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Fungal communities in Norway spruce stumps along a latitudinal gradient in Sweden ☆

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ABSTRACT

Tree stumps left after clear-cutting have replaced naturally formed logs as the most common type of coarse woody debris in managed boreal forests. It is therefore necessary to understand stump importance for the biodiversity of wood-inhabiting organisms, including fungi, and determine their role in hosting species of conservation interest. We analyzed wood from 485 Norway spruce (*Picea abies*) stumps from 41 clear-cuts at seven localities along a latitudinal gradient from northern to southern Sweden using 454-sequencing. We also collated data about the known ecology of the 86 identified macro-basidiomycetes. In total, 1355 fungal operational taxonomic units were detected, of which 19% were identified down to genus or species level. The most widespread fungi were generalists, such as *Leptodontidium elatius*, *Resinicium bicolor*, *Fomitopsis pinicola*, and *Coniophora puteana*. Five species of conservation interest were detected, but were not abundant (*Kneiffiella curvispora*, *Metulodontia nivea*, *Perenniporia subacida*, *Postia placenta*, and *Climacocystis borealis*). Fungal community composition changed with stump age and along the latitudinal gradient.

These results will enable us to better incorporate important biodiversity and conservation issues when making decisions about using stumps as resources for biofuel.

1. Introduction

Coarse woody debris (CWD) is important for forest biodiversity. About 40% of the 1800 red-listed species in Sweden with strong affinity to forests depend on CWD (Sandström et al., 2015). The amount of natural CWD in Swedish forests has decreased dramatically since the introduction of modern forestry practices in the 1920s. At the same time, the proportion and amount of CWD in the form of stumps left after clear-cutting and thinning has increased, constituting up to 75% of the CWD formed in managed forest landscapes (Dahlberg et al., 2011). Managed forests cover more than 21 million hectares in Sweden, of which 183 000 ha (0.85%) were clear-cut in 2013 (Skogsstyrelsen, 2014). Depending on tree species, site quality and management, 500–800 stumps per hectare, or 50–75 m^3 CWD, result from clear-cutting, creating a large and widespread potential habitat for CWD-dependent organisms. Stumps differ in quality compared with naturally formed CWD and they represent a uniform substrate all over the clear-cut area, being of the same age and of a similar size, smaller than logs and always having ground contact. The freshly cut surface of the stumps, corresponding to 20–40 m^2 ha^{-1}, is instantly available for fungal colonization, whereas only a small area of freshly exposed wood is available for the colonization when CWD is created naturally by small scale disturbances. Furthermore, when an area of forest is clear-cut, the mesoclimatic conditions change, whereas when natural CWD is formed, the forest canopy may remain closed.

Fungi are a species rich and diverse group of saproxylic organisms. Besides being the major decomposers of wood, they have diverse roles in the saproxylic food web: for example, as parasites or as a substrate for invertebrate fungivores grazing on their mycelia, fruit bodies or spores (Stokland et al., 2012). Based on surveys of sporocarps, it has been estimated that there are more than 2500 saproxylic fungi in Sweden (Dahlberg and Stokland, 2004). Some of them have declined owing to decreasing amounts of natural CWD and have been identified as being of conservation interest, that is, they are either considered to be indicator species (Nitare, 2005) or they have been red-listed (Sandström et al., 2015). By contrast, other species, such as *Heterobasidion* spp., have increased its frequency because of a combination of it is ability to efficiently utilize stumps and the increasing number of stumps in Sweden during the last century (Oliva et al., 2011; Cleary et al., 2013). Fungi reported to fruit on stumps are typically widespread species, whereas species of conservation interest have rarely been recorded on stumps

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(Penttilä et al., 2004; Allmér et al., 2006; Junninen et al., 2008; Berglund et al., 2011a, 2011b; Toivanen et al., 2012; Nordén et al., 2013). The use of 454 sequencing has revealed that mycelial fungal diversity is much higher in the wood then can be observed as sporocarps, from mycelia isolations or earlier molecular methods (Ovaskainen et al., 2010, 2013; Kubartová et al., 2012). Thus, it is still largely unclear which fungi are making use of stumps, to what extent fungal communities in stumps corresponds to that in naturally formed CWD and if the fungi of conservation interest are present in the stumps as mycelia or not.

Since 2000, there has been an interest in the large-scale harvest of stumps for bioenergy purposes in Finland. In Sweden, stump removal has been more restricted because of concerns about the potential environmental consequences (Persson, 2013). In 2010, stumps of predominantly Norway spruce were removed from about 10% and 1% of the annual cutting area in Finland and Sweden, respectively (Routa et al., 2013). Stump removal has also been used as a successful strategy to reduce the amount and the spread of certain root-rot fungal species, both in northern Europe and in North America (Vasaitis et al., 2008; Menkis et al., 2010; Berch et al., 2012; Cleary et al., 2013; Vasaitis et al., 2016). However, if the stumps provide a suitable habitat for threatened species, their removal could have considerable consequences for biodiversity.

The aim of this study was to identify and characterize fungal communities in Norway spruce stumps throughout Sweden using 454 high-throughput sequencing. We also determined the main environmental variables affecting the community compositions, compiled data about the distribution and ecology of the identified macro-basidiomycetes and examined whether there were any red-listed and indicator species present in the stumps.

2. Methods

2.1. Study sites and samplings

Stumps were sampled from clear-cuts in seven localities along an 1100-km-long latitudinal gradient from northern to southern Sweden (66.3°N–57.4°N, Fig. 1 and Supplementary Table S1). The gradient ranges from the northern boreal zone to the hemiboreal zone. Annual mean precipitation varies from 500 mm to 700 mm. Mean temperature in January is between −13 °C and −4 °C and mean temperature in July ranges from +15 °C to +17 °C (Ahti et al., 1968; Swedish Meteorological and Hydrological Institute, mean values for 1931–1960). The location of the clear-cuts carried out between 1990 and 2010 was selected within 30 km^2 at each locality. The localities were next to nature reserves that were expected to act as source populations. The information of the location and age of the clear-cuts was obtained from the Swedish Forest Agency, based on their annual remote sense analyses of clear-cuts, and complemented by data from the forest companies Sveaskog, Holmen skog, Korsnäs and Södra. Only clear-cuts that were previously dominated by Norway spruce forests (>70% of the volume) were sampled. We aimed to sample as old stumps as possible. However, we found that stumps formed more than 20 years ago could rarely be sampled because they were almost totally decayed. Therefore, three clear-cuts performed 3–10 years ago and three clear-cuts performed 11–20 years ago were randomly chosen at each locality (Supplementary Table S2).

Twelve spruce stumps were sampled at each clear-cut site. The stumps were positioned 50 m apart on two crossing transects. The following data were noted for each stump (shown in Supplementary Appendix S1): GPS coordinates, height, diameter, and decay stage by the knife method (scale 1–5, from the least to the most decayed (Hottola and Siitonen, 2008). Sawdust wood-samples from the stumps were collected using a 10-mm-diameter drill bit. One sample from each stump was extracted horizontally 10 cm below the stump cut surface. Each sample almost spanned the whole diameter of the stump; care was taken so as not to drill through to the far side of the stump. Any bark and the surface layer of wood were removed with a knife prior to drilling. The drill bit was carefully cleaned with paper and washed in ethanol (70%) after each stump sample had been collected. The sawdust samples were collected separately in plastic zip bags and kept frozen at −21 °C until DNA extraction. Sampling was conducted between August and October 2012: 492 samples were collected in total.

Fig. 1. Positions of the seven localities on the latitudinal gradient along Sweden (see also Table S1).

2.2. Laboratory analyses

One and a half cm^{-3} of each frozen wood sample was transferred to a 2-mL screw-cap tube together with five glass beads prior to DNA extraction. The samples were homogenized using a Fast-prep shaker (Precellys 24 Bertin Technologies) and DNA was obtained by adding lysis CTAB buffer, extracting with chloroform and precipitating with isopropanol (for details, see (Kubartová et al., 2012). DNA concentration was measured using a spectrophotometer, NanoDrop™ (Thermo Scientific). Samples with a DNA concentration greater than 20 ng μL^{-1} were further purified using a JetQuick DNA purification kit (Genomed GmbH). DNA from each sample was amplified by PCR using the internal transcribed spacer primer pair gITS7 and ITS4, which are highly specific for fungi (Ihrmark et al., 2012) (Appendix S2). The ITS4 primer was tagged with a sample-specific identifier (8-bp long, designed at the Department of Forest Mycology and Plant Pathology, SLU; listed in Appendix S3). PCRs were conducted using 50-μL reactions (5 ng of the template, 200 μM of each nucleotide, 200 nM of each primer, 0.025 U/μL of DreamTaq Green polymerase (Thermo Scientific) in buffer; 5 min at 94 °C, 27 cycles of 30 s at 94 °C, 30 s at