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Fine roots of *Picea abies* compensate for drought stress in the rainfall reduction experiment

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Abstract: This study evaluates the influence of repeated artificial drought stress on the fine root characteristics – including ectomycorrhizae – of Norway spruce [*Picea abies* (L.) Karst]. The experimental site consisted of two plots in a mature spruce monoculture stand. The water regime at parts of both plots was regulated by shelters and an isolation trench during vegetation season (spring to autumn) since 2010. Root samples were collected during autumn in 2010, 2012, and 2013. Root analyses revealed the effect of drought stress on mycorrhizal root tips changed over time. While a density of active mycorrhizae was about 34% lower in drought-stressed areas compared to nonstressed (control) areas in 2010, it increased by 15% in 2012 and by 22% in 2013 over both plots. We observed the less pronounced effect of drought on a proportion of active mycorrhizae, but it generally followed the pattern of active mycorrhizae density. The density of nonactive mycorrhizae was not influenced by drought but significantly fluctuated during the course of the experiment. Other root characteristics such as the dry mass of fine roots (< 1 mm), the specific length of fine roots (< 1 mm) and the composition of the ectomycorrhizal community (primarily dominated by *Amphinema byssoides*, *Tylospora fibrillosa*, *Tylopilus felleus,* and *Cenococcum geophilum*) were also not significantly influenced by drought. Our results indicate the ability of Norway spruce fine roots to compensate for repeated drought stress of the intermediate intensity.

Keywords: climate change, community, ectomycorrhiza, Norway spruce, WinRhizo

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Introduction

Due to ongoing climatic changes, European forests will probably face more frequent and intense weather fluctuations. Predictions show it will entail both long periods of drought and torrential rainfall (Lindner et al., 2010). Under these conditions Norway spruce [*Picea abies* (L.) Karst.] is at risk of substantially decreasing growth (Altman et al., 2017), as well as more natural infection by parasitic fungi, especially annosum root rot (*Heterobasidion* sp.) (Lindberg & Johannson, 1992) and honey fungus (*Armillaria* sp.) (Lindner et

al., 2008) or by herbivorous insects (Økland & Berryman, 2004). Norway spruce has unusually low resistance to drought compared to other common forest species such as beech or oaks, especially when grown in monocultures (Pretzsch et al., 2013).

Drought influences tree physiology on several levels and lead to a decrease of storage compounds, a decrease of leaf area index together with cambial activity, and an increase of fine roots' mortality (Bréda & Badeau, 2008). The general response to drought includes increased root to shoot ratio, the shift of fine roots into deeper (mineral) soil layers and a shorter lifespan. Main root traits such as specific root length or root tissue density remained unchanged (Brunner et al., 2015).

Mycorrhizal symbiosis seems to be one of the key factors enabling increased ecosystem resilience to climatic extremes (Kivlin et al., 2013). Mycorrhiza can alleviate drought stress due to the roots' increased absorption surface, efficient water distribution using mycelial fibers, increased hydraulic conductivity at the soil-root interface and augmented expression of aquaporins (Bréda et al., 2006; Brunner et al., 2015). In comparison to non-mycorrhizal plants, mycorrhizal plants usually have higher values of proline (osmoprotectant and an effector molecule alleviating many different kinds of stress including drought) and also lower values of lipid peroxidases which play a role in oxidative stress reactions (Porcel & Ruiz-Lozano, 2004; Bois et al., 2006). During low or intermediate drought stress, ectomycorrhizal plants are usually better supplied by water than nonmycorrhizal plants, resulting in lower soil water content near the root system. Contrary, during severe drought stress, water can flow from the plants to soil (Gryndler et al., 2004). As individual mycorrhizal fungi differ in their drought stress tolerance (di Pietro et al., 2007), the composition of mycorrhizal communities (and hence their physiological function) can be substantially influenced by drought (Shi et al., 2002). Koide et al. (2008) suggested that reduced carbon allocation via their tree host (which occurs due to drought) may have negative impacts on fungi with high demand for carbon from photosynthesis and instead favor fungi with enhanced saprotrophic potential. Common ectomycorrhizal fungus *Cenococcum geophilum* seems to be particularly resistant to drought (di Pietro et al., 2007) and possesses relatively high saprotrophic abilities (Peter et al., 2016) favoring it for drought conditions. The ecological importance of ectomycorrhizal fungi species is often regarded as being proportional to the number of ectomycorrhizal tips, but characteristics of extraradical mycelium also play a significant role (Landeweert et al., 2003). The development of fine roots and ectomycorrhizae is influenced by factors internal to the plant (e.g., host genotype, chemistry, growth rate, other present symbionts), and various environmental conditions including soil, climate and season with mutual interconnection of some of them (Buée et al., 2005; Kernaghan, 2005; Korkama et al., 2006; Piculell et al., 2008). Crucial factor is pH, which substantially influences the number of ectomycorrhizal roots (Lehto, 1994; Nowotny et al., 1998) and the representations of individual ectomycorrhizal species (Von Alten & Rossbach, 1989; Kjøller & Clemmensen, 2009).

Most studies dealing with fine roots (including ectomycorrhizae) in relation to water availability in the soil profile have been short-term (involving only one drought period) and often done under artificial conditions (Lehto & Zwiazek, 2011). The influence of long-term and repeated drought periods on ectomycorrhizae under natural conditions, however, is not well known. Cudlín et al. (2007) recommended using a number of root tips, and a density of fine roots based on dry weight, root length, or soil volume as indicators of drought impact.

The objective of this study was to determine an influence of repeated drought stress on selected characteristics of fine spruce roots, including the density of mycorrhizal root tips and the composition of ectomycorrhizal fungal community in the mature spruce stand. To achieve the goal we compared fine root characteristics of trees artificially stressed by drought with those of control unstressed trees. We assumed that drought would generally negatively influence tested root characteristics and that the community of ectomycorrhizal fungi would change in favor of more drought-tolerant species. We also expected a partial adaptation of tested trees to repeated exposure to drought over the tested period, i.e., recovery of some of the measured characteristics (e.g., the proportion of active mycorrhizal tips) while the long-standing change of other characteristics (e.g., dry mass of fine roots).

Methods

The experiment was conducted at Kostelec nad Černými lesy (the Czech Republic, 49°57'56"N, 14°51'17"E). Mean annual temperature at the location is 9°C and mean annual precipitation is 527 mm (Zajíčková et al., 2011). Soil type at the location is modal cambisol (Němeček et al., 2004) with mean pH value 3.74 (measured in water solution, according to ISO/DIS 10390, 1994). The location consisted of a uniform-age 80 years old monoculture spruce stand. The experiment involved two research plots (P1 and P2) with 40 trees per plot. Each of these research plots was divided into two parts: drought-stressed and control. The water regime in the drought-stressed parts was controlled using shelters with a plastic cover at a height about 1.8 m, which covered an area

of 25 × 25 m on each of these subplots and conducted precipitation water outside from the plots with drought regime. The drought-stressed subplots were sheltered from the beginning of May to mid-October every year during 2010–2013. The water regime of these sites was also modified by the construction of an isolation trench to prevent subsurface draining of water to them. The water potential in all four parts of the plots (both control and stressed ones) was measured using total 48 GB-2 gypsum sensor blocks from Delmhorst Instrument Co. (Montville, NJ, USA) distributed in a regular square network in soil at depth 15 cm and it was recorded automatically at 1 h intervals.

Soil samples were collected by a soil auger with an inner diameter of 6 cm to a depth of 15 cm (sample volume ca. 424 cm^3), during autumn in 2010, 2012, and 2013: ten samples per plot * year (i.e., 40 samples per year, 120 samples in total). Samples from mineral soil layers were not analyzed due to the low amount of present fine roots. After collection, the soil cores were placed in a refrigerator until further processed. Processing of all collected soil samples included root extraction according to Pešková and Soukup (2006). Spruce roots were manually extracted from the soil using tweezers and dissecting needles. Roots of other plants which could not be identified were removed.

In samples collected in 2010 and 2012, the density of active and nonactive mycorrhizae, the proportion of active mycorrhizae and dry mass of roots were evaluated together with specific root length determined using a WinRhizo instrument (Regent Instruments Inc., Canada). An overview of the analyzed root parameters is given in Table 1. All root parameters were using fine roots with a diameter up to 1 mm because these roots respond most sensitively to environmental factors, and they are better represented in a soil auger than larger-diameter roots (Pešková & Soukup, 2006). We also intended to keep analyses of fine roots consistent with previously acquired and published data (Pešková et al., 2015). After extraction, roots were stored in a fixative solution of 2.5% glutaraldehyde until further processed. Active and nonactive mycorrhizae were evaluated according to the methodology described by Pešková and Soukup (2006) (Table 1). From each sample, 20 root segments with main root length of 5 cm were randomly selected. The total length of each segment was calculated as the primary root length plus the root length of its branching systems. On each selected root segment, mycorrhizal tips were counted and assessed under a stereomicroscope at 40x magnification. Tips with a smooth surface, high turgor pressure, well developed hyphal sheaths and lacking radical hair, were classified as active mycorrhizae. Tips with wrinkled surface, low turgor pressure and without hyphal sheats were classified as nonactive mycorrhizae (Holuša et al., 2009). Afterward, dry mass of roots (at 105°C) was determined.

Table 1. Mycorrhizal and root characteristics evaluated

Abbreviation	Variable	Unit
ActM	Density of active mycorrhizae (number of active mycorrhizal tips divided by root length in centimetres)	cm^{-1}
NactM	Density of nonactive mycorrhizae (number of nonactive mycorrhizal tips divided by root length in centimetres)	cm^{-1}
% ActM	Proportion of active mycorrhizae (number of active mycorrhizal tips divided by total number of mycorrhizal tips)	%
DM<1 mm	Dry mass of roots with diameter up to 1 mm	g
spec_length	Specific root length (length of roots with diameter up to 1 mm divided by dry mass of roots with diameter up to 1 mm)	$cm*g^{-1}$
Mycorrhizal fungus species	Representation of a molecularly determined mycorrhizal fungus	%

For the evaluation of specific root length (Table 1) by WinRhizo, the dissected roots were scanned and then analyzed. The total root length was divided by root dry matter weight to determine specific root length (Konôpka & Takáčová, 2010).

Samples collected in 2013 were divided into two parts. The first part was processed as described in the above section. In the second part, the species composition of ectomycorrhizal fungi in roots from the organic soil layer was determined using morphotyping and subsequent molecular analyses. For the analyses, fine roots with diameter up to 2 mm (commonly used threshold in mycorrhizal research) were selected. These roots were stored in 35% ethanol and analyzed under a stereomicroscope (Olympus SZX12). Ectomycorrhizal tips were separated into morphotypes on the basis of branching type, color, hyphal sheath characteristics, the presence of extra-radicular mycelia and rhizomorphs (Agerer, 1987–2012). An average of 300 mycorrhizal tips was analyzed per sample though only approx. 140 mycorrhizal tips/sample were turgescent and undamaged and thus suitable for sorting to morphotypes. Representative mycorrhizal tips of each morphotype were separated and kept in 35% ethanol at 4°C until used for molecular determination.

At least two representatives of each morphotype (preferentially from different subplots) were used for subsequent molecular analyses. DNA was isolated from individual mycorrhizal root tips using the DNeasy PowerPlant Pro kit (Quiagen, USA) and amplified using polymerase chain reaction (PCR) based on their rDNA region. PCR conditions followed the work of Vohník et al. (2013). Semi-nested PCR was used with primers ITS1F and ITS4 (White et al., 1990; Gardes & Bruns, 1993) in the first run and with

primers ITS1-ITS4 in the second run. Purification and sequencing of PCR products were done by Macrogen Europe (Amsterdam, the Netherlands). The obtained sequences were BLAST searched against the nucleotide database in GenBank (NCBI) and sequence similarity ≥ 97% was used as a threshold for species identification. After molecular identification of each morphotype, the overall relative proportion of ectomycorrhizal species was calculated (unidentified morphotypes constituted less than 6% of the total number of morphotypes). The sequences were submitted to the NCBI GenBank database (accession numbers MK050091, MK050546-MK050552, and MK050633-MK050642).

Statistical evaluation of the density of active mycorrhizae, the density of nonactive mycorrhizae, and proportion of active mycorrhizae was performed using statistical software R (R Core Team, 2015). We used a linear mixed effect model (LME) fitted by restricted maximum likelihood (REML), where mycorrhizal parameters (ActM, NactM,% ActM) were used as the dependent-variables, year as a continuous variable and drought stress as a fixed factor. Both independent variables were nested in the plot variable. In the evaluation of the model were used approaches according to Crawley (2007) and Pekár & Brabec (2012).

Results

Drought stress caused by a rainfall reduction in our study reached the intermediate intensity and was not uniform over the tested plots. In autumn of 2010, soil water potential reached the lowest values in the drought-stressed part of plot P2 (−1.1 MPa) in comparison to others (−0.5 MPa in the drought-stressed part of plot P1, −0.1 MPa at control part of plot P1, −0.2 MPa at control part of plot P2) (Turčáni et al., 2010). In the following years water potential was also lower on both stressed parts compared to the control ones but due to missing data we cannot present reliable average values.

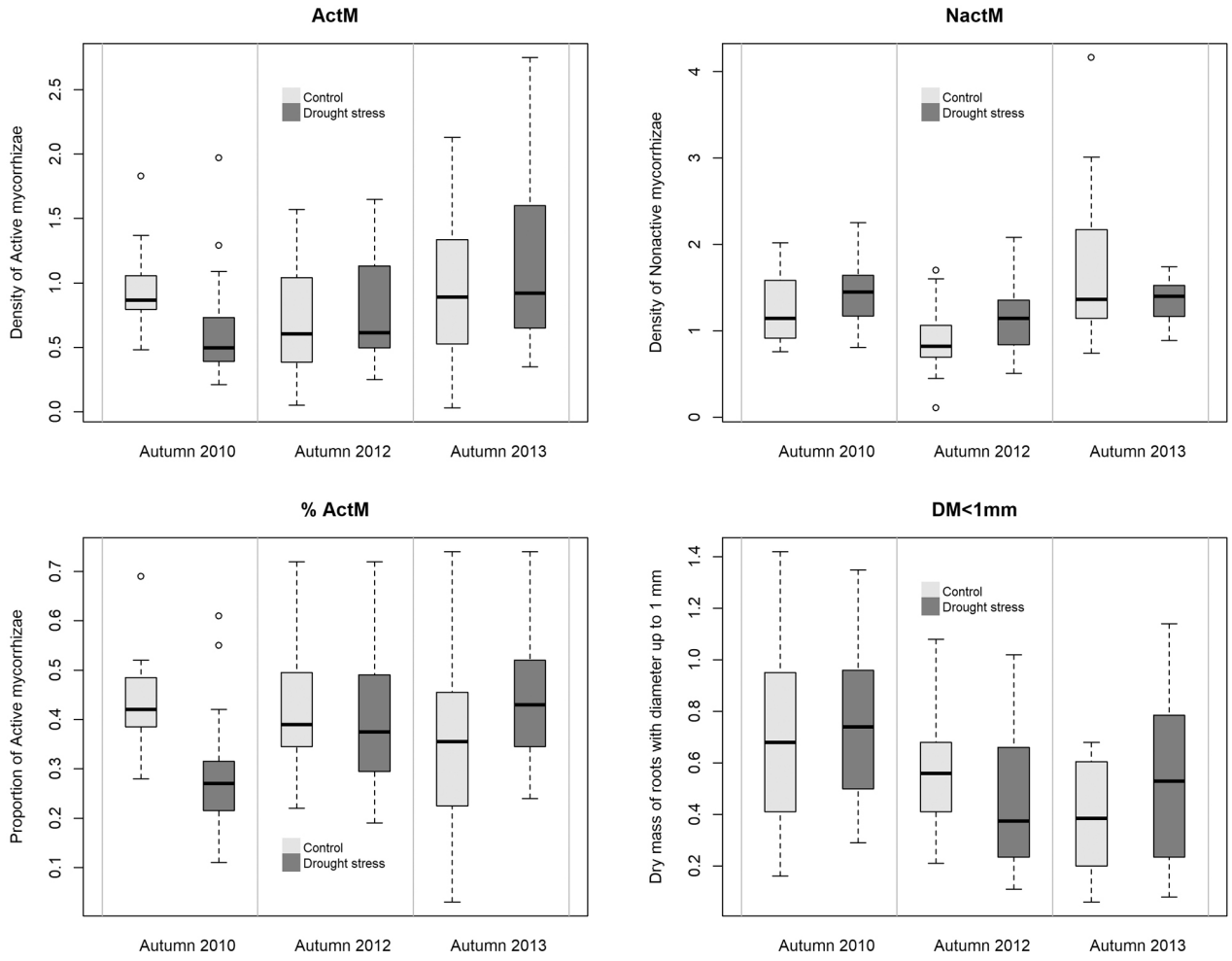

Fig. 1. Density of active mycorrhizae, density of nonactive mycorrhizae, proportion of active mycorrhizae and dry mass of roots up to 1 mm in stressed and control parts over the both plots in every sampled yeas. Every column consists of 20 samples. Central band – median, box – 1st and 3rd quartiles, whiskers – 1.5 multiple of interquartile range, dots – above 1.5 multiple of interquartile range

Table 2. Effect of tested variables (year, drought stress) on root characteristics of Norway spruce. Linear mixed effect model was used for statistical evaluation. ActM – density of active mycorrhizae, NactM – density of nonactive mycorrhizae,% ActM – proportion of active mycorrhizae, DF – degrees of freedom, F – statistical F-test values, p – singificance level: * p < 0,05; ** p < 0,01; *** p < 0,001

Dependent variable	Independent variables in model	DF	F	p
Actm	{Intercept)	1	52.83	***
	year	2	5.34	**
	drought stress	1	0.01	0.93
	year : drought stress	2	3.44	*
NactM	(Intercept)	1	851.04	***
	year	2	11.05	***
	drought stress	1	0.23	0.63
	year : drought stress	2	4.82	**
% ActM	(Intercept)	1	114.19	***
	year	2	1.47	0.23
	drought stress	1	1.38	0.24
	year : drought stress	2	6.65	**

The density of active mycorrhizae has significantly changed through the evaluated years (LME: n = 119; df = 2; $p < 0.01$; Table 2). A decrease of ActM in 2012 and an increase in 2013 was observed contrary to 2010 values. The density of active mycorrhizae in drought-stressed and control parts followed the general trend of the changes over evaluated years, so we did not detect observe the statistically significant difference between them (Table 2). On the other hand, time course of changes in ActM was significantly different between drought stressed and control parts (LME: n = 119; df = 2; $p < 0.05$; Table 2). In drought stressed plots fast decrease of ActM in reaction to water deficiency was followed by steady increase of active root tips density to the levels exceeding the original values in 2010. In the control parts only transient decrease of ActM was detected in 2012 followed by an increase of this parameter to the levels measured in 2010. Overall, the density of active mycorrhizae was about 34% lower in drought-stressed areas compared to nonstressed (control) areas in 2010, but it increased by 15% in 2012 and by 22% in 2013 over both plots (Fig. 1; Supplementary Table 1).

Density of nonactive mycorrhizae was highly different between the sampling years (LME: n = 119; df = 2; $p < 0.001$; Table 2). In 2012, it was about 25% lower than in 2010 and in 2013 about 51% greater than in 2012 over both plots. Effect of drought stress on the density of nonactive mycorrhizae was not statistically significant (Table 2), due to the high variance of both control and drought stressed values. The density of nonactive mycorrhizae differed in drought-stressed plots and control plots through the tested period of time (LME: n = 119; df = 2; $p < 0.01$; Table 2), where more pronounced changes were recorded in the control treatment (Fig. 1; Supplementary Table 1).

Table 3. Mean ± standard deviation of specific root length (spec_length) and percentage representation of individual ectomycorrhizal species of fungi in 2013. N: number of samples

Variable	Drought stress	N	Plot	2013
spec_lenght	no	10	P1	1655 ±319
	no	10	P2	1977 ±669
	no	20	P1+P2	1816 ±556
	yes	9	P1	1482 ±616
	yes	9	P2	1449 ±460
	yes	18	P1+P2	1465 ±528
A. byssoides	no	10	P1	42 ±18
	no	10	P2	44 ±22
	no	20	P1+P2	43 ±19
	yes	10	P1	50 ±21
	yes	10	P2	46 ±23
	yes	20	P1+P2	48 ±22
T. felleus	no	10	P1	11 ±11
	no	10	P2	6 ±7
	no	20	P1+P2	8 ±9
	yes	10	P1	4 ±7
	yes	10	P2	11 ±11
	yes	20	P1+P2	7 ±9
C. geophilum	no	10	P1	31 ±26
	no	10	P2	45 ±22
	no	20	P1+P2	38 ±25
	yes	10	P1	35 ±4
	yes	10	P2	28 ±22
	yes	20	P1+P2	32 ±24
T. fibrilosa	no	10	P1	16 ±12
	no	10	P2	5 ±5
	no	20	P1+P2	10 ±11
	yes	10	P1	11 ±10
	yes	10	P2	15 ±10
	yes	20	P1+P2	13 ±13

The proportion of active mycorrhizae generally followed the pattern of active mycorrhizae density, but with a less pronounced effect of drought. The difference in the proportion of active mycorrhizae between drought-stressed and control plots was highest in 2010 (Fig. 1; Supplementary Table 1). However, the difference between the compared years was not statistically significant (Table 2). No significant effect of drought stress was detected in the proportion of active mycorrhizae, probably due to the large variance of experimental data (Fig. 1; Supplementary Table 1). But two-factor interaction year × drought stress was statistically significant (LME: n = 119; df = 2; $p < 0.01$; Table 2). In the drought-stressed plots, the proportion of active mycorrhizae was gradually increased contrary to control plots, where the proportion had a decreasing trend (Fig 1; Supplementary Table 1).

Neither dry mass of roots with a diameter up to 1 mm (Fig. 1; Supplementary Table 1) nor specific

root length with a diameter up to 1 mm (Table 3) showed any statistically significant differences between drought-stressed and control parts.

Nine morphotypes were distinguished in samples collected in autumn 2013 (i.e., 4th year of the experiment). Using molecular analyses, we determined 4 species of ectomycorrhizal fungi corresponding to 5 different morphotypes which commonly occurred in the samples: *Amphinema byssoides* (Pers.) J. Erikss., *Cenococcum geophilum* Fr., *Tylopilus felleus* (Bull.) P. Karst. (comprising 2 morphotypes that were merged after molecular identification), and *Tylospora fibrillosa* (Burt) Donk. These four mycobionts dominated mostly the community and represented 97.2–100% of the discerned morphotypes on the tested plots in 2013. Other morphotypes occurred sporadically in the samples: one morphotype with low abundance (0–1.4%) comprised 3 ectomycorrhizal species (*Tylopilus felleus*, *Tylospora fibrillosa,* and *Thelephora terrestris*) and molecular analyses of the remaining three morphotypes (occurring at abundances ranging from 0–1.7%, 0–0.3% and 0–1.5%, respectively) yielded mostly sequences of low quality some of which were related to saprobic or endophytic fungi (e.g., *Ganoderma* sp., *Oidiodendron* sp., *Cryptococcus* sp. etc.) indicating these were partially rotten senescent mycorrhizae or non-mycorrhizal root tips. The representation of the discerned ectomycorrhizal species in the tested plots was quite homogeneous and not influenced significantly by drought and plot (Table 3).

Discussion

Drought stress caused by a rainfall reduction in our study reached an intermediate intensity we assume it was not detrimental to ectomycorrhizae as hyphae of mycorrhizal fungi are able to survive long periods of drought even at extremely shallow water potential values (−20 MPa) (Querejeta et al., 2003). However, using our experimental approach, we cannot exclude groundwater availability putatively attenuating the drought stress regime in deeper mineral soil layers with impact on an overall physiological status of the tested trees.

Induced drought stress leading to changes was more pronounced in Norway spruce seedlings grown under artificial conditions, whether it was a decrease of mycorrhizal colonization in seedlings as soon as exclusion of irrigation for 9 days (Möttönen et al., 2001), 6 days and subsequent 8 days (Möttönen et al., 2005) or 2–4 months in the third and the fifth year (Nilsen et al., 1998). The effect of drought on mycorrhizal colonization within Norway spruce stands (including our study) was not so apparent. On 80-years-old spruces drought induced by shelters significantly reduced the density of active mycorrhizae, but not the density of nonactive mycorrhizae neither proportion of active mycorrhizae (Pešková et al., 2015). In two 12 years-old spruce stands drought induced by shelters (approx. 40 % of control atmospheric precipitation) had no significant effect on a level of the mycorrhization (determined indirectly on the basis of chitin) between control and drought-stressed. Instead, there was observed considerable dynamics of in the experimental time course (Palátová, 2004). Less obvious effect of drought on mycorrhizae on stands in natural conditions can be due to other environmental factors (including natural dynamics of mycorrhizae), which significantly interact with drought and may blur its impact. Results from our study also show that the dynamics of the density of active and nonactive mycorrhizae differ, probably not only due to the quantitative transition of one form to the other or the influence of drought on their change. The density of active mycorrhizae compared to relatively higher and more fluctuating density of nonactive mycorrhizae can surprisingly indicate a significant fluctuation in the lifetime of active mycorrhizae. High temporal dynamics of the density of active and nonactive mycorrhizae were also observed in previous studies (e. g. Blasius et al., 1989; Pešková, 2007; Pešková et al., 2011).

We should stress that before the beginning of the experiment (spring 2010) the densities of active and nonactive mycorrhizae, the proportion of active mycorrhizae, and dry mass of roots with a diameter up to 1 mm did not differ significantly in any part of the research plots (see Lorenc, 2012; Supplementary Table 2).

Dry mass of roots with diameter up to 1 mm in this study was not significantly influenced by drought stress. It is congruent with study on 80 years old spruces in the first and the second year with the same experimental design (Pešková et al., 2015) and in roots with diameter up to 2 mm on 140 years old spruces after six weeks of water exclusion induced by shelters (Gaul et al., 2008). On the other hand, the influence of drought on root weight has been observed to be negative in roots with diameter up to 1 mm in spruces 12 years old in all four years of the experiment (Palátová, 2004), in roots with diameter up to 2 mm in trees 5 years old, exposed to 11-year reduced watering in greenhouse (Eldhuset et al., 2013) and in young spruce stands in natural conditions, monitored for four years (Konôpka et al., 2013). These contradictory results are suggesting that the response of fine root biomass to drought is not uniform. Similarly, there is a wide variation in the effect of drought on specific root length. Brunner et al. (2015) stated that specific root length usually is not significantly affected in drought-stressed trees which is in agreement with our observation. On the other hand, Puhe (2003) declared that in adverse conditions fine roots of Norway spruce might have

smaller specific root length and greater amount of the roots due to an increased quantity of necromass. Contrary to our results, Olmo et al. (2014) observed higher specific root length of very fine roots (diameter up to 0.5 mm) using seedlings of 10 tree species stressed by severe drought in a greenhouse. The discrepancies among the above-mentioned studies may be partially explained by the different diameter of the analyzed roots but may also reflect other physiological processes such as changes in the composition of associated ectomycorrhizal communities (with a concomitant effect on multiple root parameters including specific root length).

Although the identified spruce ectomycorrhizal species in our study correspond well to those observed by others in the Czech Republic (Peter et al., 2008; Vohník et al., 2013), the community richness was very low. Low diversity of detected fungi may be due to several putative reasons. Firstly, we only analyzed root tips in the upper organic layer leaving thus all ectomycorrhizal species occurring exclusively in the mineral layers undetected. Secondly, a large proportion (59% on average) of microscopically analyzed root tips was of low quality and their unequivocal assignment to a particular morphotype was not possible. After their subtraction approx. 140 root tips per root sample remained and were assigned to the particular morphotypes. With this amount of root tips theoretically, only fungi whose frequency reached at least 2.1% in the community were detected at 95% probability level according to Taylor (2002). Rarer species thus might have been overlooked though their functional importance is disputable.

While we have not detected the effect of drought stress on the composition of the ectomycorrhizal community, Shi et al. (2002) observed a significant effect of three months lasting drought (induced by plastic roofs) on the fungal community associated with *Fagus sylvatica* L. In their study ectomycorrhizal species responded differently to the drought of varied intensity. Using analogous experimental approach to our study, Nickel et al. (2018) detected changes in the ectomycorrhizal community in a mixed beech-spruce forest caused by drought with an increase of species having long rhizomorphs. The authors concluded that ectomycorrhizae responded to repeated drought by maintaining or increasing their functionality at the individual root level, but were unable to compensate for quantitative losses at the ecosystem level. On the contrary, Nilsen et al. (1998) observed only *C. geophilum* was significantly affected by drought (imposed by 2–4 months exclusion of water in the third and the fifth experimental year) in potted Norway spruces in the sixth year of the experiment. We suppose that lack of ectomycorrhizal community changes the fourth year after the drought onset in our study was caused by moderate drought stress and the compensation ability of Norway spruce (the community composition was tested four years after the onset of drought stress).

Intense and sudden stress stimulus may lead to trees damage and inhibition of their growth. Gradual onset of stress, however, allows a physiological adaptation of a plant which may prevent adverse consequences of the stress (Kozlowski & Pallardy, 2002). On ectomycorrhizal roots, it was demonstrated by Swaty et al. (2004), who reported twice as high colonization of roots by ectomycorrhizal fungi exposed to medium levels of drought stress compared to trees exposed to a low or high level of drought stress in stands of Colorado pinyon (*Pinus edulis* Engelm.). We assume fine spruce roots in our study were able to compensate for repeated drought stress of intermediate intensity which was reflected in the recovery of the density of active mycorrhizae and no detectable changes of other root characteristics including ectomycorrhizal community composition.

Overall this study demonstrates that fine roots of Norway spruce, including ectomycorrhizae, are able to endure repeated periods of moderate drought stress and compensate for it. Observed differences of root parameters between plots and years indicate that a combination of drought stress intensity and other environmental factors contributed to the mitigation of drought effect on spruce fine roots. Our results underline the necessity to study the effect of drought on a broad severity scale and to monitor both short- and long-term changes of tested tree parameters.

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