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Abstract

The introduction and cultivation of alien (i.e. non-native) plant species came out to be a global phenomenon over the last centuries. Today, the plantations of alien tree species are an economically important part of forestry in numerous countries around the world. In Europe, extensive introduction of alien tree species to the native forest ecosystems occurred in the XIX and XX centuries, due to the decreasing timber resources of native forests. Over time, some introduced alien tree species started to spread in the native ecosystems on their own, effectively compete with native tree species and modify the biotic and abiotic conditions in local environments. Although only a minority of introduced alien species could become invasive ones, biological invasions of alien tree species pose a serious challenge for nature conservation and forest management. Due to the strong negative impact on the native ecosystem, invasive tree species pose a crucial threat to native species, especially rare ones and native ecosystems, generating billions of dollars of losses.

In the last few decades, the role of symbiotic organisms, e.g., mycorrhizal fungi, was proved as crucial to the acclimation and naturalisation of alien tree species outside their native range, and mutualistic mycorrhizal fungi were revealed to be drivers of global-scale invasion of alien tree species. Mycorrhizal fungi are a key element in the forest ecosystem, because they provide trees with an efficient acquisition of nutrients e.g. phosphate and nitrogen, and increase trees' resistance to unfavourable abiotic conditions (water conditions, climate, soil physicochemical properties) and biotic interactions (pathogens, competition). In temperate and boreal forests, almost all tree species form ectomycorrhizal symbiosis, one of two main types of mycorrhiza. Etomycorrhiza, a specific symbiosis of trees, is essential for the proper development of almost all native trees in Europe.

Until now, not much is known about the interactions between alien and invasive tree species and native ectomycorrhizal fungi, especially non-invasive alien tree species, which pose a lower risk to native ecosystems than invasive trees, thus attracting less attention from researchers.

This study aimed to determine the interactions between alien and invasive tree species and native ectomycorrhizal fungi. For this purpose, North American hickory trees (*Carya laciniosa*, *C. cordiformis*, *C. ovata*) and northern red oak (*Quercus rubra*) were studied. Northern red oak belongs to the alien to Europe subgenera *Lobatae* and is considered one of the most invasive tree species in Europe due to its negative impact on native ecosystems. Hickories do not reveal the traits of invasive species and seem to have no negative impact on native forest ecosystem.

To determine how the mycorrhizal symbiosis of alien and invasive tree species is shaped under different environmental conditions, trees were studied under common garden conditions in Kórnik Arboretum and in forest areas. In the first step, the natural regeneration of *C. cordiformis* and *C. laciniosa* under the canopy of mature hickory trees in Kórnik Arboretum were analysed. This study shown the ectomycorrhizal fungi associated with hickories, which grown surrounded by hundreds native and alien ectomycorrhizal trees from different continents. In the second step, both *C. ovata* stands from surviving old experimental plantations of alien trees established in the XIX century in Central and Western Poland and surrounding native forest were examined for the comparison their ectomycorrhizal communities. In the third step, ectomycorrhizal communities were compared between mature *Q. rubra*, *C. ovata* and *C. laciniosa* trees grown together in Kórnik Arboretum. In their native range in North America, they form oak-hickory forest ecosystems.

The ectomycorrhizal fungal species associated with tested trees were identified using the morphological assessment and molecular analyses. Ectomycorrhizal root tips were classified into morphotypes based on their morphological characteristics and the DNA was isolated out of each selected morphotype. In total, 132 root samples were analysed: 72 from Kórnik Arboretum and 60 from forest stands. The fungal ITS rDNA region was amplified from DNA extracted from the individual root tip, and sequences using Sanger Sequencing. Obtained sequences were assigned to the fungal taxa based on their similarity to the reference ITS sequences deposited in UNITE and GenBank databases, and then assigned to the phylogenetic lineages of ectomycorrhizal fungi.

Statistical analyses were prepared using quantitative and qualitative parameters: percent root length colonization by mycorrhizal fungi, the share of living ectomycorrhizal root tips in relation to the dead root tips, the frequency and abundance (percentage share) of each identified fungal taxa, and phylogenetic lineages of ectomycorrhizal fungi, which were determined based on the number of ectomycorrhizal root tips represented by each identified fungal taxon. The mean and total species richness of ectomycorrhizal fungi, the Shannon diversity and Simpson dominance indices, analysis of variance (ANOVA), similarity analysis (ANOSIM), nonmetric multivariate scaling (NMDS), canonical correspondence analysis (CCA) and the Chao-2 species richness estimator were calculated using PAST, Statistica 9.0, Canoco 4.5 and EstimateS 9.0.

Altogether 137 ectomycorrhizal (ECM) fungal taxa were identified on the roots of tested alien tree species. They belong to 40 fungal genera and 29 phylogenetic lineages of ECM fungi from orders Basidiomycota (27 genera of 17 lineages) and Ascomycota (13 genera of 12 lineages).

Out of 137 ECM taxa, 40 ECM taxa were noted on the roots of natural regeneration of hickory trees in Kórnik Arboretum, 65 taxa on the roots of mature hickory stands in forest conditions, and 73 taxa on the roots of mature trees in Kórnik Arboretum: 40 on hickories and 44 on red oak.

Only native to Europe ectomycorrhizal fungal species were detected. Species richness of ectomycorrhizal fungi associated with hickory trees was higher than expected, both on hickory roots in Kórnik Arboretum and under forest conditions. On the genera level and phylogenetic lineages level, the ectomycorrhizal fungi associated with hickories in Europe correspond to those associated with hickories in their native range in North America. Thus can be suspected, that under different habitat conditions and tree ageing, hickories in Europe can establish symbiotic interactions with a rich pool of local, native ectomycorrhizal fungi, which plays a crucial role in naturalisation of alien ectomycorrhizal tree species outside their native range. Around 90% of ectomycorrhizal fungi noted on the roots of hickory trees are known to form symbiosis with oaks (*Quercus* spp.), which are relatively close related to hickories (both belong to Fagales).

The significant differences were noted between ectomycorrhizal communities of hickories and native trees under forest conditions, and red oak compared to hickories in Kórnik Arboretum. In forest conditions, hickory trees generate favourable conditions for rich pule of ectomycorrhizal fungi, most likely due to the specific microhabitats under the canopies of mature hickories, moist and shaded more than forest floor under the canopy of native trees in the surrounding. The study on the mature red oak and hickory trees under common garden conditions in Kórnik Arboretum has shown significant differences between their ectomycorrhizal communities. Significant more dead ectomycorrhizal root tips and significantly lower mean ectomycorrhizal fungal richness were noted on the roots of northern red oak, than hickory trees. Moreover, a high share of long-distance exploration type of ectomycorrhizas was noted on the roots of northern red oak (about 30% of ectomycorrhizal root tips), but low on the roots of hickories (2% of ectomycorrhizal root tips).

Presented studies have shown, that alien ectomycorrhizal tree species probably have low to no negative impact on the local ectomycorrhizal fungi in native ecosystems. However, further studies on the impact of invasive non-ectomycorrhizal tree species are needed. The influence and potential negative impact of invasive arbuscular mycorrhizal trees such as *Prunus serotina* and *Robinia pseudoacacia* on the ectomycorrhizal fungi in native forest ecosystems remain unknown.