



New reintroduction method makes conservation of fungi applicable at scale

Joette Crosier^{a,*}, Reijo Penttilä^a, Lorin von Longo-Liebenstein^b, Mattias Edman^c,
Leena Hamberg^a

^a Forest Health & Biodiversity Group, Natural Resources Institute Finland (Luke), Viikinkaari 4, 00790 Helsinki, Finland

^b Koskoontie 66, Kettula, Finland 25430

^c Mid Sweden University, Holmgatan 10, 851 70 Sundsvall, Sweden

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ABSTRACT

Wood-inhabiting fungi are an increasingly threatened component of global biodiversity, yet conservation efforts remain limited in part by non-standardized, unscalable reintroduction techniques. We present the largest fungal reintroduction program conducted to date, using a standardized and scalable method adapted from commercial mushroom cultivation. Bulk spawn of seven red-listed species (*Antrrodia crassa*, *Antrrodia infirma*, *Amylocystis lapponica*, *Skeletocutis stellae*, *Perenniporia tenuis*, *Radulodon erikssonii* and *Haploporus odoratus*) was produced and used to inoculate more than 1700 one-metre logs. Logs represented both natural host tree species and a locally available alternative, *Betula pubescens* or *Betula pendula*. In total, 53 forest plots in Finland and Sweden received inoculated logs. A subset of 465 logs was sampled to evaluate how wood type, inoculation method (sawdust versus dowel) and delivery time influenced establishment success.

We found that strain identity is a major determinant of reintroduction outcome, with substantial variation among individuals within species. Inoculation methods performed similarly for most species, indicating flexibility in practical implementation. For several species, brief pre-translocation colonization in controlled conditions improved early establishment in the field. This study provides the first evidence-based, field-tested methodology capable of supporting fungal reintroductions at operational scales. By demonstrating high establishment success across multiple threatened species and forest contexts, it offers a practical conservation tool that enables the integration of fungi into mainstream ecosystem restoration and management.

1. Introduction

Populations of wood-inhabiting fungi are declining worldwide due to habitat loss, habitat degradation, and other human-driven disturbances (IUCN, 2026). Although some species may recover naturally when suitable habitat is restored, many rare and specialist fungi remain limited by fragmentation, dispersal constraints, and reduced landscape connectivity (Abrego et al., 2015; Norros et al., 2012; Edman et al., 2004). In addition to these structural barriers, widespread reductions in high-quality deadwood substrates pose further challenges for wood-decaying fungi (Jonsson et al., 2016; Bader et al., 1995).

The most fundamental requirement for fungal conservation is the protection of remaining populations and the habitats that support them (Majdanová et al., 2023). However, conservation initiatives increasingly emphasize a more comprehensive approach that includes improved assessment, ex situ preservation such as culture banking, and species

reintroduction (Moose et al., 2019; Singh et al., 2018; Junninen and Komonen, 2011; Lonsdale et al., 2008; Molina et al., 2001; Moore and British Mycological Society, 2001; Dahlberg et al., 2010; Pasailiuk et al., 2022). Some wood-decay fungi continue to decline even as substrate availability increases (Stokland and Kauserud, 2004), particularly among red-listed species (Pasanen et al., 2014). These species are often more sensitive to forest continuity and substrate quality than to deadwood quantity alone (Majdanová et al., 2023), suggesting that targeted reintroduction may be beneficial when suitable substrate exists.

Past fungal reintroduction attempts have shown mixed outcomes. Some species successfully establish, while others fail to colonize or fail to fruit within the monitoring period, even when established (Nordén et al., 2020). Such inconsistency is likely linked not only to varying degrees of ecological knowledge, but also substantial methodological variation across studies (Wainhouse and Boddy, 2022). Establishment success may depend on initial mycelial mass, which influences fruiting

* Corresponding author at: Koskoontie 66, Kettula, 25430, Finland.

E-mail address: crosier.brittini@gmail.com (J. Crosier).

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potential (Ovaskainen et al., 2013) and competitive ability (Holmer and Stenlid, 1993). Given the diversity of fungal life histories, no universal reintroduction approach is expected to suit all species or environments. Nonetheless, conservation mycology would benefit from a shared set of best-practice guidelines.

Small-scale inoculation studies have typically used dowel spawn because of its ease of laboratory preparation and convenience during fieldwork. In mushroom cultivation, however, sawdust spawn is preferred for its scalability and cost-effectiveness. Despite widespread use of both techniques, direct comparisons of their effectiveness in conservation contexts remain limited. One study found that for *Hericium* spp. inoculated in live trees, spawn type had no impact on establishment, but that sawdust spawn had lower morphotype occurrence (Wainhouse, 2023). Some studies indicate that sawdust spawn may yield higher establishment success (Bruhn et al., 2009; Woods et al., 2006), but results are difficult to generalize due to differences in delivery methods, assessment indicators, and species studied.

Using freshly cut logs for off-site inoculation may reduce competition from resident fungi relative to on-site inoculation, but sourcing suitable wood requires careful consideration. Aspen (*Populus tremula*) and kelo (long-dead, barkless Scots pine, *Pinus sylvestris*) serve as key substrates for many threatened species, yet both have declined sharply due to modern forest management practices (Kuuluvainen et al., 2017; Hardenbol et al., 2020). Their use should therefore be minimized. Encouragingly, substrate flexibility appears to exist for some species: fungi naturally associated with kelo have successfully colonized non-kelo pine logs when inoculated under controlled conditions (Crosier et al., 2025a).

Decay strategy may further shape establishment outcomes. White-rot fungi degrade all major cell-wall components and are often regarded as capable of colonizing a broader range of wood types (Rustøen et al., 2023), although recent phylogenetic work questions this generalization (Krah et al., 2018). In this study, white-rot species, such as *Skeletocutis stellae*, *Haploporus odoratus*, *Perenniporia tenuis*, and *Radulodon erikssonii*, may therefore be expected to establish on both host and non-host logs. Brown-rot fungi, by contrast, often occur in more advanced decay stages (Rajala et al., 2012), and the three brown-rot species examined here—*Antrodia crassa*, *Antrodia infirma*, and *Amylocystis lapponica*—may have lower establishment success in freshly cut logs, particularly hardwoods, although their late occurrence in natural decay may also reflect ecological context rather than intrinsic establishment limitations.

Despite their central role in most ecosystems, fungi remain almost entirely absent from reintroduction and restoration frameworks. Their omission reflects long-standing practical barriers rather than a lack of ecological importance or threat (IUCN, 2026). Addressing these gaps is increasingly urgent as dispersal-limited specialists face heightened pressures. Effective fungal conservation requires methods that are not only ecologically sound but also scalable to the spatial and temporal realities of modern forest management. In many managed forest regions—including Northern Europe, where Finland and Sweden harvest the vast majority of all forest cover on short rotation cycles—intensive practices can rapidly remove substrates needed by wood-inhabiting fungi. Similar patterns occur in production forests globally. Even in regions with smaller-scale or less intensive forestry, fungi have had little realistic prospect of being included in restoration efforts, as previously available methods were too uncertain and costly to be implemented at meaningful scales. By developing and testing methods that can be implemented reliably at scale, this study provides an operational foundation for including fungi within global restoration and conservation frameworks—an increasingly critical need as land-use practices shift toward more sustainable approaches.

This study aims to advance fungal reintroduction methodology by adapting scalable practices from mushroom cultivation and systematically comparing factors influencing establishment success. We compare sawdust versus dowel inoculum, birch (*Betula* spp., a locally available

by-product of forest thinning) versus host wood substrates, and different durations of ex situ maintenance (none, one growing season, or two growing seasons). All experiments use freshly cut log sections (“bolts”) and subsequent translocation to forest sites to reduce competition from resident fungal communities, a recommended practice for fungal reintroductions (Nordén et al., 2020). We also evaluate whether maintaining inoculated logs under controlled, mushroom-farm-like conditions enhances early colonization by reducing moisture stress and competition.

Based on ecological theory and previous reintroduction efforts, we proposed four hypotheses. (1) All target species would successfully colonize inoculated bolts, performing at least as well as in previous reintroduction trials, demonstrating the scalability of ex situ inoculation and translocation. (2) White-rot species would establish more successfully across all wood types, including birch, than brown-rot species. (3) Sawdust spawn, the more cost-effective and industry-standard inoculum type, would perform comparably to dowel spawn. (4) Maintaining bolts under controlled conditions prior to deployment would increase mycelial establishment relative to immediate placement in forest sites.

2. Materials and methods

2.1. Experimental design: Sites, species, and translocation

We assessed inoculation and translocation methods for seven threatened wood-inhabiting fungi associated with different host tree species: *Antrodia crassa* and *A. infirma* (Scots pine), *Amylocystis lapponica* and *Skeletocutis stellae* (Norway spruce), *Perenniporia tenuis* and *Radulodon erikssonii* (European aspen), and *Haploporus odoratus* (goat willow). Strains were isolated either from fresh wild fruit bodies collected in Sweden in autumn 2022 or obtained from culture collections at Natural Resources Institute Finland and the University of Helsinki (see Supplementary Table 1). All strains were refreshed on malt yeast agar prior to inoculation.

Species and strain selection followed conservative principles consistent with IUCN conservation translocation guidelines (IUCN, 2013) and fungal-specific recommendations (Nordén et al., 2020), including the use of locally sourced material and ecologically appropriate reception sites within recent regional occurrences and containing sufficient host substrate to minimize ecological risk. We used 5 strains per species with exception to *P. tenuis* where only 2 strains from Finland were available. For each available wood type and spawn type, nine replicate bolts per strain were prepared. Birch was included for all species, and additional host-specific wood types were incorporated according to species ecology: spruce and slow-grown spruce for *A. lapponica* and *S. stellae*; pine, slow-grown pine, and kelo wood (sawdust only) for *A. crassa* and *A. infirma*; aspen for *P. tenuis* and *R. erikssonii*; and willow for *H. odoratus*. This yielded 36, 54, or 63 bolts per strain depending on the number of wood types used (full counts per species and wood type in Supplementary Table 2). Three bolts per strain per spawn type were assigned to each delivery period (immediate, after one growing season, after two growing seasons).

2.2. Spawn preparation

Grain spawn was prepared by soaking rye grain in hot water for 16 h, draining, and mixing with moistened expanded vermiculite (1.25:1 ratio; Glückspilze, Austria). The mixture was transferred into mushroom grow bags (type 10 T; Unicorn Bags, TX, USA) and sterilized at 121 °C and 15 PSI for 2 h 40 min. After cooling, each bag was inoculated with a Petri dish of actively growing mycelium.

Two types of spawn were produced: sawdust and dowels. Birch sawdust was sieved to 3.15 mm, mixed with wheat bran (2.7 kg sawdust to 400 g bran; Korpelan Mylly, Finland), and moistened to 60%. We used grooved birch wood dowels in a standard 8 × 26 mm size (Arvo-Helat Oy, Jurva, Finland). Dowels were soaked in hot water for 48 h and drained. Both substrates were sterilized in grow bags (type 3T; Unicorn

Bags) at 121 °C and 15 PSI (sawdust: 3 h 20 min; dowels: 2 h 30 min). After cooling, sawdust and dowels were inoculated with grain spawn at 20% and 40%, respectively, and incubated at 22 °C and ~45% humidity for 2–4 weeks. Fully colonized spawn was stored at +4 °C for up to two weeks before use.

2.3. Bolt preparation

Healthy trees with no visible fungal colonization (e.g., fruit bodies or internal staining) were felled during late winter 2023 and cut into 3 m segments. These were later divided into 1 m bolts (10–20 cm diameter), measured, tagged with unique identifiers, and transported to the base site, or “mushroom farm” for inoculation.

2.4. Bolt inoculation and farm maintenance

Each strain was inoculated into nine bolts per spawn type and wood type (Fig. 1c), including uninoculated controls. Bolts were inspected prior to inoculation to ensure intact bark and absence of active fungal decay. Inoculations were carried out at the mushroom farm site using an angle grinder (9565CR; Makita, Japan) fitted with specialized drill bits and an adapter (Field & Forest, USA). Sawdust spawn was inserted using a palm-style brass inoculator (Field & Forest; item PT), whereas dowels were hammered flush with the wood surface using a rubber mallet. Melted beeswax was applied to seal inoculation points.

Holes were spaced approximately 10 cm apart lengthwise and 8 cm between rows, offset to form a diamond pattern, resulting in 30–40

inoculation points per bolt. Bolts were labeled, stacked by strain on pallets (no more than four rows high), and irrigated throughout summer to maintain moisture.

2.5. Plot selection and translocation

Receptor sites were selected within each species' documented historical range and in areas lacking extant populations, based on national red-list assessments and published distribution records (Kotiranta and Niemelä, 1996; Kotiranta et al., 2009), data from the Finnish species information portal (LajiGIS / laji.fi), and expert correspondence confirming regional occurrence patterns (Reijo Penttilä, personal communications). Sites were designated for long-term protection and contained suitable quantities of appropriate deadwood substrate and continuity. In total, 53 forest plots were established in Finland and Sweden. Sites were provided by Metsähallitus, UPM, and private forest owners in Finland, and by Stora Enso and Svenska Cellulosa Aktiebolaget (SCA) in Sweden. Plots were distributed across southern, central, and northern regions of both countries, with 1–3 plots allocated for pine, spruce, aspen, or willow species per region. Species were matched to plots based on host tree association: Pine plots: *A. crassa* and *A. infirma*; spruce plots: *S. stellae* and *A. lapponica*; aspen plots: *P. tenuis* and *R. erikssonii*; willow plots: *H. odoratus*. Within each 40 × 40 m plot, bolts were randomly placed. To account for microenvironmental variation, canopy cover and ground contact were recorded for each bolt; however, both were excluded from final models due to a lack of explanatory power and to avoid model overparameterization. All inoculated bolts were delivered directly to

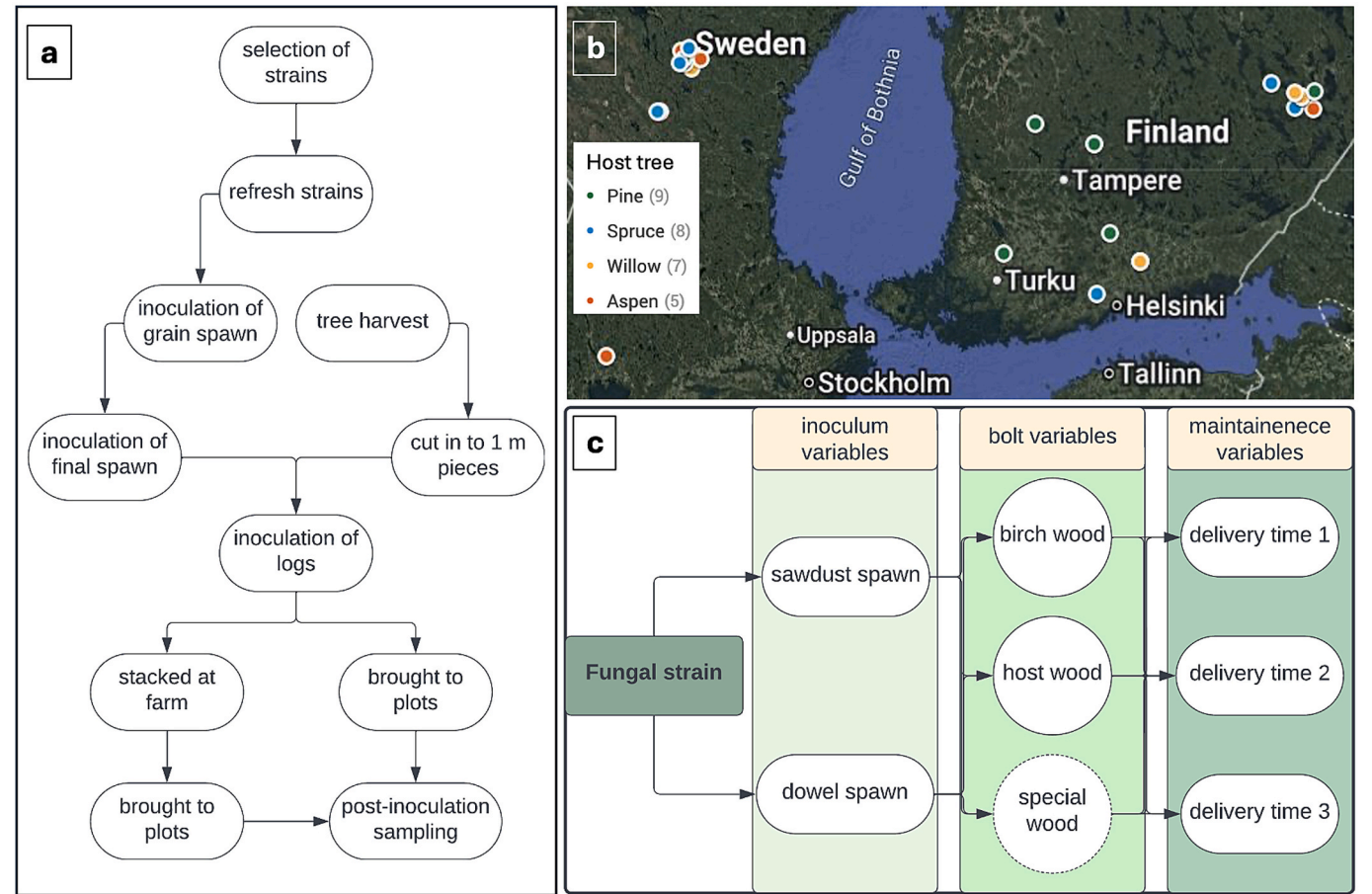


Fig. 1. Experimental workflow, plot locations and inoculation conditions. **a**, Workflow from strain selection to final sampling, including sequential steps: strain selection, spawn preparation, inoculation and sampling. **b**, Locations of sampled plots, marked by host type. The map was generated using plot-center GPS coordinates in Google Maps; some positions are offset for clarity. Plots where bolts were delivered but not sampled are not shown. **c**, Experimental conditions tested for each strain, shown in workflow sequence. Variables included inoculum type, bolt host type and delivery time point.

their designated plots during three delivery periods (June 2023, May 2024, and August–September 2024), rather than to predefined regions. At each time point, three bolts per strain per spawn type were translocated to designated plots, along with uninoculated controls.

2.6. Post-inoculation sampling and processing

After two growing seasons, bolts were sampled from a subset of plots (Fig. 1b). A total of 29 plots were sampled. Bolts from all delivery groups were included, as delivery time was an experimental variable and all bolts had been inoculated simultaneously; thus all bolts had experienced two growing seasons since inoculation at the time of sampling. Sawdust was collected by drilling 1.5 cm away from inoculation points with a 5 mm bit, allowing the material to fall directly into a clean bag. Ten standardized drill points were used and homogenized per bolt: five evenly spaced from the end to the midpoint on one side, and five corresponding points on the opposite side starting from the other end. Before drilling, a small segment of bark was removed at each sampling point using a sterilized knife. Equipment was cleaned between samples using tap water, 70% ethanol, DNAOut (Geno Technology, Inc.), flame-sterilization, and sterile water. Samples were kept cool, stored at -80°C , freeze-dried for 62–92 h (0.57 mbar at -15°C), and powdered using a ball mill (Retsch; 30×2). Approximately 2 ml of the powdered sample was stored in cryovials at -80°C .

2.7. DNA extraction, PCR, and library preparation

For each sample, 50 mg of material was placed in 2 ml tubes with 1.6 ml pre-warmed lysis buffer (Aljanabi and Martinez, 1997; Vesterinen et al., 2016) and 1.6 μl Proteinase K. Samples were incubated at 56°C for 3 h in a shaking incubator. Lysates (1 ml) were centrifuged at $10,000 \times g$ for 5 min, and DNA was purified using SPRI bead-based extraction (Vesterinen et al., 2016). Blank extraction controls were included.

PCR amplification targeted the ITS2 region using primers fITS7 and ITS4 (Ihrmark et al., 2012; White et al., 1990), each with linker-tags and heterogeneity spacers. PCRs were run in duplicate following established protocols (Kankaanpää et al., 2020), and synthetic positive controls were added at 0.02 pg/ μl . Libraries were dual-indexed, pooled, purified (GeneJet Gel Extraction Kit, Thermo Scientific), and sequenced on an Illumina NovaSeq6000 SP Flowcell (2×250 bp) at Turku Centre for Biotechnology.

2.8. Bioinformatics

Bioinformatics followed established pipelines using VSEARCH for merging and quality trimming (Rognes et al., 2016). Primers were removed with Cutadapt (Martin, 2011), allowing 20% mismatch and retaining reads 100–1000 bp. Reads were filtered to ≤ 1 expected error, dereplicated, and singletons removed.

Denoising, chimera removal, and ZOTU clustering were performed using UNOISE3 in USEARCH (Edgar and Flyvbjerg, 2015). Taxonomic assignment used SINTAX (Edgar, 2010) with the UNITE Fungi database v10.0 (Abarenkov et al., 2024), refined using BOLDigger3 (Buchner and Leese, 2020) against BOLDSystems v5. Mock community and spike-in controls were included.

2.9. Statistical analyses

Species establishment (presence–absence) was analyzed using Generalized Linear Mixed Models (GLMMs) with a binomial distribution and logit link, using glmer from the lme4 package (R Core Team: Bates et al., 2015). A detection was considered “present” when the relative abundance of a target species reached $\geq 0.1\%$ of spike-normalized read counts within a sample. This threshold was applied to exclude very low-level detections while retaining low-abundance but potentially meaningful signals, consistent with commonly applied filtering thresholds in

metabarcoding studies (Nikodemova et al., 2023). Species included: *A. crassa* ($n = 76$), *A. infirma* ($n = 67$), *A. lapponica* ($n = 47$), *S. stellae* ($n = 61$), *P. tenuis* ($n = 31$), and *R. erikssonii* ($n = 41$). *H. odoratus* and two strains with no growth were excluded. Models were fit per target species. Fixed effects were wood type, inoculum type, delivery time point, and scaled bolt volume. Random effects were strain nested within site. Due to sparse observations for *P. tenuis*, penalized logistic regression was used (Heinze and Ploner, 2024).

Relative DNA abundance, defined as spike-normalized sequencing read counts of the target species, representing its proportional DNA signal within each sample, was analyzed using Generalized Additive Mixed Models (GAMMs) via gamm in mgcv (R Core Team, 2025; Wood, 2017) with Gamma distribution and log link. Fixed effects matched those in GLMMs; bolt volume was modeled as a smooth term. Random effects included site and strain. Residual diagnostics and gam check were used to assess model assumptions.

3. Results

3.1. General detection rates

Several species achieved high detection frequencies (e.g., *P. tenuis*, *S. stellae*), whereas others exhibited lower or highly strain-dependent outcomes (e.g., *A. infirma*, *A. lapponica*, *R. erikssonii*). The detection rates of target species DNA across all tested bolts varied considerably by both species and individual strain (Fig. 2). *A. infirma* and *A. lapponica* displayed the widest range, from 0% to over 90% depending on strain, with a mean of 51% and 25%, respectively. *S. stellae* exhibited relatively more consistently high detection rates of 33% to 100%, and averaging 72%. *P. tenuis* showed the strongest establishment, with detection between 62% and 83%, mean 72%. *A. crassa* had a moderate detection rate, which ranged from 29% to 47%, with an average of 37% across strains. Finally, *R. erikssonii* had the lowest detection rates, ranging from 13% to 50%, mean 29%. *H. odoratus* was not detected in any bolts.

Detection of target species in non-inoculated bolts was observed across several treatments, but both frequency and relative abundance were substantially higher in intended inoculation treatments. In their own inoculated bolts, target species were detected in 25.9–74.2% of samples depending on species, with mean relative abundances ranging from 1.62% (*R. erikssonii*) to 37.08% (*P. tenuis*). In contrast, detections in bolts inoculated with other species were generally less frequent, ranging from 0.3 to 5.5%, with the exception of *P. tenuis* (10.2%). Mean relative abundance in non-target bolts was low for all species, ranging from $<0.01\%$ to 0.56%. Control bolts showed occasional detections of *P. tenuis* (13.4%), *A. infirma* (6.0%), and *S. stellae* (3.0%), but mean relative abundances remained low (0.05–0.29%). A full summary of relative abundance values for all target species across all samples is provided in Supplementary Table 3.

3.2. Factors influencing presence-absence of target fungi

The likelihood of species detection (presence – absence) was influenced by several factors (Table 1). Inoculum type had an impact on *A. crassa*, where using sawdust spawn was associated with a significantly lower probability of the species being detected compared to using dowels ($p = 0.003$). Wood type played a role for some of the fungal species (Table 1, Fig. 3). Detection probabilities of *P. tenuis* and *R. erikssonii* was higher in aspen wood compared to birch ($p = 0.021$ and $p = 0.016$, respectively). *A. crassa* was more likely to be detected in slow-grown pine wood compared to birch ($p = 0.022$). *S. stellae* showed higher detection in spruce compared to birch ($p = 0.025$). There was also an indicative positive effect of slow-grown spruce on the presence of *A. lapponica* ($p = 0.088$). Other wood types were not significantly different from each other. Taken together, these results indicate that substrate identity was the most consistent predictor of presence across species, while inoculum type and bolt properties showed more species-

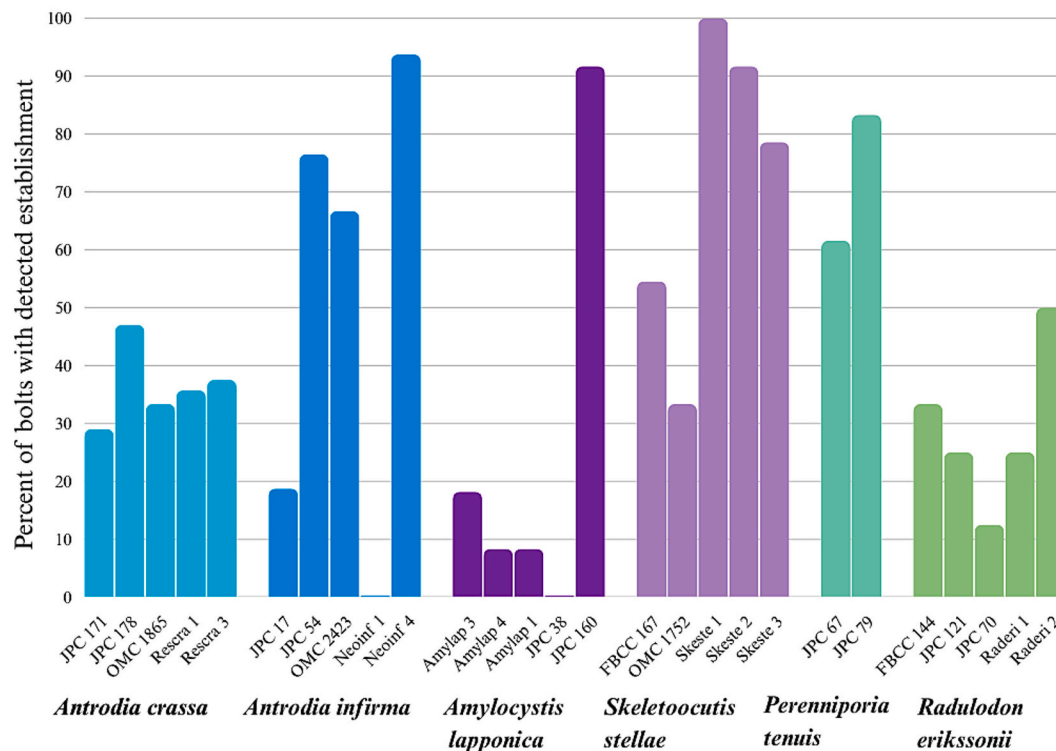


Fig. 2. Percentage of established bolts by strain for six threatened wood-inhabiting fungal species. Data include all wood types, inoculum types, and delivery time points combined.

Table 1

Effects of inoculation variables on mycelial presence or absence. Shown are the number of observations, estimated coefficients (Coeff.), standard errors (SE), and *p*-values from a binomial generalized linear mixed model (GLMM). Fixed effects include wood type ('birch' as baseline), inoculum type ('dowels' as baseline), delivery time point (immediately as the baseline, after one or two growing seasons), and bolt volume (scaled to mean zero and SD one). Strain nested within site was treated as a random factor. Statistical significance is in bold ($p < 0.05$); indicative significance ($0.05 < p < 0.1$) is underlined.

	<i>Antrodia crassa</i> <i>n</i> = 76			<i>Antrodia infirma</i> <i>n</i> = 67			<i>Amylocystis lapponica</i> <i>n</i> = 47			<i>Skeletoocutis stellae</i> <i>n</i> = 61			<i>Perenniporia tenuis</i> <i>n</i> = 31			<i>Radulodon erikssonii</i> <i>n</i> = 41		
	Coeff. ± SE	<i>p</i>		Coeff. ± SE	<i>p</i>		Coeff. ± SE	<i>p</i>		Coeff. ± SE	<i>p</i>		Coeff. ± SE	<i>p</i>		Coeff. ± SE	<i>p</i>	
Intercept	−0.715 ± 0.773	0.355		0.123 ± 1.148	0.914		−3.816 ± 2.615	0.145		1.498 ± 2.070	0.469		1.189 ± 0.903	0.158		−2.423 ± 1.355	<u>0.074</u>	
Aspen	–			–			–			–			–			–		
Kelo	−0.715 ± 0.773	0.968		−0.437 ± 1.194	0.715		–			–			–			–		
Pine	0.673 ± 0.795	0.397		1.068 ± 0.912	0.241		–			–			–			–		
Slow-grown pine	2.293 ± 1.003	0.022		<u>1.801 ± 1.057</u>	<u>0.089</u>		–			–			–			–		
Slow-grown spruce	–			–			<u>3.119 ± 1.827</u>	<u>0.088</u>		1.241 ± 1.091	0.255		–			–		
Spruce	–			–			1.477 ± 1.914	0.440		3.655 ± 1.632	0.025		–			–		
Inoculum sawdust	−2.189 ± 0.740	0.003		−0.493 ± 0.828	0.552		−0.114 ± 1.685	0.947		−1.437 ± 1.387	0.300		−1.831 ± 1.673	0.245		1.985 ± 1.608	0.217	
Deliv. after one growth season	1.027 ± 0.857	0.231		0.636 ± 0.873	0.466		1.419 ± 1.710	0.410		1.069 ± 1.395	0.444		−1.652 ± 1.175	0.153		−1.428 ± 1.456	0.883	
Deliv. after two growth seasons	−0.025 ± 0.851	0.977		0.254 ± 1.398	0.856		0.280 ± 2.208	0.899		−1.401 ± 0.610	0.610		−0.070 ± 1.767	0.970		−2.685 ± 1.748	0.187	
Volume	−0.709 ± 0.477	0.137		−0.189 ± 0.380	0.619		−1.285 ± 1.203	0.285		−0.469 ± 0.551	0.395		0.147 ± 0.418	0.735		0.358 ± 0.482	0.457	

specific effects.

3.3. Factors influencing relative DNA abundance

When a species was detected, various factors influenced the relative DNA abundance (Table 2). For *A. crassa*, it decreased with increasing

bolt volume ($p < 0.001$). For all other species, bolt volume did not show significant effects. The inoculum type only affected *S. stellae*, which showed lower relative DNA abundance when sawdust spawn was used compared to dowels ($p = 0.019$). For all other species, there was no impact of inoculum type. Wood type influenced relative DNA abundance only in *A. infirma*, where the amount of DNA was higher in slow-grown

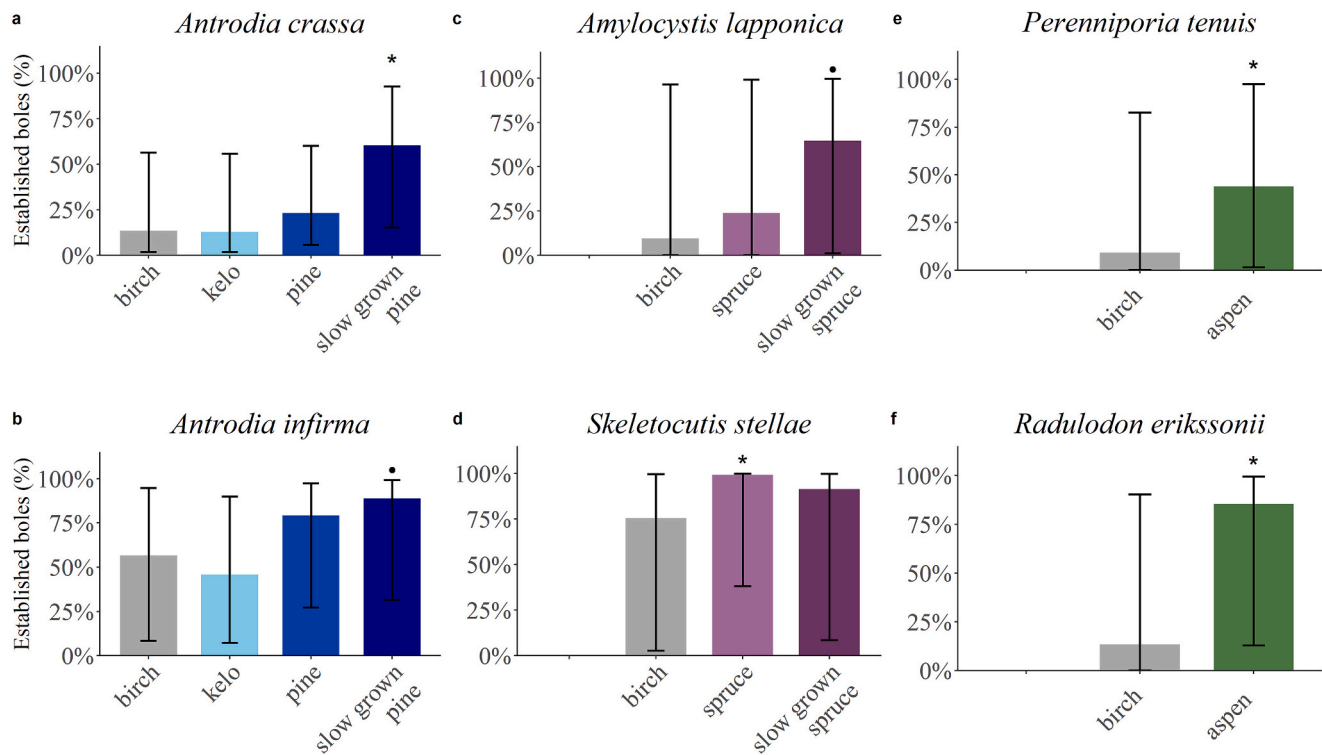


Fig. 3. Effect of wood type on species establishment in bolts. Predicted establishment success and 95% confidence intervals are based on a generalized linear mixed model (GLMM), with inoculum set to “sawdust,” delivery time set to “after one growing season”, and volume held at mean values. Significant effects are marked with “*” and near-significant effects with “•.”

Table 2

Effects of inoculation variables on relative DNA abundance. Shown are the number of observations, estimated coefficients (Coeff.), standard errors (SE), and *p*-values from a generalized additive mixed model (GAMM) fitted to continuous relative abundance data from logs where the species was detected. Fixed effects include wood type (‘birch’ as baseline), inoculum type (‘dowels’ as baseline), and delivery time (immediately as the baseline, after one or two growing seasons). Bolt volume (scaled to mean zero and SD one) was included as a smooth term. Strain nested within site was treated as a random factor. Statistical significance is in bold ($p < 0.05$); indicative significance ($0.05 < p < 0.1$) is underlined.

	<i>Antrodia crassa</i> n = 28			<i>Antrodia infirma</i> n = 43			<i>Amylocystis lapponica</i> n = 15			<i>Skeletocutis stellae</i> n = 44			<i>Perenniporia tenuis</i> n = 23			<i>Radulodon erikssonii</i> n = 14		
	Coeff. ± SE	p		Coeff. ± SE	p		Coeff. ± SE	p		Coeff. ± SE	p		Coeff. ± SE	p		Coeff. ± SE	p	
Conditional Model (Intercept)	6.961 ± 0.540	<0.001		7.633 ± 0.736	<0.001		6.277 ± 0.542	<0.001		8.796 ± 0.677	<0.001		10.340 ± 0.474	<0.001		6.242 ± 0.454	<0.001	
Aspen	–			–			–			–			–0.182 ± 0.523	0.732		–0.101 ± 1.066	0.928	
Kelo	–0.076 ± 1.155	0.948		–0.322 ± 0.628	0.612		–			–			–			–		
Pine	0.052 ± 0.495	0.916		0.258 ± 0.392	0.514		–			–			–			–		
Slow-grown pine	0.120 ± 0.565	0.834		1.034 ± 0.411	0.017		–			–			–			–		
Slow-grown spruce	–			–			0.646 ± 0.527	0.255		0.623 ± 0.449	0.173		–			–		
Spruce	–			–			0.211 ± 0.649	0.753		0.481 ± 0.434	0.275		–			–		
Inoculum sawdust	0.621 ± 0.565	0.267		0.438 ± 0.364	0.237		–0.747 ± 0.638	0.275		–1.304 ± 0.532	0.019		–0.435 ± 0.726	0.557		0.880 ± 1.033	0.427	
Deliv. after one growth season	0.545 ± 0.491	0.161		0.786 ± 0.427	0.114		1.817 ± 0.544	0.010		0.035 ± 0.517	0.947		0.556 ± 0.683	0.427		0.108 ± 0.786	0.896	
Deliv. after two growth seasons	2.410 ± 0.604	0.002		1.190 ± 0.416	0.447		3.908 ± 0.722	0.001		–0.010 ± 1.027	0.993		0.181 ± 0.684	0.795		–1.253 ± 1.030	0.269	
Volume	–1.195 ± 0.240	<0.001		–			–0.242 ± 0.269	0.395		0.276 ± 0.228	0.234		–0.348 ± 0.268	0.212		–0.320 ± 0.263	0.242	

pine wood compared to birch ($p = 0.017$). Delivery time impacted relative DNA abundance for two species. *A. crassa* counts were higher when deployed after two growth seasons ($p = 0.002$), and *A. lapponica*

showed a positive effect from delivery after one ($p = 0.010$) and two ($p = 0.001$) growth seasons. No other species were impacted by delivery time. Overall, species differed greatly in which factors influenced

abundance, but few variables showed consistent effects across multiple taxa, underscoring strong interspecific differences in establishment dynamics.

4. Discussion

4.1. Overview

Restoring overlooked functional groups is a critical yet underdeveloped frontier in biodiversity conservation. While plants and animals dominate reintroduction programs, wood-decay fungi—key drivers of habitat function—remain largely absent from restoration frameworks. This gap persists partly because practical, scalable tools have been lacking, limiting efforts to reverse their decline. Addressing this challenge is important for maintaining biodiversity and resilience, particularly as old-growth habitats decline globally. Our study provides evidence that high-volume inoculation of fresh logs can overcome dispersal and early competitive barriers, offering a pathway to integrate fungal conservation into restoration strategies. Because the methods used here closely mirror modern edible mushroom cultivation and use standardized materials and equipment, well-established industry estimates already exist for the associated costs. A trained two-person team can inoculate ~100 bolts in roughly 30 labor-hours once spawn is prepared, with tools costing approximately USD 150–200 (Mudge et al., 2013). These values provide a realistic benchmark for conservation practitioners and demonstrate that implementation of reintroducing fungi can begin at relatively low cost.

4.2. Evaluation of hypotheses

Our study's findings largely supported our hypotheses. Hypothesis 1, that all target species would successfully colonize the bolts and perform

as well or better than prior reintroduction attempts, was mostly confirmed. Most species showed establishment success equal to or exceeding previous efforts, demonstrating that ex situ bolt inoculation and translocation is a viable and scalable method. One exception was *H. odoratus*, which failed to establish, consistent with reports that even inoculation of its natural host (living willow) is challenging (Reijo Penttilä, personal communication). Hypothesis 3, that sawdust spawn would perform comparably to dowel spawn, was mostly verified. Sawdust spawn was largely as effective for mycelial establishment as dowels for most species, supporting its use as a cost-effective method at scale. Hypothesis 4, that maintaining bolts in a controlled “mushroom farm” environment prior to translocation would enhance establishment, was partially confirmed. Farm maintenance had either a positive or neutral effect on establishment success compared to immediate delivery to the forest, suggesting it is a safe and potentially beneficial practice. Hypothesis 2, that white rot species would spread more successfully across all wood types compared to brown rot species, was not directly verified. Instead, wood type preferences proved to be species specific, though some rot type trends were evident, suggesting that species-specific substrate selection may reflect niche differentiation and resource partitioning in decay succession, rather than rot type alone.

4.3. Detection patterns and comparison with previous reintroductions

The methodology used in our study matched or substantially improved establishment success compared to prior reintroduction attempts reported in earlier studies that used much less inoculum on naturally fallen, partially decaying wood—especially when wood type and inoculum were comparable (Fig. 4). For *S. stellae*, we observed high establishment rates, with three strains surpassing 75% detection (one at 100%), contrasting with previous studies that reported successful detection in only 5% of logs inoculated with some of the same strains

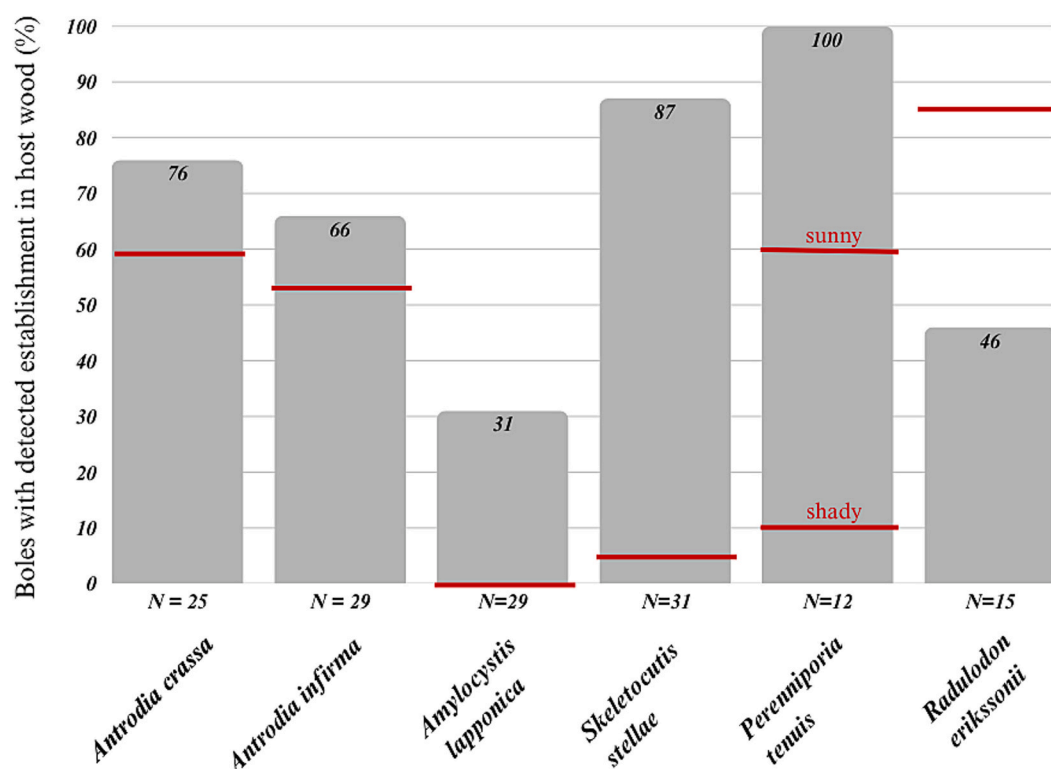


Fig. 4. Bolt establishment percentages compared with previous studies. Bars show establishment rates averaged across strains and restricted to dowel inoculations into natural host wood. Red lines indicate reference rates measured as DNA presence one or two years after inoculation, from the following sources: *Antrodia crassa* and *A. infirma* (Crosier et al., 2025a); *Amylocystis lapponica* (Abrego et al., 2016); *Skeletocutis stellae* (Saine et al., 2025); *Perenniporia tenuis* and *Radulodon erikssonii* (Penttilä et al., 2023). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

after one year (Saine et al., 2025) and similarly low detection across years 1, 2, and 4 in Abrego et al. (2016), where the highest value reached was only ~25% after four years. Similarly, *A. lapponica* was not detected at all during the first two years in earlier inoculation efforts, and even after four years detection remained low (<10%; Abrego et al., 2016), whereas with our methodology, it was already established in the first year achieving similar or better rates to what was previously found after four years, and with one strain far exceeding with over 90% detection. Both *A. crassa* and *A. infirma* also outperformed previously reported success rates when pine was used as the substrate (Crosier et al., 2025a). *P. tenuis* consistently achieved high success, outperforming previous findings in natural log inoculations (Penttilä et al., 2023). Although detection approaches were broadly comparable (DNA-based), some methodological differences among studies may influence direct comparisons. Together, these results indicate that the methodology used here can enhance colonization success in species previously considered difficult to introduce. This supports the idea that a high-volume starting inoculum in logs with low pre-existing fungal occupancy may reduce competitive barriers during early colonization (Boddy, 2001). The results align with hypotheses that several of these species have poor competitive ability and adaption to specific substrates that contribute to or exacerbate their dispersal limitation (Martikainen et al., 2000), and they are also in line with laboratory findings that larger inoculum volumes tend to improve competitive outcomes (Fukasawa et al., 2020).

Detections of target species in non-inoculated bolts likely reflect a combination of limited dispersal and incidental transfer. While such detections occurred across several species, they were less frequent and occurred at lower relative abundance compared to inoculated bolts (Supplementary Table 3). Among species, *Perenniporia tenuis* showed the highest frequency of unintended detections, and project partners reported possible early fruiting within the first and second years, suggesting that elevated propagule pressure may have contributed to its spread beyond inoculated bolts. In addition, the nature of field sampling and DNA processing may allow low-level detection of airborne or environmentally transferred DNA. Proximity of bolts during farm maintenance may also have facilitated incidental transfer under shared handling conditions. Several wood-decay fungi are capable of producing asexual propagules (e.g. conidia), which can facilitate dispersal independently of fruit body formation (Nobles, 1965; Mukhin and Votintseva, 2002), providing another plausible mechanism for these detections outside intended treatments.

4.4. Species-specific establishment patterns

Two of our species did not establish well with our methods. *R. erikssonii* performed more poorly than a prior study using older naturally fallen aspen logs (Penttilä et al., 2023). While its fruiting bodies are typically found on fallen trunks, evidence suggests its sister species, *Radulum casearium*, is a significant cause of rot in living aspen (Basham, 1958). Anecdotal observations hint that *R. erikssonii* may also kill aspen in Europe (Martini, 2016). This outcome, along with our unsuccessful attempt to establish *H. odoratus*, a heart-rot specialist of living goat willow (*Salix caprea*), suggests that species tied to living-host conditions may be less amenable to fresh-bolt translocations. This interpretation is consistent with previous failures to grow *Inonotus obliquus* (associated with living *Betula* spp.) on deadwood (Pietka, 2006). Given their ease of growth on wood substrates in the lab, the low detection of *R. erikssonii* and absence of *H. odoratus* here imply that fresh bolts may lack key conditions needed for establishment, and possibly allow the target species to be rapidly outcompeted by arriving fungi. Further investigation could focus on combative experiments.

4.5. Wood type and substrate effects

Despite their specific natural substrates, four of the six fungi did not show a strong difference in colonization between their basic host wood

and birch. This finding aligns with some lab experiments (Crosier et al., 2025b), suggesting that with inoculation, a fungus's host specificity may be less pronounced than in natural environments. Consistent with their natural habitat, *R. erikssonii* and *P. tenuis*, which are usually found on aspen in nature, established significantly better on aspen wood in our study, although they are not significantly impacted by birch compared to aspen in lab conditions (Crosier et al., 2025b). There was another pattern of wood type effect for a few species. For *A. crassa*, *A. infirma*, and *A. lapponica*, slow-grown host wood generally favored colonization over standard host wood and birch. The natural distribution of *A. lapponica*, concentrated in northern Finland, taiga and montane regions of Europe (Kotiranta and Niemelä, 1996; Holec and Kucera, 2007), may be linked to the prevalence of slow-growing trees in these climates (Oleksyn et al., 1998). However, these areas are also containing more old-growth forests, so *A. lapponica*'s presence there may have more to do with the continuity. Similar conditions apply also to *A. crassa*, *A. infirma* and *S. stellae*, which seem to be boreo-continental polypore species (Kotiranta and Niemelä, 1996). Our findings suggest that the specific properties of some wood qualities, such as those found in slow-grown trees, might play a role in the establishment of some fungi, a possibility supported by evidence that growth rate of trees influences decay capacity in wood-decay fungi (Edman et al., 2006). This warrants further investigation to understand what factors in these specific wood types promote colonization, and additionally to disentangle colonization versus fruiting trends.

4.6. Rot type and colonization patterns

Contrary to our hypothesis, rot type was unrelated to a species' ability to adapt to alternate wood types, with white-rot aspen associates strongly impacted and others similarly affected. However, rot type may impact the general ability to establish themselves in fresh wood bolts. The two most successful species overall were white-rot species: *S. stellae* and *P. tenuis*. In contrast, three brown-rot species—*A. crassa*, *A. infirma*, and *A. lapponica*—had noticeably lower average detection rates. A notable exception of brown-rot species was one *A. infirma* strain, which achieved over 90% detection. Interestingly, our brown-rot species, which generally are thought to have higher substrate affinity than white-rot species (Rustøen et al., 2023), did not show strong wood-type preferences beyond the trend of preferring slow-grown qualities. An evolutionary analysis of the *Fomitopsis* clade reveals it consists mainly of generalist species (Krah et al., 2018). This aligns with our findings that *A. crassa* and *A. infirma* may be less restricted by wood type than initially predicted. It is also possible that the ecological roles of some brown-rot species as stress-adapted colonizers could influence their apparent habitat associations, which are often inferred from fruit-body records rather than mycelial distributions. These broader ecological dynamics may partly explain why brown-rot species responded differently to early-stage substrates in this experiment. However, brown-rot species were the only ones benefiting from farm establishment before translocation. Even though a recent study found no competitive differences between white-rot and brown-rot species in low-stress lab conditions (Fukasawa et al., 2020), the field-based approach of the present study serves as a more complex natural setting for assessing these competitive dynamics, where functional trait differences in resource acquisition and stress tolerance may become more apparent. Our observations may provide evidence to the suggestion that competition under highly stressful conditions could reveal more differences and provide a direction for future research.

4.7. Impact of inoculation factors

Our findings indicate that the form of inoculum generally had a limited impact on mycelial establishment. The general lack of difference observed for most of our species suggests that for mycelial establishment in fresh wood, both sawdust and dowel inoculum forms are largely

equally effective. *S. stellae* and *A. crassa* showed a significant difference, with sawdust spawn leading to notably lower relative DNA abundance compared to dowels in the former, and lower detection rates in the latter. This is counter to some mushroom growing guides which claim dowel spawn leads to slower log colonization (Shults et al., 2025), but that regards generally easy growing fungi. It could be that growing inside the dowel provides more protection for the mycelium than sawdust.

Delivery time, referring to when inoculated bolts were delivered to the forest site (immediately, after one growing season, or after two growing seasons), impacted the colonization success of some species. Later delivery was associated with higher DNA abundance for *A. infirma* and *A. lapponica* (after one growing season), and for *A. crassa* (after two growing seasons). This suggests that the extended period at the farm prior to site delivery provided conditions beneficial for these species, likely buffering environmental stress and reducing priority effects from early-arriving competitors. These conditions may have offered protection from competitors and stabilized moisture during initial establishment (Fukasawa, 2021). No species was negatively impacted by remaining on the “mushroom farm.” Therefore, it could be recommended as a safe practice, saving resources if the reintroduction sites are remote or spread out.

4.8. Impact of strains

The findings suggest strain selection may be among the most critical factors influencing inoculation success. While many reintroduction efforts correctly emphasize using multiple strains to maintain genetic diversity, there has been little evidence or discussion in conservation mycology regarding variation in individual strain performance—beyond recent acknowledgment of possible intraspecific differences in inoculation research (Edman et al., 2024) and broader discussion of strain-level considerations in conservation inoculation frameworks (Wainhouse and Boddy, 2022). This level of uncertainty contrasts with the industrial mushroom realm, where strain selection is well-established and extensively investigated (Chakravarty, 2011; Cervera-Gascó et al., 2023). Our data provides conclusive evidence of strain importance, showing dramatic differences in establishment success within the same species. For instance, while most *A. infirma* strains had moderate to low success, one strain achieved over 90% detection. Likewise, one *A. lapponica* strain showed a remarkable detection rate over 90%, while the rest were under 20%. The range of *S. stellae* was also large, where one strain only established in a third of the bolts, and one was detected in every bolt it was inoculated in. While culture age and handling can influence many aspects of performance ability in fungi, this does not appear to explain the observed differences. Strains ranged from less than one to about four years old, maintained under identical storage conditions, and establishment patterns did not correspond to culture age. Nonetheless, such factors merit further consideration in future reintroduction work. Many studies appear to utilize strains based on availability rather than a selection process informed by performance. That said, early survival may not correlate with long-term survival in the habitat and fruit-body production, and genetic diversity should not be overly compromised for ideal inoculation qualities. All strains used in our study originated from wild populations shaped by natural selection, so strains that appeared “slow” under our conditions may be adapted to traits not captured here (e.g., drought tolerance or competitive ability). Moreover, large-scale programs that rely too heavily on a single high-performing strain risk reducing genetic diversity or biasing restored populations toward traits favored in artificial substrates. While strain performance clearly matters for early establishment, reintroduction efforts should maintain genetic diversity and avoid overemphasizing a single genotype. Still, identifying and documenting effective strains, and subsequently ensuring their proper cryopreservation for future access, would be a large advancement for conservation mycology. Studying intraspecific functional diversity can help understand characteristics that influence colonization success, patch-level persistence, and population

resilience under variable environmental conditions.

4.9. Ecological considerations and justification of reintroductions

Although fungal reintroductions necessarily target only a small subset of the full species community, their ecological footprint can be justified when declines are driven by dispersal limitation or habitat discontinuity rather than by competitive exclusion. Wood-inhabiting fungi form highly structured successional assemblages, and many threatened polypores are weak early colonizers that sometimes fail to reach suitable substrates despite habitat availability, particularly in fragmented landscapes (e.g., Boddy, 2001; Abrego et al., 2015). In these cases, assisted colonization can complement habitat restoration by enabling establishment where natural colonization is unlikely within relevant timescales. By using fresh bolts as new carrier substrates, inoculation should not displace existing communities, and decomposition processes may proceed through normal successional pathways. These considerations also relate to broader risk assessment frameworks for conservation translocations. While the present study did not include a formal IUCN-style risk assessment, key risk categories were inherently addressed through conservative experimental design, including the use of locally sourced strains and ecologically appropriate host substrates. Nonetheless, the application of standardized risk assessment protocols for fungal translocations would represent an important advance for the field, particularly as such interventions move toward larger-scale conservation implementation. These introductions represent a temporary and spatially limited intervention relative to the scale of forest processes, yet they can help recover key species whose presence enriches substrate diversity and supports the local ecosystem. Fungal species can be selected for reintroduction to address different conservation needs, including to restore missing functional groups, support specialized dependent insect or fungal guilds, or bolster severely threatened taxa. When applied judiciously and in combination with habitat protection, fungal reintroductions therefore may constitute an ecologically beneficial conservation tool.

4.10. Conclusions

These methods provide a robust framework for planning future fungal reintroductions, offering a solid foundation for scaled application. Future work should focus on determining whether these initial establishments progress toward self-sustaining populations. The next steps would include periodic fruit-body surveys of the inoculated logs. Previous work indicates that fruiting of some threatened fungi can occur in the first four years (Abrego et al., 2016). Given the high inoculum volume used here, substrate colonization may be accelerated, possibly allowing species to fruit even earlier. Surveys should also extend into the surrounding forest to assess dispersal and colonization beyond the inoculated logs, providing evidence of spread and early signs of long-term persistence. Our initial success rates for fungal establishment and survival are particularly encouraging when compared to plant reintroductions, a field with established, widely applied protocols that often report lower average first-year survival rates (Godefroid et al., 2011). Measuring reproduction rates, representing a later and more important stage, will require long-term monitoring. Limited communication beyond mycological communities has slowed fungal conservation progress (Arnolds, 2009), and improved collaboration between conservation practitioners and fungal specialists is increasingly recognized as essential (Wainhouse and Boddy, 2022). For this approach to be effectively implemented, we recommend that a multidisciplinary team of mycologists, forest specialists, project managers, and population modelers work together. This collaborative effort is essential for successfully applying the practical aspects of reintroduction while adhering to the core tenets of sound reintroduction biology.

CRediT authorship contribution statement

Joette Crosier: Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization, Writing – original draft. **Reijo Penttilä:** Supervision, Resources, Conceptualization, Writing – review & editing. **Lorin von Longo-Liebenstein:** Supervision, Methodology, Conceptualization, Writing – review & editing. **Mattias Edman:** Resources, Writing – review & editing. **Leena Hamberg:** Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Joette Crosier reports financial support was provided by Stora Enso Group. Joette Crosier reports financial support was provided by UPM. Joette Crosier reports financial support was provided by Stiftelsen Skogssällskapet. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocon.2026.112061>.

Data availability

Data will be made available on request.

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