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Short communication

Reintroduction of threatened fungal species via inoculation

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ABSTRACT

Reintroduction of locally extinct species is increasingly applied as a conservation tool for re-establishing species within their historical ranges. Thus far, this option has however not been investigated for fungi other than lichens. A large fraction of wood-inhabiting fungal species have declined because of forest loss and fragmentation, in addition to a decrease in dead wood. Here, we show the results from an experiment carried out in southern Finland, which demonstrates that inoculation is an effective method for reintroducing threatened wood-inhabiting fungi. All selected red-listed fungal species successfully established in the inoculated logs as mycelia, and three out of the seven produced fruit-bodies. Success rate was greater when the strains were inoculated in early-decay logs, including species that usually fruit in late decay stages. Inoculation can provide an effective tool for reintroducing fungal species, as the source populations remain intact and it is possible to produce massive amounts of inoculation-units with relatively low cost. Reintroductions of fungi should however be preceded by a risk assessment of the species to be reintroduced, by using source populations from nearby localities, and they should be considered complementary to the primary target of increasing the amount of their habitat. Our results suggest that the reintroductions of threatened fungi via inoculation in combination with other conservation measures can have important bearings for forest conservation and restoration.

1. Introduction

Reintroduction of threatened or locally extinct species is an important conservation tool for re-establishing species within their historical ranges (Seddon et al., 2007). Reintroductions and translocations have been carried out especially for animals (e.g. Kuussaari et al., 2015; Tosi et al., 2015) but also for plants (e.g. Weisenberger et al., 2014; Parthibhan et al., 2015). Many groups of fungi are highly vulnerable to anthropogenic changes such as habitat loss and fragmentation (Penttilä et al., 2006; Nordén et al., 2013), air pollution (e.g. Arnolds, 2001) and climate change (e.g. Kauserud et al., 2012). In spite of this, fungi have received limited emphasis in conservation biology (Heilmann-Clausen et al., 2015). For example, the potential of reintroducing threatened fungi has been not evaluated, except for lichens (see Lidén et al., 2004; Smith, 2014).

Experimental studies indicate that many fungi can be successfully introduced via inoculation. Fungal inoculations are routinely used to grow edible mushrooms (Hall et al., 2003), and to facilitate the growth of commercially important plants (e.g. Hart et al., 2015). Inoculations of wood-inhabiting fungi are used as a biological control tool against pathogenic fungi (e.g. Garbelotto and Gonthier, 2013) and as means for creating habitats for cavity breeding vertebrates (Filip et al., 2004). In a conservation context, the survival of threatened plant species has been facilitated by inoculations of mycorrhizal fungi (e.g. Zubek et al., 2009; Ferrazzano and Williamson, 2013). Furthermore, results from pilot studies suggest that some threatened fungal species can be successfully reintroduced to their habitats by inoculation (Venturella and Ferri, 1996; Pietka and Grzywacz, 2005).

Due to the drastic reduction of dead wood caused by forestry, many saproxylic species have diminished worldwide (Stokland et al., 2012). In particular, wood-inhabiting fungi have declined due to the reduction of natural forest areas and the loss of dead wood in managed forests (Junninen and Komonen, 2011). As a consequence, in Finland for

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example, over 40% of the polypore species have been red-listed according to the IUCN criteria (Rassi et al., 2010).

Many threatened wood-inhabiting fungi are dispersal limited (see Norros et al., 2012) and depend on landscape-level connectivity to retain viable populations (Penttilä et al., 2006; Nordén et al., 2013; Abrego et al., 2015). This decreases the efficiency of protected area networks, as small and isolated conservation sites hold less threatened species than they potentially could, some of the species being possibly absent simply due to dispersal limitation (Abrego et al., 2015). To counteract declines of saproxylic organisms, many restoration and conservation programs have focused on increasing the volume of dead wood in forests (Jonsson et al., 2005; Halme et al., 2013). However, the positive effect of dead-wood restoration for red-listed species has in many cases remained small (Pasanen et al., 2014) or realized only with long delay (Penttilä et al., 2013). Whether or not restored habitats are helpful for conserving species depends on whether the focal species are able to colonize them, which in turn depends on the proximity of the restoration areas to source populations (Kouki et al., 2012). In cases where natural colonization is unlikely, one alternative for re-establishing threatened species into restored and isolated protected sites is to artificially reintroduce them (Seddon et al., 2007).

The objective of the present study was to test the potential of inoculation as a tool for the reintroduction of red-listed wood-inhabiting fungal species. We developed laboratory and field protocols for inoculations, and tested their potential for fungal reintroduction by inoculating seven red-listed and regionally rare wood-inhabiting fungal species into a forest area in southern Finland, and by following their establishment success, both as mycelia and/or as fruit-bodies, for seven years after the reintroductions.

2. Materials and methods

Seven red-listed wood-inhabiting fungal species (Fig. 1) associated with Norway spruce (*Picea abies*) were selected for the reintroduction experiment with the criteria that i) the species had not been previously found from the reintroduction area, but were native species to the region (Appendix 1), ii) source populations were available within 300 km from the reintroduction area.

In autumn 2008, fungal fruit-bodies of the focal species were collected from various old-forest localities in southern and Central Finland (see Appendix 2 for the names of the localities and Appendix 3 for the stored voucher cultures). In the laboratory, we transferred small pieces of the fruit-bodies to agar plates to allow for mycelial growth and transferred the mycelia to *Picea abies* wood plugs (see Appendix 2 for details on the laboratory procedures).

The reintroduction area was located within Rörstrand, a spruce-dominated 80 ha natural-like forest abundant in dead wood, located in Sipoo, southern Finland. In Rörstrand, we delimited a 200 m × 200 m reintroduction area (coordinates: 60.45°N, 25.20°E), which had been intensively studied in our earlier work (e.g. Ovaskainen et al., 2013) but from which the focal species were not previously found. We selected randomly 100 spruce logs of 20–42 cm in diameter and representing decay classes 1–4 (range 1–5 from recently dead to very decomposed wood; Hottola and Siitonen, 2008). The numbers of selected logs in decay classes 1 to 4 was 19, 31, 34 and 16, respectively, and each log was marked to allow its monitoring.

In spring 2009, we drilled ten holes in each of the selected logs, five on the top part of the dead tree and five on the basal part, each 1 m apart from each other. We introduced one species into each drilling hole by inserting an inoculated wood plug. To each log, we inoculated 2–4 species to different randomly chosen drilling holes, using different strains of the same species if such were available. Each species was inoculated in total into 40 logs (Appendix 4).

For determining the absence of the focal species before the inoculations as well as their establishment success afterwards, all inoculated logs were surveyed for fruit-bodies in the autumns of 2008, 2009,

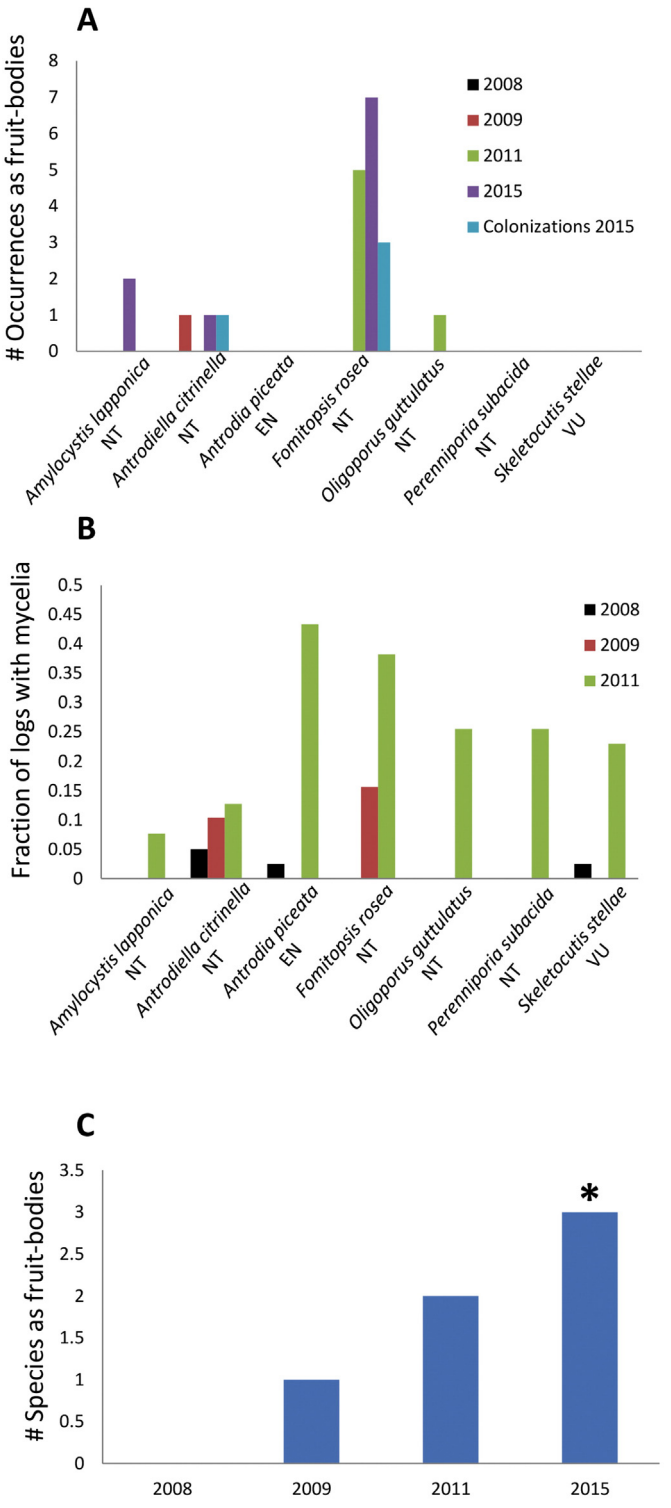

Fig. 1. Inoculation success rates for the species included in this study. **A** shows the number of logs in which each focal species was observed as fruit-bodies either as established individuals (fruit-bodies on inoculated logs) or as colonizations (fruit-bodies on non-inoculated logs). **B** shows the fraction of logs out of those logs to which the focal species was inoculated, in which each species was observed as mycelia. **C** shows the number of focal species that were recorded as fruit-bodies in each of the surveys. In Panel C, we have marked with an asterisk those cases in which the observed number of focal species was significantly greater than expected by the background colonization rate ($p = 0.01$ for 2015). For each focal species, the Finnish Red List categories according Rassi et al. (2010) are indicated in the figure (EN- Endangered, VU - Vulnerable, NT - Near threatened). The inoculations were carried out in the spring 2009.