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Ectomycorrhiza affect architecture and nitrogen partitioning of beech (*Fagus sylvatica* L.) seedlings under shade and drought

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ABSTRACT

The aim of this study was to investigate the influence of ectomycorrhizal fungi (EMF) on the architecture of and nitrogen (N) partitioning in young beech (*Fagus sylvatica*) plants in response to different light regimes and water deprivation. We hypothesized that EMF modify biomass partitioning and architecture of young beech plants by increased N uptake in comparison with non-mycorrhizal (NM) plants and that therefore, the drought responses of EM and NM plants diverge. We anticipated that full light-exposed plants were more drought tolerant due to improved water status and nutrition, whereas shade-acclimated EM plants were more drought susceptible because of decreased mycorrhizal colonization. To test these hypotheses seedlings were grown in native or sterilized forest soil. To avoid effects of soil pretreatment NM and EM plants were transplanted into sand-peat culture systems and exposed to shade, drought or the combination of both factors. Shade resulted in reduced root biomass production decreasing the root-to-shoot ratio. Mild drought stress (pre-dawn water potential [Ψ_{pd}] = −1.3 MPa) did not affect biomass partitioning. EMF colonization did not increase plant biomass, but had strong effects on root architecture: the numbers of root tips as well as the absolute and specific root lengths were increased because of formation of thin roots, especially in the diameter classes from 0.2 to 0.8 mm. In contrast to our expectation N uptake of well irrigated EM plants was not increased despite their larger potential for soil exploitation. Overall, EM plants exhibited higher amounts of carbon fixed per unit of N taken up than NM plants and shifted N partitioning towards the roots. Beneficial effects of EMFs were apparent under mild drought but the responses differed depending on the light availability: shaded EM plants showed a delay in the decrease of Ψ_{pd}; light exposed EM plants showed increased N uptake compared with NM beeches. These results indicate that EMFs are involved in mediating divergent responses of beech to drought depending on the light availability.

1. Introduction

European beech (*Fagus sylvatica* L.) is an economically and ecologically important forest tree species (Ellenberg and Strutt, 2009). In the ecogram of temperate trees, beech is positioned as a relatively drought sensitive but shade tolerant species (Ellenberg and Strutt, 2009). Beech seedlings are especially shade tolerant and show morphological and physiological adaptations in response to changes in light climate (Tognetti et al., 1994, 1998; Johnson et al., 1997; Collet et al., 2001; Parelle et al., 2006; Druebert et al., 2009). Shade leads to acclimation of light capture with large thin leaves, high chlorophyll contents and saturation of photosynthetic carbon assimilation at low light intensities (e.g., Gansert and Sprick, 1998; Druebert et al., 2009). Furthermore, the root-to-shoot ratio decreases because carbon is preferentially invested into leaf rather than root production in the shade (Burschel and Schmaltz, 1965; van Hees and Clerkx, 2003; Löf et al., 2005). These adjustments optimize light capture, but may affect drought susceptibility. Indeed, drought-exposed beech seedlings in the understory showed lower pre-dawn water potentials and had higher mortality rates than those in gaps (Robson et al., 2009). However, when beech was exposed to drought under controlled conditions root growth was less affected in shade than under high light levels (Madsen, 1994; Tognetti et al., 1994; van Hees, 1997; Löf et al., 2005) indicating that the responses of beech to interacting drought and shade are not yet fully understood.

In addition to light and water, other abiotic and biotic factors influence beech growth and performance. An important aspect in this regard is nitrogen (N) availability. N is usually a limiting factor in forest ecosystems and it has been suggested that the perennial life style of woody plants may be an adaption to these conditions (Rennenberg and Schmidt, 2010). Only woody plants form

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associations with ectomycorrhizal fungi (EMF) on their roots, which increase the surface by extended extraradical mycelia, thereby, facilitating nutrient uptake (Read and Perez-Moreno, 2003; Gobert and Plassard, 2008). EMF also influence root architecture inducing dichotomous branching of the tips, which in turn increases soil exploration (Osmont et al., 2007). Furthermore, EMFs improve plants' water status, thus, ameliorating plant performance under drought (Lehto and Zwiazek, 2011).

The vital root tips of beech trees in forests are usually 100% colonized by EMF (Rumberger et al., 2004; Buée et al., 2005; Grebenc et al., 2009; Pena et al., 2010; Lang et al., 2011). In contrast, young planted beech seedlings show less colonization, e.g., 26–100% in sterilized soil and inoculated with *Lactarius subdulcis* after 6–18 months (Beyeler and Heyser, 1997) or 5–10% in forest soil after two seasons (Zeleznik et al., 2007). Among other factors, low colonization rates can be caused by carbon limitation imposed for example by shading (Druebert et al., 2009). In controlled experiments studying the influence of drought on beech N nutrition, the mycorrhizal status of beech was low or absent (Winkler et al., 2010). Since drought reduces carbon allocation to the below ground compartment (Ruehr et al., 2009; Winkler et al., 2010), it may exert negative effects on EMF colonization and functioning and, thereby, influence the performance of young beech trees. Previous studies addressing the influence of shade and drought on beech stress responses and nutrition did not consider possible interactive effects of EMF.

The aim of this study was to investigate the influence of EMF on architecture and N allocation of young beech plants under shade or full irradiation in combination with sufficient irrigation or water shortage. We hypothesized (i) that unstressed young beech, colonized by a natural community of EMF exhibit higher N uptake than nonmycorrhizal (NM) trees and that this will modify biomass partitioning and plant architecture and (ii) that drought responses of EM and NM plants diverge depending on the light conditions: light-exposed EM plants are expected to be more drought tolerant due to an improved water status and better nutrition than NM plants; shade-acclimated plants may not be able to sustain EM and, therefore, become as drought susceptible as NM plants. To test these hypotheses, beech seedlings were grown in sterilized or native forest soil. After establishment of ectomycorrhizas, EM- and NM-plants were transferred to sand cultures, exposed to two different levels of light and subjected to moderate water shortage. Pre-dawn water potentials (Ψ_{pd}), root architecture, total and specific leaf area as well as biomass of leaves, stems and roots were determined. To assess N-uptake and partitioning, the plants were labeled with ^{15}N and used to measure total and newly acquired N in leaves, stems and roots.

2. Materials and methods

2.1. Plant material and growth conditions

Beech nuts (provenance: Forstsaatgutstelle Oerrel, Niedersachsen, Germany) were germinated on moist filter paper at 4 °C in darkness for four weeks. When the radicles had reached a length of 1–2 cm, the seed coats were removed and the seedlings were sterilized in a solution of 1 mL fungicide Proplant (Stähler, Stade, Germany) and 100 mg tetracycline (Duchefa Biochemie, Haarlem, Holland) in 1 L distilled water for 24 h at room temperature. Afterwards, they were rinsed three times with tap water. Sterilized seedlings were planted in 1 L pots filled with forest soil and transferred to the greenhouse.

The soil was obtained from a mature beech stand in the Tuttlingen forest (47°58′43″N, 08°44′53″E, see Dannenmann et al., 2009 for more details) by collecting the Ah horizon (20 cm depth). The soil was sieved through a mesh (1 cm width). Half of the soil was kept in darkness at 4 °C until planting. The other half was sterilized three times by autoclaving at intervals of seven days, at 121 °C and 0.11 MPa (HST 6 × 6 × 6, Zirbus Technology, Bad Grund, Germany). The pH values of the sterilized and the original soil were 6.46 (±0.07) and 7.02 (±0.02), respectively. Soil nutrient elements were determined by inductively coupled plasma atomic emission spectroscopy (Spectro Analytical Instruments, Kleve, Germany) after the dried soil powder was ashed in 65% HNO_3 at 170 °C for 12 h (Heinrichs et al., 1986). The soil element composition (means of $n = 5$, per treatment ±SE) was unaltered ($P > 0.05$) by sterilization: C (99.47 ± 1.69 mg g^{-1} dry soil), N (5.85 ± 0.11 mg g^{-1} dry soil), P (0.92 ± 0.01 mg g^{-1} dry soil), S (0.79 ± 0.01 mg g^{-1} dry soil), K (10.75 ± 0.17 mg g^{-1} dry soil), Ca (12.58 ± 0.17 mg g^{-1} dry soil), and Mg (7.35 ± 0.11 mg g^{-1} dry soil), with the exception of Mn which increased from 0.92 ± 0.02 to 0.99 ± 0.02 mg g^{-1} dry soil (+7%, $P = 0.019$).

The seedlings were grown in a greenhouse under ambient conditions and irrigated at field capacity. After four months, eight seedlings grown in sterilized and original soil, respectively, were harvested for biomass determination, nutrient element analyses and evaluation of the mycorrhizal status (see below). The roots of seedlings planted in original soil were about 40% colonized by a community mainly composed of *Cenococcum geophilum*, *Tomentella punicea*, *Tuber rufum*, and two further unknown *Tuber* sp., whereas roots of seedling in sterilized soils were completely nonmycorrhizal (Pena, 2011). The EM and NM beeches were then transferred individually to 660 mL pots filled with a mixture of fine sand (0.4–0.8 mm), gross sand (0.7–1.2 mm) and peat (4:5:1). The pots were placed in a greenhouse with ambient light at 16 h day length achieved by additional lighting and maintained at 20 °C and 55% relative air humidity. The EM and NM beech plants were exposed to two light levels: (L) full light, with a photosynthetic active radiation (PAR) of 200 μmol m^{-2} s^{-1} (lamps series 3071, Schuch, Worms, Germany) and (S) low irradiance with 35–40 μmol PAR m^{-2} s^{-1} at plant height obtained by installing a green double layer polyethylene shading net (5 mm × 5 mm mesh Mayer, Rellingen, Germany) above the seedlings. The irradiance levels were measured using a quantum photometer Li-185B with a quantum sensor Li-190SB (LiCor INC., Lincoln, USA). The plants were irrigated at 2-h intervals during the light phase, with the first irrigation starting two hours after the beginning of the light phase and the last one two hours before the day ended. Each plant received eight mL of a Hoagland-based nutrient solution after Dyckmans and Flessa (2001) [0.4 mM NH_4Cl, 0.05 mM $NaSO_4$, 0.1 mM K_2SO_4, 0.06 mM $MgSO_4$, 0.13 mM $CaSO_4$, 0.03 mM KH_2PO_4, 0.005 mM $MnSO_4$, 0.005 mM $FeCl_3$, 0.15 μM $ZnCl_2$, 0.1 μM MoO_3, 0.064 μM $CuCl_2$ (pH 3.9)] per irrigation event. The soil moisture content was measured with ThetaProbe ML2X soil moisture sensors equipped with a HH2 readout unit (Delta-T Devices Ltd, Cambridge, UK) and was maintained above 0.040 m^3 m^{-3}. At regular time intervals, five samples of drainage solution from each treatment were collected for pH measurements. The mean pH of the drainage solution did not vary with the growth conditions and averaged 4.32 ± 0.17. Since low pH and soil sterilization affect Mn availability, the Mn concentrations of six-months old plants from all growth conditions were controlled and amounted 0.9 ± 0.1 mg Mn g^{-1} dry mass in NM and 0.2 ± 0.1 mg Mn g^{-1} dry mass in EM plants, which is the range of values found in beech under field conditions (0.1–1.7 mg g^{-1}, Duquesnay et al., 2000; Heilmeier et al., 2000; Jochheim et al., 2007).

2.2. Drought stress and ^{15}N application

After six weeks in sand culture, half of the seedlings from each growth regime were subjected to drought stress for 16 days. During the drought treatment the volume of the watering solution