No difference in ectomycorrhizal morphotype composition between abandoned and inhabited nests of wood ants (*Formica polyctena*) in a central European spruce forest

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ABSTRACT

In temperate forest ecosystems, ectomycorrhizal (EcM) fungi substantially support coniferous trees by nutrients, but importance of EcM for tree nutrition might be lower in nutrient-rich hotspots, such as in wood ant nests. In this study, we focused on differences in EcM morphotype composition between natural nutrient-rich hot spots of various quality (inhabited vs. abandoned wood ant nests) and the surrounding forest floor. In a secondary Norway spruce forest typical for central Europe, we took samples from different belowground parts of five inhabited and five abandoned nests (centre and rim) as well as from the forest floor 20 cm from the nests (run-off zone) and > 3 m from the nests (forest soil). The substrates were subjected to analyses of the EcM morphotype composition, moisture and nutrient contents. Wood ant nest substrates had several times increased nutrient contents compared to the forest soil, but there were no significant differences in moisture. Available nutrient contents were higher in inhabited nests than in abandoned nests. The EcM morphotype composition differed between the nest samples and the forest floor samples but not between the inhabited and abandoned nests suggesting that, apart from the level of nutrient enrichment and their availability, moisture is another important factor affecting EcM community composition.

1. Introduction

Coniferous forest soils are usually acidic (Brady and Weil, 2002) due to fulvic acids contained in high concentrations in coniferous litter (Berg and McClaugherty, 2008). When released from the litter during decomposition, fulvic acids leach nutrients from the organic soil layer to lower soil horizons, which subsequently makes the surface layers nutrient-limited. Trees growing in such forests are mostly limited by low availability of nitrogen (N) (McGroddy et al., 2004) and phosphorus (P) (Batjes, 1997). However, this limitation can be alleviated by symbiotic associations of roots with ectomycorrhizal (EcM) fungi (Smith and Read, 2008) or by exploitation of nutrient-rich hot spots (Hutchings and de Kroon, 1994; Shemesh et al., 2010).

Most temperate and boreal tree species form symbiosis with EcM fungi which often extend the root exploitation area due to the production of the extraradical mycelium (Plassard and Dell, 2010) and help to gain otherwise inaccessible organically bound nutrients in exchange for plant photosynthetically bound carbon (C) (Smith and Read, 2008). In addition, EcM fungi increase root resistance against a wide range of both abiotic and biotic stressors such as drought, heavy metals, herbivores and soil pathogens (Smith and Read, 2008).

An alternative way to gain the necessary nutrients is to exploit nutrient-rich hot spots in coniferous forest soil, such as decomposing stumps (Palviainen et al., 2010), buried organic horizons (Werdin-Pfisterer et al., 2012) or wood ant nests (Frouz et al., 2016). In suitable forest ecosystems, wood ants (*Formica* s. str.; Hymenoptera: Formicidae) build large and numerous mounds from the mixture of plant material and mineral soil cemented by ant saliva and resin particles (Kristiansen and Amelung, 2001; Frouz and Jílková, 2008; Frouz et al., 2016; Risch et al., 2016). Nest material is kept drier compared to the surrounding soil due to water-repellent surface layer and good aeration of the nests (Seifert, 1996). Large amounts of food in the form of aphid

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honeydew and insect prey are accumulated in the nests due to ant foraging activities (Horstmann, 1974), which leads to the depletion of the close vicinity of nests (i.e. the run-off zone). The food and the plant material are decomposed by microorganisms which are most active in the centre of the aboveground parts of nests where the highest moisture and temperature is maintained (Coenen-Staβ et al., 1980; Frouz, 1996, 2000). This leads to the release of mineral nutrients increasing nutrient-enrichment and pH of the nests and the run-off zone (Frouz et al., 2005; Kilpelainen et al., 2007; Jurgensen et al., 2008; Jílková et al., 2011) and thus spatial heterogeneity in forest ecosystems (Kilpelainen et al., 2007; Finér et al., 2013). Available nutrients can be further exploited by tree roots growing at higher densities in the belowground parts of the nests (Ohashi et al., 2007) and in the nest close surroundings (Jílková et al., 2017), which leads to changes in tree growth in the nest vicinity (Frouz et al., 2008; Jurgensen et al., 2008).

Wood ant nests are often inhabited for several decades (Hölldobler and Wilson, 1990) but can be abandoned when their occupants move to a new territory (Kristiansen and Amelung, 2001) or due to the damage by wild animals and clear cutting (Risch et al., 2016). In the abandoned nests, the high temperature and low moisture is no longer maintained by the ant activity and the food and the plant material are no longer accumulated. However, the nutrient concentrations in the nest material remain high for several years or even decades after the abandonment (Kristiansen and Amelung, 2001; Kristiansen et al., 2001; Frouz et al., 2005, 2016) due to accelerated decomposition of the nest material (Domisch et al., 2008). In addition, contrary to the inhabited nests where active pruning by ants and low moisture prevent roots from growing into the nest material (Woodell and King, 1991), the whole nest structure can be exploited by roots after the abandonment (Kristiansen and Amelung, 2001).

The occurrence and diversity of EcM fungi as well as the amount of their soil mycelium are often negatively influenced by increased nutrient availability because plants growing in nutrient-rich substrates tend to allocate carbohydrates elsewhere than to their fungal symbionts (Treseder, 2004; Børja and Nilsen, 2009). As a consequence, EcM fungi may become C-limited (Read, 1991). However, the EcM community may eventually be rearranged and become dominated by fungi adapted to high nutrient levels because different EcM species play different roles in acquiring N and P from distinct sources (Wallander et al., 1997; Cox et al., 2010).

Because previous studies investigating EcM community composition aimed mostly at artificially created nutrient-rich hot spots with very high nutrient concentrations (e.g., Helmisaari and Hallbäcken, 1999; Treseder, 2004; Grønli et al., 2005; Børja and Nilsen, 2009), our knowledge about naturally occurring nutrient-rich hot spots and their exploitation by EcM roots is largely limited. For example, our preliminary study focusing on the EcM morphotype composition in spruce seedlings growing in wood ant nests was limited to an in vitro incubation experiment (Jílková et al., 2015). Therefore in the current study, we focused on the differences in the EcM morphotype composition between inhabited and abandoned wood ant nests and their surroundings in relation to the root biomass and substrate moisture and nutrient contents under natural conditions. Our hypotheses were that: 1) moisture is higher in abandoned nests but nutrient contents are similar in inhabited and abandoned nests, 2) the root biomass is higher in abandoned nests due to increased moisture and absence of pruning by the ants, and 3) the EcM morphotype composition differs between the two types of the nests and their surroundings due to changes in moisture and nutrient contents and absence of pruning by the ants.

2. Materials and methods

2.1. Study site and sampling procedures

Samples from the nests and their surroundings were collected in June 2015 in a ca. 100-year-old secondary forest dominated by Norway spruce (*Picea abies* (L.) H. Karst.) with some sparse grass understory, situated in the southern part of the Czech Republic, about 20 km northeast from the city of Tábor (49°27′47″N, 15°50′12″E; elevation ca. 700 m above sea level). At the collection site, the average annual temperature is 7.1 °C and the annual precipitation is 586 mm. The forest contains several hundred nests of the wood ant *Formica polyctena* Förster scattered over an area of ca. 10 ha, with the average density of 12.5 nests per ha.

Five inhabited and five abandoned nests of similar size (ca. 1 m³) were selected. The same abandoned nests as in our previous study (Jílková et al., 2015) were sampled; they were abandoned during the 2011/12 winter, i.e. already for 3.5 years. Taking advantage of our long-term experience with the nests in this area, we selected those with the substrate moisture content ranging from 20 to 35%, i.e., covering most of the moisture range available (Frouz, 1996, 2000). The samples were taken with a soil corer (diam. 4 cm, depth 10 cm) at four locations: 1) the centre of the nests (from the central bottom part of the nests at the same level as the surrounding soil surface); 2) the soil rim of the nests; 3) the run-off zone (ca. 20 cm from the nests) to which the nutrients from the nests are leached; and 4) the surrounding soil (> 3 m from each of the nest in the surroundings). To sample the belowground parts of the nests, we firstly carefully uncovered the aboveground parts of nests with a shovel. The samples were taken randomly in six replicates per nest and location (i.e. from six different directions from the nest centre) and transported immediately to the laboratory for processing.

2.2. Sample analyses

Three sample replicates per nest were pooled and used for the determination of root biomass and substrate properties. The roots were hand-picked, washed under running tap water and dried at 60 °C for one day to obtain root dry weight. The remaining substrate samples were then sieved through a 5-mm sieve and thoroughly mixed. Field-moist substrate samples were used for measurements of microbial respiration and microbial biomass (C_{mic}) and ergosterol, ammonium (NH_4^+), nitrate (NO_3^-) and moisture content. Air-dried substrate samples were used for organic matter (OM), total carbon (C_{tot}), total nitrogen (N_{tot}), total phosphorus (P_{tot}), available phosphorus (P_{avail}), calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^+) content analyses.

Microbial respiration was determined as CO_2 absorbed in 1 M NaOH solution after a three-day incubation in air-tight vessels by titration by 0.01 M HCl (Page, 1982). C_{mic} was determined using the fumigation-extraction method (Vance et al., 1987). For ergosterol extraction, field-moist substrates were freeze dried and crushed and total ergosterol was extracted and analysed following Šnajdr et al. (2008). Briefly, the samples (0.500 g) were sonicated with 3 ml of 10% KOH in methanol at 70 °C for 90 min. A 1-ml volume of distilled water was added and the samples were extracted three times with 2 ml of cyclohexane, evaporated under nitrogen, re-dissolved in methanol and analysed isocratically using an HPLC chromatograph with methanol as the mobile phase and a flow rate of 0.8 ml min^{-1}. Ergosterol was identified by UV detection at 282 nm. Ergosterol was used as an indicator of the EcM fungal biomass because previous studies including both saprotrophic and EcM fungi have reported strong positive correlations between the ergosterol content and the number of EcM root tips (e.g. Priha et al., 1999). NH_4^+-N and NO_3^--N were extracted from the substrate samples using a 0.0125 M CaCl$_2$ solution. NH_4^+-N was determined by a modified Berthelot reaction (Schinner et al., 1996). The determination was based on the reaction of sodium salicylate with NH_3 in the presence of sodium dichloroisocyanurate; this reaction generates a green complex. Sodium nitroprusside was used as a catalyst. NO_3^--N was assessed by UV absorption at 210 nm (Schinner et al., 1996). Controls were performed by reducing nitrate with copper-sheathed granulated zinc. To determine their moisture, substrates were dried at 105 °C for 12 h.