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Colonization of new land by arbuscular mycorrhizal fungi

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ABSTRACT

The study describes the primary assembly of arbuscular mycorrhizal communities on a newly constructed island Peberholm between Denmark and Sweden. The AM fungal community on Peberholm was compared with the neighboring natural island Saltholm. The structure of arbuscular mycorrhizal communities was assessed through 454 pyrosequencing. Internal community structure was investigated through fitting the rank-abundance of Operational Taxonomic Units to different models. Heterogeneity of communities within islands was assessed by analysis of group dispersion. The mean OTU richness per sample was significantly lower on the artificial island than on the neighboring natural island, indicating that richness of the colonizing AM fungal community is restricted by limited dispersal. The AM fungal communities colonizing the new island appeared to be a non-random subset of communities on the natural and much older neighboring island, which points to high colonization potential of certain – probably early successional – mycorrhizal fungi, likely assisted by migratory birds.

1. Introduction

The 130 ha artificial island Peberholm was constructed in 1998 from seabed sediments and deeper limestone strata and serves as part of the Øresund tunnel and bridge connection between Denmark and Sweden. The island was left open to be colonized by natural dispersal, thus providing an unique opportunity to study colonization by the plant root symbiotic arbuscular mycorrhizal fungi during large scale primary succession. The fungi colonize the majority of terrestrial plant species and have profound effects on both ecosystem properties and on plant community dynamics and diversity (Streitwolf-Engel et al., 1997; van der Heijden et al., 1998a; van der Heijden et al., 1998b; Fitter, 2005). Several studies have described community structure of arbuscular mycorrhizal fungi, including re-establishment of communities after disturbance (Lekberg et al., 2011), but so far, few studies have addressed their primary succession.

Primary succession is an initial community assemblage by a functional group, and can be described by the species relative abundance. Within plant communities a change in species abundance distribution models from a geometric to a log-normal distribution are seen through succession, allegedly as a result of increased competition and niche partitioning (Whittaker, 1972). Geometric models (May, 1975) reflect extremely uneven abundances of organisms with a fractional distribution, whereas the log-normal distribution (Preston, 1948) reflects that species abundances are normally distributed with an excess of species having an intermediate abundance. Dispersal limitation of a log-normal distributed source community may lead to a local geometric community assemblage (Hubbell, 2001).

Dispersal limitation and environmental filtering are two of the main processes shaping the structure of species assemblages (Vellend, 2010; Hájek et al., 2011). Local communities get enriched through dispersal from sources in the region, but may at the same time become impoverished through selection against certain species (Shipley, 2009, Ch 1). Selection, again, may go through either environmental selection or competitive exclusion. Both dispersal limitation and environmental filtering will result in non-random subsets of the regional species pool being present in any given community. Likewise, both processes will yield an imprint in the relative abundance of species' in local communities.

Microorganisms are often assumed to have virtually unlimited dispersal capacity, which then shifts the balances between the mentioned processes entirely towards environmental filtering

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("everything is everywhere, but the environment selects" (Baas Becking, 1934; Finlay, 2002)). A study of primary succession of root-associated fungi at the front of retreating glaciers in Norway, showed a high diversity even at the early stages of succession (Blaalid et al., 2012). Noteworthy, almost all the frequent taxa were found in all exposed zones, indicating that dispersal limitation in this setting played a minor role, but in a similar study, Jumpponen (2003) found that fungi dependent on aerial dispersal dominated newly exposed areas. This suggests that fungal dispersal traits could be an important issue in understanding their primary succession and accumulating evidence supports the view that microbial dispersal is indeed limited and the balance between dispersal and environmental filtering worthy of study (Telford et al., 2006; Casteleyn et al., 2010).

AMF belong to the phylum Glomeromycota and are characterized by their relatively large resting spores. The spores are generally not well adapted to long distance passive dispersal by wind, though Warner et al. (1987) found that spores potentially can be dispersed up to 2 km by wind. Similarly, small mammals may serve as vectors for mycorrhizal spores over shorter distances, but whether mammal dispersal generally occurs among AMF – as it is known from hypogeous ectomycorrhizal fungi (Johnson, 1996) – is not known. The present study was conducted on an island with limited impact of mammals, though the island serves as a resting place for migratory birds such as geese. However, no information on birds as vectors for AMF dispersal is available in the literature. Life history traits related to dispersal may play a significant role in primary succession of these universally distributed fungi. In perennial vegetation, a significant fraction of the AM community can consist of non-sporulating taxa (Rosendahl and Stukenbrock, 2004), whereas sporulating species seem to be prevalent in annual agricultural crops (Rosendahl et al., 2009). The sporulating species are found in a number of monophyletic lineages which should result in less phylogenetic clustered communities on newly colonized land compared to a random species assembly.

In this study we compare the AMF communities at Peberholm with the 4000 yr old adjacent island Saltholm, which has comparable soil types. Species abundance distribution models were employed to describe the AM fungal communities. The focus was on patterns in community assembly, taxonomic and phylogenetic diversity. The hypothesis, in accordance with Whittaker (1972), was a geometric rank abundance species distribution at Peberholm and a log normal distribution at Saltholm. We expected the communities on Peberholm to be less phylogeneticly clustered, and have a lower species richness compared to Saltholm, due to dispersal limitation and to environmental filtering.

2. Materials and methods

2.1. Sampling locations

Two locations in Southern Scandinavia were studied, The primary location was *Peberholm* (55.60°N, 12.75°E), an artificial island completed in 1998 as a part of the Øresund connection between Denmark and Sweden. Sampling took place in October 2010, 12 yr after the completion of the island. The island measures approximately 4.0 × 0.4 km and is divided longitudinally by a traffic corridor (a four-lane motor highway and a twin-track railway). pH in the soil is relatively high: 8.1 (range 7.7–8.4) and soil organic matter low (6%), as the island was made from calcareous sand, clay and Cretaceous limestone, which was excavated from a 10 m deep trench in the seabed, into which the connecting tunnel elements were lowered. The island was left to natural colonization and has very restricted human access. Plant colonization has been surveyed annually, and at present more than 500 species of vascular plants have been recorded (Örneberg, 2003). Most of the plant species found on Peberholm are known to form symbiotic relations with AM fungi. The reference location *Saltholm* (55.66°N, 12.76°E), covers an area of 16 km^2 and is approximately 4000 yr old. Similar to Peberholm the island is founded on limestone, but covered by a 5–10 cm humus-rich layer.

2.2. Sampling and sample preparation

The vegetation on the recent island of Peberholm is typical of newly formed communities on raw soils. It consists predominantly of patches of herbaceous vegetation (forbs and grasses, and mainly AM plants), interspersed by areas with little or no vegetation. Shrubs of *Salix* and *Hippophaë* are scattered here and there. A list of the dominating plant species in the four sites at Peberholm can be found in Supplementary Materials, Table S2. The vegetation on Saltholm consists of cattle-grazed salt meadow grassland with predominantly AM forbs and graminoids. Saltholm has never been subject to arable farming. Due to the high degree of spatial variation in the vegetation on Peberholm, a stratified random sampling strategy was used. Pronounced difference in vegetation physiognomy was visually identified within 50 m diameter sites. Five circles, each with a diameter of 5 m, were placed upon each site to best encompass variation in abiotic environment and vegetation within the site. In each of the 5 m circles, ten soil cores were taken, each with a diameter of 4 cm and 10 cm in depth. Roots from the ten soil cores were later pooled into one sample. At Peberholm, the 10 subsamples were taken in patches with plant cover, while ensuring samples were obtained from the most prevalent plant species present. At Saltholm, where the vegetation cover was continuous, the corresponding ten samples were taken at random. Four sites on Peberholm and two sites on Saltholm, each with five samples, resulted in a total of 30 samples. Samples were collected in June 2011. Coordinates of individual samples from Peberholm and Saltholm can be found in Supplementary Materials, Table S1. Additionally, goose droppings were sampled at Peberholm for PCR detection of AM fungal propagules.

Spores from 25 g sieved (2 mm) soil of each pooled sample were collected after sucrose centrifugation (Daniels and Skipper, 1982) on 63 and 100 μm sieves, and spores were counted for each sample.

Roots from each sample were rinsed, cut into 5 mm pieces and thoroughly mixed in tap water. A sub-sample of the roots was cleared with KOH and stained for fungal colonization using trypan blue (Brundrett et al., 1996). Colonization degree was determined using the gridline intercept method (McGonigle et al., 1990). The remaining root material was freeze dried before subsequent DNA extraction. DNA was extracted from 30 mg of freeze dried, milled roots from each sample by a CTAB/chloroform/isopropanol method (Gardes and Bruns, 1993). The DNA pellet was dissolved in 50 μl TE buffer (10 mM Tris–HCl (pH 8.0), 1 mM EDTA), and stored at −20 °C until use.

2.3. PCR, cloning and sequencing

Samples were prepared for tag-encoded amplicon pyrosequencing in a two-step nested PCR procedure. Amplification of the desired nLSU-D2 region with the primers glo454 (Lekberg et al., 2011) and NDL22 (van Tuinen et al., 1998), and a subsequent amplification of the amplicons from the first run with the same primers, but with the addition of linkers and tags for the sequencing. First round of amplification was run with 30 cycles, the second with 25 cycles, and both with the following thermocycling parameters: 1 min 95 °C, 30/25 cycles of 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C (+4 s per cycle) with a final extension step of 2 min at 72 °C. Both PCR-amplifications were done in a 30 μl final