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Fungal communities in veteranised oak branches are not a replacement for naturally occurring dead wood communities

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ABSTRACT

Veteranisation is a promising management technique for dead wood creation at sites where dead wood, and ancient and veteran tree abundance is limited; it aims to replicate the microhabitats associated with ancient and veteran trees in younger trees by controlled physical damage of woody tissues. Five veteranisation treatments were applied, using a chainsaw, to healthy branches within the crowns of three mature oak trees. Treatments consisted of variations of cambium girdling, lopping, and lopping with girdling in combination; in addition, two branches that had died naturally were selected from each tree for comparison. Veteranised branches were harvested after 14 months. The fungal community composition was determined in different parts of the branch and their 3-dimensional structure mapped in representative branches. *Stereum gausapatum* was the most frequently isolated basidiomycete from veteranised branches, which occurred in all treatment types. The most commonly isolated ascomycete of veteranised branches was *Ophiostoma quercus*, which caused distinctive pockets of discoloured wood and was associated with half girdle treatments. There were significant differences in fungal species composition between veteranised branches and branches that had died naturally. This compositional difference may influence the development of later stage fungal communities, managers must consider these community compositional differences when prescribing veteranisation.

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Dead wood; veteranisation; fungal communities, conservation, oak

Introduction

European forest habitats are amongst the most fragmented and disturbed globally, with only 0.7% of European forest cover being considered primary and without anthropogenic disturbance (Sabatini et al., 2018). Dead wood is an integral component of these forest ecosystems where its availability is central to maintaining forest productivity, nutrient and carbon storage and recycling, providing critical habitat, refugia and nutrient sources for a variety of organisms, including wood decay fungi, saproxylic invertebrates, lichens, bryophytes, mammals, and birds (Lonsdale et al., 2008; Stokland et al., 2012). Despite the widely acknowledged importance of dead wood in supporting forest

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

biodiversity and the functioning of forest ecosystems, substantial dead wood resources are becoming a rarity in European landscapes, with a 90% decrease in coarse woody debris (CWD; Ø >10 cm) compared to pre-industrial levels (Dahlberg et al., 2010). Declines in dead wood availability have coincided with concurrent reductions in large old habitat-rich living trees (ancient and veteran trees), leading to some saproxylic groups being considered amongst the most threatened groups of organisms in Europe (Stokland et al., 2012). For example, 18% of European saproxylic beetles are considered threatened by the IUCN (Cálix et al., 2018).

Saproxylic fungi are the major agents of wood decomposition and play a pivotal role in mediating the availability of dead wood to other taxa through enzymatic modification of wood structure. They, therefore, exert important bottom-up constraints on which species can utilise dead wood. Despite this critical role in the dead wood creation process, fungi are seldom the focus of conservation efforts (Dahlberg et al., 2010). They are the forgotten ecosystem engineers of the current conservation paradigm, usually hidden within substrates and remaining largely unseen, except for when they fruit, making them difficult to survey.

Saproxylic fungi are dependent on spatial and temporal continuity in the availability of dead wood resources, which can be fulfilled by ancient and veteran trees. Such trees are keystone ecological structures that contribute to ecosystem functioning disproportionately in relation to their area of occupancy and biomass (Manning et al., 2006). They maintain ecological continuity across space and time by providing colonisable habitat to a diverse range of taxa, enhancing ecosystem connectivity, and providing structural enrichment to sites (Manning et al., 2006). As trees age, the probability of accumulating damage and dysfunction increases. Often this dysfunction provides crucial microhabitats (e.g. wounds, cavities, crown dead wood, bark fissures and bark necroses) for a wide range of taxa (Kraus et al., 2016; Siitonen, 2012). The decline in microhabitat rich ancient and veteran trees threatens ecological continuity and is expected to have negative consequences on the persistence, richness, and colonisation rates of saproxylic species (Mestre et al., 2018; Nordén et al., 2013; Norros et al., 2012; Penttilä et al., 2006). Furthermore, rare or specialist taxa with narrow niche spaces that are already limited in their potential to colonise new sites are likely to be more severely impacted by increasing habitat isolation (Nordén et al., 2013; Norros et al., 2012; Penttilä et al., 2006).

Dead wood creation has emerged as a promising approach for saproxylic conservation and for preserving ecological continuity, with the explicit goal of increasing the availability of dead wood habitat (Sandström et al., 2019). Veteranisation has been developed as a management tool which aims to recreate microhabitats associated with old trees through the controlled damage of suitable young living candidate trees, without killing the tree (Bengtsson et al., 2012; Pešout et al., 2020). Although veteranisation is gaining acceptance as a management technique and is widely applied, it often lacks an empirical basis, and many attempts have not been sufficiently monitored nor evaluated (But see, Bengtsson & Wheater, 2021; Menkis et al., 2020). Previous dead wood habitat creation in trees has used chainsaw felling, girdling, wounding or uprooting of whole trees, and has shown that artificially created dead wood may harbour a higher overall richness of fungal species but that such communities exhibit different species compositions (Komonen et al., 2014; Pasanen et al., 2014, 2018). Therefore, suggesting

veteranisation may not replicate the ecological quality of naturally occurring ancient and veteran tree habitat.

As veteranisation works are frequently conducted by arboriculturists, understanding whether these works deliver the intended conservation outcomes has a broad scope for guiding the day-to-day decision making of tree managers. We assessed this by applying five types of veteranisation treatments that may be prescribed in tree crowns, consisting of variations of lopping distal foliage and girdling the vascular cambium, and applied them to branches within the crown of mature oak trees. We compared this to early decay stage dead wood that had arisen naturally within the crown. Using a culture-based approach, differences in the composition and structure of fungal communities between creation treatments and naturally dead wood are compared. Specifically, we test the hypothesis that artificial dead wood creation in oak (*Quercus robur*) leads to the development of fundamentally distinct fungal communities than those present within dead wood that has arisen naturally. Oak was selected as it is one of the most important native tree species within the United Kingdom harbouring high levels of biodiversity with 2300 associated species (326 species with obligate associations) (Mitchell et al., 2019). It is well-known for producing long-lasting dead branch habitat, comprising very durable heartwood. The well-characterised oak branch basidiomycete community, with a limited number of main decay species, make oak an ideal model system for elucidating the effect of artificial crown dead wood creation on fungal communities (Boddy & Rayner, 1981, 1983a).

Materials and methods

Experimental design

In August 2018, three mature oak trees were selected from within an oak-dominated mixed stand located within the Forest of Dean (Table 1; grid ref: SO 58,043 08716). Each tree was visually inspected, which indicated no significant dysfunction, ill-health, stem- or root-associated fungal sporocarps, nor the abundance of microhabitats that are particularly associated with ancient and veteran trees (Kraus et al., 2016). From each tree, six apparently healthy second-order branches, with a minimum basal diameter of 10 cm were selected. Branches were randomly assigned one of five treatments or a no treatment control (Figure 1). Treatments were intended to inhibit xylem function in different ways to lead to different rates of wood drying within each branch which is known to effect fungal community development. The following five treatments were applied: (a) lopping of distal foliage; (b) complete cambium girdling (ring barking) immediately distal to the branch union; (c) lopping of distal foliage combined with complete cambium girdling immediately distal to the branch union; (d) cambium girdling of the top 180°circumference immediately distal to the branch union; (e) cambium girdling of the bottom 180°-circumference immediately distal to the branch union. At the same time as treatments were applied, two naturally occurring early decay stage dead wood branches from each tree were removed for comparison to veteranised branches. To ensure branches were as comparable as possible, early decay stage dead branches selected conformed to the criteria of decay class 1 as defined by Heilmann-Clausen and Christensen (2003) e.g. wood was physically hard, and bark was intact and sound. Veteranised branches and no treatment controls were harvested after 14 months in the field. Extensive decay columns

Table 1. Summary of tree and branch biometric data and the treatments applied to each branch. Vet: veteranised branch.

Tree Identity	Tree DBH	Tree height (m)	Branch identity	Branch diameter (cm)	Branch height (m)	Branch type	Treatment
1	1050	18	1.1	10	11	Vet	Lopping and Girdle
			1.2	18	14	Vet	Girdle bottom
			1.3	10	12	Vet	Lopping
			1.4	19	13	Vet	Control
			1.5	12.5	13	Vet	Girdling
			1.6	12.5	14	Vet	Girdle top
			1.DW1	11	11	Dead wood	-
			1.DW2	15	7	Dead wood	-
2	780	17	2.1	15	14	Vet	Lopping and girdle
			2.2	10	14	Vet	Girdle
			2.3	12.25	13	Vet	Control
			2.4	12.5	14	Vet	Lopping
			2.5	11.5	12	Vet	Girdle bottom
			2.6	17.5	12	Vet	Girdle top
			2.DW1	15	9	Dead wood	-
			2.DW2	9	10	Dead wood	-
3	1150	18	3.1	13	14	Vet	Girdle top
			3.2	11	9	Vet	Control
			3.3	10.5	16	Vet	Lopping and girdle
			3.4	12	9	Vet	Girdle
			3.5	13.5	9	Vet	Lopping
			3.6	11	8	Vet	Girdle bottom
			3.DW1	21	10	Dead wood	-
			3.DW2	13	4	Dead wood	-

can form in as little as one growing season; therefore, 14 months was deemed sufficient for the development of early successional fungal communities (Boddy & Rayner, 1983b).

Treatment application

Prior to treating each branch, chainsaws were sterilised to prevent the transfer of fungal inoculum between branches. Firstly, the chainsaw side cover, guide bar, and chain were removed, and these components alongside the body and clutch housing were then scrubbed to remove any build-up of material, sprayed, and wiped down with 10% bleach followed by 70% isopropanol. Chains were then assembled onto the guide bar and transferred to sealed pre-sterilised bags. The guide bar assembly was then re-attached to the body while enclosed within the sterile bag and pulled up to the climber on rope. Sterilised bags were kept on the guide bar assembly until immediately prior to treatment application.

Girdling treatments were performed by running the edge of the bar through the bark and cambium tissues perpendicular to the direction of the branch. Lopping and lopping with girdle treatments were applied from within reach of a safe working position at the second order branch union resulting in a mean lopped branch length of 120 cm (S.D = 21 cm) and a mean wound diameter of 10.25 cm (S.D = 1.4 cm).

At harvest, branches were removed from the tree at the base and lowered to the ground using rigging techniques to prevent damage and placed on plastic tarpaulin to avoid contact with the soil. The presence of fungal sporocarps was noted. Branches were

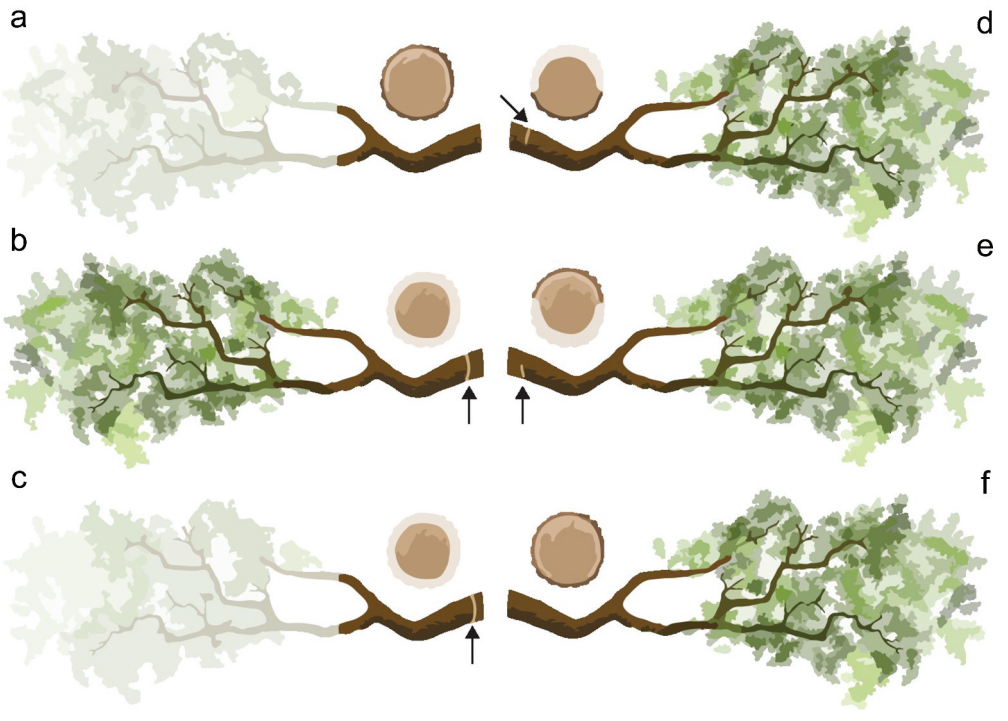

Figure 1. Dead wood creation treatments applied to oak branches. Faded regions indicate branch material removed. (a) lopping of distal foliage, (b) complete cambial girdling immediately distal to the branch union, (c) lopping of distal foliage combined with complete cambial girdling immediately distal to the branch union, (d) cambial girdling of the top 180° immediately distal to the branch union, (e) cambial girdling of the bottom 180° immediately distal to the branch union (f) no treatment control. Arrows indicate half or full girdle treatments.

cut into approximately 15 cm lengths using a sterilised chainsaw and transported to the laboratory for further sampling.

Culture-dependent sampling of fungal communities

In the laboratory, the transverse face of each section was inspected for the appearance of decay, and photographed using a DSLR camera (Canon, Japan). Fungal isolations were made directly from the transverse face of each wood section based on the appearance of decay. The isolation region was surface sterilised with 10% domestic bleach. Then approximately 2.5 cm^2, 1 mm thick, sections of wood were aseptically removed using a 25 mm chisel that had been sterilised by dipping into 70% ethanol then heated in a flame. Subsequently, using a second 6 mm chisel sterilised as previously described, 5–7 chips of 2–3 mm were aseptically excised from directly beneath the previously removed 2.5 cm^2 section and placed on to 2% malt extract agar (MEA; pH = 5.3 ± 0.2, 20 g l^{-1} malt, 15 g l^{-1} agar no. 2, LabM, UK).

Plates were incubated in the dark at 20°C to allow the development of fungal mycelia. Cultures were inspected regularly and passed through successive rounds of sub-culturing onto fresh media until pure cultures were obtained. Cultures were

grouped into morphotypes based on similar mycelial morphology. To aid the qualitative interpretation of each branch section, estimates of the number of genetic individuals were obtained via somatic incompatibility testing. All conspecific isolates within a 15 cm branch section were paired pairwise on MEA and the fusion of mycelia between isolates indicated that isolates were the same genetic individual.

The species identity of morphotype groups was ascertained by DNA sequencing of the full internally transcribed spacer region (ITS). Firstly, a representative isolate from each morphotype group was selected and 4–5 mm^2 of mycelium was aseptically removed using a sterilised 1 mm diameter needle and placed into a sterile 2 mm collecting tube.

To extract and amplify fungal DNA the Extract-N-Amp™ Plant Tissue PCR Kit (Sigma Aldrich, USA) was used, following the manufacturer's guidelines. Extracted DNA was amplified via the polymerase chain reaction (PCR) using the primer pair ITS1f (5"-CTTGGTCATTTAGAGGAAGTAA-3' (Gardes & Bruns, 1993); and ITS4 (5'-TCCTCCGCTTATTGATATGC-3' (White et al., 1990); (Sigma Aldrich, USA). The PCR was performed under the following conditions: 95°C for 3 min, 35 cycles of: 94°C for 30 sec, 56°C for 30 sec, 72°C for 1 min, followed with a final 10 min elongation at 72°C. Gel electrophoresis was performed to check PCR products using 1.5% agarose gels stained with Sybr Safe (Invitrogen, USA). PCR products were purified using QIAquick Purification Kit (Qiagen, Germany) following the manufacturer's guidelines and DNA concentrations quantified via Nanodrop (Thermo Fisher, USA). Purified PCR products were Sanger sequenced in the 3'-5" direction by Eurofins Genomics (UK). Sequences were compared to published sequences in the UNITE curated fungal database to assign a taxonomic classification to each sequence (Kõljalg et al., 2013).

Multivariate community analysis

All statistical analysis was performed using R statistical programming language in R version 4.1.2 (R Core Team, 2021) using the *Vegan* package (Oksanen, 2017). All quantitative analysis was performed on binary transformed count data at the branch level, species represented by only one or two isolates were removed from the analysis and a Sørensen's distance matrix computed. This treatment of the data and choice of distance matrix reduces the influence of rare taxa which can be highly influential in multivariate community analysis. Therefore, differences identified in this analysis will more aptly capture true differences in the core community composition and not just differences being driven by poorly represented rare taxa. Principal Coordinate Analysis (PCoA) was performed to visualise the fungal community structure within each branch. Significant differences in fungal community composition between natural occurring dead wood and veteranised branches were tested using a permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Branch type and tree ID were used as predictor variables. A second PERMANOVA was performed using only the veteranised branches with treatment type replacing branch type as a predictor variable.

Results

General patterns of species occurrence

In total, 117 branch sections from 24 branches including 6 naturally occurring dead branches were surveyed for the presence of wood-inhabiting fungi. From 542 attempted isolations, 339 pure cultures were obtained corresponding to 79 species, 8 Basidiomycota and 71 Ascomycota. Across all branches 77% of species were represented by one or two isolates; a smaller proportion of Basidiomycota isolates (13%) were represented by one or two isolates than Ascomycota isolates (85%). Mapping the spatial arrangement of dominant isolates (Figure 3; Figure 4) revealed that basidiomycetes occupied larger territories than ascomycetes. No isolates were derived from the control branches.

Excluding isolates made once or twice only, 18 species were identified (Basidiomycota, 7 species; Ascomycota, 11 species) across all branches. A total of 8 species were identified from branches that had died naturally. Veteranised branches had a total of 11 species. From the veteranised branches, lopping treatments had the highest number of species (9 species) (Table 2). Basidiomycete communities in all veteranised branches were dominated by *Stereum gausapatum*, which occurred ubiquitously across treatment types and in naturally occurring dead wood (Table 3). *Stereum hirsutum* was also frequently isolated from veteranised branches; however, it was absent from all dead branches. *Mucidula mucida* was the most frequently isolated basidiomycete from naturally occuring dead wood branches but was absent from all veteranised branches. While most ascomycete species were sporadically isolated, *Ophiostoma quercus* was consistently the most frequently isolated from veteranised branches.

General patterns of decay and qualitative analysis

All veteranised branches had firmly attached and intact bark at the time of harvest. Excluding lopping treatments, most branches were foliated at the time of removal and many branches had epicormic shoots (Figure 2a), indicating that despite complete or partially girdling damaging the vascular cambium, branches maintained some functionality in water conduction. *S. gausapatum* sporocarps (Figure 2b-c) were found on 60% (9/15) of

Table 2. Number of fungal species isolated from branches of each treatment type and from branches that had died naturally (excluding singleton and doubleton taxa).

Substrate type	Treatment	Sections sampled	total species No.	No. basidiomycetes	No. ascomycetes	Mean total No. species /section	SD No. species/ section
Dw	-	42	8	3	5	1	0.68
Vet	BG	7	4	2	2	2	0.78
Vet	G	14	5	2	3	1	0.97
Vet	L	17	9	4	5	2	1.61
Vet	L+G	18	6	4	4	2	1.03
Vet	TG	10	5	1	2	2	0.78
Total	-	108	18	7	11	2	1.2

Dw: Natural dead branches, Vet: Veteranised branches, BG: bottom girdle, G: full girdle, L: lopping, L + G: lopping and girdle, TG: top girdle.

Table 3. General patterns of occurrence of wood decay fungi within veteranised branches.

	Stereum gausapatum	*Stereum hirsutum*	*Vuilleminia comedens*	*Phlebia rufa*	*Mucidula mucida*	*Bjerkandera adusta*	Isolate 1.1.2.4	*Ophiostoma quercus*
Phylum	Basidio	Basidio	Basidio	Basidio	Basidio	Basidio	Basidio	Asco
Decay type	white	white	white	white	white	white	white	-
Sporocarp present	+	-	-	-	-	-	-	-
Trees present	1,2,3	1,2,3	1,3	1	1,2,3	2	1	1,2,3
No. branches colonised	15	8	2	1	4	1	2	13
No. sections occupied	39	18	4	3	21	3	4	27
Mean number of mycelial individuals/ occupied section	3	2	1	1	-	1	1	-
Distance from branch base found (cm, min – max)	0–120	0–105	30–165	45–75	0–215	30–60	45–60	0–75
Diameter of colonised branches (cm, min – max)	7–16	10–16	10–11	12	7–14	14	11–12	9–16
Treatments present	BG, G, L, L +G, TG	BG, G, L, L+G, TG	-	L	-	L+G	L+G	BG, G, L, L +G, TG
Present in dead wood	+	-	+	-	+	-	-	-

Basidio: Basidiomycota, Asco: Ascomycota, BG: bottom girdle, G: full girdle, L: lopping, L + G: lopping and girdle, TG: top girdle. DNA extraction from isolate 1.1.2.4 was not of sufficient quality for molecular identification.

branches in which it was isolated (Branch ID: 1.1, 1.DW.2, 1.3, 1.5, 2.1, 2.2, 2.DW.2, 3.3 and 3.5), no other sporocarps were detected. Visual inspection of the branch sections indicated that the extent of decay had not progressed past areas in close proximity to veteranisation wounds, with the appearance of decay in transverse branch sections decreasing with increasing distance from the wound. Across all branches, most decay was of the white rot type with different treatments giving rise to qualitatively different patterns of decay (Figure 2d-h; Figure 3). In general, full girdle, lopping, and lopping with girdling were more effective at producing dysfunctional sapwood and had visibly larger decayed areas. The transverse face of sections adjacent to lopping cuts were completely colonised (Figure 2d) whereas girdle treatments resulted in a central area of undecayed heartwood (Figure 2e), and in half girdle treatments decay was restricted to areas adjacent to the wound (Figure 2f). Several lopping treatments, alongside one complete girdle, had dense patterns of interaction zone lines (Figure 2d). Somatic incompatibility tests between isolates from these decay regions revealed that these interaction lines demarcated the territorial boundaries of several mycelial individuals of either the same or different species. Observations made 2–3 h after cutting the veteranised branches into sections showed that some decayed regions began exuding a black fluid that stained the adjacent wood surface (Figure 2g). Removal of the surface wood revealed small dark pockets of discoloured wood dispersed radially across the outside edge of the transverse cut face that occasionally appeared to be interrupted by medullary rays (Figure 2h). Isolations from these decay pockets revealed *O. quercus* to be consistently associated with this pattern. In contrast, decay patterns from naturally dead branches were more homogenous across the transverse face of each section and was exclusively of the white rot decay type (Figure 2i).

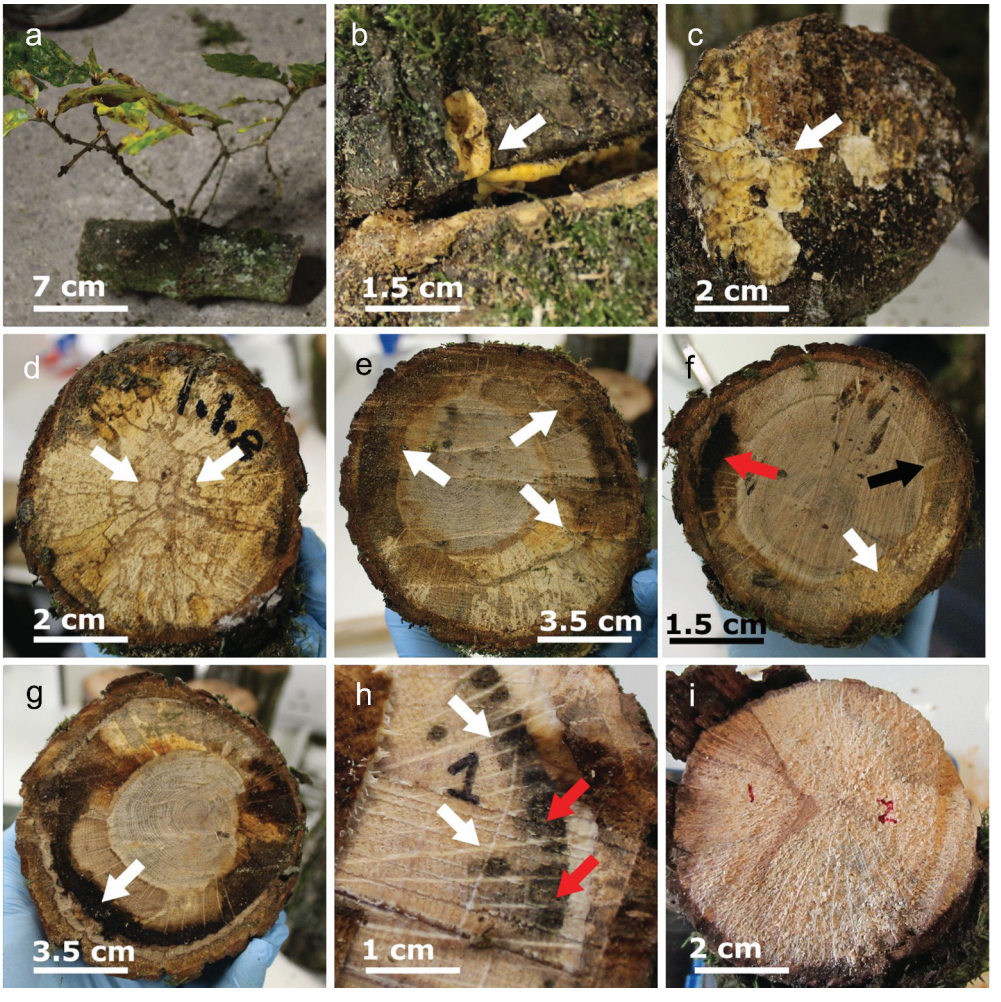

Figure 2. General patterns of fungal colonisation and characteristics of branches. (a) Epicormic growth from a veteranised branch. (b) full girdle wound with a sporocarps of *Stereum gausapatum* (arrowed). (c) lopping wound with a sporocarps of *S. gausapatum* (arrowed). (d) interaction zone lines between conspecific and allospecific *Stereum* species mycelial individuals on the transverse face of a lopped branch. White arrows indicate interaction zone lines. (e) full girdle treatment with a central undecayed heartwood region enclosed within peripherally decayed sapwood (white arrows). (f) half girdle treatment with decayed regions restricted to areas immediately adjacent to wounding (white arrows). The formation of a heartwood wing (HW) between the intersection of decayed and functional sapwood is shown (black arrow). Red arrow indicates black staining. (g) characteristic staining (arrowed) from veteranised wounding developed 2–3 hours after branches were cut into section leading to staining of adjacent wood surface. (h) characteristic decay pattern associated with several girdle treatments. *Ophiostoma quercus* was consistently isolated from this pattern. Red arrows indicate pockets of decayed material whereas white arrows indicate intact and apparently undecayed medullary rays. (i) transverse section of a dead wood branch with homogenous white rot decay pattern. All scale bars are approximate.

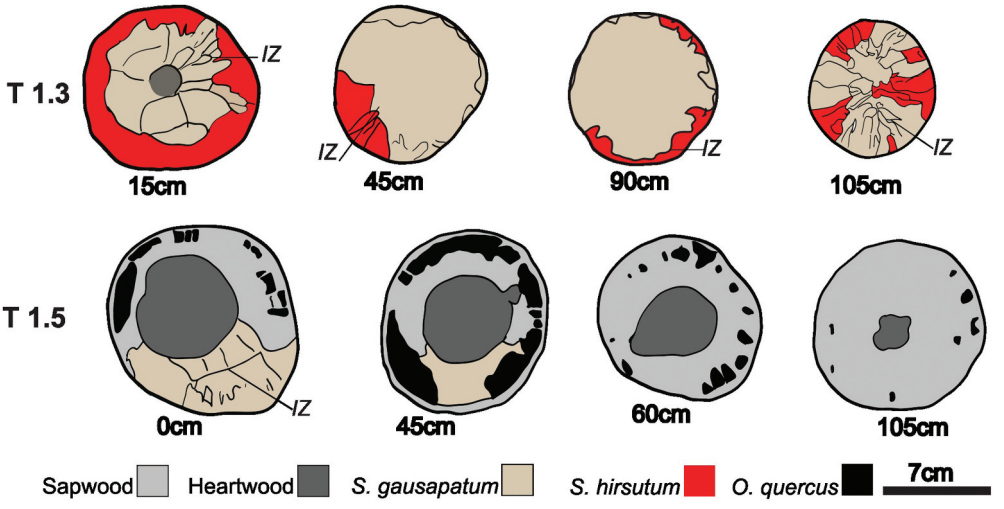

Figure 3. Cross-sectional illustrations of community spatial structure of dominant decay fungi isolated from veteranised branches that had been lopped and girdled (T 1.3) or bottom girdled (T 1.5). in T 1.3 *Stereum gausapatum* and *Stereum hirsutum* were the dominant basidiomycetes. Heartwood was only present in basal branch sections where it formed a barrier to colonisation. Interaction zone lines (IZ) developed in branch sections adjacent to wounding, indicating possible colonisation of the wounds via basidiospores as opposed to the development of mycelia from latently present propagules. In T 1.5 *S. gausapatum* was the dominant basidiomycete; however, it occupied smaller territories with decreasing distance from the branch base and substantial areas of sapwood were undecayed in all sections. It was unable to colonise the heartwood. The ascomycete *Ophiostoma quercus* was isolated from all branch sections and was associated with a black staining. Length along branch of each cross section is shown below each section. Scale bar is approximate.

Community composition

Visual inspection of the PCoA ordinations revealed a clear difference in fungal community composition between veteranised branches and the naturally occurring dead branches (Figure 5a). PCoA ordination of the veteranised branches alone revealed no clear patterns of community differentiation between treatment types (Figure 5b). PERMANOVA indicated that there was a significant difference in community structure between veteranised and naturally occurring dead branches (Substrate Type, $F_{1,20} = 13.19$, $R^2 = 0.41$, $P < 0.001$), but not between trees (Tree, $F_{2,20} = 0.05$, $R^2 = 0.72$, $P < 0.71$). When veteranised branches were analysed separately, PERMANOVA indicated there was no significant difference in community composition between treatment types (Treatment type, $F_{4,14} = 1.27$, $R^2 = 0.34$, $P = 0.324$) or between trees (Treatment type, $F_{2,14} = 0.86$, $R^2 = 0.12$, $P = 0.552$). Overall, these results indicate distinct fungal community compositions between veteranised and naturally occurring dead branches but no compositional differences between different veteranisation treatments.

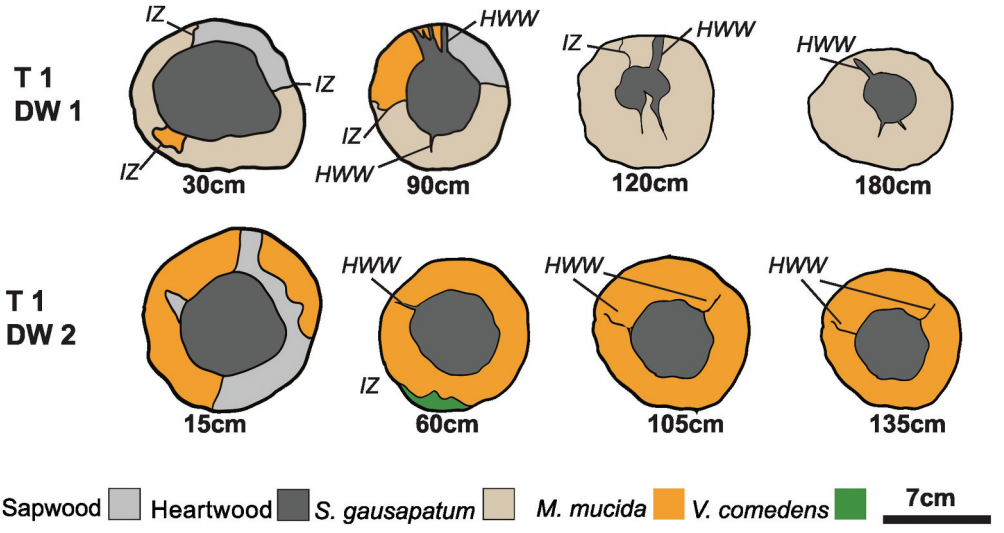

Figure 4. Cross-sectional illustrations of community spatial structure of dominant decay fungi isolated from naturally occurring dead wood branches (DW 1.1) (DW 1.2). in DW 1.1 *Stereum gausapatum* was the dominant basidiomycete isolated from all sections and caused an extensive white rot of the decayed sapwood. *Mucidula mucida* was isolated up to 90 cm from the branch base and an interaction zone line (IZ) demarcated the territorial boundaries between *S. gausapatum* and *M. mucida*. in DW 1.2 *M. mucida* was the basidiomycete isolated from all sections and caused an extensive white rot of the decayed sapwood. The sapwood of basal section was only partially colonised with undecayed areas; however, the sapwood of more distal sections was decayed throughout. At 60 cm from the branch base, *vuilleminia comedens* and *M. mucida* were isolated from the same sampling point but no interaction zone line was formed; therefore, the exact boundaries of the territories between the two individuals could not be ascertained. The heartwood was a substantial barrier to colonisation and was undecayed in all sections. Heartwood wings were present in both branches. Length along branch of each cross section is shown below each section. Scale bar is approximate.

Discussion

Using culture-dependent methods, we found significant differences in fungal community composition between chainsaw veteranised dead branches and naturally arising dead wood in the crown of mature oak trees (Figure 5). While different treatment types typically led to a simple basidiomycete community dominated by *Stereum* spp. there was considerable variation in the ascomycete community (Table 3).

Differences in fungal community composition between both standing and felled created dead wood, and naturally occurring dead wood have been reported in dead wood creation studies of conifer tree species, *Picea abies* (Norway spruce) and *Pinus sylvestris* (Scots pine), in Northern European boreal forests (Komonen et al., 2014; Pasanen et al., 2014, 2018). Previous studies have at least partially relied on sporocarp observations to make inferences on the impact of dead wood creation on fungal diversity (Komonen et al., 2014; Pasanen et al., 2014, 2018). Most species in the present study were detected solely via isolation of mycelia from wood, with only *S. gausapatum* occasionally producing sporocarps (Table 3; Figure 2). Sporocarp surveys alone

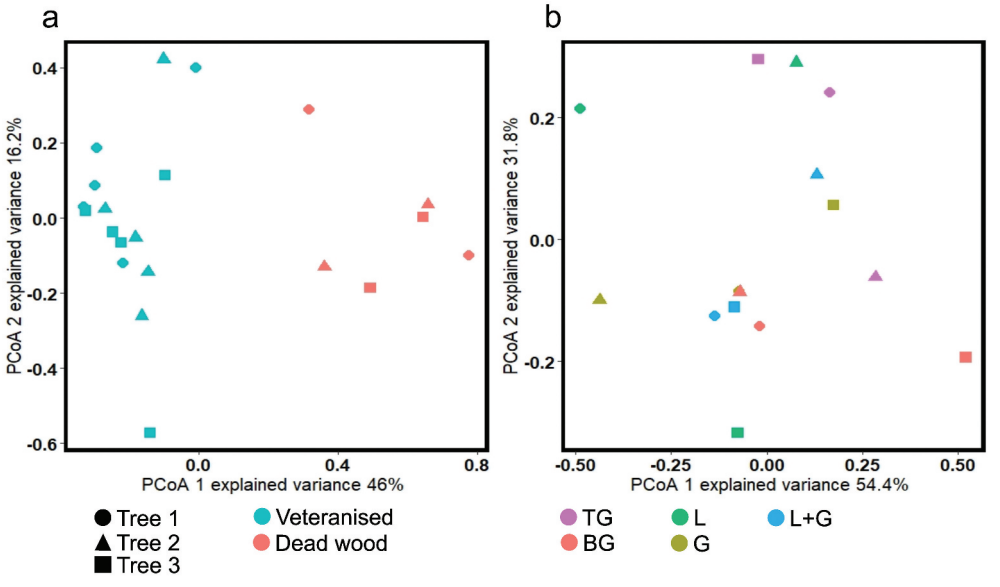

Figure 5. (a) Principal coordinate analysis of fungal community structure from veteranised branches ($n = 15$) and branches that had died naturally ($n = 6$) using Sørensen's similarity coefficients calculated from presence-absence occurrence data. (b) Principal coordinate analysis of fungal community structure of 15 veteranised branches using Sørensen's similarity coefficients calculated from presence absence occurrence data. Colour indicates treatment type. BG: bottom girdle, G: full girdle, L: lopping, L + G: lopping and girdle, TG: top girdle.

considerably underestimate the true fungal diversity within dead wood, missing many species present as vegetative mycelia but not fruiting during the survey period (Allmér et al., 2006; Ovaskainen et al., 2013). For example, unlike other basidiomycetes identified in this study, *M. mucidula* produces ephemeral sporocarps only in the autumn. In the present study, it occupied considerable territories within dead wood that had arisen naturally but was absent from all veteranisation treatments (Table 3). Previous studies are, therefore, measures of the differential reproductive success of fungal species associated with particular substrates rather than complete representations of fungal community composition (Ovaskainen et al., 2013; Runnel et al., 2015). Here, by utilising culture-dependent methods, we avoided biases associated with using sporocarp surveys alone to assess community composition. Moreover, vegetative mycelial individuals absent as sporocarps were dominant members of the decay community responsible for significant compositional differences between naturally occurring and created dead wood.

By making isolations into artificial culture, we were able to detect significant differences between fungal communities in natural and created dead branches over a substantially shorter temporal scale than in previous studies (Komonen et al., 2014; Pasanen et al., 2014, 2018). These differences likely arise from differences in: (i) the source of fungal inoculum; (ii) the drying regime of sapwood; and (iii) death of living cells. Following natural death, drying will gradually occur, allowing the development of fungi that were latently present in functional sapwood (Boddy, 2001; Boddy et al., 2017; Chapela & Boddy, 1988), whereas veteranisation wounding will additionally allow

colonisation by fungi arriving from outside the branch when compared to branches that have died in the absence of mechanical damage. Many of these latter fungi will have ruderal traits, such as prolific and rapid production of spores, rapid germination, and growth. These species utilise relatively simple organic compounds and have a relatively poor combative ability against other fungi (Boddy et al., 2017). The rapid death of living tissues additionally provides a ready supply of non-structural carbohydrates that are easily assimilated (Hulme & Shields, 1970), this benefits many of the ascomycetes which have ruderal characteristics and might account for their preponderance following veteranisation wounding. *S. gausapatum*, which was ubiquitous across treatment types and readily formed fruit bodies on several branches following veteranisation wounding, appeared to develop from both latently present propagules as well as via air-borne basidiospores. For example, the numerous interaction zone lines associated with lopping treatments is regarded as evidence for colonisation via air-borne basidiospores (Figure 3) (Coates & Rayner, 1985). The dominant occurrence of common or ruderal trait selected fungal species on created dead wood has been reported previously, for example, *Bjerkandera adusta*, which was also identified on veteranised branches in the present study (Coates & Rayner, 1985; Lindhe et al., 2004). *B. adusta* has previously been described as arriving at newly colonisable resources via air-borne basidiospores, a colonisation pathway afforded to both *S. gausapatum* and *B. adusta* in the present study due to sapwood exposure following veteranisation treatments. Veteranised branches had higher species richness than branches that had died naturally, again reflecting the arrival of airborne spores on wounded branches (Table 2). However, differences in sampling effort may also account for this difference in species richness. Nonetheless, similar results have been reported in other dead wood creation studies (Komonen et al., 2014; Pasanen et al., 2014, 2018).

The ascomycete community within veteranised branches was highly variable with most species only occurring sporadically. Species richness of ascomycetes far exceeded that of basidiomycete in our veteranisation treatments, similar to previous reports of veteranised oak stems (Menkis et al., 2022). The insect-vectored species, *O. quercus*, dominated the ascomycete community being strongly associated with half girdle treatments; however, medullary rays appeared partially resistant to colonisation. High moisture content and lignified secondary cell wall properties of parenchyma cells likely explain this apparent resistance (Schwarze et al., 2003). *O. quercus* has been implicated as a contributing factor to European oak decline disease complexes, although its status as a pathogen has been debated (Harrington et al., 2001; Taerum et al., 2018). The occurrence of *O. quercus* following wounding supports the notion that the species is unlikely to be a primary pathogen but rather an opportunistic coloniser of partially dysfunctional sapwood.

Heartwood was a substantial barrier to fungal colonisation. Oak heartwood's low pH and comparatively large amounts of antimicrobial polyphenolic compounds make it an inhospitable environment for most fungi. Interestingly, the prevalence of well-developed heartwood wings were identified in several branches that had died naturally but were absent or less developed in veteranised branches (Figure 4) (Boddy & Rayner, 1981). Within heartwood wings, vessels are occluded by tyloses and gums forming a physical barrier to the extension of hyphae (Boddy & Rayner, 1981). When oak branches decline naturally, typically columns of sapwood, rather

than all the sapwood, becomes dysfunctional in water conduction, and the cambium dies around only part of the branch circumference. The heartwood wings form from the centre of the branch to the junction of living and dead cambium, separating functional from dysfunctional sapwood. In doing so it is possible that heartwood wings increase the mechanical stability of partially decay compromised branches that are still photosynthetically functional. In cut branches, loss of water-conductive function of sapwood is likely too quick and too widespread for heartwood wing development.

Notably, several basidiomycetes previously reported from oak branches were absent or scarce in the present study (Boddy & Rayner, 1983a), probably reflecting local absence and the number of branches sampled. Interestingly, *M. mucida* was not observed on any branches investigated by Boddy and Rayner (1983a). Although *M. mucida* may just have been absent from their sites, it is typically considered a specialist coloniser of *Fagus sylvatica* but was locally very abundant as fruit bodies at the time of branch harvest in the current study. The timing of veteranisation wounding may have reduced its ability to colonise veteranised branches via basidiospores, as *M. mucida* typically fruits in the autumn and our veteranisation treatments were carried out in August. The timing of veteranisation treatments may have important effects on community development. For example, if target species that disperse via basidiospores are not present at the time of wounding, when they do arrive, they may be outcompeted by species that have already established. Additionally, different microclimates within wood resulting from the season, may alter fungal community development (Chapela & Boddy, 1988). Future experiments should address potential seasonal effects.

It is impossible to know exactly when the branches that had died naturally became dysfunctional nor their cause of death, but there was no evidence that it was pathological. However, branches were clearly in decay class 1. Different tree tissue mortality factors can give rise to different saproxylic species assemblages. In this study, lopping treatments may be most representative of storm damage that often snaps limbs, whereas girdle treatments resemble the shading out of branches. While these treatments lead to qualitatively different decay patterns, we identified no statistical difference in fungal community composition between lopping and girdling treatments (Figure 5).

Conclusions and management implications

This study adds to an existing, but limited, evidence base for the efficacy of dead wood creation. Here, we demonstrate that early successional fungal communities in created dead wood branches significantly differed from early decay stage branch communities following natural death. This result is in broad agreement with previous dead wood creation studies of coniferous trees (Komonen et al., 2014; Pasanen et al., 2014, 2018). Our results in veteranised oak branches, along with those of others conducted in different wood types and tree species, demonstrate that not all dead wood is of equivalent ecological quality regarding fungal community composition. Tree managers should be aware of this limitation when making management decisions surrounding dead wood creation. Due to differences in fungal community composition between veteranised branches and naturally occurring dead branches, veteranisation

techniques such as those used here should not be considered a complete replacement for the loss of decay habitat. Nonetheless, when the explicit management aim is to increase fungal abundance and dead wood availability, dead wood creation including branch veteranisation can support a diverse range of fungal taxa (Komonen et al., 2014; Pasanen et al., 2014, 2018).

Decay features of ancient and veteran trees of high ecological quality develop over long temporal scales that will often far exceed the lifespan of tree managers, highlighting the irreplaceable nature of these trees. Therefore, the protection and care of existing ancient and veteran trees must be considered a primary conservation objective for tree managers as opposed to retroactively attempting to restore habitats through means of chainsaw applied veteranisation. Furthermore, the felling of mature trees for spurious safety reasons must also be avoided as these individuals will be the ancients of the future and are the greatest conservation resource tree managers have.

Finally, we stress that veteranisation techniques should only be applied to young trees. They should not be applied to ancient and veteran trees, where pruning best practice should be followed. For ancient and other veteran trees, management aims should focus on maximising their life spans and limiting stress factors contributing to their decline.

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