Supplementary Information for the paper

Furfurylation protects timber from degradation by marine wood boring crustaceans

Lucy S. Martin, Stanislav Jelavić , Simon M. Cragg and Lisbeth G. Thygesen

Wood treatments

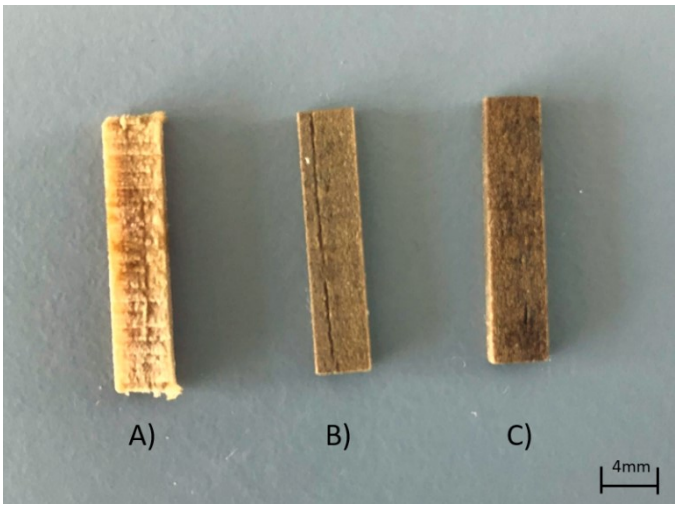

Fig. S1: Colour change in treated Pinus radiata wood used in feeding trials from a) untreated to b) treatment 1 and c) treatment 2. Wood sized 2mm x 20mm x 4mm.

Feeding trials

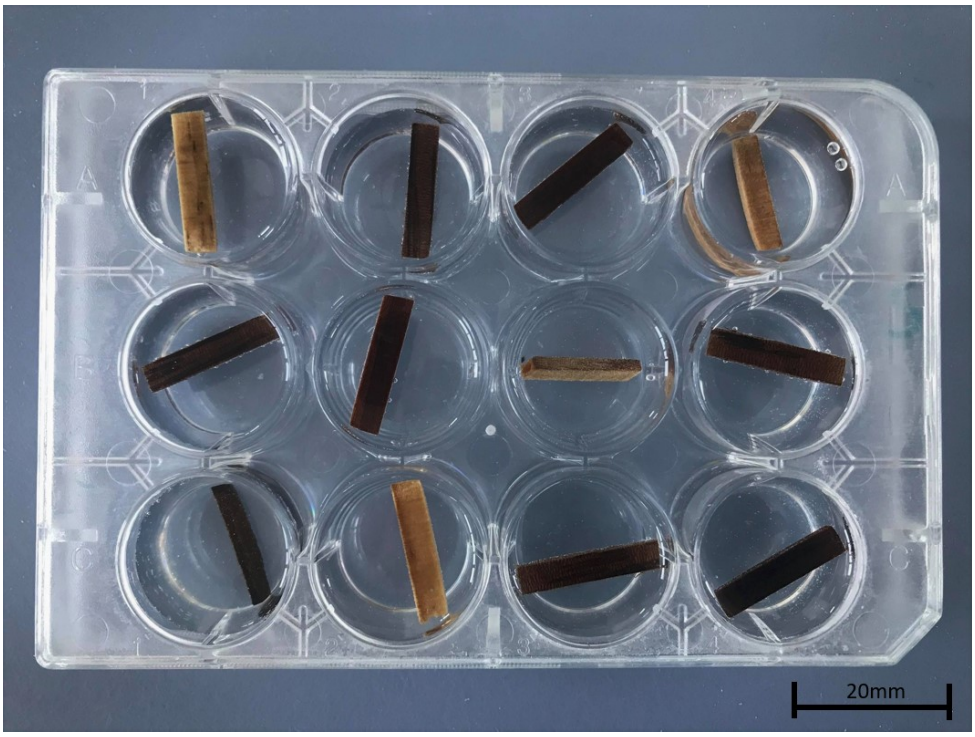

Fig. S2: In 12 multi-well cell culture plates, 5ml seawater (32-35ppt), one gribble (Limnoria quadripunctata) and one stick of either untreated, treatment 1 or treatment 2 wood (4mm x 20mm x 2mm) was added. At least one stick of each treatment was added per row and per column. In row one from left to right, untreated, treatment 1, treatment 2, untreated. In row 2, treatment 1, treatment 2, untreated, treatment 1. In row 3, treatment 2, untreated, treatment 1, treatment 2. Wells are 20mm in diameter.

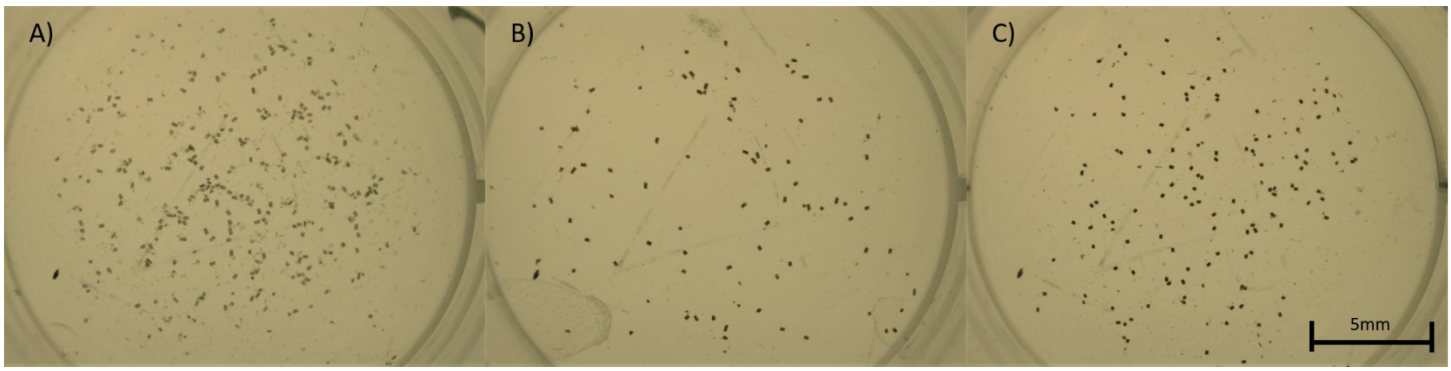

Fig. S3: Images from Leica microscope camera MC170 HD of wells containing faecal pellets from Limnoria quadripunctata after feeding on different treatments of wood (Pinus radiata). A) Faecal pellets from untreated wood. B) Faecal pellets from treatment 1 wood. C) Faecal pellets from treatment 2 wood. Well diameter is 20mm.

AFM-IR absorption maps

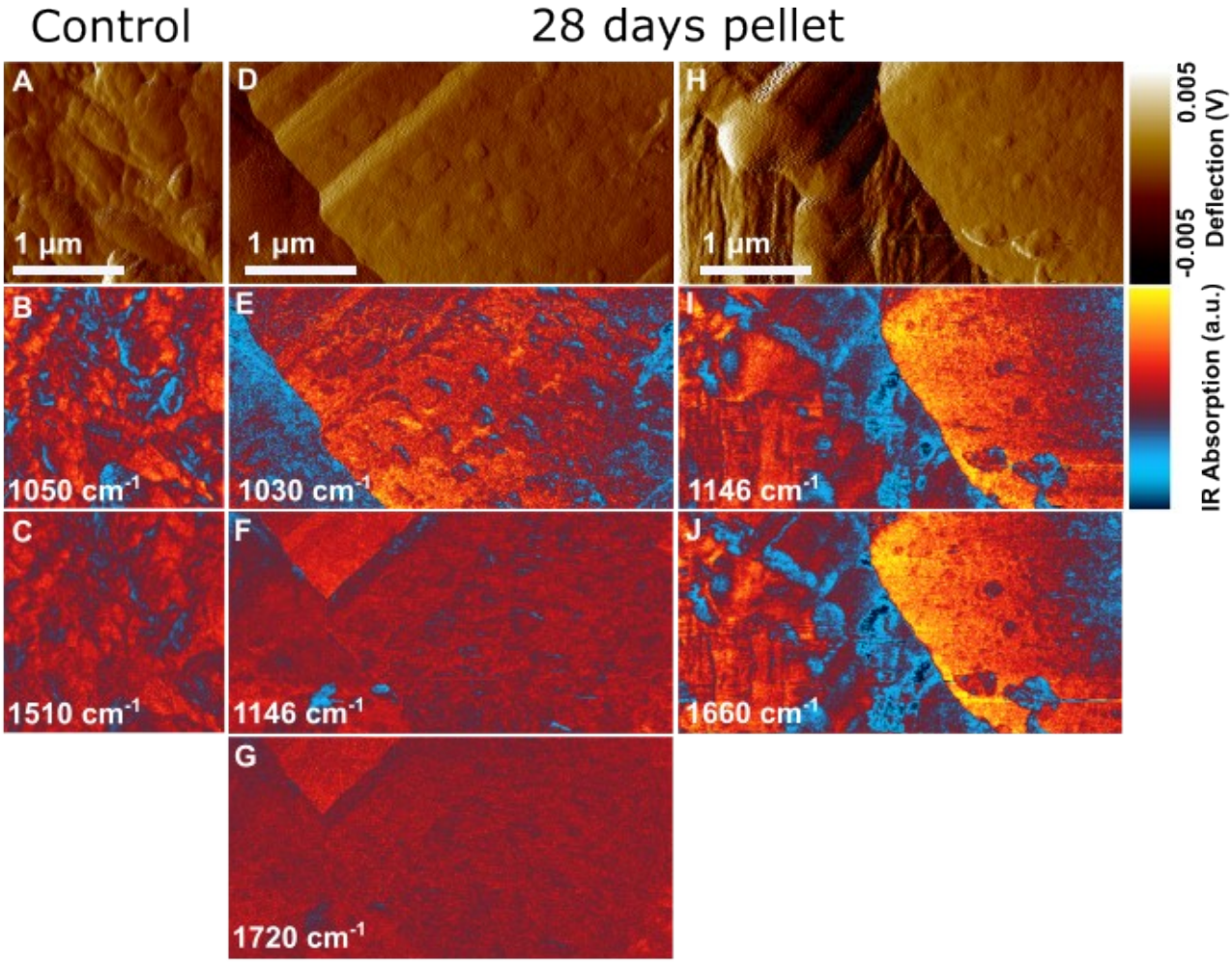

Fig. S4: Deflection AFM image of control pine (A) and corresponding IR absorption maps (B-C), and deflection images of two areas from two different faecal pellets collected after 28 days from furfurylated pine (D and H) and their corresponding IR maps (E-G and I-J). The deflection scale bar on the top-right is the same for all deflection images and the IR absorption scale bar is the same for all maps. A small displacement in x-y direction between images and maps is a result of a thermal drift, an uncontrolled movement of the AFM tip due to expansion and contractions of mechanical parts within the AFM during the analysis. The surface of wood is more homogeneous than the surface of pellets. This is seen as a lack of distinct topographic features in a deflection map A and similar distribution of absorption maxima and minima for both C-O at 1050 cm^{-1} (B) and aromatic rings at 1510 cm^{-1} (C) bands. On the other hand, deflection images of pellets (D and H) show topographic features as a consequence of various orientations of consumed wood pieces that were blended in a digestive tract of gribble. These features are not present in control wood. In addition, these features show different IR signals that cannot be explained by topography only, most clearly recognized as a poor correlation in distribution of signal maxima and minima between maps E, F and G.

PCA of ATR-IR spectra

Principal component analysis of the ATR-IR spectra confirmed our observations regarding the variations in spectral features between wood analysed as a function of treatment, leaching and feeding. In addition, by comparing the scores and loading plots, we identified additional differences between groups of spectra. The scores plot (Fig. S5A) suggested that the largest variation in spectral features (captured by PC1, 66% of the variance between spectra) was between the three groups of spectra from **unleached** wood, leached wood and faecal pellets, while the difference between untreated and furfurylated wood dominated the next principal component (PC2, 15% of the variance). That **the leached** wood grouped together in the scores plot implies that the compositional variation was negligible between the leached wood before being used for the feeding trials and the same wood left after feeding it to gribble, indicating that IR did not indicate that the wood continued to leach during the feeding trials. The result that the spectra from faecal pellets grouped separately from spectra of wood confirmed that the gribble metabolism modifies the composition of the wood on which it feeds. The score plot also suggest a smaller spectral variation between untreated and furfurylated wood compared to the variations between unleached wood, leached wood, and pellets. This confirms earlier results[1] that furfurylation does not affect wood spectra to any large exten**t**, as the molecular bonds formed blend in with already existing bonds within and between the wood cell wall biopolymers. In untreated wood subjected to leaching and digestion, the loading plot (Fig. S5B) confirmed an increase in signal between 1700 and 1600 cm^{-1} in wood subjected to leaching and digestion and a decrease in the bulk carbohydrate signal (C-O) between 1100 and 1000 cm^{-1}. Combined, these confirm a likely increase in the amount of lignin degradation products and a decrease in cellulose in these samples. Fig. S5B also shows negative peaks at positions of some of the bands known to be associated with furfuryl alcohol homopolymers[2], i.e. 1180 and 1562 cm^{-1} and possibly 791 and 734 cm^{-1} (observed in the loading plot at 789 and 733 cm^{-1}). This agrees with furfurylated samples having smaller scores for this factor than untreated wood samples.

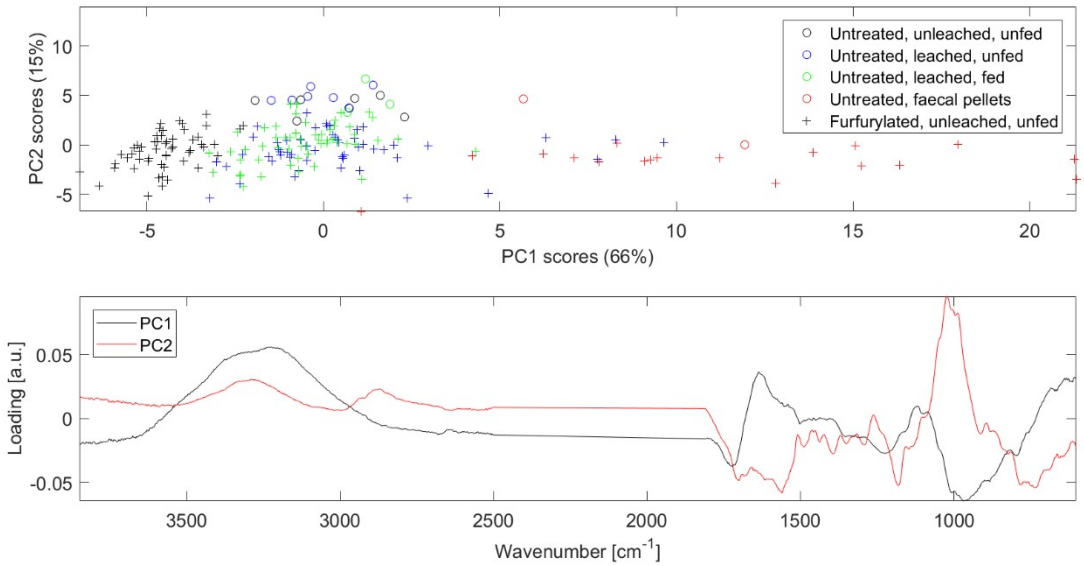

Fig. S5: Scores and loadings for the first two principal components (PC1 and PC2) of a Principal Component Analysis of ATR-IR spectra obtained from wood samples and faecal pellets from feeding trail II, treatments 1 and 2 combined. In the scores plot spectra of untreated wood are represented by circles and spectra of furfurylated wood by plus signs. Colours represent the step during the study on which the wood was assessed, as indicated for untreated wood samples in the legend.

AFM-IR sample positions

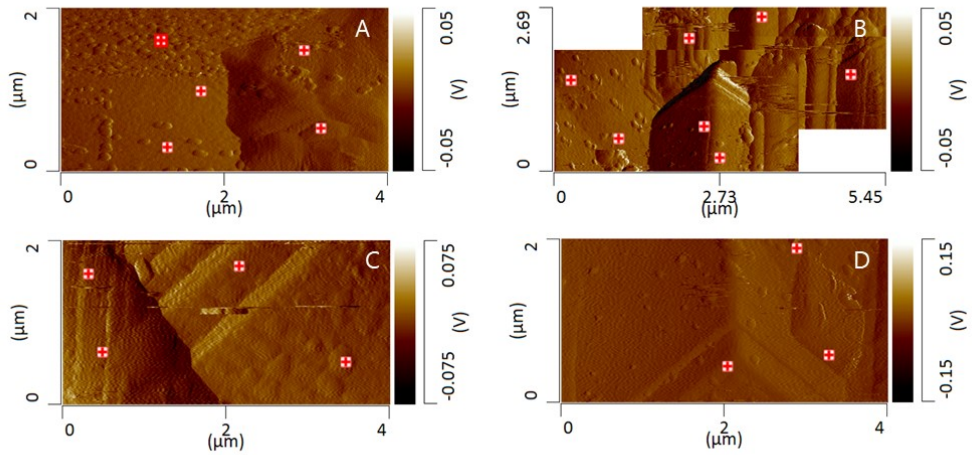

Fig. S6: Examples of deflection AFM images of faecal pellets from Trial I corresponding to some of the AFM-IR spectra in Fig. 9 (positions marked with red crosses). A is untreated wood after 7 days, B is furfurylated wood after 28 days, C is untreated wood after 28 days and D is furfurylated wood after 28 days.

References

1. Thygesen, L. G., Ehmcke, G., Barsberg, S. & Pilgard, A. Furfurylation result of Radiata pine depends on the solvent. *Wood Science and Technology* **54**, 929-942, doi:10.1007/s00226-020-01194-1 (2020).
2. Barsberg, S. & Thygesen, L. G. Poly(furfuryl alcohol) formation in neat furfuryl alcohol and in cymene studied by ATR-IR spectroscopy and density functional theory (B3LYP) prediction of vibrational bands. Vibrational Spectroscopy 49, 52-63, doi:10.1016/j.vibspec.2008.04.013 (2009).