










# 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 ( $144 \pm 26 \text{ m}^2$  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  $-1.8 \text{ MPa}$  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<sup>3</sup> water/dm<sup>3</sup> 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<sup>2</sup>) 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 [ $\lambda$ -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 (<sup>2</sup>H<sub>2</sub>O) 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).

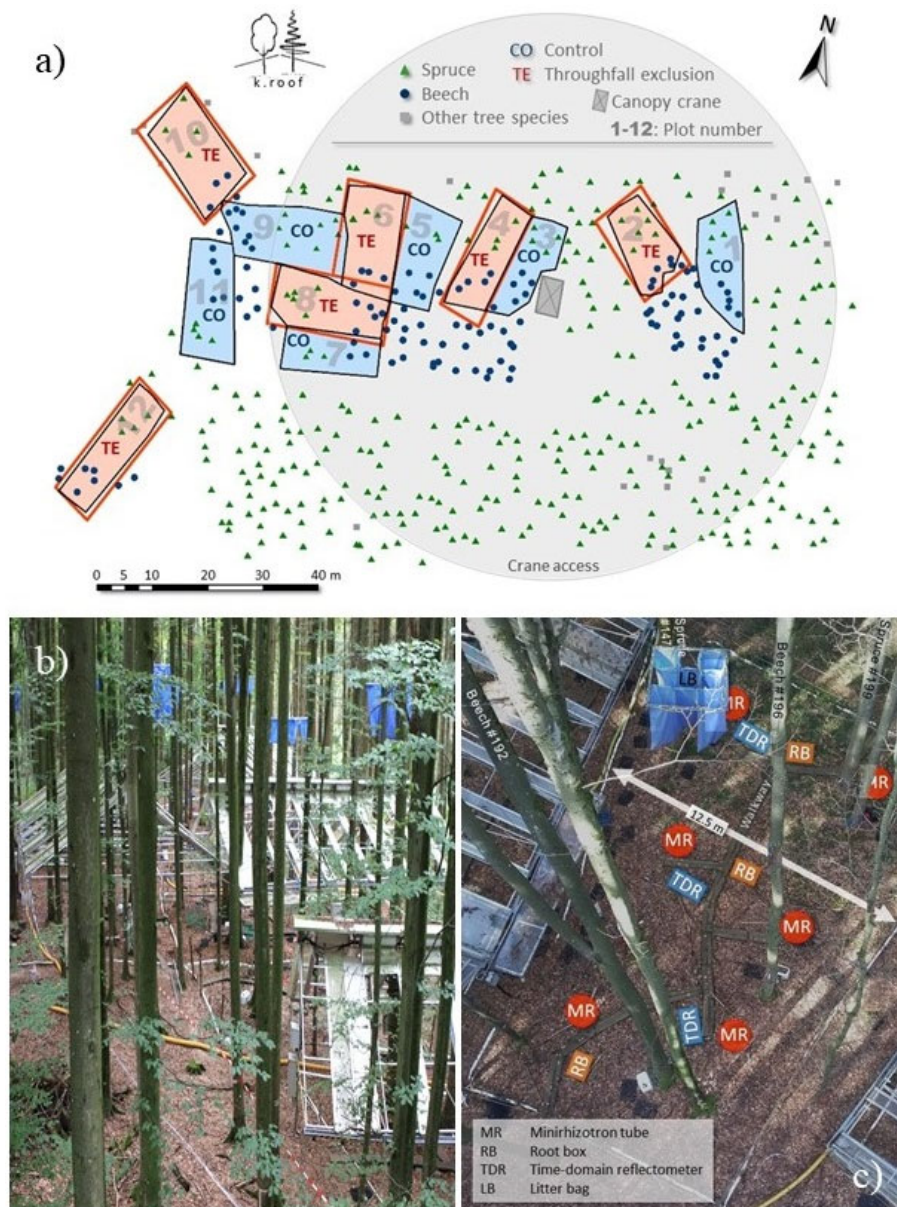


Fig. 1. Map of Kranzberg (top, a), view of the roofs from the crane (bottom left, b) and overview of installed structures per plot (bottom right, c).

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 ( $120 \times 80 \times 14$  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 ( $30 \times 40$  cm). The roofs were fabricated from light penetrating acrylic sheets secured by an aluminum skeleton. Each roof extended  $\sim 40$  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  $\pm$  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).

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

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  $69 \pm 7\%$  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<sup>2</sup>), 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  $12.1^\circ \pm 7.8^\circ\text{C}$ ), VPD (mean  $3.7 \pm 3.8$  hPa), global radiation (mean of  $151.5 \pm 96.8$  W/m<sup>2</sup>), and monthly sum of rainfall ( $61.1 \pm 32.0$  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 mid-day ( $WP_M$  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).

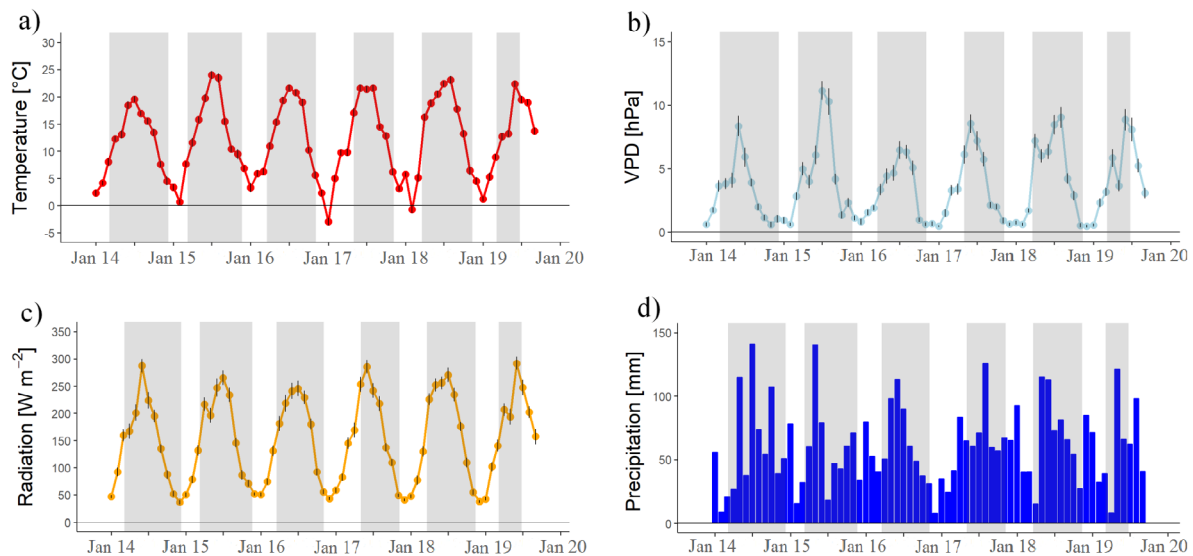


Fig. 2. Meteorological data (mean  $\pm$  1 SD) from 2014 to 2019 (Kroof I and II): monthly means of temperature (a), vapor pressure deficit (b) and global radiation (c) and monthly sum of precipitation (d), with gray areas showing the time of roofs activated.

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  $7.4 \pm 3.6$  vol.-% in the top layer (0–7 cm depth),  $13.5 \pm 2.0$  vol.-% in 10 to 30 cm,  $19.1 \pm 5.7$  vol.-% in 30 to 50 cm, and up to  $25.8 \pm 2.4$  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

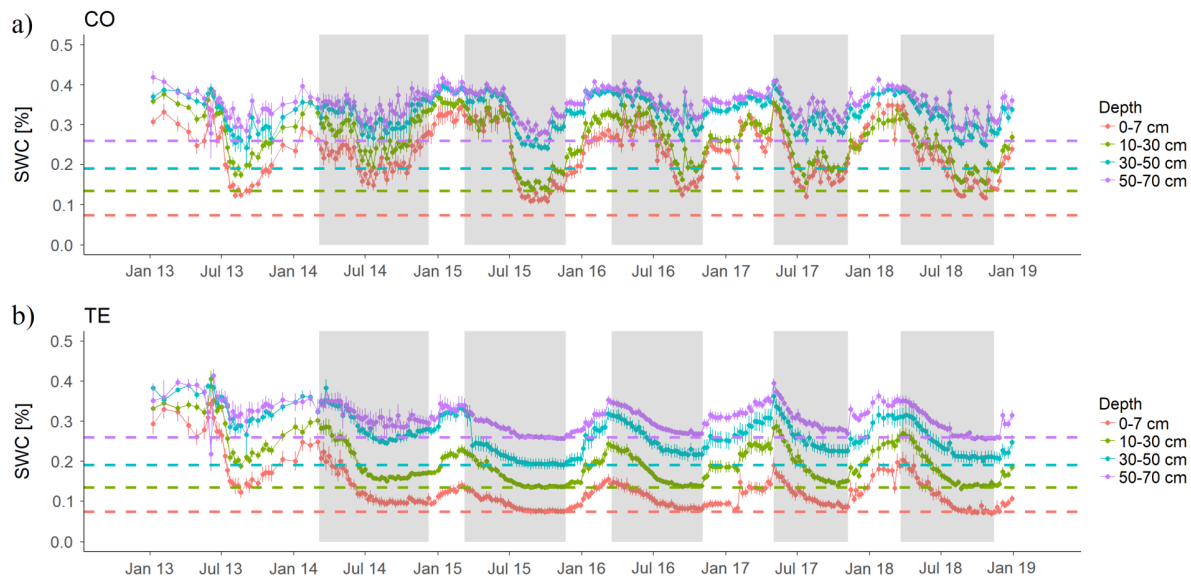


Fig. 3. Volumetric soil water content (SWC, mean  $\pm$  1 SD) from 2014 to 2018 on CO (a) and TE plots (b) in four different depths (red 0–7 cm, green 10–30 cm, blue 30–50 cm, and purple 50–70 cm) with gray areas showing the time of roofs activated and horizontal dashed lines (same color code for depths) 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  $O_f + A_{ii}$  horizons) and lower section ( $A_{1B_v}$ ). 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, fungal 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  $84 \pm 139$  s and TE  $3368 \pm 3390$  s) and spruce (CO  $1315 \pm 1546$  s and TE  $3267 \pm 3222$  s), while the drought impact on the wettability was less strong at 10 cm depth (beech CO  $168 \pm 328$  s and beech TE  $714 \pm 1030$  s; spruce CO  $716 \pm 656$  s and spruce TE  $409 \pm 728$  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  $1111 \pm 97$  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<sup>2</sup> of water (approximately  $2034.5 \pm 537.3$  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 ( $WD_{\text{soil}}$  in L/m<sup>2</sup>).

$$WD_{\text{soil}} = (SWC_{\text{CO}} - SWC_{\text{TE}}) \times \text{soil volume} \quad (1)$$

$WD_{\text{soil}}$  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 ( $WD_{\text{toplayer}}$  of  $14.40 \pm 3.62$  L/m<sup>2</sup>). 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 ( $WD_{\text{plot}}$  in L).

$$WD_{\text{plot}} = WD_{\text{soil}} \times \text{area} + WD_{\text{toplayer}} \times \text{area} + (15 \text{ L/m}^2 \times \text{area}) \quad (2)$$

On average, we added 12,849  $\pm$  2801 L (this amount corresponds to a precipitation event of  $\sim 90$  L/m<sup>2</sup>) 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  $0.25 \pm 0.07$  mm/h and water drop penetration time of  $3317 \pm 2864$  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.

Treatment	Plot	Species	Shallow soil		Deep soil		<i>n</i>
			WDPT ± SE (s)	Repellency category	WDPT ± SE (s) - deep	Repellency category	
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
	4		Beech	21 ± 3	Slightly	1 ± 0	Non-repellent
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  $WD_{plot}$ ) and the actual amount of water added to each plot (actual added water).

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

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,  $WP_{PD}$ , 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 data-points (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  $\pm$  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  $11.9^\circ \pm 2.8^\circ\text{C}$  in September 2014 and  $10.7^\circ \pm 3.0^\circ\text{C}$  in October 2014 (Table 4). Soil temperature was slightly, however not significantly, higher in the CO plots (average of  $14.1^\circ$ – $11.5^\circ\text{C}$  from August to October 2014) than in the TE plots (average of  $13.5^\circ$ – $11.1^\circ\text{C}$  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 ( $\pm 1$  SD) of soil and air temperature (in  $^{\circ}\text{C}$ ) in 2014.

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

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

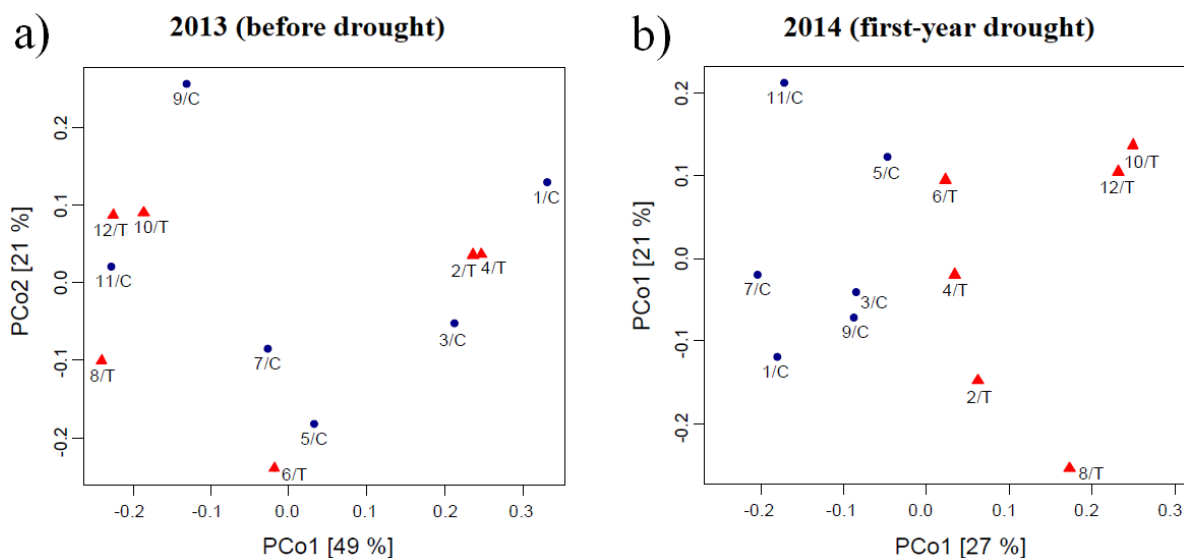


Fig. 4. Principal coordinate analyses showing the ectomycorrhizal (ECM) community before (a, autumn 2013) and at the end of the first throughfall exclusion period (b, autumn 2014). Red triangles represent plots assigned for throughfall exclusion and blue circles represent plots assigned for untreated 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 except 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  $23 \pm 3$  vol.-%,  $-0.37 \pm 0.09$  MPa and  $23 \pm 3$  vol.-%,  $-0.64 \pm 0.17$  MPa, beech and spruce, respectively), except for the natural drought year of 2015 ( $21 \pm 3$  vol.-%,  $-0.86 \pm 0.20$  MPa and  $21 \pm 3$  vol.-%,  $-1.08 \pm 0.16$  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  $18 \pm 3$  vol.-%,  $-0.66 \pm 0.18$  MPa and  $18 \pm 2$  vol.-%,  $-0.98 \pm 0.31$  MPa for beech and spruce, respectively, even in the drought year 2015 ( $17 \pm 2$  vol.-%,  $-1.30 \pm 0.24$  MPa and  $16 \pm 3$  vol.-%,  $-1.63 \pm 0.14$  MPa, respectively).

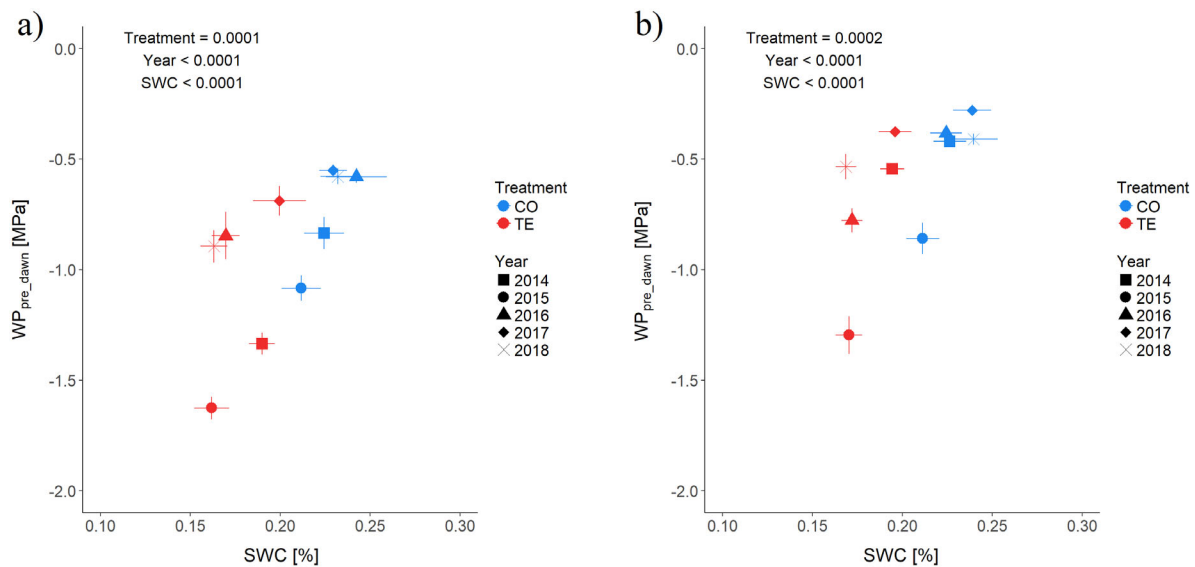


Fig. 5.  $WP_{PD}$  of summer of Phase I vs. SWC of spruce (a) and beech (b, mean  $\pm$  1 SD, with treatment CO, blue; TE, red; year 2014, rectangles; 2015, circles; 2016, triangles; 2017, diamonds; 2018, saltires). For both species the treatment, year and the SWC had a significant influence on the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

*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  $\mu\text{m}$  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  $\mu\text{m}$  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:  $12.79 \pm 8.85 \times 100/\text{m}^2$  window) and spruce (mean for both treatments:

$4.59 \pm 4.19 \times 100/\text{m}^2$  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 ( $29.71 \pm 26.21 \times 100/\text{m}^2$  window) compared with CO ( $41.92 \pm 33.86 \times 100/\text{m}^2$  window), and in 2015, the number of root tips was reduced by 85% in TE ( $2.19 \pm 2.50 \times 100/\text{m}^2$  window) vs. CO ( $14.98 \pm 7.86 \times 100/\text{m}^2$  window); however, TE was not significantly different from CO ( $p$  value for treatment: 0.1774). Spruce (Fig. 6c) reduced the number of root tips by 56% in the first year after drought (2014;  $8.00 \pm 4.98 \times 100/\text{m}^2$  window) compared with CO ( $18.27 \pm 12.12 \times 100/\text{m}^2$  window), and in 2015, the root production in drought plots ( $1.09 \pm 1.18 \times 100/\text{m}^2$  window) was reduced by 90% compared with CO ( $11.36 \pm 8.08 \times 100/\text{m}^2$  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).

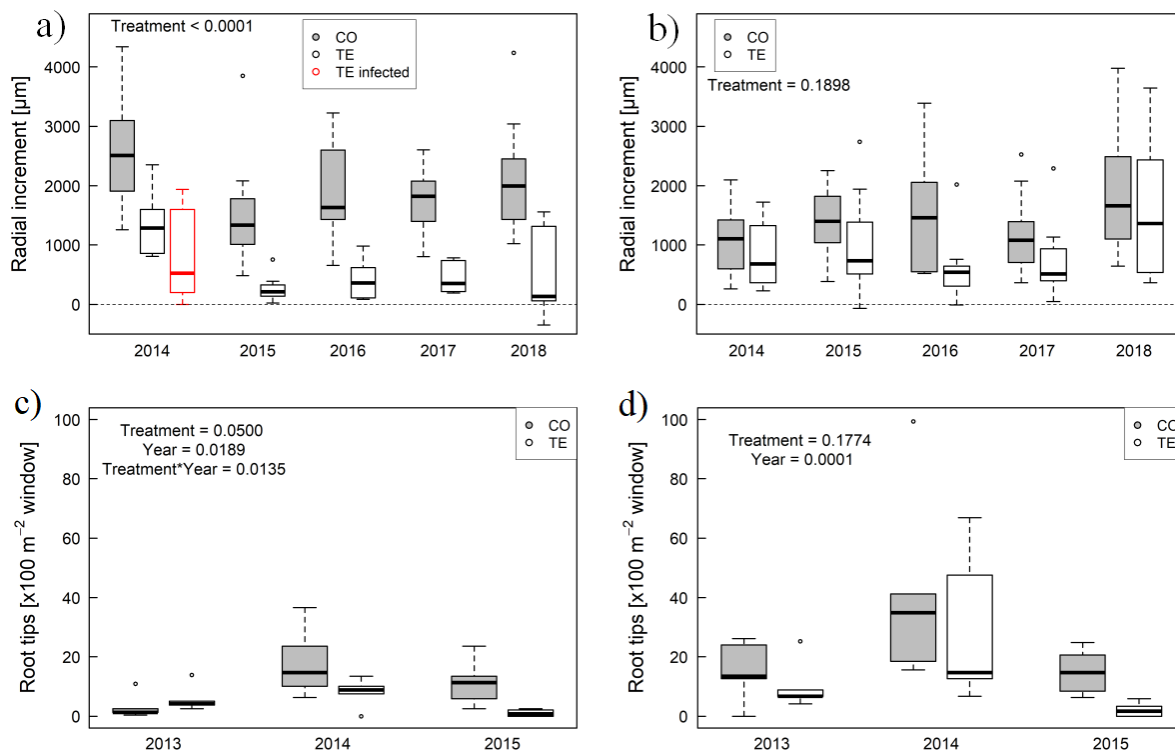


Fig. 6. Radial growth increment of spruce (a, treatment showed a significant influence) and beech (b, no influence of the treatment) and number of root tips for spruce (c, treatment and year showed a significant influence) and beech (d, only the year showed a significant influence, with CO, gray; TE [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  $24.5 \pm 2.5$  vol.-%). Correspondingly,  $WP_{PD}$  of CO trees was similar over all timepoints (Fig. 7a,b), with a  $WP_{PD}$  of  $-0.48 \pm 0.11$  and  $-0.63 \pm 0.08$  MPa for beech and spruce, respectively. Before watering and irrespective of tree species, TE ( $18.54 \pm 2.28$  vol.-%) plots had significantly lower soil moisture than CO ( $26.57 \pm 1.82$  vol.-%). Pre-dawn leaf water potential indicated mild leaf drought stress  $-0.79 \pm 0.07$  and  $-0.93 \pm 0.07$  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

( $0.61 \pm 0.07$  cells/d) was 2.4 times higher than in spruce ( $0.25 \pm 0.04$  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 ( $0.39 \pm 0.06$  cells/d) and 69% lower for spruce ( $0.08 \pm 0.01$  cells/DOY) compared with CO trees. The rate slightly increased upon watering in TE beech (maximum of  $0.47 \pm 0.34$  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

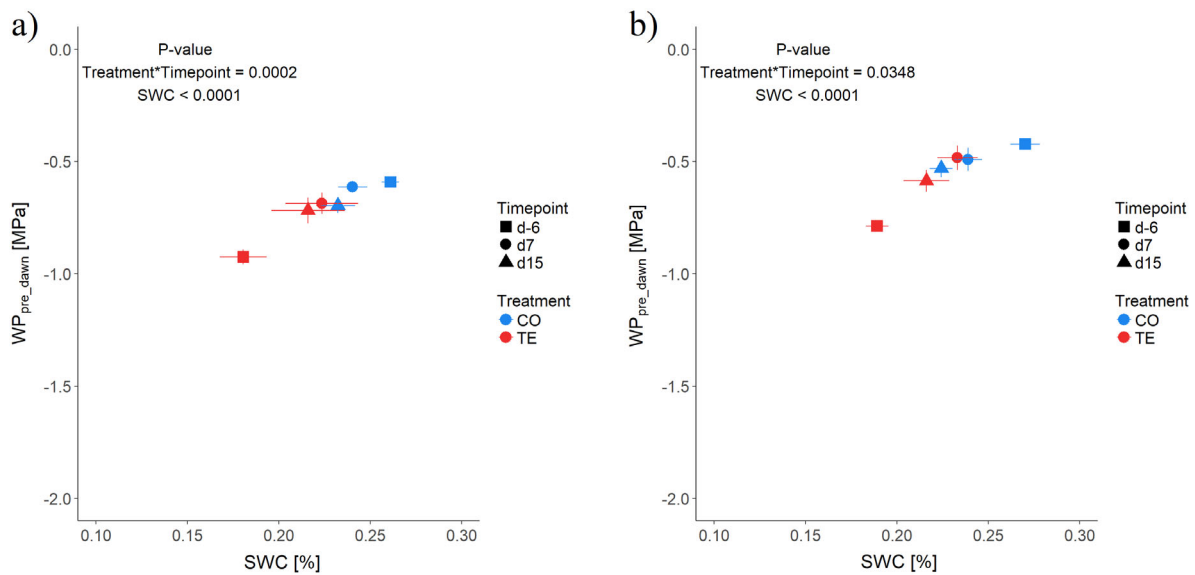


Fig. 7. Pre-dawn water potential vs SWC of spruce (a) and beech (b) in 2019 during the watering phase (mean  $\pm$  1 SD, for both species, the interaction of treatment and timepoint was significant as well as the SWC, with treatment CO, blue; TE, red; day after watering –6, circles; 7, triangles; 15,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

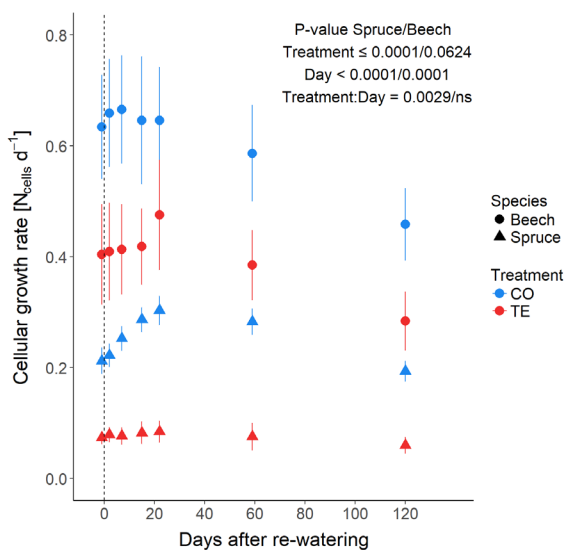


Fig. 8. Cellular growth rate (mean  $\pm$  1 SD) of the growing season in 2019 during the watering phase for spruce (triangles) and beech (circles, with treatment CO, blue; TE, red). For spruce, a significant influence of the treatment and day, as well as their interaction was found, while for beech, 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 throughfall 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 micro-meteorological 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<sup>2</sup>. 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 (Vandenkoornhuysen 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  $0.25 \pm 0.07$  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<sup>2</sup> 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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