Temperature effects on mycorrhizal diversity and fine root sistem of beech seedlings

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Introduction

Mycorrhiza is the main spatial and temporal linkage between different constituents in a forest ecosystem. Knowledge on the ectomycorrhizal types-species composition, their abundance, physiology and ecology is necessary in order to understand the functioning of a forest ecosystem, and it is applied in the mycobioindication of stress in forest soils (Kraigher et al, 1996). European beech (*Fagus sylvatica* L.) is the main naturally occurring, economically and ecologically important tree species in more than 60% of Slovenian forests, therefore beech seedlings were used in the study of temperature effects on its roots and mycorrhiza.

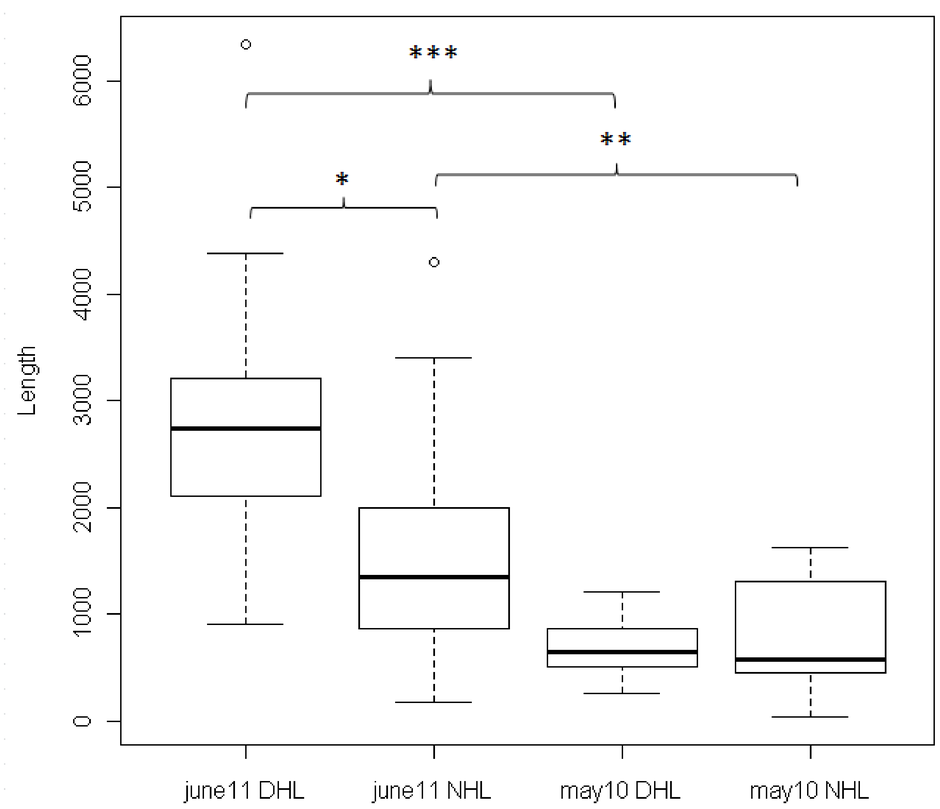
Description of experimental conditions

- Beech seedlings were obtained from the tree nursery Omorika Muta d.o.o., and planted into transparent rhizotrons in November 2010.
- They were grown at four different temperature regimes (15-25° C (NHL), 15-25° C and cooling of roots (DHL), 30-50° C, outside air temperature in Ljubljana)
- 17 seedlings were grown in covered transparent rhizotron boxes (2x30x50 cm) at each temperature regime
- Both 15-25° C regimes included an elevated CO_2 atmosphere

Methods

We have monitored the occurrence of types of ectomycorrhiza (ECM) on beech seedlings grown in rhizotrons at four different temperature conditions (15-25° C, 15-25° C and cooling of roots, elevated temperature in a greenhouse, outside air temperature).
Fine root growth (in the cooled and not-cooled treatments) was studied using Rootfly©, types of ECM were identified by anatomical-morphological method (Agerer, 1991), sequencing of the ITS1-5.8S rDNA-ITS2 ribosomal region and construction of phylogenetic trees (Grebenc et al, 2010).
Phylogenetic analysis was performed on a combined dataset of *Scleroderma* ITS1-5.8S rDNA-ITS2 sequences discovered in this study and retrieved from Genbank and UNITE online nucleotide databases. *Tremellogaster surinamensis* (EU718127) ITS1-5.8S rDNA-ITS2 sequence was used as an outgroup. Sequences were aligned with MAFFT v6 (Katoh and Toh, 2008) online multiple sequence alignment application (http://mafft.cbrc.jp/alignment/server/) using L-INS-i alignment strategy. Phylogenetic tree was constructed with MEGA v5.05 (Tamura et al, 2011) using Neighbour-Joining method and Tamura 3-parameter model of nucleotide substitutions considering Gamma distribution of variable sites. Robustness of nodes was assessed with a 1000-replicate Bootstrap analysis.
Statistical analyses were made with statistical programme R Version 2.12.2.

Results



- There were significant differences (p=0,01075) in root length (mm) between seedlings growing at 15-20ºC with additional (DHL) and without additional (NHL) cooling of root sistem in June 2011.
- There were also significant differences in root length (mm) of seedlings from May 2010 to June 2011 in both treatments DHL (p<0,001) and NHL (p=0,00847). In both cases roots in June 2011 were significantly longer than in May 2010, as expected.
- There were no significant differences in root length (mm) between seedlings growing at DHL and NHL in May 2010 , because all seedlings were young and of the same age at the begining of spring growth.
- DHL – 15-20ºC with additional cooling of root sistem for 4-5º C, NHL – 15-20ºC with no additional cooling of root sistem for 4-5º C
- * p<0.5, ** p<0.01, *** p<0.001

Phylogenetic analysis regrouped all of the *Scleroderma* ITS1-5.8S rDNA-ITS2 sequences discovered in this study into the same clade which also contained five *S. areolatum* and two *S. verrucosum* sequences. Low level of intra-clade genetic differences suggests that this clade is homogenous and probably corresponds to a single species. According to available information it was impossible to unambiguously determine which, if any, of the two above mentioned *Scleroderma* species this clade actually represents.

Conclusions

Summer and spring growth results confirm the hypothesis that soil temperature influences growth and development of beech fine roots. Different temperature conditions influenced the fine root length. At lower belowground temperature and in the same air temperature roots of seedlings grew faster than roots of seedlings without additional cooling of the root sistem.

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R version 2.12.2 (2011-02-25)
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