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Diversity and community structure of ectomycorrhizal fungi in a wooded meadow

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ABSTRACT

Wooded meadows are seminatural plant communities that support high diversity of various taxa. Due to changes in land use, wooded meadows have severely declined during the last century. The dominant trees in wooded meadows acquire mineral nutrients *via* ectomycorrhizal fungi. Using anatomotyping and sequencing of root tips, interpolation and extrapolation methods, we studied the diversity and community structure of ectomycorrhizal fungi in two soil horizons of both managed and forested parts of a wooded meadow in Estonia. Species of *Thelephoraceae*, *Sebacinaceae* and the genus *Inocybe* dominated the whole ectomycorrhizal fungal community of 172 observed species. Forested and managed parts of the wooded meadow harboured different communities of ectomycorrhizal fungi, whereas soil horizon had a negligible effect on the fungal community composition. Diverse soil conditions and host trees likely support the high richness of ectomycorrhizal fungi in the wooded meadow ecosystem. Direct sequencing integrated with interpolation and extrapolation methods are promising to identify the fungi at the species level and to compare species richness between communities of ectomycorrhizal fungi.

Introduction

Wooded meadows are seminatural, sparsely wooded ecosystems that have developed due to hay-making or sheep grazing in low-productive areas in Europe. A few retained trees have created heterogeneous light conditions and soil nutrient gradients. Such patchiness, together with shallow, nutrient-poor soils, support high small-scale species richness of plants in wooded meadows (Kull & Zobel 1991).

During the last century, industrialization and urbanization have strongly affected land use and reduced the importance of traditional farming methods, driving vast countryside areas to abandonment (Vitousek 1994; DeFries 2002). Seminatural meadows and wooded meadows have been most affected among seminatural ecosystems (Kukk & Kull 1997; van Dijk 2002). Wooded meadows covered immense areas especially at low-productive coastal and mountainous sites throughout Europe. At present more than 99 % of the wooded meadows have been abandoned, and have developed naturally into thickets, bushlands and marshlands (Kukk & Kull 1997). Of similar ecosystems, North American oak savannas have declined 5000-fold due to cessation of prescribed burning in the last few hundred years (Nuzzo 1986).

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In addition to high plant diversity, wooded meadows support many threatened orchids and agarics (Kukk & Kull 1997; Kalamees 2004; Watling 2005). Of the rare fungi, fruit bodies of calciphilous *Boletus* spp. and *Entoloma* spp. are prominent in wooded meadows, but absent in nearby forests and strongly overgrown wooded meadows. Autumnal fruiting of both rare and common fungi is considerably reduced if the meadows remain unmown in summer (Kalamees 1979). Similarly, plant species richness declines in a few years after cessation of management due to shading by rapidly growing tree seedlings and bushes. Restoration of wooded meadows is costly and plant species require decades to re-establish if they are lost from the seed bank (Stampfli & Zeiter 1999).

Ectomycorrhizal (EcM) fungi provide mineral nutrition to the dominant trees (*Quercus robur*, *Betula* spp., *Tilia cordata*, *etc.*) in North European wooded meadows. EcM fungi differ in enzymatic activities (Courty *et al.* 2005) and deliver species-specific benefits to their host plants (van der Heijden & Kuyper 2003), which render their biodiversity of high importance to plant nutrition. EcM fungi are highly diverse in most ecosystems, comprising tens of observed species. Similar or even larger number of species remains undetected due to rarity and aggregated distribution of EcM fungal species on adjacent root tips (Horton & Bruns 2001; Taylor 2002). The natural mechanisms promoting high fungal diversity have remained unclear, but it seems likely that differential preference for soil conditions and host plants play the key role (Bruns 1995).

Rarefaction (interpolation) provides a powerful alternative for species richness comparisons between sites and experimental conditions. When sample size is unequal among treatments, rarefaction facilitates biodiversity comparisons by interpolating randomized species accumulation curves to the same sample size, including calculation of confidence intervals (Gotelli & Colwell 2001; Taylor 2002). Similarly, extrapolation (i.e. estimation of the amount of unobserved species) methods enable comparisons between sites and studies that employ different sampling schemes. Extrapolation methods exploit the relative number or abundance of the rarest species, or predict the plateau of species accumulation curves to estimate the total number of unseen species (Colwell & Coddington 1994; Keating *et al.* 1998).

In this study we intended to determine the community composition of EcM fungi in a single wooded meadow. We hypothesized that the community structure and species richness differ between the managed and unmanaged (hereafter forest) parts of the wooded meadow and between soil horizons. Using direct sequencing of EcM root tip DNA and species richness extrapolation, we demonstrate an unusually high below-ground diversity of EcM fungi. The forest and wooded meadow harbour different communities of EcM fungi.

Materials and methods

Study site

Sampling was performed in a traditionally managed wooded meadow and an adjacent forest at Tagamõisa, Saaremaa Island, northwestern Estonia (58°27′N, 22°00′E). The Tagamõisa wooded meadow was selected, because it comprised contrasting managed and forested areas with known history. The study area arose from the Baltic Sea *ca* 5000 y before present and lies *ca* 8 m above sea level. The climate is dry maritime with mean annual rainfall of 550 mm and mean annual temperature of +5.8 °C. The Tagamõisa wooded meadow originates at least 300 y ago (T. Ojala, pers. comm.). Haymaking and sheep grazing facilitated the development of a highly diverse flora (up to 67 species m^{-2}), including several rare orchids (Kukk & Kull 1997). The wooded meadow was partly abandoned during World War II, resulting in rapid forestation and decline in plant species richness. Several inconsistent restoration attempts resulted in partial recovery of the wooded meadow during 1970 to 1990. Since the mid-1990s, most of the area had been mown annually in July and slightly grazed in late August (Kukk & Kull 1997). The forested area has remained unmanaged for >60 y.

Vernal flooding has resulted in mound development especially in the forest. The soil is classified as a Mollisihumi-Rendzic Leptosol (FAO *et al.* 1998), with a thick O-horizon (up to 7 cm depth) and a sandy A-horizon (from 5–7 to 25–35 cm depth) on maritime sand (below 25–35 cm depth) and limestone (below 90 cm depth). Both the wooded meadow and forest were dominated by downy birch (*Betula pubescens*), common oak (*Quercus robur*), hazel (*Corylus avellana*), European aspen (*Populus tremula*), black alder (*Alnus glutinosa*) and small-leaved lime (*Tilia cordata*). Of these, *P. tremula*, *B. pubescens*, *C. avellana*, and *A. glutinosa* dominated the undergrowth. Ground vegetation of the managed meadow was predominantly composed of a dense turf of *Poaceae* spp. and *Cyperaceae* spp. The forested area was covered by sparse *Vaccinium myrtillus*, *Hepatica nobilis*, *Oxalis acetosella*, and *Carex* spp.

To obtain reference sequences for species-level identification of EcM fungi, we performed irregular fruit body surveys in summer and autumn, 2003 to 2005. The most abundant agarics included *Lactarius pubescens*, *L. evosmus*, *Leccinum* spp., *Boletus luridus*, *Inocybe rimosa* and *I. maculata*. Of taxa with resupinate fruit bodies, *Tomentella terrestris*, *T. cinerascens*, and *Amphinema byssoides* were the most common. *Peziza michelii* and *Hymenogaster* spp. were the most abundant cup fungi and taxa with hypogeous fruit bodies, respectively. *Boletus* spp. fruited exclusively in the wooded meadow, whereas *T. cinerascens*, and *A. byssoides* were found only in the forest.

Sampling

Eight plots (6 m diam) were established in both the wooded meadow and forest (Fig 1). Plots were randomly distributed between 15–60 m from the well-defined community edge and at least 25 m from each other. Half of the plots were established around randomly selected individual birch trees >50 y old (hereafter designated birch plots) and the rest around randomly selected spots. Five to six 15 × 15 cm soil cores were taken at random from each plot using a spade and a sharp knife. The soil cores were separated into two root samples: the O-horizon sample to 5 cm depth and the A-horizon sample from 15–20 cm depth. In cases where root samples comprised an insufficient amount (<30 cm in length) of living roots, another soil core was taken instead. EcM roots were manually separated from the soil and non-EcM roots after soaking the