Effects of drought stress on photosynthesis, rhizosphere respiration, and fine-root characteristics of beech saplings: A rhizotron field study

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Abstract

Soil drought influences the C turnover as well as the fine-root system of tree saplings. Particularly during the period of establishment, the susceptibility to drought stress of saplings is increased because of incompletely developed root systems and reduced access to soil water. Here, we subjected beech saplings (*Fagus sylvatica* L.) to different levels of drought stress.

Beech saplings were planted in rhizotrons, which were installed in the soil of a Norway spruce forest before bud burst. Soil moisture was manipulated in the following year during May to September. We measured photosynthetic net CO_2 uptake, volume production of fine roots, and rhizosphere respiration during the growing season. Biometric parameters of the fine-root system, biomass, and nonstructural carbohydrates were analyzed upon harvest in October.

Photosynthesis and rhizosphere respiration decreased with increasing drought-stress dose (cumulated soil water potential), and cumulative rhizosphere respiration was significantly negatively correlated with drought-stress dose. Fine-root length and volume production were highest at moderate soil drought, but decreased at severe soil drought. The proportion of fine-roots diameter < 0.2 mm and the root-to-shoot ratio increased whereas the live-to-dead ratio of fine roots decreased with increasing drought-stress dose.

We conclude that the belowground C allocation as well as the relative water-uptake efficiency of beech saplings is increased under drought.

Key words: drought stress / European beech / fine roots / rhizosphere respiration / rhizotrons

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1 Introduction

The response of tree saplings to extended drought periods is of relevance for future forest management as the intensity and frequency of summer droughts is expected to increase during the next decades (*IPCC*, 2007). Drought stress affects the C budget and growth of trees, interactions with other environmental factors such as light intensity, air humidity, and temperature may influence the drought effect on the C budget (*Irvine* et al., 2005; *Meir* et al., 2008; *Ruehr* et al., 2009; *van der Molen* et al., 2011).

The effects of soil drought on photosynthesis have been intensively studied. Due to stomatal closure and reduced CO_2 assimilation, drought reduces the amount of available C within the plant (*Gollan* et al., 1986). Also the allocation of assimilated C to different plant organs is affected, *e.g.,* being retarded under drought (*Ruehr* et al., 2009). Plant belowground responses to drought have been studied less explicitly, given the complexity of the root–soil system. A thorough assessment of drought impacts on the plant–soil system requires a holistic view on the involved response mechanisms (*Leuschner* et al., 2001; *Gaul* et al., 2008).

Many plants have the ability to acclimate function and morphology of their root system to water deficiency in the soil (*e.g., Joslin* et al., 2000; *Ostonen* et al., 2007; *Metcalfe* et al., 2008). Promoted fine-root production under drought may foster water uptake by increasing root surface area and by exploitation of moister soil regions (*Santantonio* and *Herrmann*, 1985; *Gaul* et al., 2008). Drought-induced formation of thin and widely forked fine roots (diameter < 2 mm) therefore reflects an improved water-uptake efficiency. However, limited availability of carbohydrates and nutrients or insufficient penetrability of dry soil can restrict root production (*Joslin* et al., 2000; *Metcalfe* et al., 2008, *Bengough* et al., 2006). Moreover, enhanced fine-root mortality is a common phenomenon under severe drought (*Janssens* et al., 2002; *Meier* and *Leuschner*, 2008a, b). Crucial is the balance between fine-root production and mortality, being susceptible to drought in either way, so that findings may become contradictory regarding fine-root turnover under water limitation (*Meier* and *Leuschner*, 2008a; *Joslin* et al., 2000).

Maintenance and growth of roots represent an important C sink of trees and result in respiratory losses in the form of CO_2 (*Eissenstat* and *Rees*, 1994; *Hanson* et al., 2000; *Jansens* et al., 2002). The measurement of root respiration is difficult due to the fact that most fine roots are associated with mycorrhizal fungi and that roots release exudates, mucilage,

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and other organic compounds into the rhizosphere. As a consequence of this methodological difficulty, respiration by roots and heterotrophic organisms which directly depend on the C supply by live roots is often summarized as rhizosphere respiration (*e.g., Kuzyakov*, 2002; *Borken* et al., 2006). Because of the dependency on root C transfer, rhizosphere respiration is prone to drought stress (*Irvine* et al., 2005; *Högberg* and *Read*, 2006; *Borken* et al., 2006).

An important component of the C budget are nonstructural carbohydrates (NSC). Drought can lead to an accumulation of NSC when impaired nutrient uptake limits the formation of plant tissues (*Körner*, 2003). Thus, the amount of NSC may reflect the drought status of trees, although such compounds are perpetually consumed by enhanced fine-root production (*Gaul* et al., 2008), respiratory metabolism and osmotic adjustment, eventually leading to a decline in NSC concentrations in later stages of drought (*McDowell*, 2011).

The term "drought stress" is not well defined in the literature. Volumetric soil water content, water-filled pore space or a qualitative comparison of different measures of soil drought may provide orientation. Such definitions, however, do not characterize plant-available water and restrict the comparability between studies. Here, we suggest the cumulated soil water potential as a conferrable and tree-relevant measure of drought stress, accounting for the time dimension of stress and, hence, a dose-related responsiveness.

European beech (*Fagus sylvatica* [L.]) is a dominant tree species in Central Europe and will play a crucial role in future silviculture, even though it is known to be drought-sensitive, especially during early stages of establishment (*Bolte* and *Roloff*, 1993; *Bréda* et al., 2006). As a species with a pronounced phenotypic plasticity (*Meier* and *Leuschner*, 2008b), its response to soil drought has been extensively examined (*Maniero* and *Kazda*, 2006; *Nahm* et al., 2007; *Fotelli* et al., 2009; *Schall* et al., 2012). However, the plasticity of planted beech saplings in terms of the C budget together with morphologic traits is barely known under drought conditions.

We conducted a rhizotron experiment with integrated analyses of photosynthesis, shoot respiration, fine-root production, and rhizosphere respiration of beech saplings at differing soil water availability. Additionally, we assessed biometric parameters and NSC contents of fine roots. We hypothesized that drought stress decreases rhizosphere respiration, increases fine-root production and leads to an accumulation of NSC. Furthermore, we hypothesized that beech saplings adjust fine-root morphology to drought towards enhanced effectiveness of water soil exploitation.

2 Materials and methods

2.1 Experimental setup

The experiment was conducted on a cleared area (10 m^2) within a thinned out mature Norway spruce stand (140 trees ha^{-1}) in the Fichtelgebirge, NE Bavaria, Germany (50°8′ N, 11°52′ E, 775 m asl). Rhizotrons (size: 30 cm × 45 cm × 6 cm^3 total root-observation area = 0.27 m^2 per rhizotron) were constructed to observe the growth of fine roots and to measure CO_2 fluxes from the soil compartment and the shoot of beech saplings (Fig. 1). Side walls of the rhizotrons were made of transparent polyvinylchloride (PVC). In spring 2009, the rhizotrons were filled with homogenized and sieved (2 mm) soil from the Bw horizon of the study site (Haplic Podsol, sandy loam, pH [H_2O] = 4.6, effective cation-exchange capacity: 48 $mmol_c\,kg^{-1}$, base saturation: 12%, C content: 1.27% (*Hentschel* et al., 2007). Bulk density was adjusted to 1.1 $g\,cm^{-3}$ by compaction, yielding a soil volume of 7.2 L in each rhizotron. One 2-y-old beech sapling (*Fagus sylvatica* L.) was planted into each rhizotron (n = 24). The bare-rooted saplings of NE-Bavarian provenance were obtained from a local nursery. We installed nine additional control rhizotrons without beech saplings for assessment of the CO_2 flux from decomposition of soil organic matter (SOM). Each rhizotron was equipped with a FDR soil-moisture sensor (ECH_2O 20, Decagon Devices, USA) that was vertically installed to integrate volumetric water contents (VWC) from 10 to 30 cm soil depth. The soil surface was covered with a sandy quartz layer

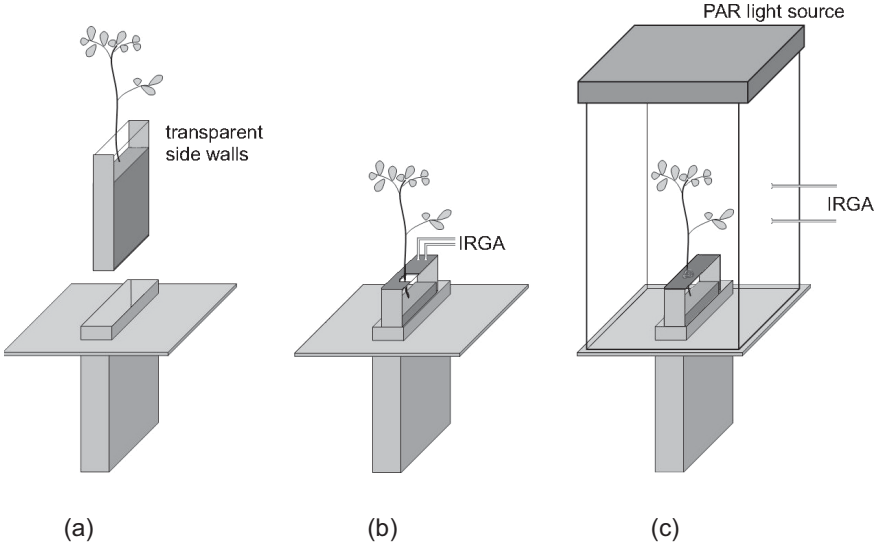

Figure 1: Schematic illustration of the rhizotron setup: (a) general design of rhizotron and slot, (b) sealed rhizotron during measurement of rhizosphere respiration, (c) chamber setup for measurement of photosynthesis and aboveground respiration, chamber wall is nontransparent.

of 4 cm thickness to minimize water losses by evaporation. The rhiztotrons were placed into slots which were embedded in the forest soil to maintain a natural temperature gradient. Potential small-scaled variations in light and temperature were compensated by random rearrangement of the rhizotrons every 2 weeks. Throughout the rest of the year 2009, the rhizotron soil was held at a soil water potential > –0.02 MPa by adding natural rain water. Drainage of the soil was enabled by small holes in the bottom of the rhizotrons. Rhizotrons were covered with wood chips to prevent freezing of the soil during the winter.

In 2010, six weeks after budburst (end of June), a translucent roof (height 1.5 m) was built over the rhizotrons to exclude natural throughfall and to manipulate soil water contents. VWC was logged hourly during the period of throughfall exclusion.

2.2 Adjustment of soil water potential and quantification of drought stress

Three treatments of soil water availability (n = 8) were established: (1) no water limitation, (2) moderate, and (3) severe water limitation corresponding to mean target soil water potentials of –0.03 MPa, –0.4 MPa, and –1.0 MPa, respectively. As shown by preliminary experiments with the same beech provenance, –0.4 MPa represents a level of beginning drought symptoms whereas –1.0 MPa already caused irreversible drought damages at beech saplings. For technical reasons, we chose –0.03 MPa for treatment A rather than field capacity. The rhizotrons were assigned randomly to the treatments. Before the start of individual soil-water manipulation, there were no significant differences in shoot diameter and height, abundance of visible roots on the rhizotron side walls, photosynthesis rate, and soil respiration between the treatments.

Every 1–2 days, soil water potential was measured in the rhizotron soil at dawn. A tensiometer was used for soil water potentials > –0.3 MPa (T5 tensiometer, UMS, Germany); measurements were carried out *in situ* at 20 cm soil depth. Soil water potentials < –0.3 MPa were assessed using a dewpoint potentiometer (WP4, Decagon Devices, USA). For this purpose, soil samples were taken from 20 cm soil depth of each rhizotron and measured in a climate chamber at 20°C.

Spline fittings of nonlinear relationships between soil water potentials and corresponding signals of the FDR sensors (mV) were used to estimate hourly soil water potentials of each rhizotron. The drought-stress dose for individual beech saplings was defined as the cumulated soil water potential during the growing season:

$$DSD = -\int \Psi(t)\, dt,$$

where DSD is the drought-stress dose (MPa d) and $\Psi(t)$ is the individual time course of soil water potential from budburst to harvest (MPa).

When target soil water potentials were reached after throughfall exclusion, further water losses were compensated by adding deionized water to each rhizotron. The irrigation water was gradually injected through the quartz layer into the soil with a syringe in order to assure a homogeneous distribution of soil moisture. Depending on the transpiration of the beech saplings, the irrigation was conducted by one to 3-day intervals at around sunset. The same target soil water potentials were achieved in the rhizotrons without beech saplings by ventilating the soil *via* a tube connected to the deep soil horizons.

2.3 Stomatal conductance

As an indicator of drought stress, stomatal conductance of single leaves (n = 2 per plant) was measured 14, 26, and 64 d after the beginning of the drought treatment at ambient temperature around noon (LiCOR 6400, Licor, USA). The photon-flux density was adjusted to 280 μmol m^{-2} s^{-1}.

2.4 CO_2-flux measurements

Soil CO_2 efflux was measured on 12 dates from May to October 2010 using the dynamic closed-chamber technique. The soil compartment of the rhizotrons was sealed by a lid and an elastic sealant (Terostat, Henkel, Germany) fitted around the beech stem (Fig. 1b). CO_2 concentration in the rhizotron headspace (volume 0.95 L) was measured every 10 s over 4 min with an infrared gas analyzer (LiCOR 820, Licor, USA). Soil CO_2 efflux was calculated from the slope of the linear regression between CO_2 concentration and incubation time. Rhizosphere respiration arose from the difference in soil CO_2 efflux between planted rhizotrons and rhizotrons without saplings (control).

Net CO_2-uptake rate by photosynthesis was measured with a chamber (volume = 35 L) immediately after the measurement of soil CO_2 efflux. To overcome different light intensities within a day and during the season, we used a light source that was placed on the chamber top plate (area 900 cm^2) and provided photosynthetically active radiation with a constant photon-flux density of 250 μmol m^{-2} s^{-1} (Fig 1 c). The chamber side walls were covered with aluminum foil to exclude daylight and to prevent the chamber air from heating up by radiation. Net CO_2 uptake was recorded after an equilibration period for light acclimation of > 3 min until a linear decrease of CO_2 concentration was observed. During the measurement time of 4 min, the temperature increase of the chamber air was < 1.5°C. Three ventilators inside the chamber ensured sufficient mixing of air during the CO_2 measurement.

Shoot respiration (dark respiration) was assessed on two dates (July 10, August 1) using an opaque chamber in the absence of light. Measurement of CO_2 concentration and flux calculation were carried out in analogy to that of soil respiration.

2.5 Root observation

From mid-May until the end of September 2010, both transparent side walls of each rhizotron were photographed on eight dates. Visible roots were analyzed by means of fine-root length and diameter using a specific software (Winrhizo Tron, Regent Inc., Canada). Neither dead nor mycorrhizal roots were identified. We calculated the fine-root volume production between two sessions (session i and session i-1) with

$f = v_{\text{session i}} - v_{\text{session i-1}}$,

where f is the fine-root volume production and v is the total fine-root volume determined at the respective session.

2.6 Root and shoot properties after harvest

In October 2010, the complete root system of the beech saplings was extracted by washing with tap water. Fine roots (diameter < 2 mm) were separated from coarse roots. Live and dead fine roots were distinguished by means of root color and root tip turgescence. Morphological properties of all live fine roots were determined by scanning (400 dpi resolution) and a digital-image-evaluation software (Winrhizo, Regent Inc., Canada). Specific root length (m g^{-1}), relative fine-root length distribution by fine-root diameter (relative diameter-class length, *Zobel* et al., 2007) and specific root tip density (g^{-1}) were calculated based on results of the evaluation software and dry mass of fine roots. The latter was determined by freeze-drying immediately after morphological analyses. The fresh leaves were scanned (600 dpi) immediately after harvest to determine the total leaf area (SigmaScan 5, Systat Software Inc., USA). The leaves and all other plant material was oven-dried at 40°C until constant weight. Root-to-shoot biomass ratio was calculated from the dry mass of all roots and the complete shoot including the foliage. All parameters expressed per unit plant biomass are also based on dry mass.

NSC analysis of freeze-dried fine roots was conducted according to *Fleischmann* et al. (2009). Water-soluble sugars were separated by hot-water extraction at 85°C. Starch was extracted after enzymatic digestion of the remaining pellet with amylase and amyloglucosidase. Analyses were performed with high performance liquid chromatography using a CARBOsep CHO-820 calcium column (Transgenomic, UK).

2.7 Statistical analysis

Differences between the treatments were analyzed using Tukey's HSD test after analysis of variance ($n = 8$); normality was assumed when data passed the Shapiro-Wilk-test ($p > 0.1$). In case of nonnormally distributed data, a Kruskal-Wallis-test was followed by the nonparametric Wilcoxon multiple comparisons test. Additionally, the influence of the individual drought-stress dose on plant parameters irrespective of the treatment collective was assessed by linear regression and characterized by the coefficient of determination (r^2) and the p value of the slope, as well as by Spearman's correlation coefficient. All statistical analyses were performed using R 2.13.0 (R Development Core Team, 2011).

3 Results

3.1 Time course of soil water potential and stomatal conductance

After the beginning of soil-water manipulation in June 2010, soil water potential decreased in all rhizotrons (Fig. 2). Mean target soil water potential for treatment A (–0.03 MPa) was reached after 2 weeks. Thereafter, individual irrigation started. The transient increase in soil water potential at the beginning of August affected all treatments and was due to extreme precipitation from the end of July to the beginning of August. Despite the roof, the rhizotrons were significantly rewetted by lateral rain input, fog, and dew deposition. Air temperature and radiation were considerably low so that transpiration did not counterbalance this unintended water input during this period. The drought level from end of July was therefore not re-attained until the end of August. Among the rhizotrons of treatment C, however, minimum individual water potentials of < –1.5 MPa were achieved during a warm period in September.

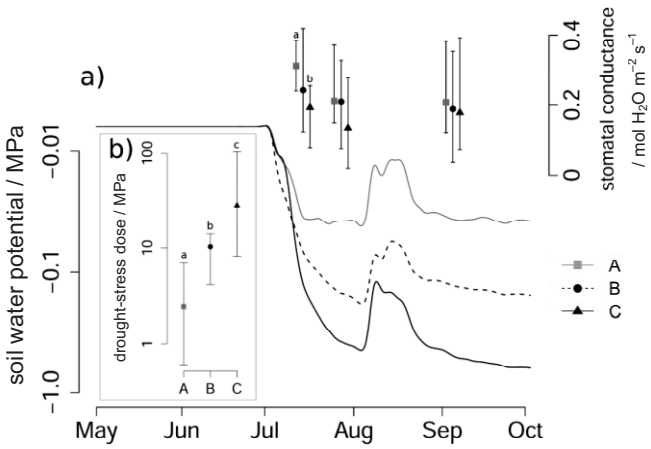

Figure 2: (a) Time course of mean soil water potential during the growing season of 2010 and stomatal conductance for the three treatments with A = no water limitation, B = moderate drought stress, and C = severe drought stress. (b) Mean drought stress doses (cumulated water potential) for the three treatments calculated for the growing season 2010 from budburst to destructive harvest. Whiskers represent minimum and maximum values; different letters indicate significant differences between the treatments at $p < 0.05$.

In July, mean stomatal conductance was consistently enhanced at high soil water availability (Fig. 2). Stomatal conductance in treatment C was significantly smaller at the first measurement date and exhibited a minimum of < 0.04 mol H_2O m^{-2} s^{-1}. Reduction of stomatal conductance also occurred in treatment A between the first and second measurement date, but rates were > 0.15 mol H_2O m^{-2} s^{-1}. No significant differences among the treatments were detected in the end of August.

3.2 Net photosynthesis, aboveground respiration, and rhizosphere respiration

In early summer, mean net photosynthesis rate increased along foliage development in all treatments (Fig. 3). A 28% reduction of mean net photosynthesis in treatment C compared to treatment A was observed at the end of July when mean soil water potential in this treatment was close to a local minimum. After wetting in August, photosynthesis recovered in the absence of treatment differences. Small net photosynthesis rates occurred in all treatments as result of leaf senescence in September. Shoot respiration

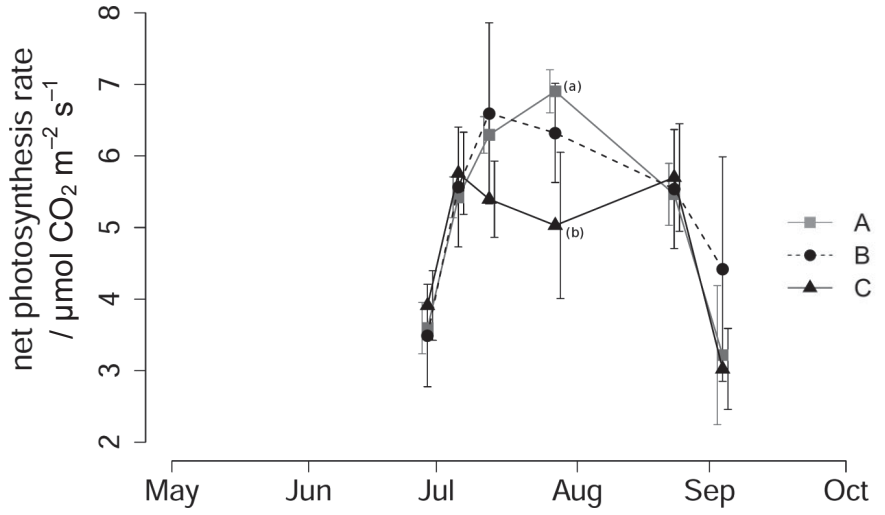

Figure 3: Net photosynthesis rate during the growing season of 2010 for the three treatments (mean ± SE, n = 8). Different letters in parentheses indicate differences between the treatments at $p < 0.1$.

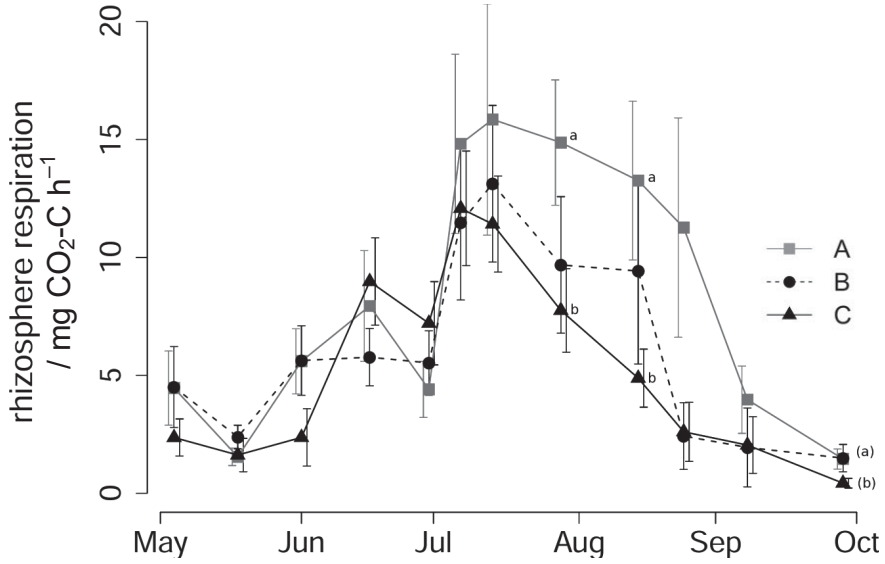

Figure 4: Time course of rhizosphere respiration per rhizotron during the growing season of 2010 for the three treatments (mean ± SE, n = 8). Different letters indicate significant differences between the treatments at $p < 0.05$, letters in parentheses refer to a significance level of $p < 0.1$.

was not different among the treatments and accounted on average for 11 ± 3.4% of the net photosynthesis rate (averaged over both measurement dates and all treatments, not shown).

Rhizosphere respiration (net soil CO_2 efflux per rhizotron) followed a typical seasonal pattern and peaked during the first 2 weeks of July in all treatment (Fig. 4). Thereafter, rhizosphere respiration decreased in all treatments, but it was always smaller in the drought treatments. Cumulative rhizosphere respiration (calculated from budburst to harvest) relative to individual root biomass at the end of the growing season negatively correlated with the individual drought-stress dose (p = 0.016, r^2 = 0.26, Fig. 5). Mean CO_2 efflux from control rhizotrons ranged between 0.8 and 5 mg CO_2-C h^{-1} throughout the season. Maximum difference between the treatments was achieved mid of July with 4.9 ± 1.6 and 2.9 ± 1.3 mg CO_2-C h^{-1} for treatment A and C, respectively (difference not significant, data not shown).

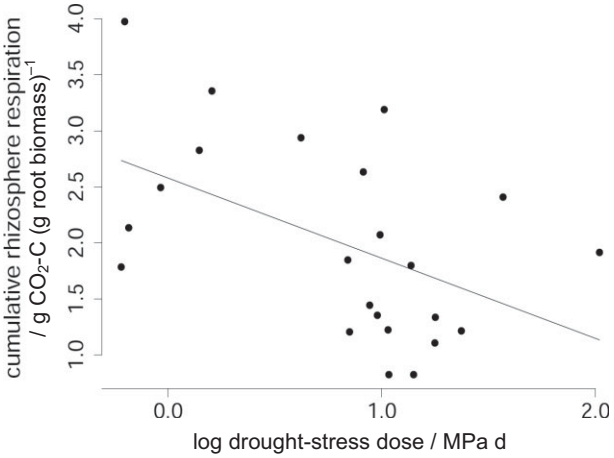

Figure 5: Cumulative rhizosphere respiration relative to individual root biomass correlated to the individual drought-stress dose (cumulated water potential) (p = 0.016, r^2 = 0.26, n = 22).

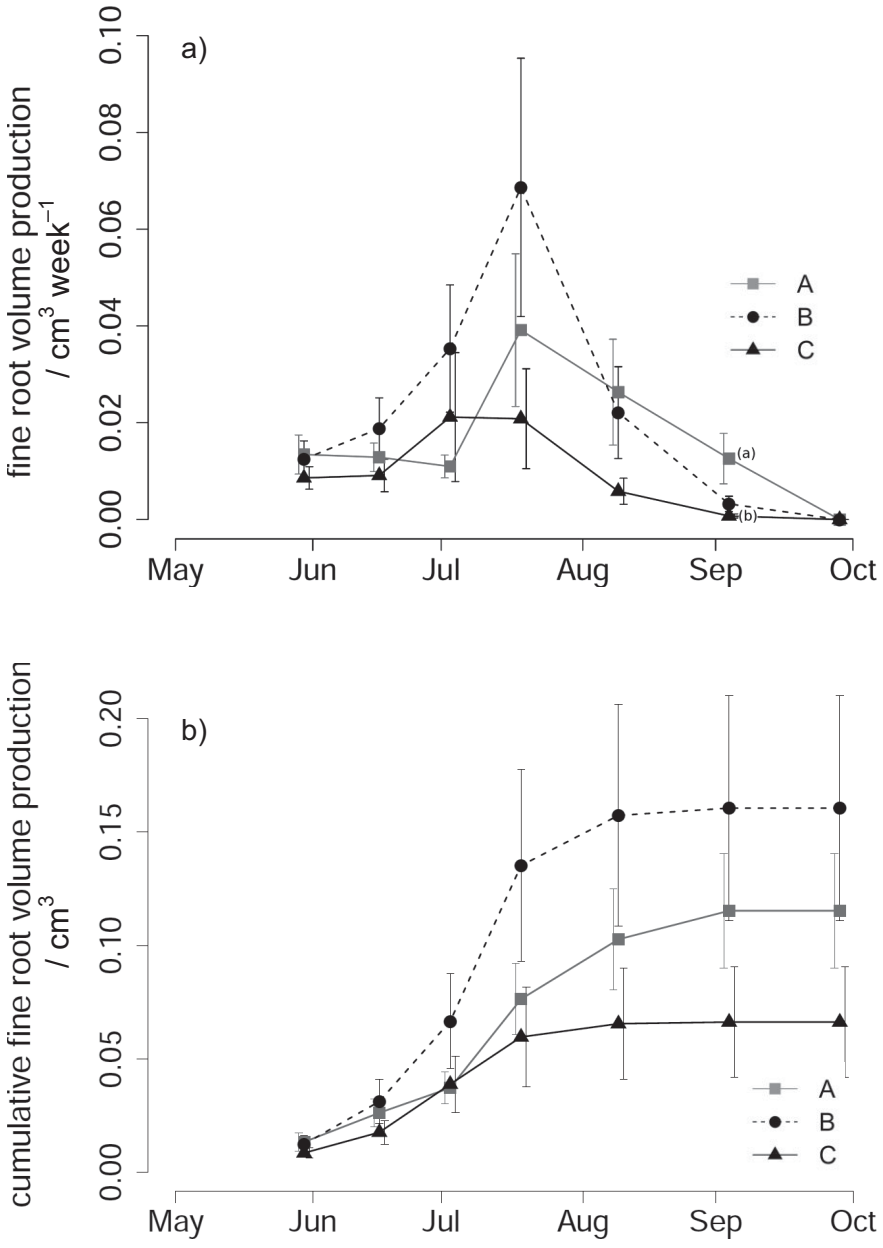

Figure 6: Fine-root volume production (a) and cumulative fine-root volume production (b) per rhizotron during the growing season of 2011 for the three treatments (mean ± SE, n = 8). Different letters in parentheses indicate differences between the treatments at $p < 0.1$.

3.3 Fine-root production

In all treatments, fine-root volume production was variable and increased from May through July (Fig. 6a). A subsequent decrease from August until stagnation in September occurred in all rhizotrons. As opposed to treatments A and C, fine-root production in treatment B peaked during mid-July (Fig. 6a). Although not significant, this treatment reached the highest cumulative mean fine-root production (calculated from bud-burst to harvest, Fig. 6b).

3.4 Fine-root NSC, root-to-shoot biomass ratio, and fine-root live-to-dead ratio

The fructose concentration of fine roots was positively correlated with the drought-stress dose (Tab. 1). Concentrations of total NSC and starch also tended to increase with increasing drought-stress dose, but the relationships were not statistically significant. In spite of high variability, the root-to-shoot biomass ratio increased with the drought-stress dose. With the amount of fine-root necromass being enhanced under drought, the fine root live-to-dead ratio was negatively correlated with the drought-stress dose.

3.5 Fine-root morphology

The drought treatments did not affect specific fine-root length (Tab.1). Fine-root length distribution by fine-root diameter revealed that the relative diameter-class length of roots < 0.2 mm significantly increased with increasing drought stress (Fig. 7). Accordingly, fine roots > 0.4 mm in diameter contributed less to the total fine-root length in the drought

Table 1: Means of plant parameters of the beech saplings after harvest 2010 for the three treatments (standard error in parentheses) and results of the correlation analysis with individual drought-stress dose. Different letters indicate significant differences between the treatments at $p < 0.05$, letters in parentheses refer to a significance level of $p < 0.1$. Coefficient of determination (r^2) and p value of the slope (p) are obtained from linear regression, ρ denotes Spearman's correlation coefficient; n.s. = not significant.

	Treatment A	Treatment B	Treatment C	r^2	p	ρ
Plant biomass / g	18.1 (2.28)	15.3 (2.45)	17.7 (1.8)	–	n.s.	–
Total leaf area / m^2	0.041 (0.006)	0.036 (0.006)	0.043 (0.004)	–	n.s.	–
Fine-root biomass / g	3.31 (0.35)	2.61 (0.53)	2.95 (0.48)	–	n.s.	–
Fine-root live-to-dead ratio	19.11 (5.04) a(b)	6.80 (1.27) a	7.91 (1.83) (b)	0.12	0.099	–
Root-to-shoot biomass ratio	1.59 (0.04)	1.69 (0.13)	1.81 (0.11)	0.13	0.091	0.39
Specific root length / m g^{-1}	21.9 (2.51)	24.8 (4.74)	24.1 (2.16)	–	n.s.	–
Specific root tip density / 10^3 g^{-1}	8.68 (1.44)	12.52 (2.76)	13.53 (2.7)	–	n.s.	–
Fine-root fructose concentration / mg g^{-1}	3.49 (0.28) a	4.36 (0.36) b	5.40 (0.34) c	0.29	0.0091	0.54
Fine-root starch concentration / mg g^{-1}	35.4 (6.9)	35.9 (5.8)	52.2 (7.0)	–	n.s.	0.38
Fine-root NSC concentration / mg g^{-1}	52.0 (4.5)	63.0 (7.8)	70.6 (13.8)	–	n.s.	0.50

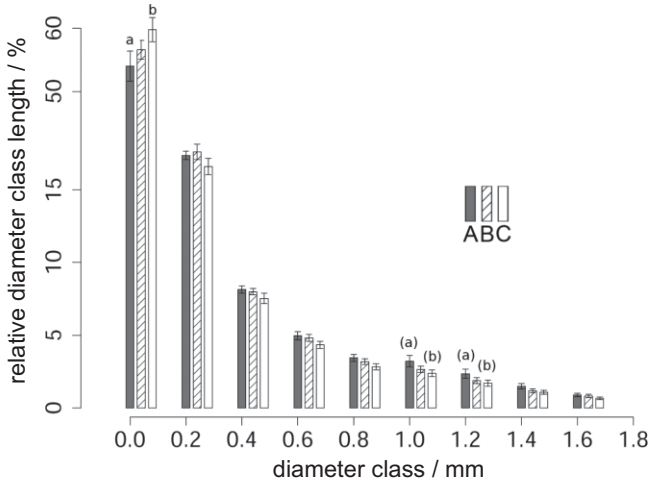

Figure 7: Fine-root diameter-class lengths of the beech fine roots for the three treatments after harvest in autumn 2010 (mean ± 1 SE, $n = 8$). Different letters indicate significant differences between the treatments at $p < 0.05$, letters in parentheses refer to a significance level of $p < 0.1$.

treatments. Specific root tip density was not significantly correlated with drought stress (Tab. 1).

4 Discussion

4.1 Drought treatment

The broad coincidence between stomatal conductance and soil water potential reflects the response of beech saplings to the differences in soil-water availability. Proceeding stomata closure with decreasing water availability is a strategy to minimize transpiration and can therefore be used as an indicator of the plant's water status (*Gallé* and *Feller*, 2007). Stomatal conductance < 0.05 mol H_2O m^{-2} s^{-1} signalizes severe drought stress of some saplings in treatment C and with delay in treatment B (*Flexas* et al., 2006). However, the large variability within treatment B and C indicates that some saplings were barely stressed by water deficiency. In September, similar mean stomatal conductance is attributed to the beginning of autumnal leaf senescence in all treatments.

4.2 Photosynthesis and aboveground respiration

The intraannual dynamics of the photosynthetic rate at constant irradiance result from different stages of leaf development and the seasonal course of temperature. Maximum photosynthetic activity is achieved in July soon after leaf formation in early summer. The reduction of photosynthesis is not as strong as reported by *Gallé* and *Feller* (2007) who observed a total breakdown of photosynthesis of beech saplings under drought. We assume that high air humidity attenuated the effect of soil drought at our site.

After rewetting in August, stressed plants re-attained their initial level of photosynthesis, with the rapid recovery indicating stomatal limitation of photosynthesis (*Tognetti* et al., 1995). Despite low soil water potentials in August/September, photosynthesis was similar in all treatments. As mentioned above, high air humidity allowed photosynthetic CO_2 uptake on nonstress level. Later in the season, gas exchange was apparently dominated by autumnal senescence. Aboveground respiration was not affected by drought so that the ratio of respiration to photosynthesis might have increased as observed in other studies (*Flexas* et al., 2006; *Atkin* and *Macherel*, 2009; *Ruehr* at al., 2009). However, our photosynthesis measurements do not provide a direct measure of C input as we cannot exclude that relative photosynthesis reduction of drought-stressed saplings was stronger under higher irradiance than that used during chamber measurements.

4.3 Rhizosphere respiration

Dynamics of rhizosphere respiration followed net photosynthesis at nonlimiting water availability, underlining the tight coupling between aboveground and belowground processes and the dependence of rhizosphere respiration on assimilate availability (*Irvine* et al., 2005; *Högberg* and *Read*, 2006).

While photosynthesis of drought-stressed saplings recovered after rewetting, rhizosphere respiration remained low and lagged behind the control for the rest of the growing season. We cannot exclude that rhizosphere respiration was under- or overestimated by subtracting soil CO_2 effluxes of planted from unplanted rhizotrons. The variation in rhizosphere respiration within the treatments is mainly triggered by differences in sapling biomass, but there is also some variation in CO_2 evolution from decomposition of SOM. Despite the methodological uncertainty, we conclude that a greater proportion of the assimilated C was translocated to the root system with increasing drought-stress dose and that rhizosphere respiration was rather limited by competing C sinks than by decreased C assimilation. Furthermore, our method does not distinguish between microbial respiration and root respiration which might respond differently to drought. The observed pattern could therefore be attributed to a decline in root respiration or heterotrophic respiration of microorganisms which rely on substrate transfer from roots.

4.4 Fine-root production

As rhizotron images only display visible roots on transparent side walls, an extrapolation of fine-root production to the whole root system is difficult (*Joslin* et al., 2000). Presuming similar initial fine-root biomass prior to the soil-water manipulation, we interpret the unimodal response of fine-root production as a drought effect. The promotion of fine-root growth at moderate drought is in accordance with other studies (*i.e.*, *Leuschner* et al., 2001) and is understood as a strategy to improve water uptake. Such a response was absent under severe drought with reduced fine-root production. We explain this by reduced assimilate availability corresponding to the decline in rhizosphere respiration. Furthermore, increased physical soil resistance at severe drought is assumed to limit root growth (*Bengough* et al., 2006). Root growth of loblolly pine has been reported to cease between –0.3 and –1.2 MPa (*Torreano* and *Morris*, 1998) which corresponds to the moisture range between treatment B and C in our experiment. However, the effect of soil density on root growth is not only soil-specific, but also species-specific (*Siegel-Issem* et al., 2005). We did not observe compensatory root growth during severe soil drought but we cannot exclude that rewetting would have promoted root growth after a certain recovery time.

4.5 Root-to-shoot biomass ratio, fine-root live-to-dead ratio, and NSC

Increasing concentration of fructose in the fine roots is interpreted as a response to soil drought as this sugar lowers the osmotic potential in the plant as a prerequisite of enhanced water uptake (*Kameli* and *Lösel*, 1993). Although not significantly correlated, accumulation of total NSC as well as the enhanced proportion of starch perhaps reflected restricted assimilate investment into tissue growth, accompanied by decoupling of rhizospheric respiration from photosynthesis (*Irvine* et al., 2005).

However, the increase of the root-to-shoot biomass ratio with increasing drought-stress dose indicates that, in relative terms, C allocated to the belowground compartments was yet rather invested for biomass increment than for respiration. We could not directly calculate fine-root turnover from the repeated root observations due to the lack of dead roots on the rhizotron side walls. Fine-root live-to-dead ratio has been discussed to serve as a proxy for this parameter (*Godbold* et al., 2003; *Zang* et al., 2011). The decrease in the live-to-dead ratio in treatment B and C resulted from drought-induced fine-root dieback (*cf. Gaul* et al., 2008; *Leuschner* et al., 2001). Root necromass, on the other hand, is also controlled by root decomposition which is retarded under drought (*Gaul* et al., 2008). We can therefore not definitely conclude that fine-root turnover was accelerated under drought.

4.6 Fine-root morphology

There was no marked effect of drought on fine-root morphology. Nevertheless, a tendency towards an increased proportion of fine roots with diameter < 0.2 mm is interpreted as a strategy to enhance the root surface area per unit of C investment. As drought was limited to 2 months in our experiment, a rapid response of fine roots becomes apparent. Assuming that a longer period of drought would have led to more pronounced results, we corroborate other studies showing a particular plasticity of the fine-root system of European beech (*Meier* and *Leuschner*, 2008b).

5 Conclusions

Planted beech saplings were sensitive to drought stress. Photosynthesis was less affected than rhizosphere respiration indicating a shift in assimilate utilization under drought. As an instantaneous response, fine-root growth was promoted at moderate soil drought, but decreased at severe drought. Even upon incipient drought, increase of belowground C allocation and fine-root mortality became apparent. Morphological fine-root parameters indicated enhanced effectiveness in soil-moisture exploitation under drought.

The results of our study refer to the *status quo* after the drought treatment. We cannot exclude compensatory effects after rewetting and in the subsequent growing seasons as described by *Olesinski* et al. (2011). However, this study gives useful information on the behavior of planted beech saplings upon soil drought and provides a reference for drought-stress quantification in future experiments.

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