

Supplementary information for:

Wood decay under anoxia by the brown-rot fungus *Fomitopsis pinicola*

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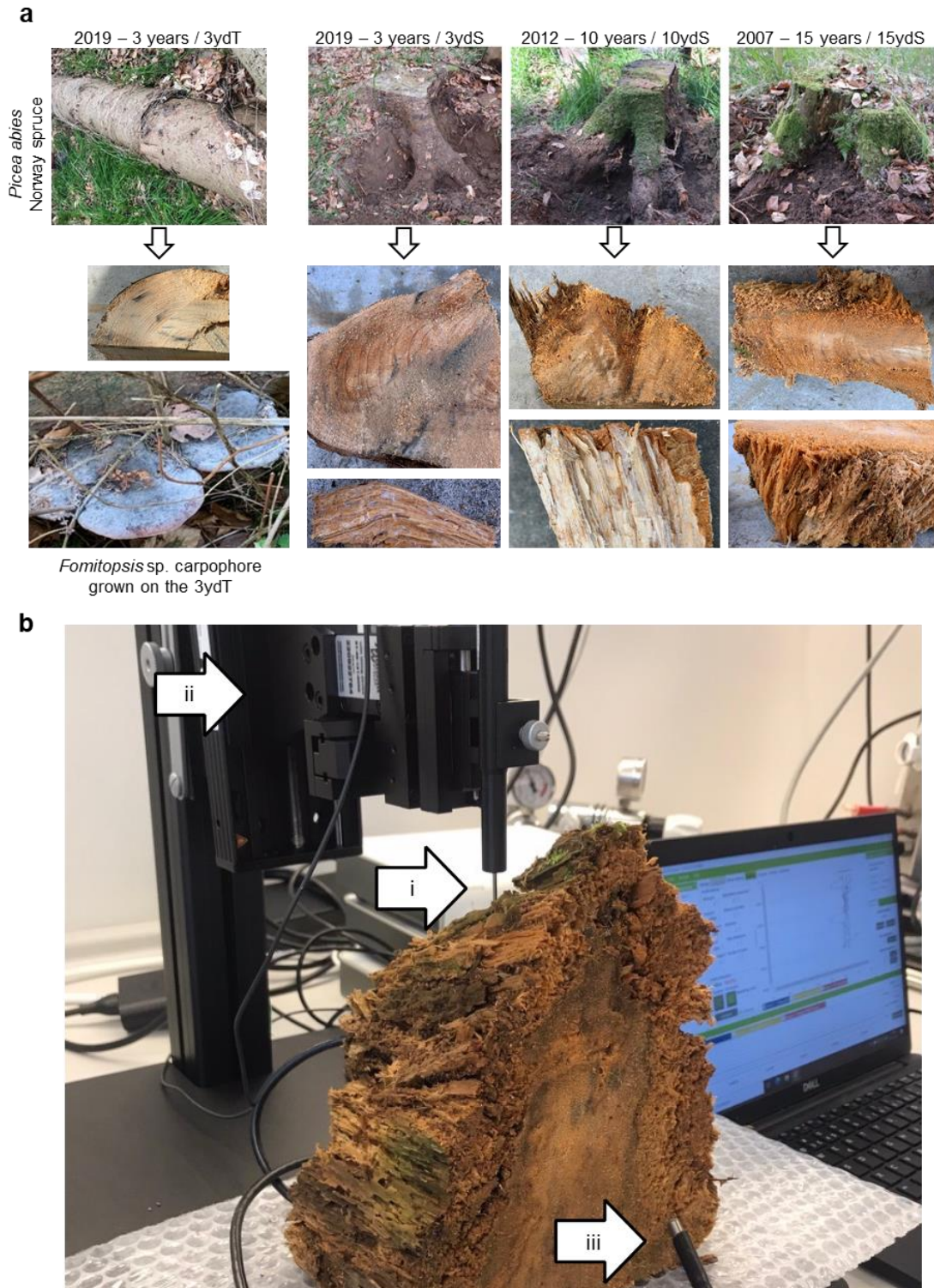
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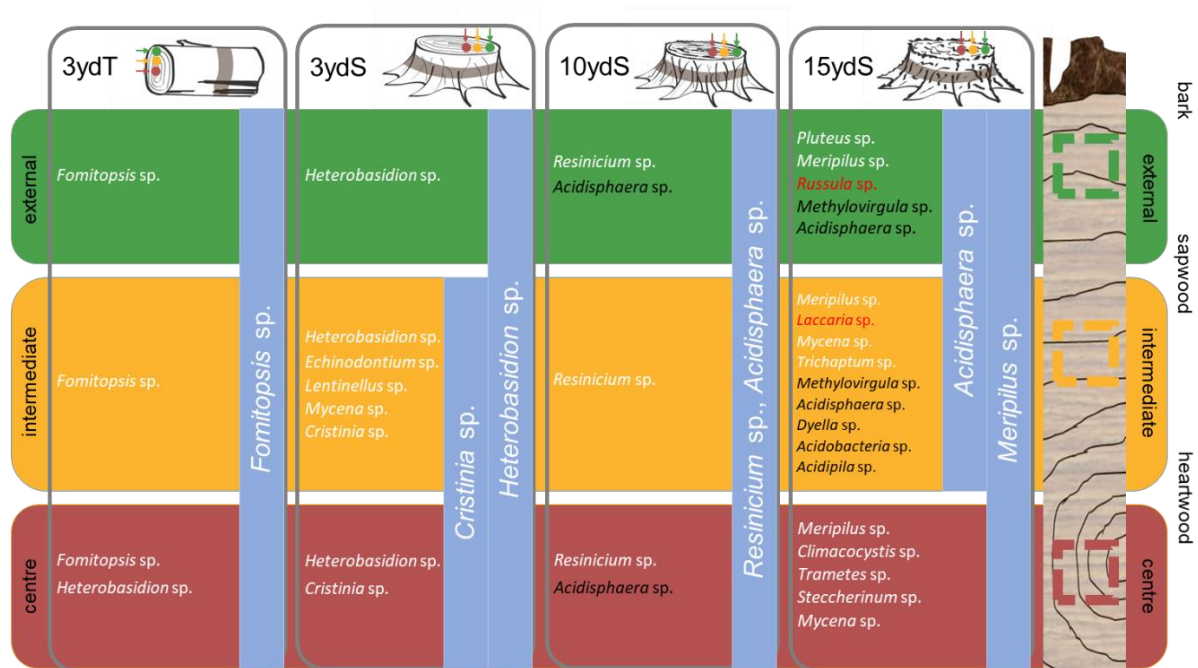
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Supplementary Fig. 14 | Protein profile during the growth of *F. pinicola* in normoxia and anoxia.

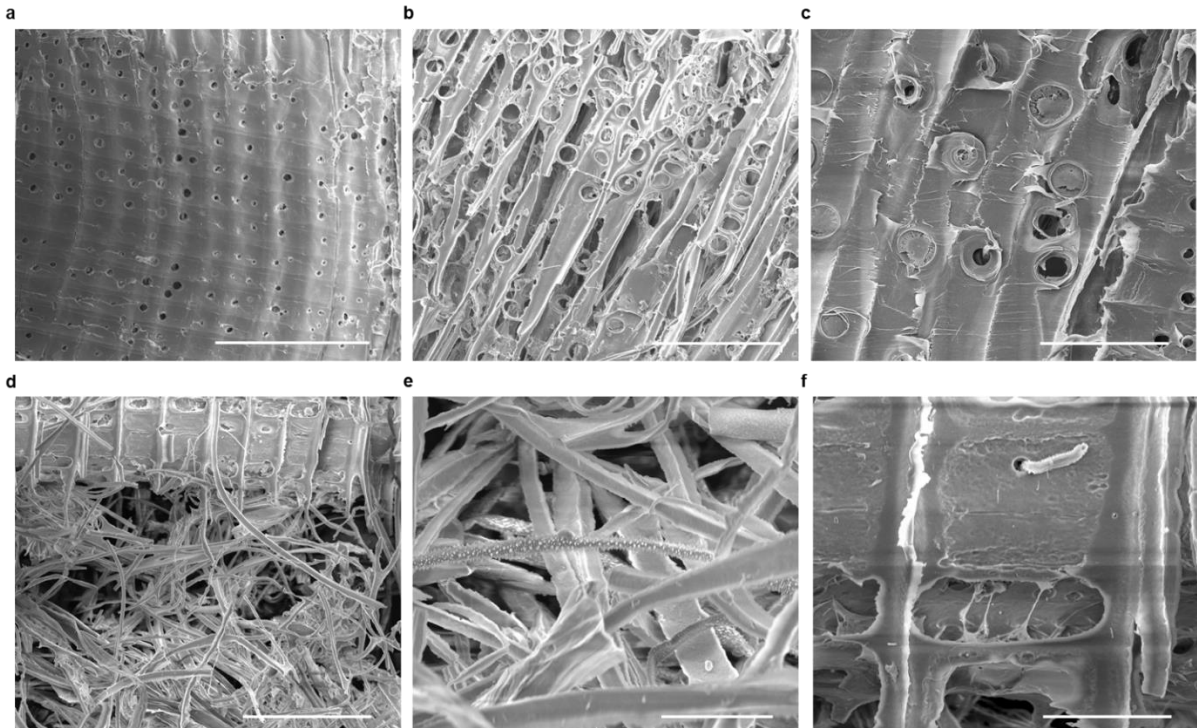
Supplementary Fig. 15 | Validation of xylanase and glucanase activities from the *F. pinicola* secretomes using cyclophellitol-derived glycoside activity-based probes.



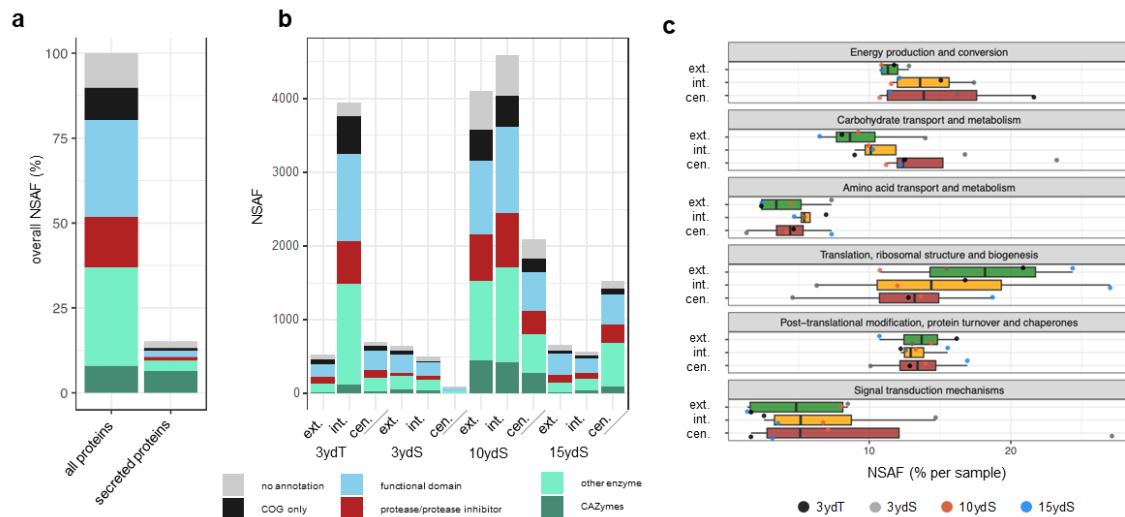
Supplementary Figure 1 | Wood sampling collected from the Stensholt Vang forest and determination of the O₂ profile in the wood pieces. a, *Picea abies* trees of different stages of decay were sampled: 3 years decayed trunk (3ydT), 3 years decayed stump (3ydS), 10 years decayed stump (10ydS), 15 years decayed stump (15ydS). **b,** Dioxygen concentrations in *P. abies* wood discs (herein 15ydS) were determined using an optical micro sensor mounted in a surgical needle. i, the sensor, and ii, motorised stage mounting the sensor, moving into the wood in 1 mm steps. iii, O₂ calibration correction by temperature measurement by a thermometer.



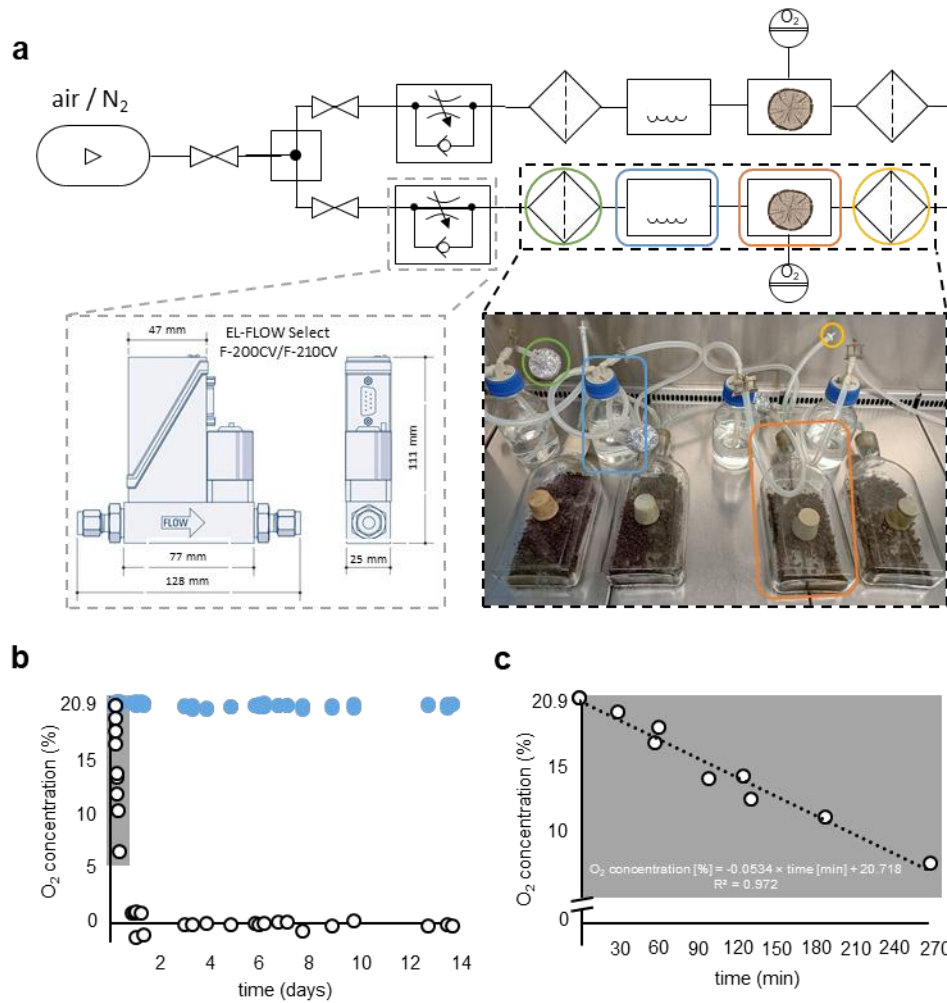
Supplementary Figure 2 | Genera detected in the different samples by metaproteomics using tandem mass spectrometry. Samples were taken from the external (dark green), intermediate (dark yellow), and central (dark red) parts of the trees after removing the part that was exposed to air (approx. 0.2 m, at the level of the brown ring, top scheme). Bacterial genera are written in black, decaying fungi in white, and mycorrhizal fungal genera in red. Taxa detected in multiple layers of the same trees are highlighted in the blue boxes. 3ydT, 3ydS, 10ydS, 15ydS as in Supplementary Fig. 1.



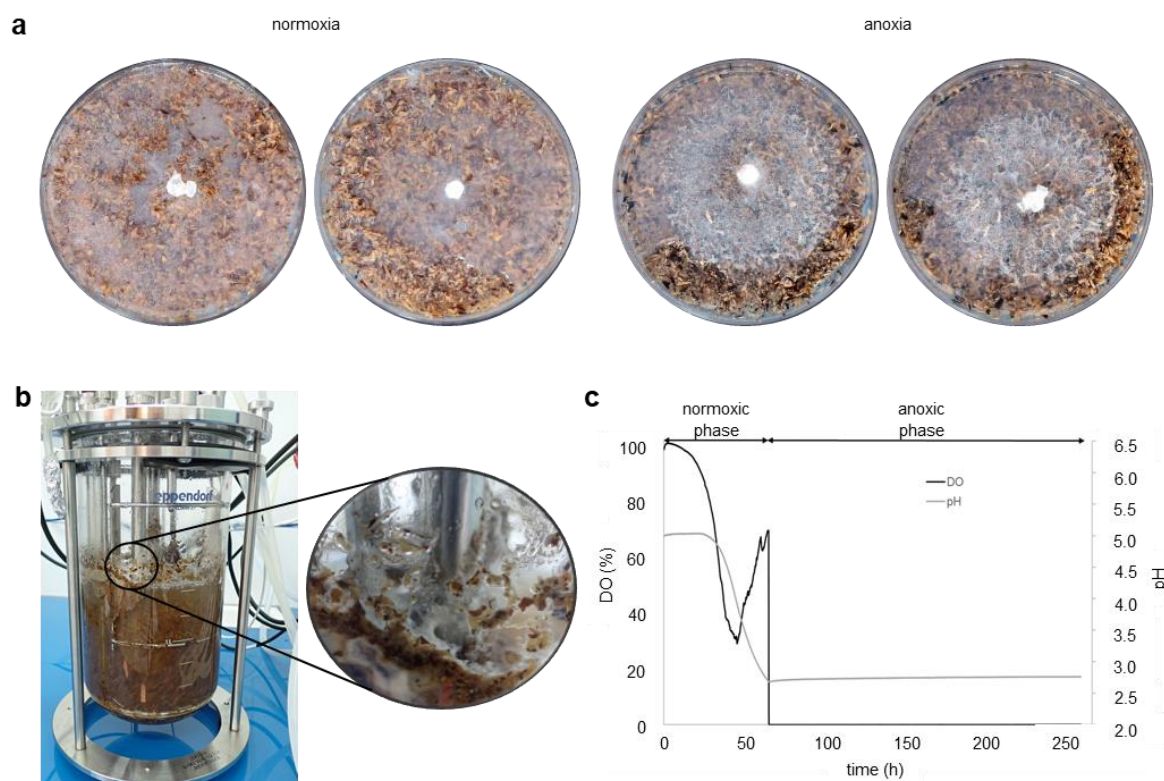
Supplementary Figure 3 | Scanning Electron Microscopy of the 3-years decayed trunk (3ydT) sample. **a**, Radial section of the tracheary tissue with crossfield pits. **b**, Radial section of the tracheary tissue with bordered pits. **c-f**, *F. pinicola* mycelium incorporated in the wood structures. **c**, Hyphae entering the pit chamber in a bordered pit. Degradation at the torus. **d**, Extensive degradation of tracheid cell walls probably leaving only S3 layer and middle lamella. **e**, Mineral structures, likely calcium oxalate crystals, according to previous reports¹. **f**, Fungal mycelium (very likely *F. pinicola*) penetrating through a crossfield pit. Scale bars in a, b, d = 100 μm , c = 50 μm , e, f = 20 μm .



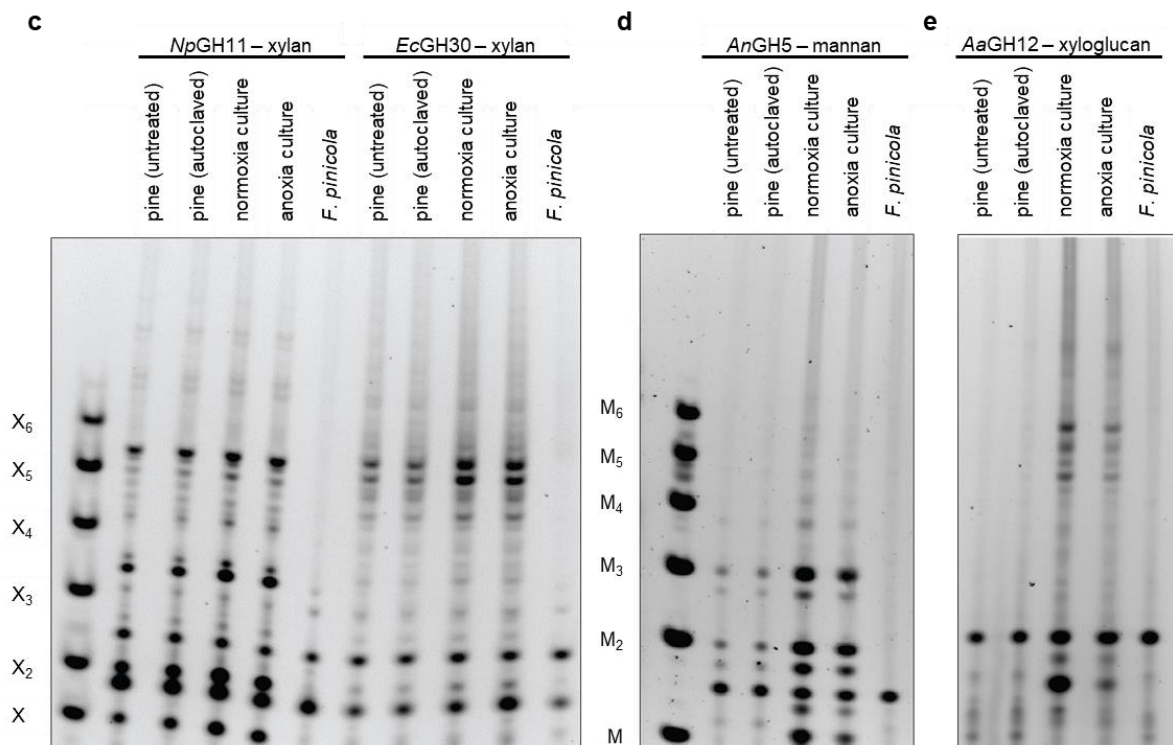
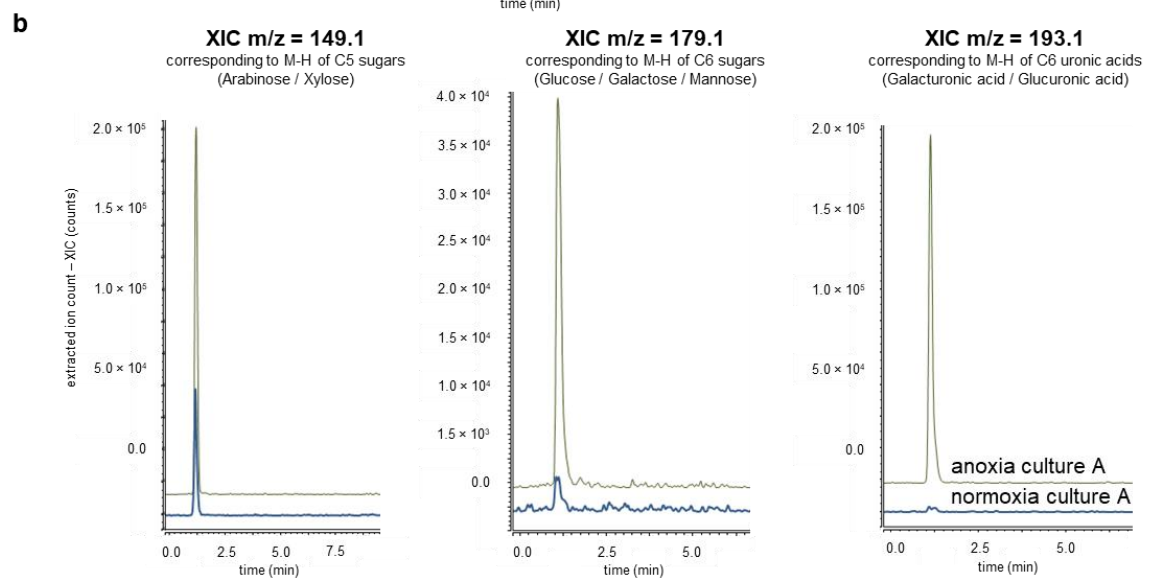
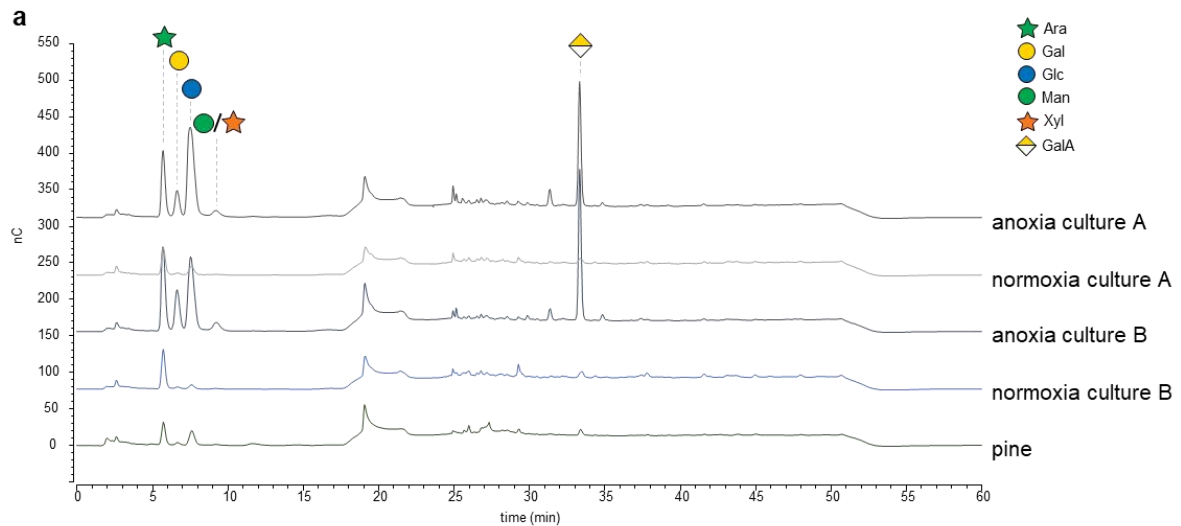
Supplementary Figure 4 | Abundance and putative function of the proteins detected in the decaying trunk and stumps by mass spectrometry. **a**, Overview of the putative functions expressed (in NSAF percentage) among all the samples. **b**, Details of the protein signal detected (in NSAF) and their putative function in each sample. **c**, Most represented COG annotation in the samples. NSAF: normalized spectral abundance factor, CAZyme: carbohydrate-active enzyme, other enzyme: all enzymes detected except CAZymes, functional domain: at least one PFAM functional domain was detected and the protein was not annotated as enzyme nor protease, COG only: match with a cluster of orthologous genes (COG) and the protein does not pertain to other categories. cen.: wood centre. int.: intermediate wood part. ext.: external wood part close to the bark. For details see Supplementary Fig. 2.



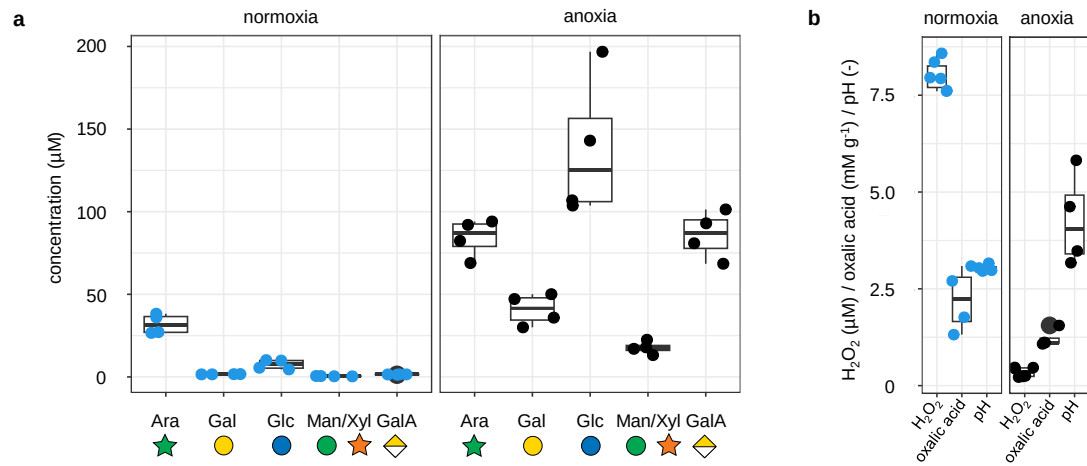
Supplementary Figure 5 | Experimental setup to control O₂ levels during solid-state cultures. a, The gas flow (air or pure N₂) was regulated by a digital thermal mass flow controller for gases (dashed grey rectangle), sterilised through a filter (green ring), humidified (blue rectangle), pumped into Roux flasks containing the fungus-wood mixture (orange rectangle) and blown out from the culture through a second filter (yellow ring). **b,** O₂ concentration during the cultivation of *F. pinicola* in the O₂-controlled set-up in normoxia (air, blue) and in anoxia (N₂, white). **c,** O₂ concentration within the first 4.5 h of the cultivation in the anoxia-induced cultures (flushed by N₂), and plot of the linear decrease reaching a stable anoxic (0.0% O₂) condition in 6.5 h.



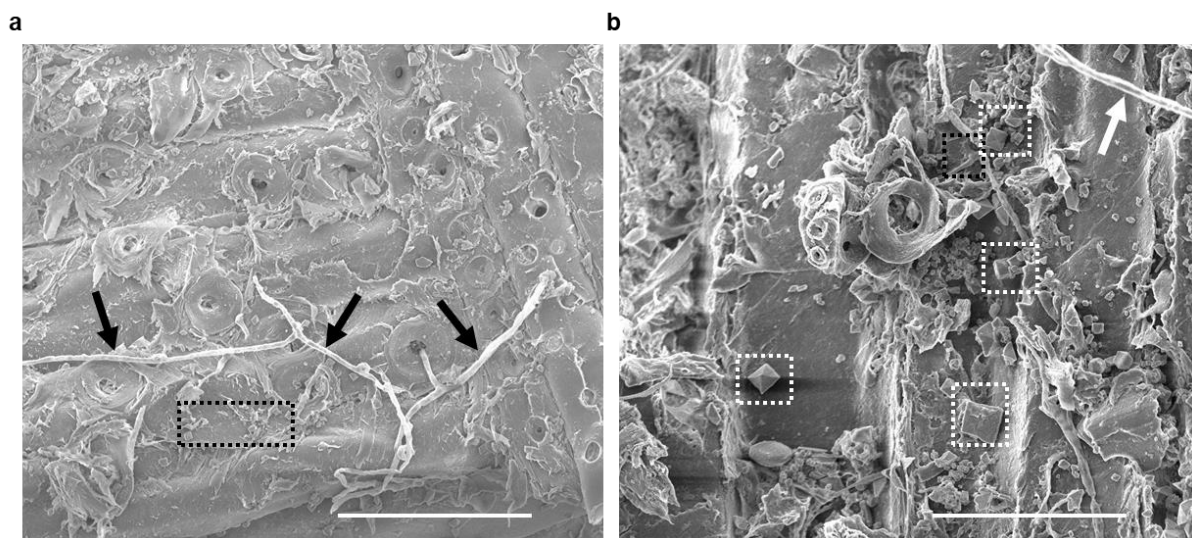
Supplementary Figure 6 | Validation of anoxic growth of *F. pinicola* on wood in an anaerobic jar and a bioreactor. **a**, Photographs of *F. pinicola* cultivated in an independent fermentation setup on a pine-agar mixture for 22 days at 30 °C in air (normoxia) and in an anaerobic jar (anoxia). **b**, **c**, Growth and physico-chemical parameters of *F. pinicola* in a bioreactor at 25 °C with autoclaved pine, continuously flushed with air (normoxia) or N₂ (towards anoxia). **b**, Culture after 11 days at the end of the fermentation with the preponderant fungal biomass grown on solid support at the N₂-liquid medium interface. **c**, Dissolved O₂ (DO, black line) and pH (grey line) in a stirring tank reactor during an initial normoxic phase followed by N₂-induced anoxia.



Supplementary Figure 7 | Wood carbohydrate analysis. **a**, Sugar monomers detected by HPAEC-PAD after soft extraction from *P. abies* wood (pine) at the end of the cultivations in normoxia and anoxia. Culture A and culture B represent two biological duplicates. **b**, LC-MS chromatograms confirming the identification of the sugar monomers and D-galacturonic acid from a culture in normoxia (blue), and from a culture in anoxia (green). **c-e**, Analysis of main hemicelluloses in pine. Saponified alcohol insoluble residue (AIR) from *P. abies* wood was hydrolysed with **c**, *NpGH11* endoxylanase and *EcGH30* glucuronoxylanase, **d**, *AnGH5* endomannanase, or **e**, *AaGH12* xyloglucanase. Hydrolysis products were analysed by polysaccharide analysis with carbohydrate gel electrophoresis (PACE). Xylo- and manno-oligosaccharide standards: X-X₆ and M-M₆, respectively.

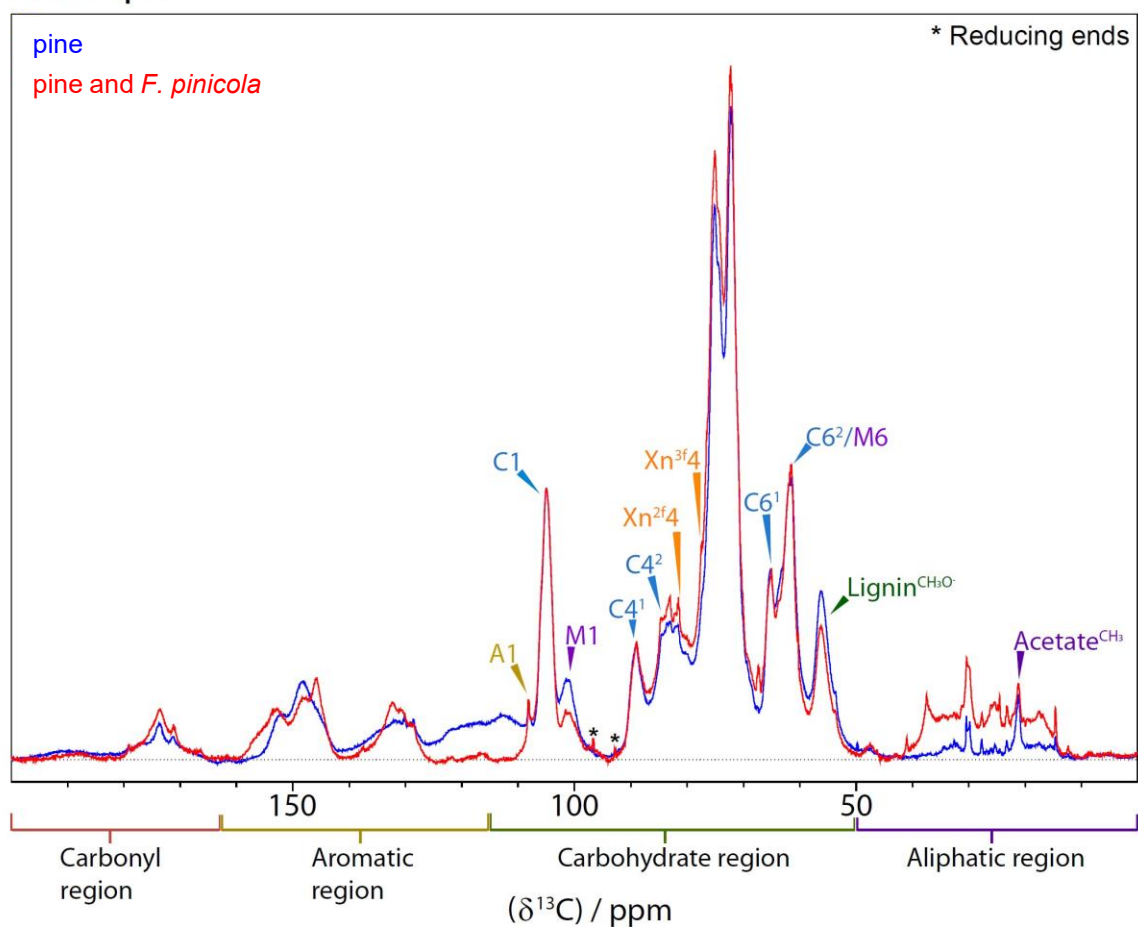


Supplementary Figure 8 | Quantification of sugar monomers, D-galacturonic acid, Fenton markers and oxalate in the soluble fractions of cultures grown in normoxia and anoxia. a, Sugar monomers, and D-galacturonic acid quantified by HPAEC-PAD (see Supplementary Fig. 7a). **b,** Two key Fenton markers hydrogen peroxide and pH, as well as oxalic acid/oxalate, which is a precursor for the enzymatic formation of hydrogen peroxide in the presence of dioxygen by the oxalate oxidase. Data originates from biological replicates ($n \geq 4$) for each condition.



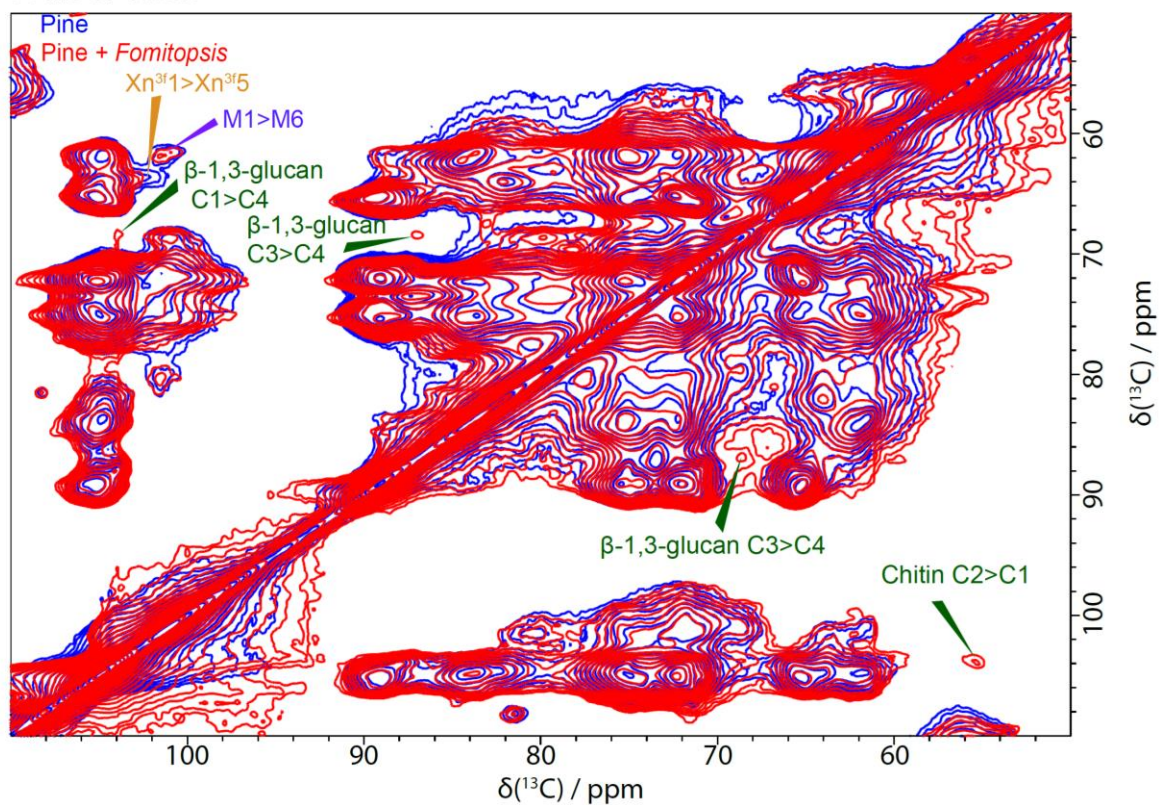
Supplementary Figure 9 | Scanning Electron Microscopy of the cultures in normoxia and anoxia.
a, *F. pinicola* mycelium (indicated by arrows) decaying wood in normoxia, and anoxia in **b**. Mineral structures, likely calcium-oxalate crystals are framed by dotted lines in both conditions, scale bars 50 μm .

20 s 1D q-DP



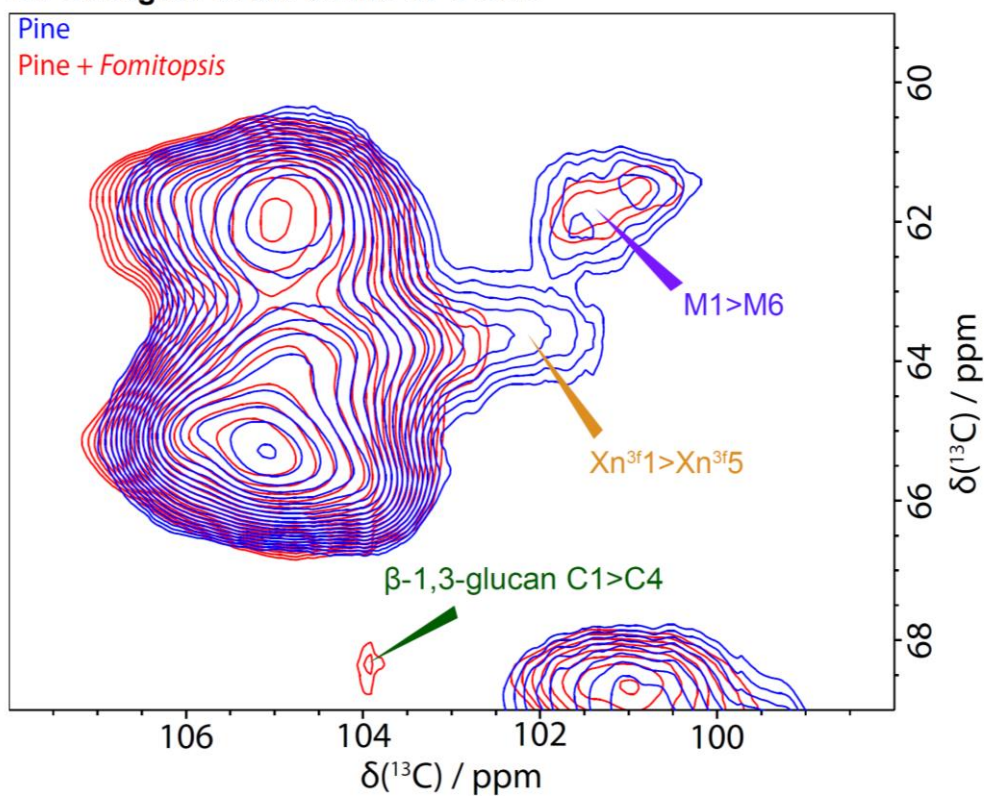
Supplementary Figure 10 | Comparison of the 1D 20s quantitative DP-MAS ssNMR spectra of pine wood (blue) and *F. pinicola* decaying pine wood (red). Asterisks indicate newly formed reducing ends at 93 ppm and 97 ppm. A, arabinose; C, cellulose; M, mannose, Xn^{2f}, 2-fold xylan; Xn^{3f}, 3-fold xylan, Lignin^{CH₃O⁻}, lignin methoxyl group; Acetate^{CH₃}, acetate methyl group.

30 ms CP PDS

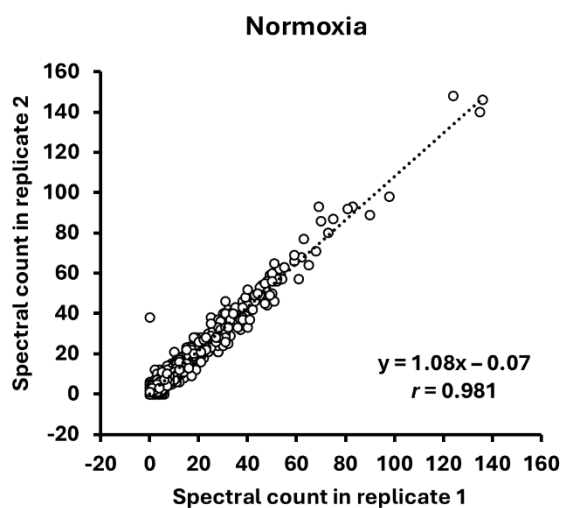
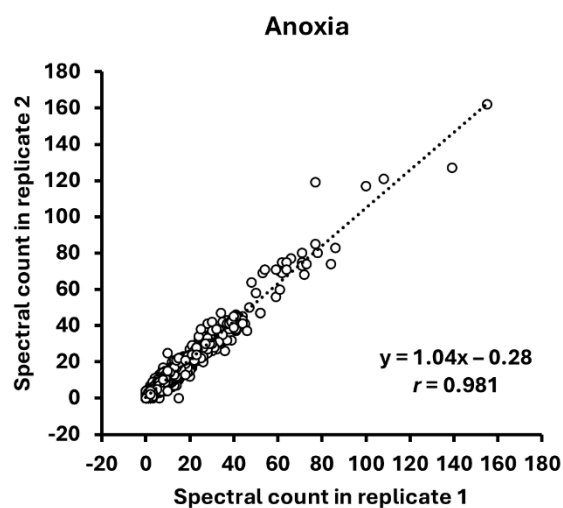


Supplementary Figure 11 | Overlay of pine (blue) and *F. pinicola* decaying pine wood (red) ssNMR 30 ms CP PDS spectra. Arrows indicate the β -1,3 glucan C1-C4 peak at (103.8, 68.3) ppm and the decreased amounts Xn^{3f} and mannose in the decaying pine wood. The spectra are normalized to the cellulose C4¹ peak at 89 ppm.

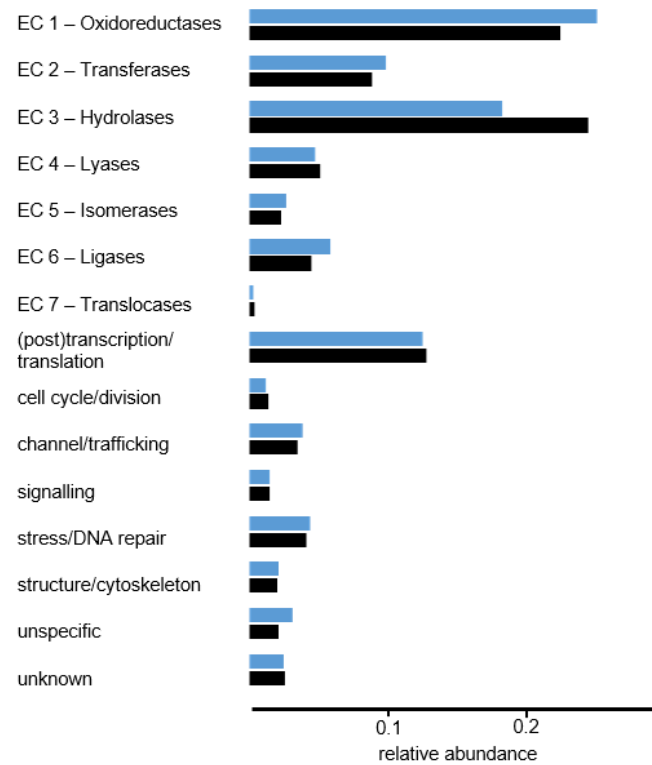
C1-C6 region of the 30 ms CP PDSD



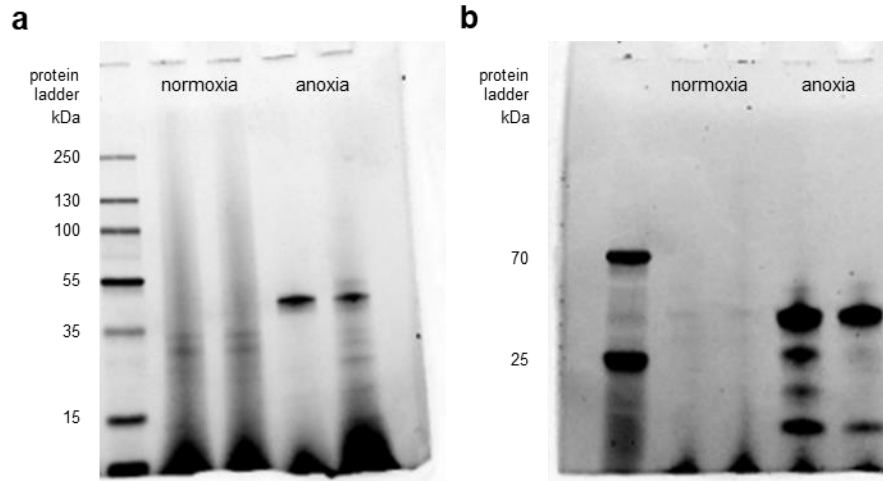
Supplementary Figure 12 | Overlay of the cellulosic C1-C6 region of the 30 ms CP PDSD ^{13}C NMR spectra of pine (blue) and *F. pinicola* decaying pine wood (red) showing the xylan Xn^{3f}1-Xn^{3f}5, mannan M1-M6 and β -1,3 glucan C1-C4 cross-peaks. There is a significant decrease in the mannan M1-M6 and Xn^{3f} conformation in the decaying pine wood. The signal for β -1,3-glucan C1-C4 cross-peaks is absent in the control pine wood.

a**b**

Supplementary Figure 13 | Assessment of reproducibility of protein abundance in biological replicates obtained in normoxia (a) and anoxia (b). For each protein, represented by a dot, the spectral count value obtained in replicate 1 was plotted against the spectral count value obtained in replicate 2. Linear regression was determined. The equation parameters are indicated, with Pearson correlation value (r).



Supplementary Figure 14 | Protein profile during the growth of *F. pinicola* in normoxia and anoxia. In blue, protein profile in normoxia with 1235 proteins in >26.7k spectral counts, and in black the profile in O₂-depletion with 1155 proteins in >24.3k spectral counts. The relative abundance represents the relative spectral counts of all proteins pooled by their predicted function.



Supplementary Figure 15 | Validation of xylanase and glucanase activities from the *F. pinicola* secretomes using cyclophellitol-derived glycoside activity-based probes. SDS-PAGE gels with fluorescence signals imaging glycoside activity in the secretomes of *F. pinicola* (biological replicates) at the end of the cultivations (14 days) in normoxia or anoxia. **a**, *Endo*- β -xylanase (Cy5⁺-ABP-Xyn, SY-F230)⁵² and **b**, *Endo*- β -glucanase fluorophore-labelled probes (Cy3⁺-ABP-Cel, CB644)⁵² show enzyme-bound fluorescence signals of the hydrolases using Cy5⁺, and Cy3⁺ filter/laser sets in a 10% SDS-PAGE (3 μ L protein ladder, 15 μ L sample) in an Amersham ImageQuant 800 Imager (Cytiva, USA, software ImageQuantTL v10.2 – 499).

Supplementary references

1 Füchtner, S., Alfredsen, G., Thygesen, L. G. Oxalate found in wood cell wall during incipient brown rot degradation. *Int. Biodeterior. Biodegrad.* **177**, 105531 (2023).