPa and PPFD of 584 ± 126 µmol m^{-2} s^{-1}) and more moist (475 mm of precipitation) (Fig. 2 a-d).

Plant available soil water

In 2013 before the start of the drought experiment, early in the growing season plant available water in the soil (to 70 cm depth) was the same between CO and TE plots, at about 20 vol.-% (p-value > 0.05, Fig. S1). In the mid of the growing season, plant available water declined to about 5%. Overall, TE plots showed almost identical water availability as CO plots before drought was imposed (Fig. S1) (p-value > 0.05, see the proximity of the blue and red symbols in Fig. S1). Thus, the initial situation of the overall water balance was the same on CO and TE plots in 2013, i.e. before the start of the drought experiment in 2014.

In 2019 before drought stress released by watering, soil water availability was significantly higher in CO compared to TE plots, with CO plots (10 to 20 vol.-%) showing about twice as high PAW in all depths than TE (5 to 10 vol.-%, Fig. 3), except for the deepest layer which was less different between CO and TE. This was also reflected in the REW, which ranged between 50 to 75 % for CO and only 0 to 25 % for the TE plots (Fig. S2 a & c). With the start of the watering, a slight increase of PAW was found in the CO plots, corresponding to the small amount of water added to the CO plots (Fig. 3 a). Immediately upon watering, a strong increase in the PAW was found in TE plots for the three lower soil depths (10-70cm, Fig. 3 c), with REW values almost doubling (Fig. S2 c). At the same time, REW in the shallowest soil depth increased only slightly (0-7 cm, Fig. 3c). After the watering and the subsequent opening of the throughfall roofs from D14 on, CO and TE plots had similar PAW (Fig. 3 b & d), with TE plots displaying even a slightly higher REW than CO in summer 2020 (Fig. S2 b & d).

Pre-dawn leaf water potential (Ψ_{PD})

Before watering (D-1 and D-6), Ψ_{PD} showed significant differences between CO and TE for both species ($p\text{-value} < 0.05$, Fig. 4). On CO plots, beech was at -0.48 ± 0.11 MPa over the whole study period. (Fig. 4a). Beech trees on the TE plots had significantly lower Ψ_{PD} than CO before the watering, reaching -0.80 ± 0.08 MPa. An immediate increase in the Ψ_{PD} of TE beech trees to -0.43 ± 0.05 MPa was already observed at D1, which remained stable afterward during 2019 (-0.53 ± 0.13 MPa) and June 2020 (D365, -0.37 ± 0.07 MPa, Fig. 4a).

In spruce, CO trees were at -0.63 ± 0.08 MPa in 2019 and slightly higher in June 2020 (D365, -0.49 ± 0.04 MPa, Fig. 4b). Before the watering, TE spruce trees were significantly lower than CO with Ψ_{PD} values of -0.92 ± 0.12 MPa (Fig. 4b). With the start of the watering, a continuous increase was found in spruce from D1 (-0.92 ± 0.09 MPa) to D2 (-0.77 ± 0.06 MPa) to D4 (-0.69 ± 0.08 MPa) when Ψ_{PD} reached the same level as in CO trees. From D4 on TE spruces remained on the same Ψ_{PD} level of -0.69 ± 0.11 MPa in 2019 and slightly increased to -0.48 ± 0.04 MPa in June 2020 (D365, Fig. 4b).

Daily averaged xylem sap flow density at breast height (u_{daily})

In beech, average u_{daily} was by 30 ± 16% ($p\text{-value} < 0.05$) lower in TE compared to CO trees before watering (mean over D-70 to D0 of 5.13 ± 2.61 and 7.29 ± 3.33 L dm^{-2} d^{-1}, respectively; Fig 5a). Within the first three months after watering, the average u_{daily} of TE beech remained significantly lower (by 26 ± 15%, $p\text{-value} < 0.05$) in TE compared to CO (mean over D1 to D90 of 6.98 ± 3.04 and 7.87 ± 2.18 L dm^{-2} d^{-1}, respectively, Fig 5a). Only during senescence, when u_{daily} of CO beech started to decline rapidly, the differences between CO and TE beech diminished slightly. Before watering a similar pattern was found for spruce with stronger significant reductions of 63 ± 7 % in TE compared to CO trees (mean over D-85 to D0 of 1.61 ± 0.52 and 4.42 ± 1.44 L dm^{-2} d^{-1}, respectively, Fig. 5c). However, upon watering, an immediate increase was observed in TE spruce, but average u_{daily} was still lower by 40 ± 12% ($p\text{-value} < 0.05$) than in CO spruce (mean over D1 to D110 of 2.30 ± 1.03 and 3.81 ± 1.63 L dm^{-2} d^{-1}, respectively; Fig 5c).

During the growing season 2020, the differences between CO and TE in u_{daily} disappeared for both species. For CO beech u_{daily} was 7.36 ± 3.33 L dm^{-2} d^{-1}, while TE beech showed slightly higher values of 8.38 ± 4.00 L dm^{-2} d^{-1} (no significant difference, though, Fig. 5 b). In spruce, the u_{daily} of CO and TE trees was very similar, i.e. 3.91 ± 2.01 and 3.53 ± 1.91 L dm^{-2} d^{-1}, respectively (Fig. 5 d). In spruce, the differences between CO and TE further decreased, with both treatments showing similar u_{daily} values ($p\text{-value} > 0.05$).

Turgor loss point (Ψ_{TLP}) and osmotic potential (π_O) in leaves

Throughout the experiment, Ψ_{TLP} and π_O of CO trees in both species remained relatively stable (Ψ_{TLP}: -2.40 ± 0.16 MPa and -2.43 ± 0.13 MPa; π_O: -1.63 ± 0.15 MPa and -1.61 ± 0.14 MPa for beech and spruce, respectively, Fig. 6). In TE trees, both species displayed significantly lower Ψ_{TLP} (p-value < 0.01) and π_O before the watering (i.e. D-6) compared to CO trees with Ψ_{TLP} about 0.70 MPa lower in TE compared to CO trees (Fig. 6a, c). A similar reduction by 0.91 MPa and 0.77 MPa was found for π_O in TE beech (p-value < 0.001) and TE spruce (p-value < 0.01) compared to controls, respectively (Fig. 6b, d). In TE trees within the first week upon watering, no increases in both parameters were observed. Ψ_{TLP} and π_O of TE trees reached control values only on D22 and D56, respectively, in beech and spruce. (Fig. 6). In June 2020 (D365), no differences were found in Ψ_{TLP} and π_O between CO and TE in both species (p-value > 0.05).

Recovery time (RT), resilience and resilience velocity (V_R) of tree water-relations after drought stress

Drought stressed beech trees reached and even overcompensated Ψ_{PD} relative to CO trees already during D1, reflecting a very short RT (1 day), very high V_R (0.4470 ± 0.0737 day^{-1}) and full resilience in this parameter (Tab. 1 and Fig 7a). In spruce, RT for Ψ_{PD} was 7-15 days until full resilience (i.e. a resilience value of 1) was reached (Fig. 7a), resulting in a significantly slower V_R of 0.0347 ± 0.0056 day^{-1} than in beech ($p < 0.001$, Tab. 1). Thus, both species demonstrated full resilience in Ψ_{PD}, but with a longer RT and slower V_R in spruce compared to beech (Tab. 1).

In u_{daily} of beech, resilience of TE trees remained relatively constant and below full resilience throughout 2019 with mean values hardly increasing upon watering from 0.70 ± 0.03 to 0.73 ± 0.03 (means ± CI_{95}) (Fig. 7 d). One year after the watering, with a RT of c. 300 days, TE beech trees showed full resilience in u_{daily} (i.e. 1.11 ± 0.02 CI_{95}) and even surpassed the controls throughout the growing season (Fig. 7d). The very long RT resulted in a low VR for beech in the recovery of u_{daily} with 0.0010 ± 0.0006 day^{-1} (Tab. 1). Conversely to beech, the resilience of spruce in u_{daily} almost doubled upon watering from 0.35 ± 0.03 to 0.61 ± 0.03 (means ± CI_{95}). Nevertheless, it hardly reached full resilience in 2020 with a mean resilience of 0.89 ± 0.02 CI_{95} (Fig. 7 d). With an RT of more than 365 days and a VR of 0.0016 ± 0.0008 day^{-1}, spruce did not fully recover even in 2020 (Tab. 1).

A different pattern was found for Ψ_{TLP} and π_O, with both species showing similar behavior. While both species again reached full resilience upon drought release (Fig. 7b & c), the RT was intermediate between Ψ_{PD} and u_{daily}, with 22 days for Ψ_{TLP} and 22-56 days for π_O to fully resilience (Tab. 1). This led to a rather low V_R for both species combined of 0.0037 ± 0.0007 $days^{-1}$ in Ψ_{TLP} and 0.0056 ± 0.0006 day^{-1} in π_O (Tab. 1).

Discussion

In 2013 before the start of the experiment, the water balance was similar between the designated CO and TE plots with similar soil moisture values throughout the growing season. This indicated that water consumption (soil evaporation plus transpiration) and tree water status (e.g. Ψ_{PD}) were similar for all plots (Emanuel, D'Odorico, & Epstein (2007) and Lundblad & Lindroth (2002). Therefore, we can use measurements on the CO plots as control values, displaying the natural situations trees TE would have experienced without artificial drought during the experimental time until 2019/20, including such elements as age/ontogeny and related effects (e.g. changes in stand structure, Cornish and Vertessy 2001, Binkley et al. 2002, Dorado Liñán et al. 2012).

Effects of drought and subsequent watering on mature trees' water relations

Pre-dawn water potential (Ψ_{PD}) and xylem sap flow density (u_{daily}) at breast height

Before watering, low PAW on the TE plots resulted in levels of Ψ_{PD} of -0.8 to -1.0 MPa, indicating moderate drought stress for beech and spruce already in early summer. Under the moderate drought, u_{daily} was reduced in both species, with beech being less affected than spruce. As the Ψ_{PD} was far away from the $\Psi_{50/88}$ of about -4 MPa (Tomasella et al. 2018), the occurrence of embolisms in spruce and beech was unlikely. The weaker reduction in u_{daily} of beech might be related to its more anisohydric strategy compared to spruce, as carbon gain would outrank water loss in more anisohydric species (Klein, 2014). Additionally to drought stress, low u_{daily} may reflect decreases in the fine root biomass, which was found since the beginning of the drought in 2014 for both species on TE plots (Zwetsloot & Bauerle, 2021), and a lower water demand/supply for transpiration (Gebauer et al., 2015; Solberg, 2004), or changes in the cellular anatomy (e.g. smaller vessels, Schuldt et al., 2016). In fact, somewhat reduced vessel diameter has been documented for branches of beech individuals used in this study (Tomasella et al 2018). Therefore, it is likely that the small decrease in u_{daily} in TE beeches was caused by anatomic differences of the xylem coupled with slightly closed stomata of the TE trees. Conversely in spruce, no differences in the cellular anatomy of the xylem were found between CO and TE (Tomasella et al., 2018). However, the strong decrease in u_{daily} of spruce on the TE plots is likely explained by a higher reduction in fine root abundance in spruce than in beech

(Nickel et al., 2018; Zwetsloot & Bauerle, 2021) and strongly decreased stomatal conductance in spruce during drought (data not published).

According to H1, TE trees would increase their Ψ_{PD} immediately after the watering to a similar level as CO plants, which was the case in beech, but not in spruce. Therefore, we accept H1 only for beech but not for spruce. Moreover, directly after watering, both species did not show full resilience in u_{daily} and even in the following growing season spruce did not recover completely which is in line with H2.

In beech, Ψ_{PD} increased on the day after the watering and was even higher than in the CO trees. The results of beech are in accordance with an earlier experiment on mature (Cermak et al., 1993) and also juvenile, potted trees of several species (Ruehr et al., 2019), which also displayed a very quick recovery of the Ψ_{PD}. Besides the lower drought effect on u_{daily} before watering, the constant u_{daily} in beech upon watering but increased Ψ_{PD} may be a result of reduced total leaf area (i.e. lower overall water demand) in 2019 due to smaller leaves. The difference appears not to be related to changes in stomatal density as no significant differences were found in the stomatal density between CO and TE in 2017 for beech and TE trees showed an even slightly higher stomatal density than CO (data not published). The full recovery in the next growing season of u_{daily} in beech could be connected to the newly built fine roots, which potentially recovered to the level of CO trees, due to the high resilience of growth in beech found in other experiments (Vanhellemont et al., 2019). Additionally, the leaf area might also have recovered in 2020, as no drought stress was present during leaf maturation (Cermak et al., 1993). The overcompensation of TE beech compared to CO in 2020 of u_{daily} could be related to the slightly higher water availability on the TE plots in 2020 compared to CO (Fig. 3). This higher water availability of the soil is probably related to the slightly reduced u_{daily} of TE spruces compared to CO spruces even in 2020.

In spruce, it took 7-15 days until full resilience in the Ψ_{PD} was reached, with a continuous increase during this time. So, while both species showed full resilience and complete rehydration of the leaves upon watering, the recovery time was much longer and the resilience velocity was much slower in spruce compared to beech. The delay in the recovery of Ψ_{PD} in spruce was unprecedented, as in experiments with juvenile trees also on isohydric species (Duarte et al., 2016) and gymnosperms (Ruehr et al., 2019) showed a fast recovery of the Ψ_{PD} after drought release. One possible explanation for the delay in spruce could be the lack of vessels in gymnosperms (Lüttge, Kluge, & Bauer, 2005) that limits water transport velocity in the xylem compared to angiosperm beech trees. However, this alone may not explain the time lag of several days in the recovery of the Ψ_{PD}, even in mature trees. An additional explanation could be the delay in stomatal opening of the more isohydric spruce, due to its proposed hormonal stomatal regulation under/after drought, which reacts slower than the hydraulic control in anisohydric species (Brodribb & McAdam, 2013). In contrast to the $\Psi_{PD,}$ u_{daily} at breast height in TE spruce increased immediately by about 20%. The delay in spruce Ψ_{PD} recovery, but increased u_{daily} at breast height during the first days upon re-watering could be the re-saturation of the stem xylem water reservoir, which gets depleted under drought (Borchert, 1994). This internal water storage is especially important for isohydric species under drought (Hartmann et al., 2021; Jiang et al., 2021). However, TE spruce u_{daily} did not completely reach the level of CO spruce u_{daily} in 2019. This indicates probably a reduced leaf area, which could be related to the evergreen strategy of spruce. As TE spruce had still needle ages/twigs built under drought, which were smaller than CO needles/twigs (personal observation), it may take several years for the leaf area to recover. Additionally, during the five years of summer drought, the fine root system of spruce showed lower growth than beech (Nickel et al., 2018; Zwetsloot & Bauerle, 2021), limiting the water uptake of spruce after watering. Moreover, most fine roots of spruce were in the shallowest soil layer (Zwetsloot & Bauerle, 2021), which was re-saturated the latest, reflecting the high water repellency in shallower soil layers (Gimbel, Puhlmann, & Weiler, 2016). Therefore, spruce could not make use of the additional water and re-saturate as quickly as beech. Also, both species lost a significant amount of fine roots under drought (Zwetsloot & Bauerle, 2021), therefore a quick recovery of u_{daily} seems unlikely, but fits to the recovery found within the next growing season when new fine roots could have been built. Altogether, re-saturation of the stem xylem water reservoir, lower/delayed demand of water from the canopy (reduced leaf area) and an impaired root system would be the most likely explanation for the delayed recovery of spruce compared to beech.

Turgor loss point (Ψ_{TLP}) and osmotic potential (π_O) in leaves

The observed decrease in Ψ_{TLP} in TE compared to CO trees of about 0.7 MPa in beech and spruce alike, allowed the TE trees to remain turgescent at lower soil water potentials (Bartlett et al., 2012, 2014). As the π_O showed a similar decrease, the changes in Ψ_{TLP} seemed to be based on osmotic adjustments (Bartlett et al. 2012 and citations within). A slightly bigger difference between CO and TE in the Ψ_{TLP} in 2019 (~0.7 MPa) than in 2016 (~0.5 MPa, Tomasella et al., 2018) would indicate a similar acclimation over the years despite ongoing drought in the sampled trees (Blackman, 2018). Furthermore, the ability of beech and spruce to reduce their Ψ_{TLP} under drought (Bartlett et al., 2014) could be used to predict their drought tolerance potential better than leaf or wood structure parameters (Maréchaux et al., 2015; Zhu et al., 2018).

Just as beech and spruce showed similar drought acclimation in their osmoregulation, they also showed a similar behavior after watering. Both species reversed their osmotic adjustments (i.e. Ψ_{TLP} and π_O) within several weeks to full resilience. Therefore, we rejected H3, which stated that the more anisohydric beech would be quicker in the reversal of osmotic adjustments. Depending on the species, developmental stage and cell type, different kinds of osmolytes are accumulated by plants during drought-induced osmoregulation (e.g. sugars, amino acids or sugar-alcohols) (Cusuman, 2001; Zhang, Nguyen, & Blum, 1999). The basic biochemistry behind the accumulation and remobilization of osmolytes is likely to be conserved among different plant genera, but details about the underlying mechanisms are still mostly unknown for higher plants (Burg & Ferraris, 2008; Hare, Cress, & Van Staden, 1998; Hellebust, 1976; Morgan, 1984). Also, no clear pattern can be found for the osmoregulatory potential of an-/isohydric species. A recent meta-analysis revealed temperate conifers to have higher plasticity in π_O than temperate deciduous trees (Bartlett et al., 2014), while Hartmann et al. (2021) pointed out, that more isohydric species are rather limited in their osmoregulatory potential compared to anisohydric species. Another important aspect of the increase in the π_O in leaves is the remobilization of former osmolytes. During stress release, remobilized osmolytes (e.g. sugars, sugar-alcohols and amino acids) could be an additional carbon source next to photosynthesis and re-mobilization of reserves, helping to meet high C sink stress upon drought release (Gallé, Haldimann, & Feller, 2007; Ruehr et al., 2019).

Differences in resilience and recovery time between physiological water relation parameters

In the first two years after drought release, both species displayed high resilience in their water relations. However, beech often seemed to outrun spruce during the recovery process with shorter RT and higher V_R. Some of these differences could be explained by the “opposite” drought stress mitigation strategies of the species (anisohydric vs. isohydric) and differences in their basic anatomy/physiology, such as deciduous angiosperm vs. evergreen gymnosperm. Spruce was often more restrained than beech under the drought treatment, showing severely impaired growth (Pretzsch et al., 2020; Zwetsloot & Bauerle, 2021), physiology (e.g. Ψ_{PD}, Grams et al., 2021) and mycorrhization (Nickel et al., 2018). Nonetheless, as both species received the same treatment (i.e. five years of summer drought, Grams et al. 2021) differences in the drought stress level were solely species dependent and reflected the different drought stress vulnerability and acclimative potential of beech and spruce. In principle, recovery times to full resilience seem to depend not only on assessed parameters but also on species. Solely physiological and biochemical related parameters, such as Ψ_{PD} or osmoregulation, recover much faster (days to weeks) than parameters that are connected to morphological acclimation such as xylem sap flow density.

Conclusions

The findings of our experiment reveal the drought stress resilience and recovery time in a mature forest stand after recurrent summer droughts. While both species displayed full resilience in almost all measured parameters, the recovery time was often shorter in beech than in spruce.The struggle of spruce to recover from repeated drought displays how intense and demanding the recovery period can be. Under ongoing climate change with a supposedly higher frequency and intensity of stress events, a short recovery time might be crucial for the fitness level and/or survival of mature trees. Thus, faster reacting species, such as beech, could benefit at the expense of slower reacting species, such as spruce. Additionally, the stress intensity is also defining the recovery costs and time (Ruehr et al., 2019). If beech already benefitted under drought from the mixture with spruce, due to the lower water demand of spruce, beech would have had an advantage for the recovery period too.

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Figures

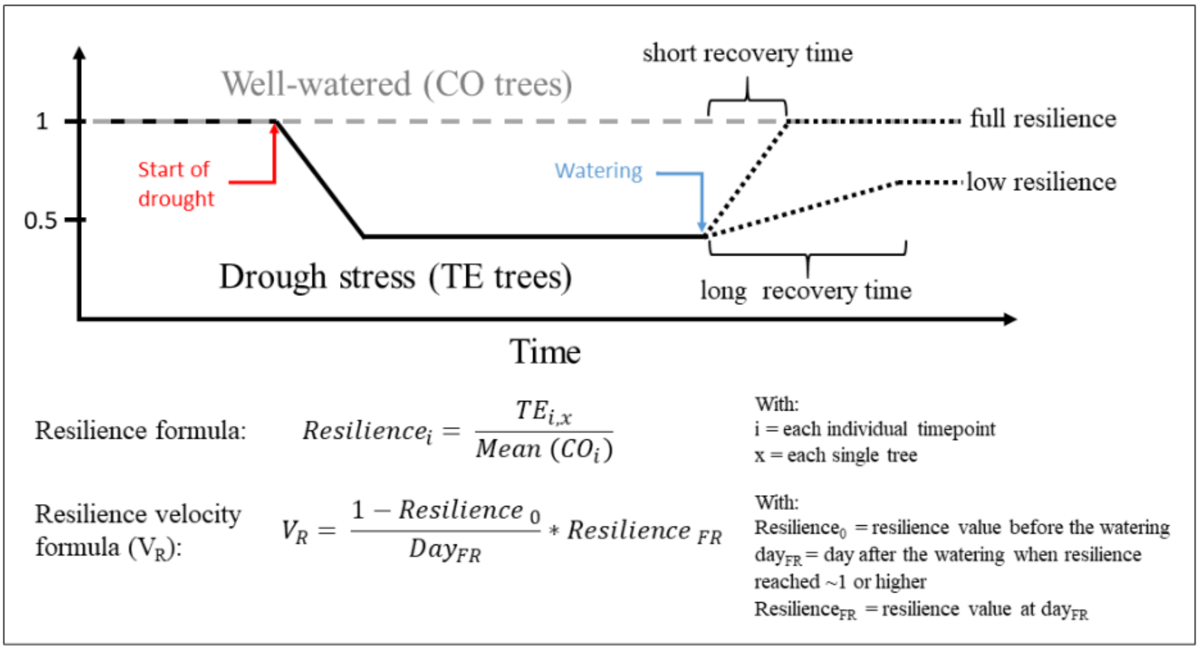

Figure 1: Schematic diagram on the resilience and recovery time of trees during drought and drought release. The horizontal dashed gray line reflects the level of the control trees (CO, set to 1) and the black line reflects the throughfall-exclusion (TE = drought stress) trees. The dotted black lines give putative recovery and resilience scenarios for TE trees upon watering.

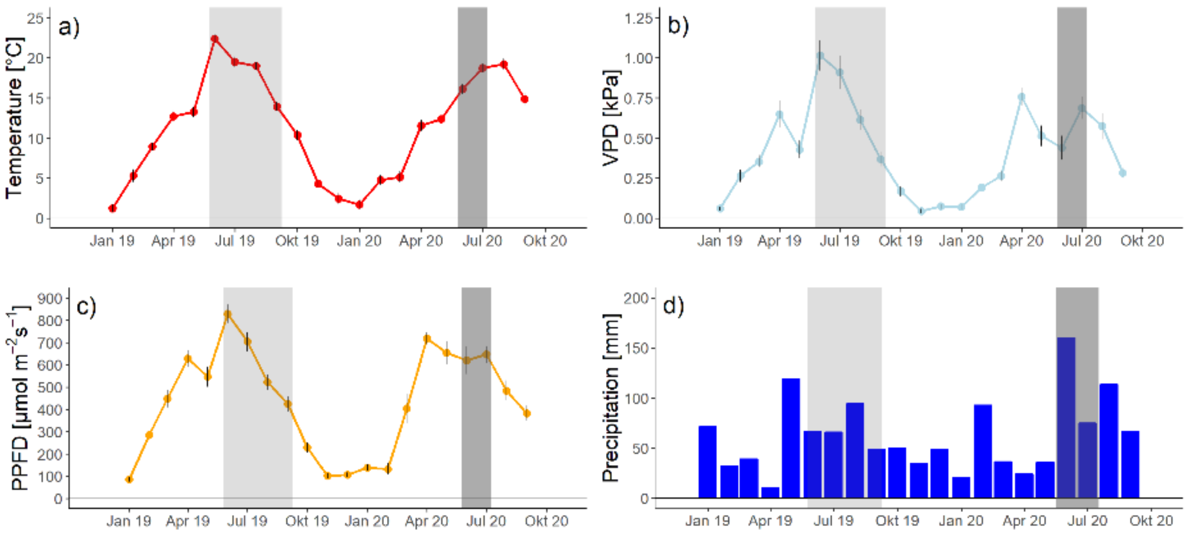

Figure 2: Monthly climate parameters (a: air temperature in °C, b: vapor pressure deficit (VPD in kPa), c: photosynthetic photon flux density (PPFD in µmol m^{-2} s^{-1}) and d: precipitation in mm) in 2019 and 2020. Data are shown as monthly mean ± 1SE (Temperature, VPD and PPFD) and sum (Precipitation). Gray areas show the main measurement time in 2019 (light) and 2020 (dark).

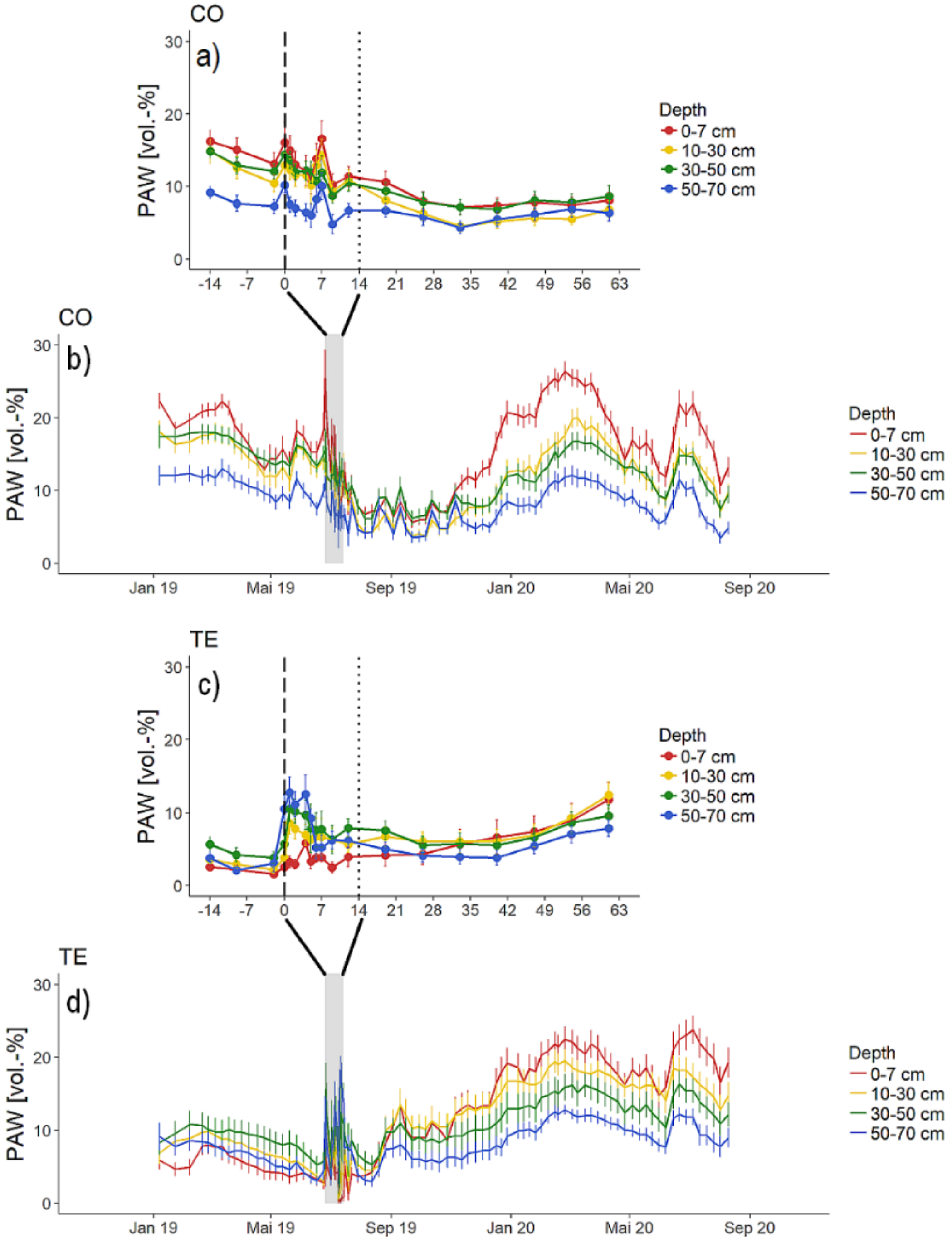

Figure 3: Plant available water (PAW in vol.-%) per volume of soil in CO (a & b) and TE (c & d) plots in four different depths (0-7 cm = red, 10-30 cm = yellow, 30-50 cm = green and 50-70 cm = blue) during 2019 and 2020. b & d show PAW during drought release by watering day (dashed lines) and permanent opening of the roofs on TE plots on D14 (dotted lines). Data are given as mean ± 1SE.

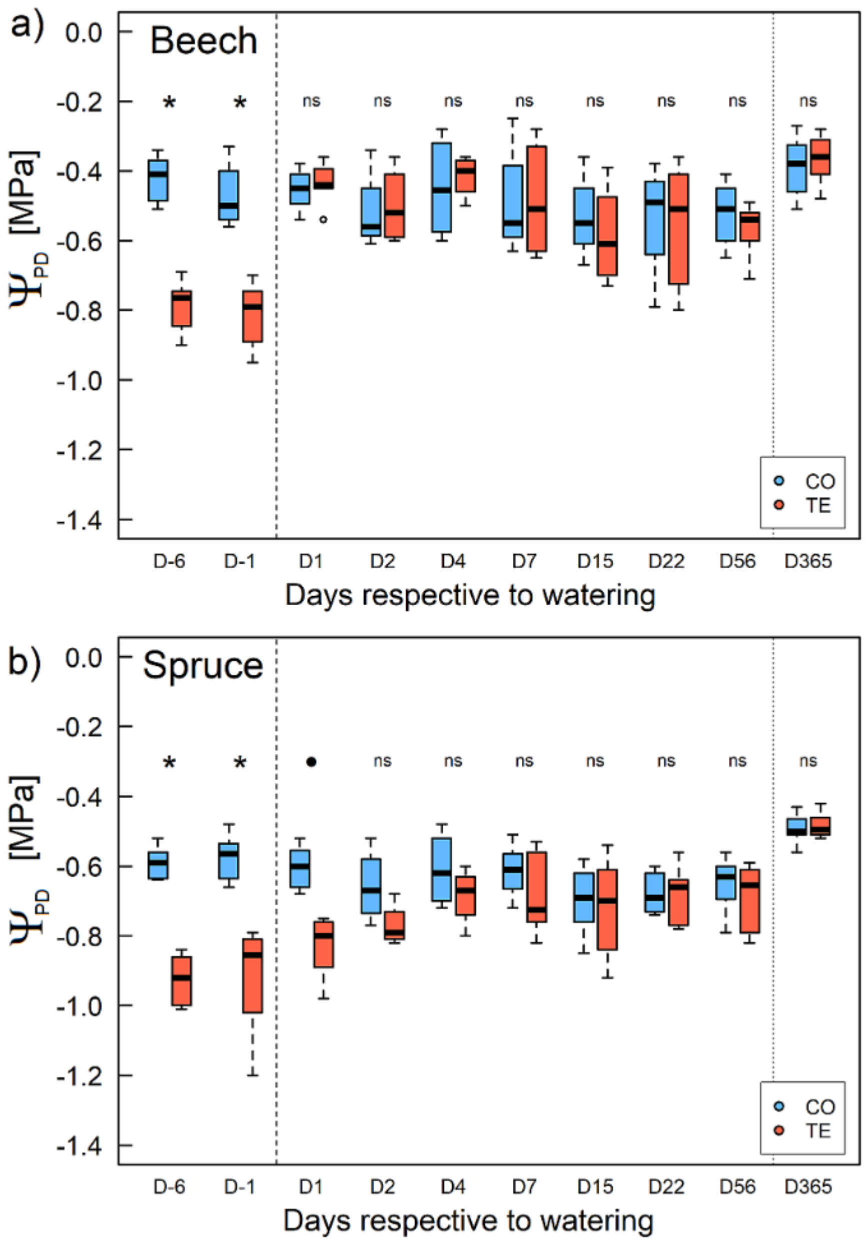

Figure 4: Pre-dawn leaf water potential (Ψ_{PD} in MPa) for beech (a) and spruce (b) with CO = blue and TE = red filled boxplots. The dashed line represents the watering day (D0) and the dotted line the end of 2019. July 4th 2020 accounts for D365 for all three campaigns.

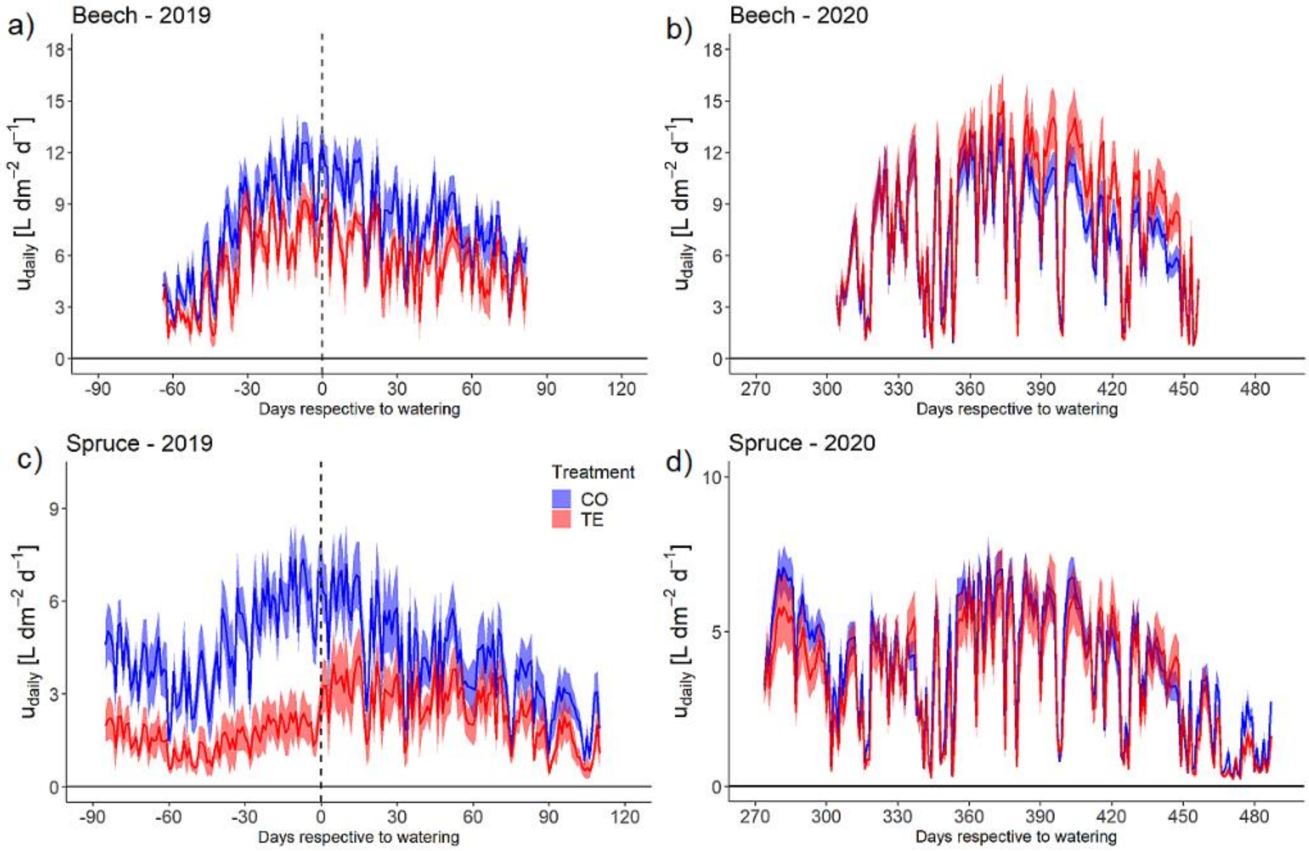

Figure 5: Daily sap flow density (u_{daily} in L dm^{-2} d^{-1}) of beech (a & b) and spruce (c & d) in 2019 around the time of watering (from D-90 to D110, dashed line shows the day of watering, a & c) and one year after watering in 2020 (b & d). Blue = CO trees and red = TE trees. Data are given as mean ± 1SE (blue and red ribbon). July 4th 2020 accounts for D365 for all three campaigns.

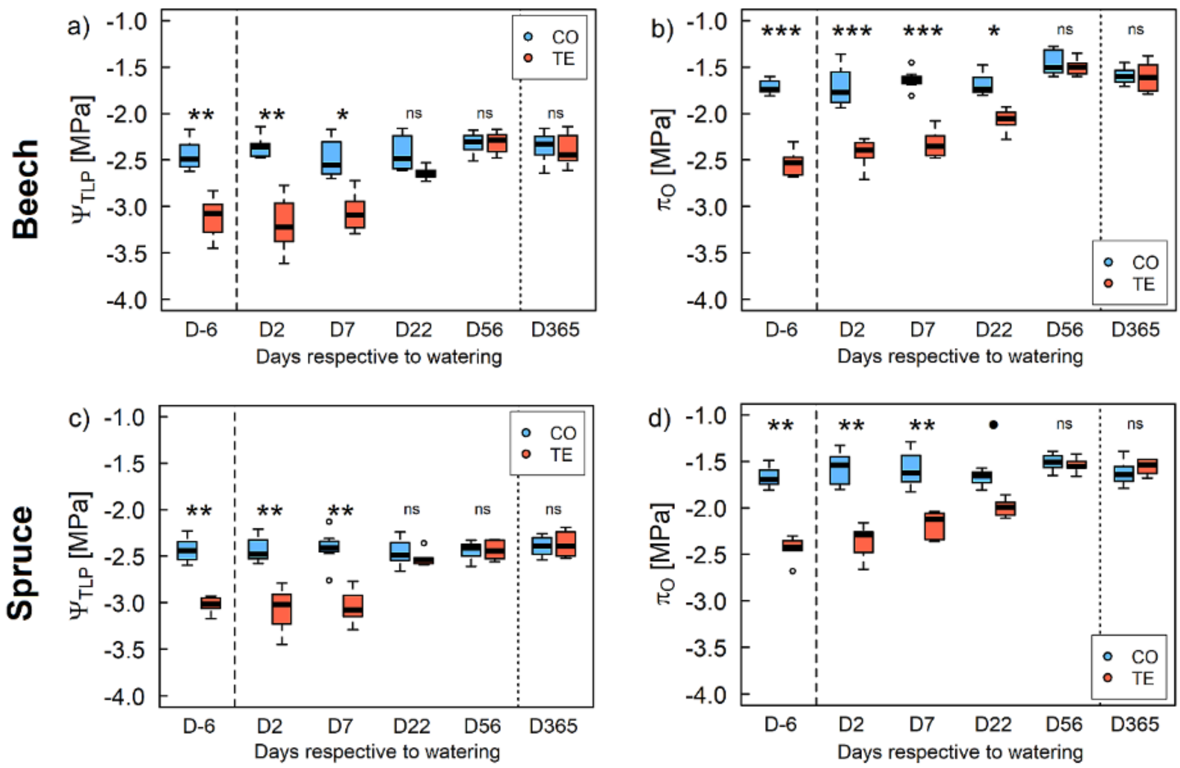

Figure 6: Turgor loss point (a & c in MPa) and osmotic potential at full turgor (b & d in MPa) for beech (a & b) and spruce (c & d) with CO = blue and TE = red filled boxplots. Dashed line represents the watering day and dotted line the end of 2019. July 4th 2020 accounts for D365 for all three campaigns.

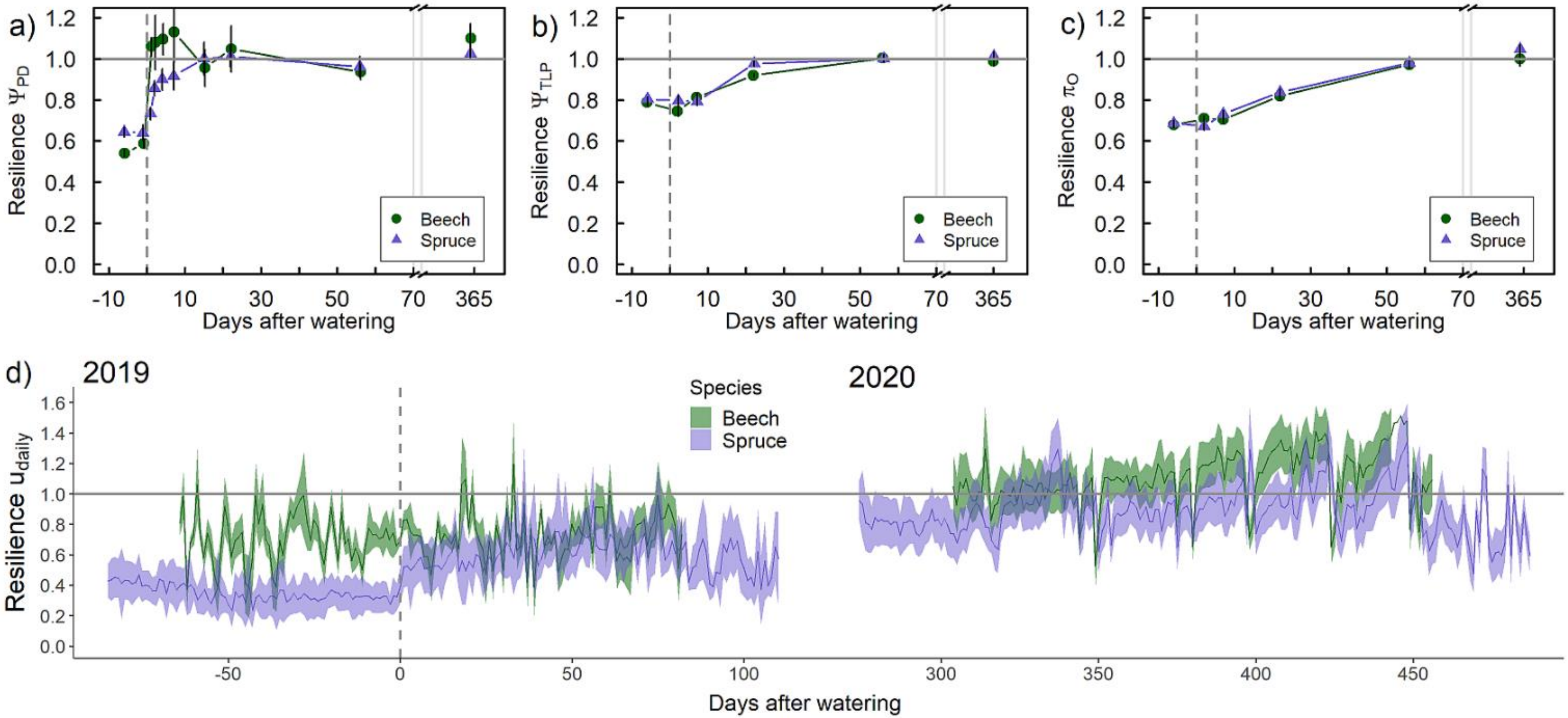

Figure 7: Resilience of TE trees upon drought release in pre-dawn water potential (Ψ_{PD}, a), turgor loss point (Ψ_{TLP}, b), osmotic potential (π_O, c) and sap flow density (u_{daily}) during 2019 and 2020 (d). Green circles and purple triangles represent beech and spruce trees, respectively. The horizontal gray line symbolizes the CO values (i.e. full resilience), the vertical dashed gray line symbolizes the day of watering and vertical solid light-gray lines indicate an axis break for better visibility. Data are given as mean ± 1SD. July 4th 2020 accounts for D365 for all three campaigns.

Tables

Table 1: Recovery time (RT) and resilience velocity (V_R) of beech and spruce for the pre-dawn water potential (Ψ_{PD}), turgor loss point (Ψ_{TLP}), osmotic potential (π_O) and daily sap flow density (u_{daily}). Asterisks show significant differences between beech and spruce for V_R. Data are given as the mean ± 1SD.

	Beech		**Spruce**		
	RT [days]	**V_R [days^{-1}]**	**RT [days]**	**V_R [days^{-1}]**	
Ψ_{PD}	1	0.4470 ± 0.0737	c. 7-15	0.0347 ± 0.0056	***
Ψ_{TLP}	c. 2-56	0.0038 ± 0.0009	c. 22-56	0.0035 ± 0.0004	ns
π_O	c. 56	0.0057 ± 0.0006	c. 56	0.0055 ± 0.0005	ns
u_{daily}	c. 300	0.0010 ± 0.0004	>365	0.0016 ± 0.0005	ns

Supplement

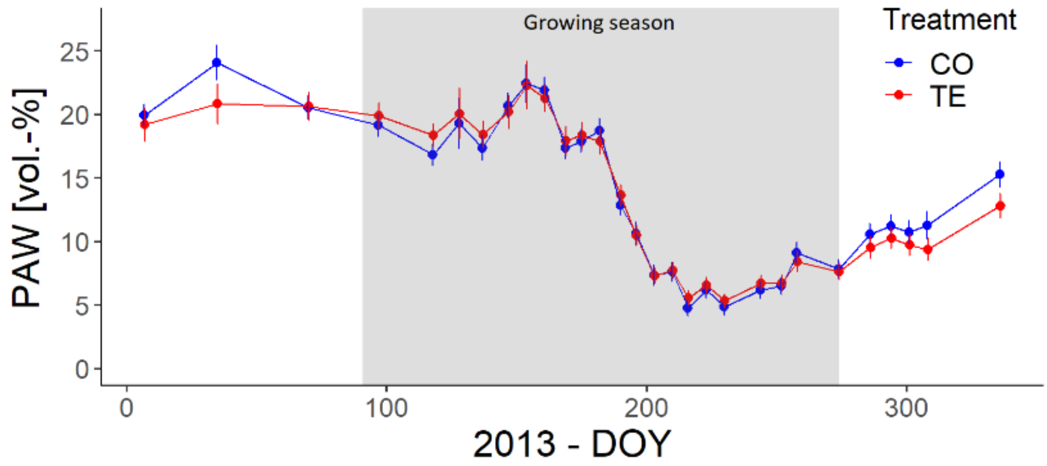

Figure S 1: Plant available water (PAW in vol.-%) per volume soil averaged over the first meter soil depth (with blue = control and red = throughfall-exclusion) in 2013. Symbols show the mean ± 1SE and the gray area indicates the growing season.

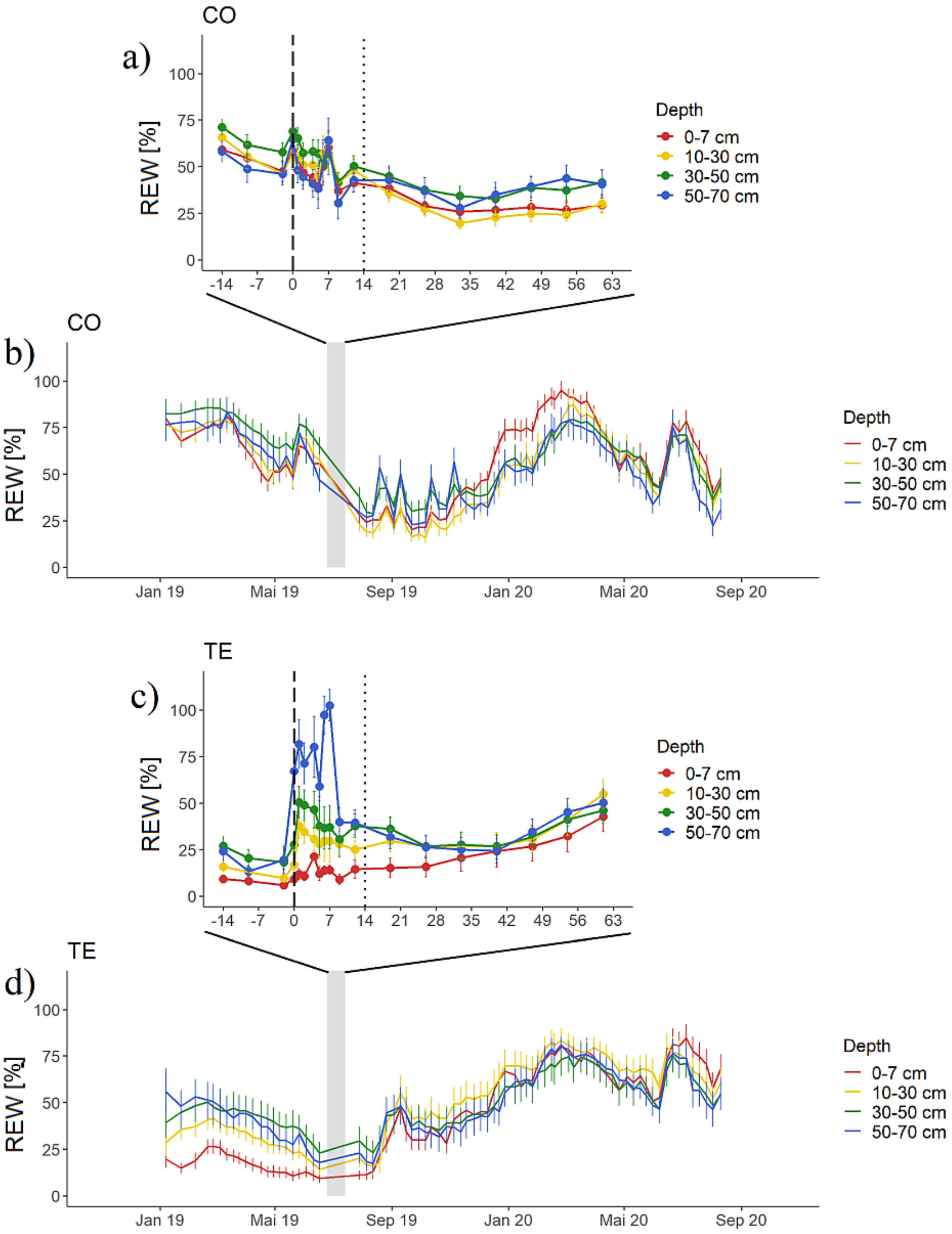

Figure S 2: Relatively extracable water (REW in %) expressed as percentage of the field capacity (θ_{fc}) for CO (a & b) and TE (c & d) plots of four different depths (0-7 cm = red, 10-30 cm = yellow, 30-50 cm = green and 50-70 cm = blue) in 2019 and 2020. Both small plots (b & d) show REW with respect to the watering day (dashed lines) and permanent opening of the roofs on TE plots on D14 (dotted lines). Data are given as mean ± 1SE.