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Influence of season and edaphic factors on endorhizal fungal associations in subtropical plantation forest trees of Northeastern India

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ABSTRACT

Investigations on arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungi are limited in trees of subtropical forests compared to other terrestrial ecosystems. We assessed the seasonal dynamics of endorhizal fungal symbioses in four dominant tree species (*Alnus nepalensis*, *Castanopsis hystrix*, *Emblica officinalis*, *Schima wallichii*) in two plantation forest stands of Northeastern India, a biodiversity hotspot part of the Indo-Burma region. All tree species examined had a dual association of AM and DSE fungi and such fungal symbioses are reported for the first time in *A. nepalensis*, *C. hystrix* and *S. wallichii*. Intermediate-type AM morphology was common in three tree species, while *Paris*-type AM morphology was found in *S. wallichii*. Colonization of AM and DSE fungi varied significantly across tree species and seasons. A total of 18 AM fungal spore morphotypes were isolated from the air-dried natural rhizosphere soils and trap culture experiments. Spore density of AM fungi was highest in summer, whereas AM colonization in roots was highest during the rainy season. In contrast, root colonization with DSE fungi was highest during summer, except for *E. officinalis*. A significant negative correlation occurred between certain AM fungal and soil variables. In contrast, DSE fungal measures were not correlated to any of the soil properties examined. Correlation analysis indicated a certain degree of competition between AM and DSE fungi. Occurrence of AM and DSE fungal associations in economically important indigenous tree species indicates the possibility of utilizing them in future conservation programs.

1. Introduction

Impact of accelerated ecological devastation on natural biodiversity loss has increasingly drawn attention in the tropics, where deforestation and modern agricultural practices most threaten the flora and fauna. Therefore, a special focus is now laid on biodiversity hotspot regions which are highly complex and species–rich ecosystems both in above and below ground biota (Haug et al., 2013). One example of a major hotspot of global biodiversity are the tropical and subtropical forests of Northeastern (NE) India along the Indo-Burma border region (van Dijk et al., 2004). Although the highly diverse flora and fauna of this region have been well documented (Ramakantha et al., 2003), the microbial groups and particularly arbuscular mycorrhizal (AM) fungi (AMF, phylum Glomeromycota) have not attracted much attention as yet (Sharma and Jha, 2014). The hilly ethnic tribes of NE India rely heavily on forest resources for their subsistence due to which natural forests are over exploited for timber, fuel wood and slash and burn agriculture (Jhum) (Ramakrishnan, 2007). The Forest Departments manage/rehabilitate the sites disturbed by Jhum by raising mono- or polyculture plantations of *Alnus nepalensis* D. Don (family Betulaceae), *Castanopsis hystrix* Hook. f. & Thomson ex A. DC. (family Fagaceae) and *Schima wallichii* Choisy (family Theaceae), but with low success (Bhatt and Tomar, 2002; Poffenberger, 2006).

Arbuscular mycorrhizal fungi are essential components of sustainable plant-soil systems and play a crucial role in ecosystem functioning (Smith and Read, 2008). The ability of AMF to colonize roots and provide nutrients to the host differs among morphological AM types, i.e. *Arum*-, *Paris*- or Intermediate type (Dickson, 2004; Dickson et al., 2007). Extraradical hyphae of AMF proliferate in the surrounding root-soil systems, absorb nutrients of low mobility, such as phosphorus (P), which otherwise would be inaccessible to the plants, and translocate them to host roots. AM fungal hyphae

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are also important for conservation of soil through formation of stable aggregates in degraded habitats (Miller and Jastrow, 1990). Soil disturbances have been reported to cause a sharp decline in the number of active AM propagules and the intensity of root colonization (Jasper et al., 1989). Short-term consequences of slash and burn practices on spore production and intraradical colonization of AMF have also been observed (Aguilar-Fernández et al., 2009). Since AMF are sensitive to physical, chemical and biological conditions of the soil, studies assessing the distribution and quantity of AMF are important to understand the plant-fungus-soil interaction (Becerra et al., 2007).

Another group of non-pathogenic root colonizing fungi, the dark septate endophytes (DSE; phylum Ascomycota), are characterized by melanized septate hyphae and compactly arranged intracellular microsclerotia. Like AMF, DSE fungi are also common in natural forest ecosystems colonizing up to 80% of fine roots in forest stands (Grünig et al., 2008). They coexist with AMF in a wide range of hosts at different latitudes and altitudes and potentially confer benefits to the plants by mineralizing nutrients in the rhizosphere and enhancing their stress tolerance (Mandyam and Jumpponen, 2005). Since the investigation of DSE fungi in roots of more than 600 plant species by Jumpponen and Trappe (1998), there is a renewed interest in studying the occurrence, taxonomic diversity and host range of these fungi in different biome types and climate regimes (Mandyam and Jumpponen, 2005; Knapp et al., 2012).

Many workers are of the opinion that AM and DSE fungal inoculation technology can be used in restoration and reforestation of fragile and degraded habitats (Pattinson et al., 2004; Newsham, 2011; Tawaraya and Turjaman, 2014). It has been suggested that both early- and late-successional woody tropical species are highly responsive to AM fungal inoculation (Zangaro et al., 2003; Middleton and Bever, 2012). Further, meta-analyses by Hoeksema et al. (2010) also indicated that woody plants are more responsive to mycorrhizal inoculation than nodulating and nitrogen-fixing herbaceous plants and grasses. Some tree species simultaneously associate with both ectomycorrhizal (EcM) fungi and AMF. The cooccurrence of AMF and EcM fungi in the same root system shows that the capability to form AM has been maintained to some degree in plant taxa which are typically EcM (Moyersoen and Fitter, 1999). However, the role of AMF in such tree species has been a matter of controversy during the past decades due to inconsistent results (Kariman et al., 2014). Studies have shown positive effects of AMF on the growth of tree species that have the potential to form ectomycorrhizae under P deficient conditions (Adjoud et al., 1996; Chen et al., 2000; Kariman et al., 2014), and a succession of these mycorrhizal types has been reported during different stages of plant growth (Santos et al., 2001). Investigations on the influence of DSE fungi on plant growth further indicate that inoculation with these fungi can increase biomass as well as nutrient content of plants (see Newsham, 2011 and references therein). In view of the potential importance of these fungi for vegetation conservation and sustainable forestry, a detail survey of indigenous AM fungal diversity and presence of DSE fungi in this hotspot region would provide a basis for rehabilitation of abandoned ecosystems.

Many researchers have investigated the seasonal and habitat differences in community composition of AMF in different ecosystems around the globe (Öpik et al., 2006). Unlike for AMF, occurrence and seasonal distribution of DSE fungi are not well resolved (Muthukumar and Udaiyan, 2002b; Newsham, 2011). To understand the structure, function and basic biology of these endorhizal fungi in natural habitats, it is essential to explore the spatial variations affecting plant-fungus associations (Uma et al., 2012). For example, abundance of arbuscules indicates fungal benefit to the host, whereas abundance of hyphae and vesicles indicate carbon drain from the host (Sanders and Fitter, 1992). Nevertheless, information on the temporal dynamics of AM and DSE fungal colonization in the plants growing in subtropical forests are still sporadic (Zhang et al., 2004; Knapp et al., 2012). Wang and Qui (2006) pointed out, that the first area that deserves more attention in current mycorrhizal research is the examination of as many plant species belonging to different biome types for their fungal symbiotic status. This would provide opportunities to understand how different root associated fungi occur in an ecosystem. We determined the seasonal distribution of AMF spore morphotypes in rhizosphere soils, and colonization levels of endorhizal fungi as well as AM morphology in roots of four dominant woody trees in subtropical plantation forest stands of NE India.

2. Materials and methods

2.1. Study site

The study was conducted at two sites (hereafter referred to as stand A and B) of the subtropical mixed plantation forest. Both stands are located within 40 m distance on the gentle slopes of the hillock along the Taphou Naga Hill range (Location: 25° 17′ 13.″–25° 17′ 26.5″N; 94° 01′ 24.7″–94° 01′ 43.8″E), at elevations between 1132 and 1154 m a.s.l., 2 km north-west of Senapati District Headquarter and 62 km north of Imphal, the capital city of Manipur, India. Manipur Hills along with Naga and Mizo hill ranges consist of mainly tertiary strata and came into existence as a result of Tertiary folding of sedimentary strata in the shallow Tethys Sea, about 40–90 million years ago (Yadava, 1990). Geographically, about 90% of the total area of Manipur is covered by hill ranges which are the offshoots of eastern Himalayas with about 1813 km^2 of the central valley area (Sehgal et al., 1993). The climate of the area is monsoonic and the year is divisible into three distinct seasons characterized by warm moist summers with occasional mild precipitation (April–June), wet rainy months (July–September) and cool dry winters (November–February). March and October comprise the transitional periods between winter and summer, and rainy and winter seasons, respectively. At both forest stands, the mean minimum and maximum temperature during the study period ranged between 1.1 °C and 14.2 °C and 24.4 °C to 34.2 °C, respectively (Fig. 1). The mean monthly averages of relative humidity (RH) varied from 57.8% to 95.6%. The total annual rainfall was 1240 mm of which 73% occurred between April–September 2013. According to USDA classification, the soil is Ultisol developed from shale and sandstones on gently sloping narrow valleys to steep side slopes of hills and is heterogeneous in nature (Sehgal et al., 1993). The soil of stand A was shallow, i.e. 10–20 cm deep, rocky type, blackish in colour and sandy loam with regard to texture (sand 68.1%, silt 16.2%, clay 15.7%), while that of stand B was 20–30 cm deep, grayish in colour and loamy sand (sand 74.2%, silt 15.3%, clay 10.4%). Since both stands are from the same parent material, the changes in soil texture and colour are due to variation in geomorphic landscapes. Soil pH at both stands was slightly acidic. *Alnus nepalensis* was the dominant tree species at stand A, along with the woody plants *S. wallichii* and *Ficus hispida* Linn. In stand B, *C. hystrix* was the most common plant species and coexisted with *Emblica officinalis* Gaertn. (Euphorbiaceae) and *Quercus serrata* Thunb. *A. nepalensis*, *C. hystrix* and *S. wallichii* are the fast growing dominant colonizers of denuded habitats and eroded hill slopes of NE India. They are predominant in both natural and managed ecosystems and were used successfully as pioneer plants in forest regeneration and for the reclamation of nutrient-depleted soils (Bhatt and Tomar, 2002; Ramakrishnan, 2007). *Emblica officinalis* is a medium sized deciduous tree species which is especially important for its high medicinal values. Details of vegetation parameters are shown in Table 1.