Supplementary Information Appendix for

Lignocellulose pretreatment in a fungus-cultivating termite

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SI Appendix, SI Materials and Methods

Sample Preparation. For the fungus comb collection, nine colonies of the fungus-cultivating termite *O. formosanus* harboring the king and queen were collected in March 2013 from a forested area in Hangzhou City, Zhejiang Province, China. The entire colony, including fungus combs, was wrapped in plastic film and transported to the laboratory within 6 h of excavation. The colonies were maintained in complete darkness at 27 ± 1 °C and 85% relative humidity using our laboratory artificial rearing system as has been previously described (1). After the colony became stable in the laboratory, nine colonies were randomly divided into three groups and subsequently each group assigned to one of three feeding experiments. The three feeding experiments used fresh poplar wood (*Populus tomentosa* Carr.), rice straw (*Oryza sativa*), and Chinese red pine (*Pinus massoniana* Lamb.); all these plant materials were separately pre-ground to pass through a 2 mm screen.

The termites in all nine colonies pick their forages, but only the colonies fed on poplar wood has been successful in building new fungal combs; the combs within the colonies fed on rice straw or Chinese red pine were discarded and removed by the termites themselves after 4 or 6 days of initial comb-building by termites. The poplar fungus comb was collected from poplar wood feeding colonies at the timepoint that the bottom layer of fungus comb (i.e., mature comb) started to be eaten by the old workers. A total of 7 poplar-based fungus combs were harvested from three colonies of *O. formosanus* after about 45 days of feeding.

Each fungus comb material was separately collected by three age groups according to clearly distinguishable differences in color and fungal development. Considering the continuous lignocellulose substrate flow in the fungus-cultivating termite system, we collected fresh fungus combs immediately following new fecal droplets of young workers deposited on the comb (Movie S1). These fecal droplets were dark brown without the presence of obvious fungal mycelia (Fig. 1*Di*). Middle-aged fungus combs were yellowish brown covered by dense fungal mycelia and fungal nodules (Fig. 1*Dii*). Mature fungus combs were gray containing little recognizable mycelia and no fungal nodules (Fig. 1*Diii*) (2, 3). Each comb sample was carefully collected under stereo-microscopes (Nikon Microscopy, Japan) and immediately pooled, lyophilized and stored at -20 °C until analysis. The wood particles were collected from the food store in each colony and pooled as for the original wood control, because our previous study revealed that the *O. formosanus* young workers always ingested the lignocellulosic materials from the food store accumulated by the old workers (1).

Scanning Electron Microscopy and Compositional Analysis. The three age group fungus comb samples together with control wood sample (both *in situ* and lyophilized state) were rinsed with phosphate-buffered saline (PBS; 137 mM NaCl, 4.3 mM Na_2HPO_4, 2.7 mM KCl, 1.47 mM KH_2PO_4; pH 7.4) and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) overnight. Samples were washed three times in 0.1 M phosphate buffer (pH 7.0) for 15 min each and dehydrated with a graded series of ethanol (50%, 70%, 80%, 90% and 95% for 15 min each, 100% for 20 min). After adding a 1:1 (v/v) mixture of ethanol and isoamyl acetate to each sample, the mixture was incubated for 30 min. Samples were then transferred to pure isoamyl acetate, incubated for 1 h and

dehydrated in a Hitachi Model HCP-2 critical point dryer with liquid CO_2. The samples were observed with a Siron 200 scanning electron microscope (FEI, Hillsboro, OR).

To observe the wood particles from the gut, the experiment was carried out by following the same procedures as described in our earlier report (4). Briefly, worker termites both of young and old age groups (30 individuals per sample) were selected at random and dissected to collect wood particles. The worker termites of two age groups were surface sterilized with 70% ethanol and washed in PBS, and intact guts were immediately dissected using sterile, fine-tipped forceps. The guts were then sliced longitudinally using a sterile razor, and their contents were gently squeezed into PBS. The suspensions were subjected to the sample preparation steps described as above and then observed with scanning electron microscopy (SEM).

Moreover, the poplar fungus comb material and original poplar wood were taken for compositional analyses of total sugars, Klason lignin, and acid-soluble lignin (ASL) using two-step acid hydrolysis procedure of National Renewable Energy Laboratory (NREL, Boulder, CO) (5). High-performance anion exchange chromatography (HPAEC) was used to quantify the monosaccharides in the hydrolysate of each sample, using a Dionex ICS-3000 HPAEC system (Dionex, Sunnyvale, CA) equipped with an analytical CarboPac^{TM} PA-20 column (4 × 250 mm, Dionex) in combination with the CarboPac PA-20 guard column (4 × 50 mm, Dionex) at the 30 °C column temperature. The procedure of determination was similar to that described by Sun et al. (2015) (6), but with modifications. Briefly, the monosaccharides and glucuronic acid were separated in 2 mM NaOH for 16 min at a flow rate of 0.4

mL/min, followed by a 100 mM NaOH and 50 mM NaAc for 15 min. A 10 min elution with 2 mM NaOH was then used to re-equilibrate the column before the next injection. The sugars including glucose, xylose, glucuronic acid, arabinose, galactose, and mannose were all purchased from Sigma-Aldrich (USA) and used as standards. Each sample analysis was biologically replicated twice.

Gel-state 2D-NMR Spectroscopy. Both the original poplar wood and fungus comb (300 mg per sample) were ground using a Retsch PM 100 planetary ball mill (Retsch, Haan, Germany) equipped with a 50 mL zirconium dioxide (ZrO_2) vessel, loaded with ten 10-mm-diameter ZrO_2 ball bearings. The control sample was milled at 600 rpm with alternating grinding (5 min) and interval pauses (5 min) for a total time of 7 h and the fungus comb samples at 300 rpm as described previously (7, 8). Approximately 100 mg each of ball-milled sample was suspended in 0.75 mL of DMSO-d_6 in the NMR tube and sonicated for 1 h in an ultrasonic bath until the gel became apparently homogeneous. One-bond ^{1}H–^{13}C correlation (HSQC) NMR spectra were acquired at 25 °C on a Bruker AVANCE 600 MHz instrument equipped with a cryogenically cooled 5 mm TXI $^{1}H/^{13}C/^{15}N$ gradient probe. The central DMSO solvent peak (δ_C = 39.5 ppm, δ_H = 2.49 ppm) was used as the chemical shift reference. HSQC spectra were acquired using a standard Bruker pulse program 'hsqcetgpsisp2.2' using the following parameters: spectral width of 12 ppm in F_2 (^{1}H) dimension with 2048 data points (TD1) and 220 ppm in F_1 (^{13}C) dimension with 256 data points (TD2); the number of scan (NS) was 128 with a 1 s interscan delay and the total acquisition time of 15 h. NMR data processing used typical matched Gaussian apodization in F_2 and a squared cosine-bell in

F_1. HSQC cross-signals from lignin and some polysaccharides in the poplar wood were assigned by comparing them with previously reported data (9, 10). Semiquantitative (comparative) analysis was conducted by comparison of integrals from the well-dispersed, non-overlapping, two-dimensional contours using Bruker's TopSpin v. 3.2 (Mac) software. Lignin sidechain integrals were calculated based on the lignin methoxyl contour, normalized to the absolute methoxyl content, following a similar procedure to that of Yelle et al. (2011) (8). The procedure for determining methoxyl content of lignin samples was similar to that described by Girardin and Metche (1983) (11) (details provided in Methoxyl Analysis below).

Py-GC/MS and Py/TMAH-GC-MS Analysis. Pyrolysis-GC/MS was performed as modified by Ke et al. (2011) (12). Approximately 1 mg of each sample and 0.005 mg internal standard (3,5-dimethoxyphenol) were placed in a quartz tube and maintained in place with glass wool. The pyrolysis processes were performed with a pyrolysis autosampler (Model 5200; CDS Analytical, Inc., Oxford, PA, USA) connected to an Agilent GC/MS system (GC 7890A; Agilent Technologies, Inc., Bellevue, WA, USA) equipped with a HP-5MS non-polar capillary column (30 m in length, 250 μm in i.d. and 0.25 μm in film thickness) and an Agilent 5975C mass-selective detector. The sample was first pretreated at 210 °C for 3 min, then pyrolysed at a temperature of 610 °C for 1 min. The column used helium as carrier gas (1 mL/min), and the oven temperature was ramped from 40 °C to 280 °C at 6 °C per minute. The mass spectrometer was in electron impact ionization mode at 70 eV and the ion-source temperature was 230 °C. For Py/TMAH-GC-MS, 1 mg of sample was mixed with approximately 5 μL of TMAH (25% in methanol, w/w) and the pyrolysis process was performed as

described above. Compounds were identified by fragmentography and by comparing their mass spectra with those of compounds in the NIST library and reported in the literature (13). Each sample analysis was biologically replicated twice. The integration of peak areas were calculated for the lignin derived products, the summed areas were normalized to 100, and the data are expressed as the means determined for two replicates ± SE and expressed as percentages.

Comparative Studies on the Intestinal Transit and Duration Time between *Odontotermes formosanus*a and *Copototermes formosanus*. The laboratory colonies of fungus-cultivating higher termites *Odontotermes formosanus*a and wood-feeding lower termites *Copototermes formosanus* were used for timing check of wood particles through the gut. Neutral Red dye was used, both because red dye in the intestines was easy visible through the cuticle of termites that fed on the stained food and less toxicity (14). A total of 50 individuals have been recorded respectively for each termite spices during this experiment.

For *O. formosanus*a, poplar (*Populus tomentosa* Carr.), wood particles (around 0.2 cm) were stained with Neutral Red dye to estimate the time of gut passage. After marked reddish termite workers have been detected, and then check the fungus comb very 15 min to see if there are red fecal droplets appeared. In contrast, for *C. formosanus*, their favorite food Chinese red pine *Pinus massoniana* Lamb. block was stained with Neutral Red dye to estimate the time of gut passage. After marked reddish termite workers have been detected, and then check the Petri dish very 30 min to see if there are red fecal droplets appeared.

Methoxyl Analysis. The procedure for determining methoxyl content of lignin samples was similar to that described by Girardin and Metche (1983) (11), but with modifications. Briefly, to a 40 mL head-space vial, approximately 30 mg of each ball-milled sample was added followed by 5 mL of 57% hydriodic acid via a volumetric pipette. A few carborundum crystals were added to each vial to suppress bumping and purged with nitrogen. The vials were sealed with Teflon/silicone septa and crimped caps and reaction mixture were heated in a welled hot-plate to 140 °C for 60 min. Immediately after this reaction period, the reaction vials were cooled in an ice-water bath and 10.0 mL of chloroform was added through the septa to extract the organic phase. The vials were shaken vigorously for 1 min and stored in a freezer (approx. -20 °C) to allow complete phase separation. The chloroform phase (uppermost layer containing methyl iodide) was then subjected GC-MS quantitative analyses using a Varian (Walnut Creek, CA, USA) model 3800 gas chromatograph interfaced to a Varian model 4000 ion trap mass detector, operated in full scan mode at 70 eV in the range m/z=135-175. The transfer line was maintained at 250 °C. A Restek (Bellfonte, PA, USA) Rxi-1MS column (30m × 0.25mm × 0.25 um) was temperature programmed as follows: 35 °C to 45 °C at 2 °C/min, to 250 °C at 50 °C/min. A CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 10-ul liquid syringe was used for 1-ul injections. The injector was operated in 50:1 split mode at 200 °C. Quantitation was performed on peak areas of quant ion m/z=142 for CH_3I using Varian MS Workstation software v6.9. Processing was performed using triplicate injections of external standards, injected before and after, 5 replicate injections of each sample and with response factor RSD 0.03% and correlation coefficient 0.999%. Each sample analysis was replicated twice. Methoxyl content was calculated by the following equations:

(1) OMe (mg) = [Ave μg/μL CH_3I found with GC] × [10 mL total volume] × [1 mmole CH_3I/141.95 mg CH_3I] × [1 mmole OMe/1 mmole CH_3I] × [31.03 mg OMe/1 mmole OMe]

(2) %OMe = [OMe (mg)]/[weight of dry sample (mg)] × 100

(3) %OMe/lignin = [%OMe]/[Klason lignin + acid soluble lignin on total weight basis]

Analysis of Carbohydrates in Extractives and Extractive-free Material. The polysaccharides region of HSQC 2D-NMR spectra for the poplar fungus comb samples show significant cleavage of polysaccharides. We further determine the monosaccharides and oligosaccharides liberated from the polysaccharides by fungus comb microbes. Considering the soluble of monosaccharides and oligosaccharides, we separated the each fungus comb samples into two phases: extractives and extractive-free. The monosaccharides and oligosaccharides of the comb extractives were then examined without acid hydrolysis. The compositional analysis of extractive-free comb was determined using standard two-step acid hydrolysis procedure described above.

An ICS-3000 HPAEC system with an AS50 autosampler, comprising a Carbopac PA-20^{TM} column (4 × 250 mm, Dionex, Sunnyvale, CA, USA) for monosaccharides and a Carbopac PA-100^{TM} column (4 × 250 mm, Dionex) for oligosaccharides were used. The monosaccharides analysis procedure is followed as described above. The analytical CarboPac^{TM} PA-100 column (4 × 250 mm, Dionex) in combination with the CarboPac PA-100 guard column (4 × 50 mm, Dionex) were used for oligosaccharides analysis at the 30 °C column temperature. The oligosaccharides were separated in 100 mM NaOH for 60 min at a flow rate of 1 mL/min, followed by

separation in a 100 mM NaOH and 50 mM NaAc for 20 min. A 3 min elution with 100 mM NaOH was then used to re-equilibrate the column before the next injection (15). The oligosaccharide including 1,4-β-D-Glucooligosaccharides and 1,4-β-D-Xylooligosaccharides comprising of cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexaose as well as xylobiose, xylotriose, xylotetraose, xylopentaose and xylohexaose were all purchased from Megazyme (Wicklow, Ireland) and used as standards. Each sample analysis was biologically replicated three times.

Unknown Compounds Isolation and Identification. Fraction Unknown I and Unknown II were manually collected from ICS-3000 HPAEC system equipped with a CarboPac PA-100 column (4 × 250 mm, Dionex) for further analysis. HPLC-RI analysis of these fractions was conducted on a Waters 2320 system, using a Sugar column (HPLC condition: flow rate, 0.6 mL/min; temperature, 40 °C; eluted by MeCN: 0.2% TEA solution = 7:3). HPLC-MS detection was carried out on an Agilent 1100 HPLC system coupled with Bruker Esquire 3000plus mass spectrometer to obtain the initial mass information of the two unknown compounds. Finally, the samples were check by high-resolution mass spectrometer (Shimadzu ion-trap-TOF) and their accurate molecular weights were determined as 485.2783 and 830.3821, respectively (16). However, there were no corresponding compounds fitting these molecular weights based on SciFinder searches.

SI Appendix, SI Text

Scanning Electron Microscopy Study. Wood particles from the fresh comb displayed many droplets accumulating on the surface with smaller fiber sizes. Wood fibers collected from the mature comb revealed numerous microfibrils exposed on the surface area with bacterial cells attached (SI Appendix, Fig. S1E). Wood particles dissected from guts of old workers had rod shaped bacteria attached to the surface and were significantly smaller and thinner (SI Appendix, Fig. S1F), compared to particles found in young workers, with average particle sizes of 8.3 ± 0.4 μm, confirming that the old workers perform most of the final digestion in the overall process (1, 17). Moreover, our SEM examination of lyophilized fungus comb samples showed significant reduction in length of the fibrils when going from the original wood to mature comb (SI Appendix, Fig. S2 and S3). Also, the average particle size of wood fibers in the mature comb (14.4 ± 0.4 μm) was significantly smaller than that in the middle-aged comb (29.0 ± 2.0 μm) and in the fresh comb (44.6 ± 2.4 μm), possibly due to the capacity of the fungus-comb microbiome for lignocellulose degradation.

SI Appendix, Figures

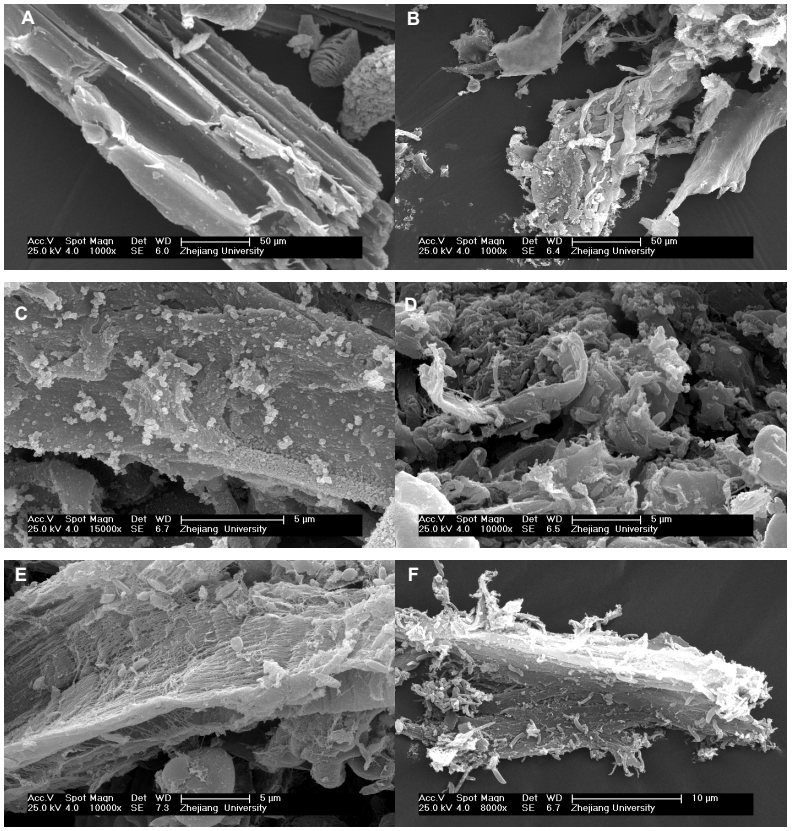

Fig. S1. Scanning electron micrographs of the poplar woody particles and microbes from food processing pathway of *Odontotermes formosanus*. (A) Original poplar wood. Wood particle in the gut of young worker termite (B), from fresh fungus comb (C), middle-aged fungus comb (D), mature fungus comb (E) and in the gut of old worker termite (F).

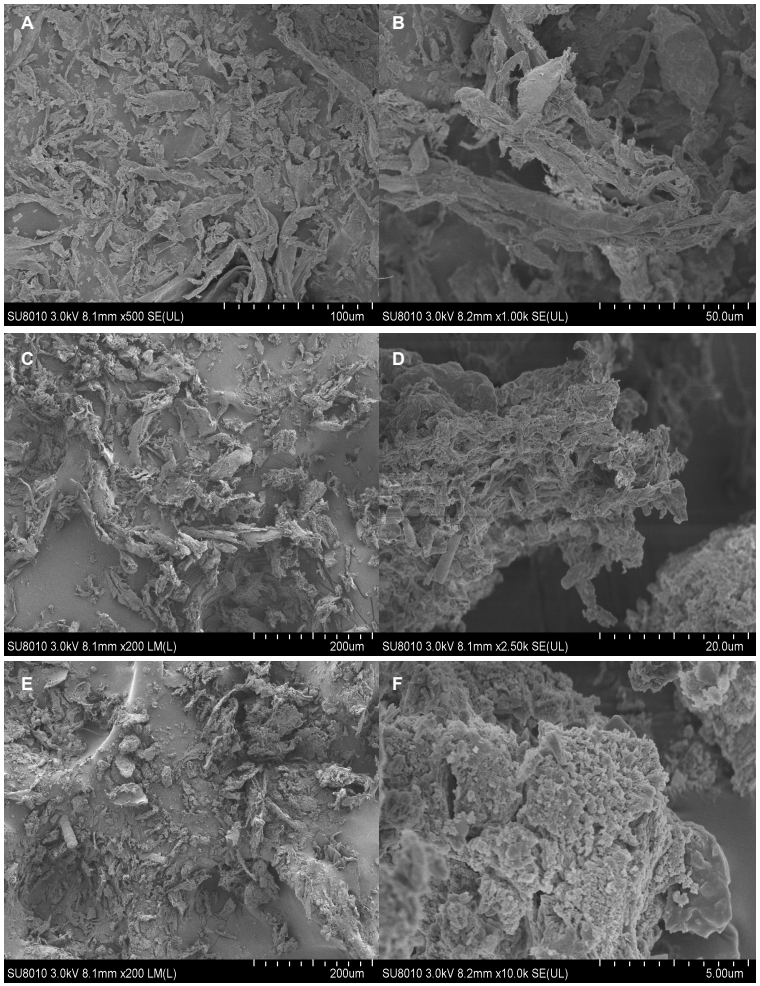

Fig. S2. Scanning electron micrographs of the poplar woody particles from fungus comb samples, which were subjected for chemical analysis. Global view (A) and detail view (B) of woody particles from fresh fungus comb. Global view (A) and detail view (B) of woody particles from middle-aged fungus comb. Global view (A) and detail view (B) of woody particles from mature fungus comb.

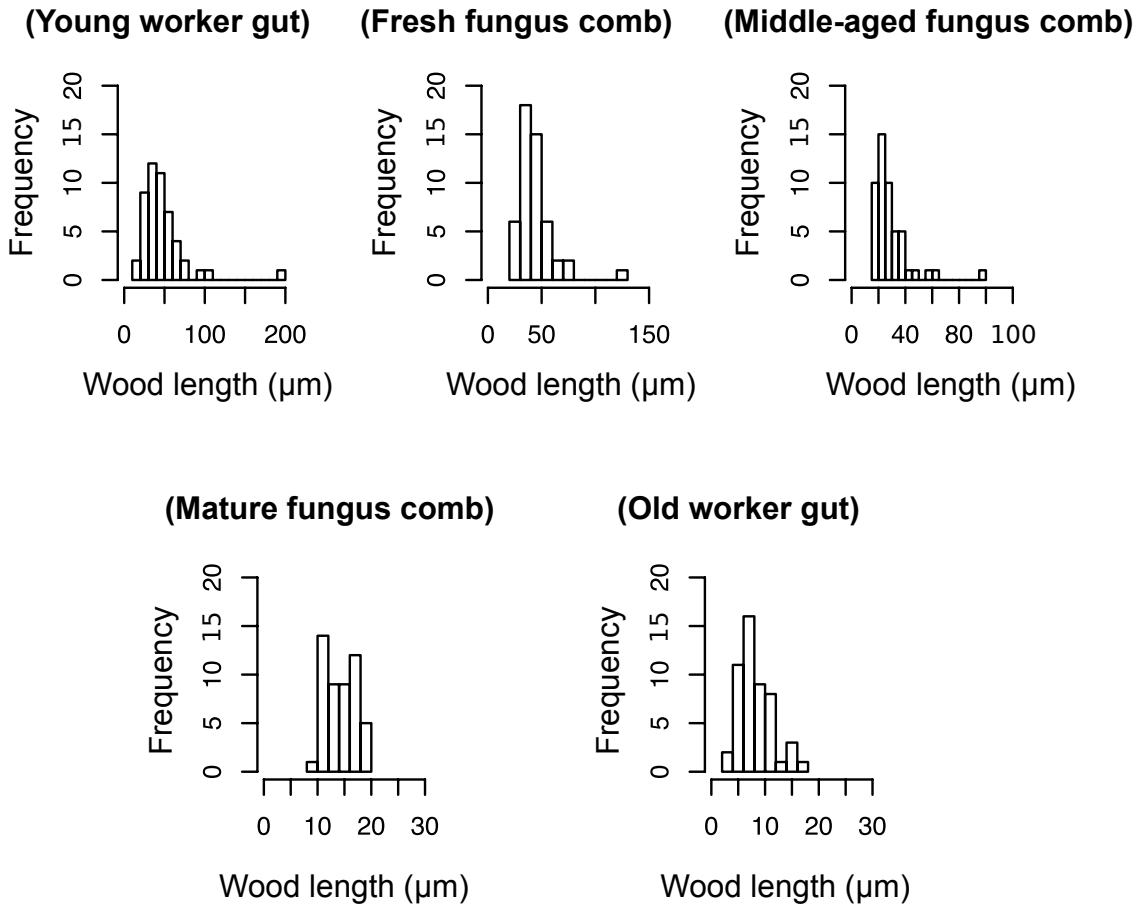

Fig. S3. Histogram comparison of the length distribution of wood fibers in the young worker gut (top left), fresh fungus comb (top middle), middle-aged fungus comb (top right), mature fungus comb (bottom left), and old worker gut (bottom right) obtained from *Odontotermes formosanus*. The wood length data obtained SEM.

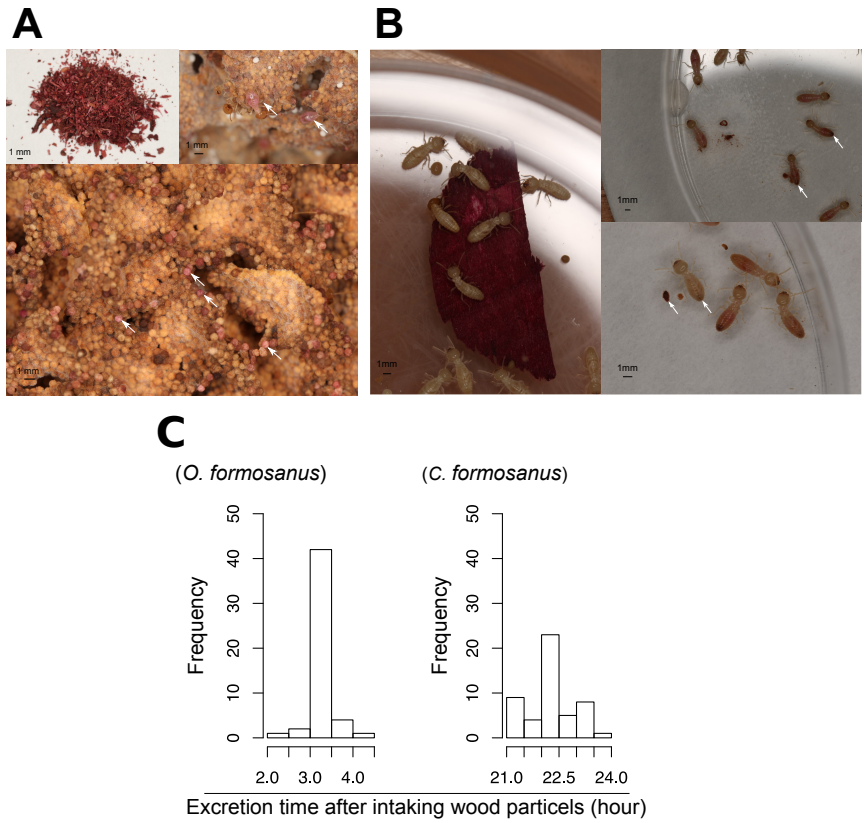

Fig. S4. Comparative intestinal transit time between fungus-cultivating higher termites *Odontotermes formosanus* and wood-feeding lower termites (non-fungus-cultivating species) *Copototermes formosanus* using neutral red marked wood. (A) The wood-stain marker feeding experiments in *O. formosanus*. The termites forage of neutral red stained poplar wood (Upper left). The young worker termites with reddish abdomen (white arrows) have fed stained wood (Upper right). The reddish fecal droplet (white arrows) has detected on the fungus comb by following check every 15 min (Lower). (B) The wood-stain marker feeding experiments in *C. formosanus*. The worker termites are feeding on neutral red stained pinewood (Upper left). The worker termites with reddish abdomen have fed stained wood, and the termites present dark reddish pot on the end of abdomen (white arrows) will produce feces soon (Upper right). The reddish fecal droplet has detected on the petri dish by following check every 30 min (Lower). (C) Histogram comparison on duration time of intestinal transit between *O. formosanus* young worker (Left) and *C. formosanus* (Right).

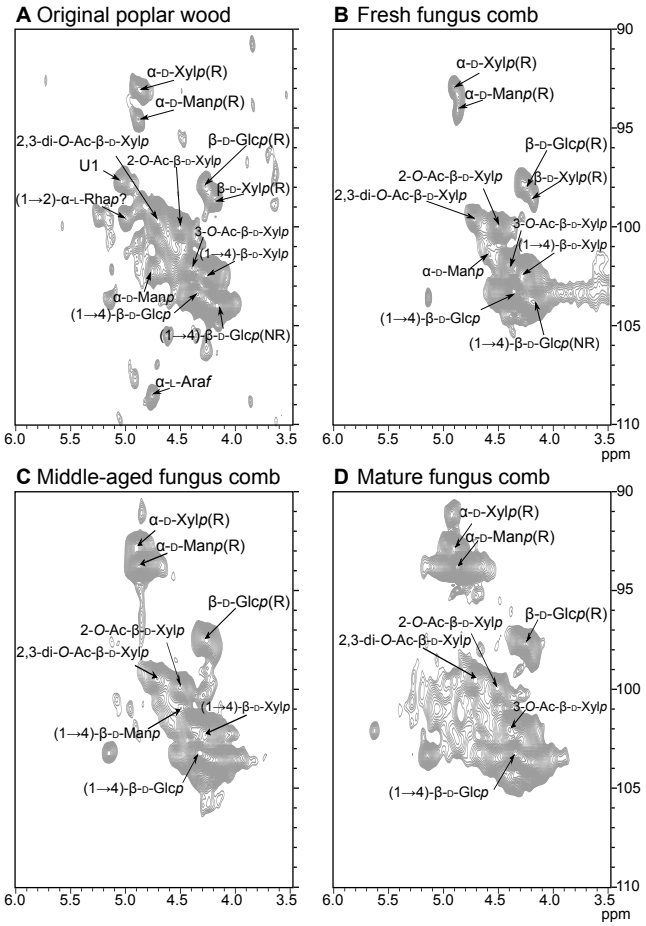

Fig. S5. $^{1}H–^{13}C$ HSQC NMR spectra of cell wall gels from samples. Polysaccharide anomeric region is shown for original poplar wood **(A)**, fresh fungus comb **(B)**, middle-aged fungus comb **(C)** and mature fungus comb **(D)**. See SI Appendix, Table S4 for signal assignments.

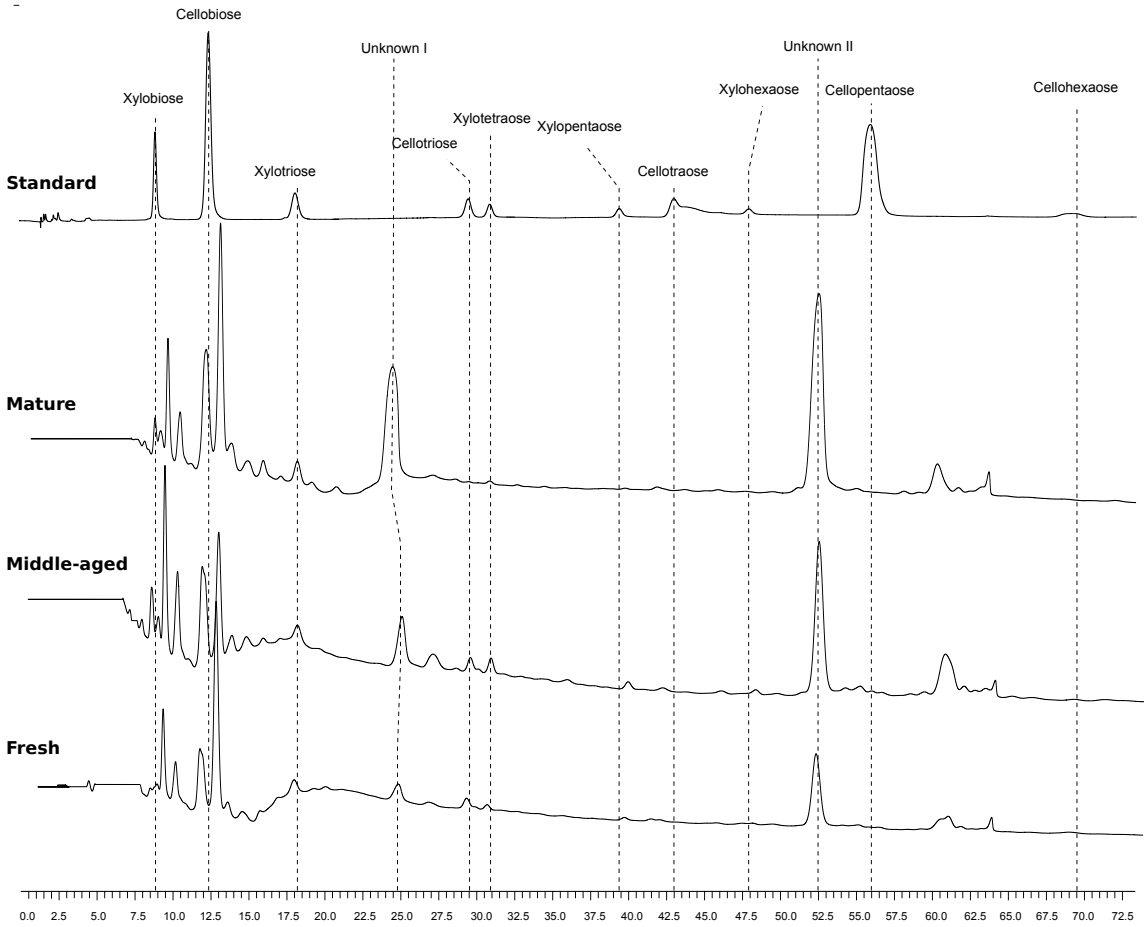

Fig. S6. HPAEC chromatograms of oligosaccharide products in the non-hydrolyzed extractive fractions of the fresh fungus comb, middle-aged fungus comb and mature fungus comb in solvent water. Notably, unknown I and unknown II peaks have increased intensity across comb samples.

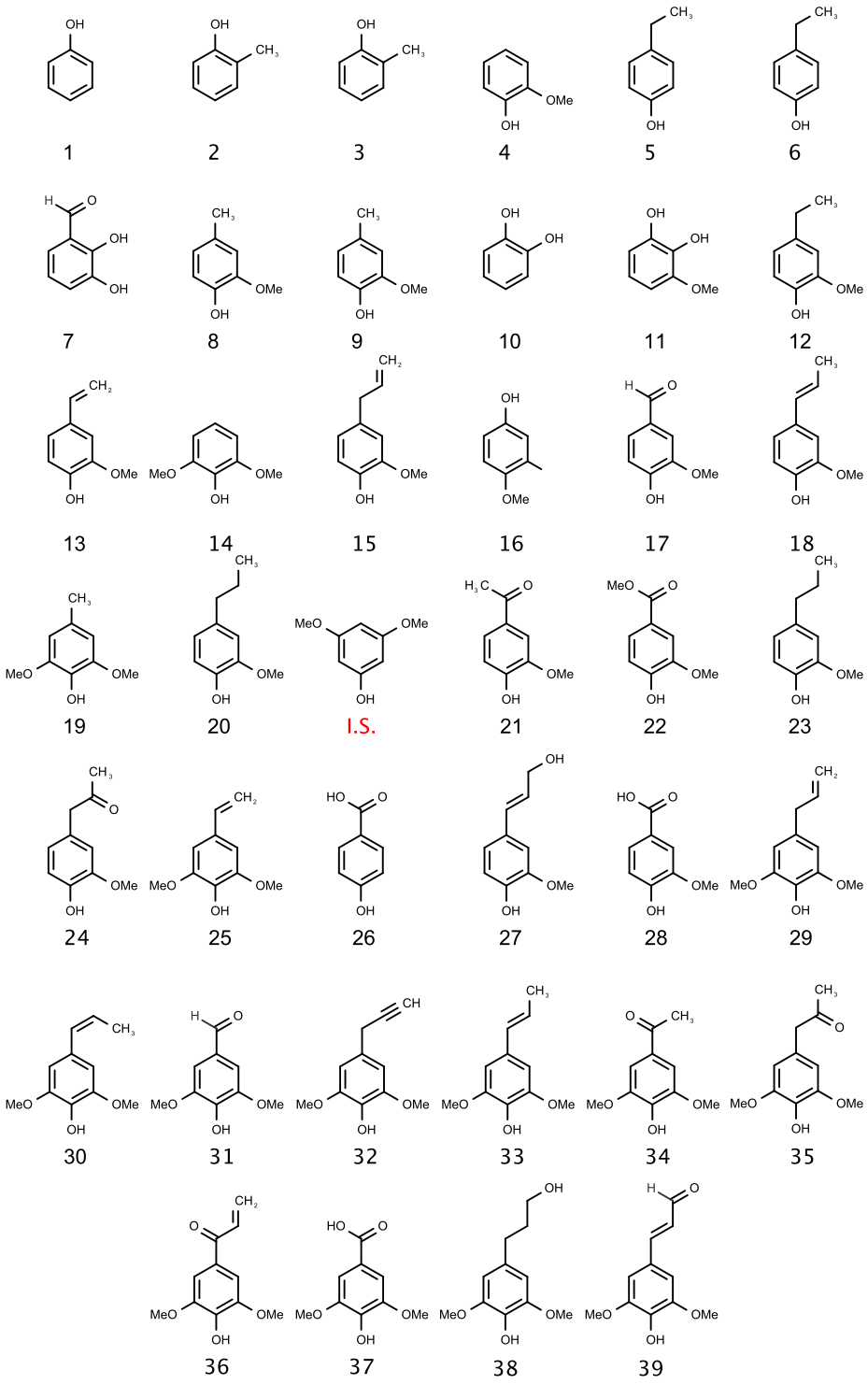

Fig. S7. Structures of the lignin derived compounds released from the Py-GC-MS. The numbers correspond to peak numbers in Figure 4A and Table S5.

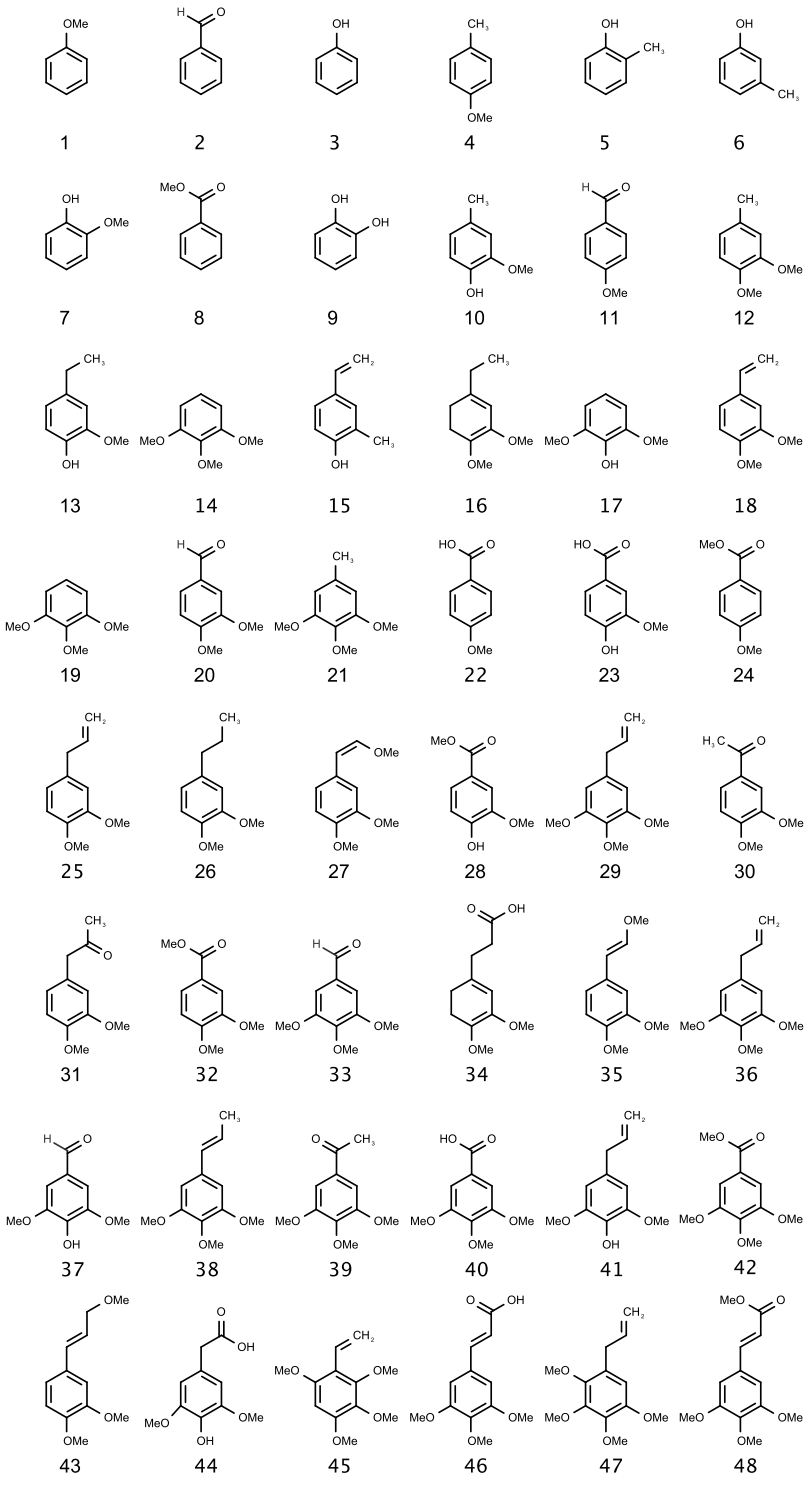

Fig. S8. Structures of the lignin derived compounds released from the TMAH-Py-GC-MS. The numbers correspond to peak numbers in Figure 4B and Table S6

SI Appendix, Tables

Table S1. Chemical composition of the original poplar wood and fungus comb of different age.

Sample	Klason lignin (mg)*	ASL† (mg)	Glucose (mg)	Xylose (mg)	Glucuronic acid (mg)	Arabinose (mg)	Galactose (mg)	Mannose (mg)	Total‡ (mg)
Poplar wood	0.19±0.02	0.03±0.01	0.50±0.03	0.22±0.00	0.02±0.01	0.01±0.00	0.01±0.00	0.02±0.00	1.00
Fresh	0.11±0.01	0.02±0.00	0.40±0.01	0.12±0.01	–§	–	0.02±0.00	0.01±0.00	0.68
Middle-aged	0.06±0.00	0.01±0.00	0.38±0.02	0.10±0.00	–	–	0.02±0.00	0.01±0.00	0.58
Mature	0.05±0.00	–	0.41±0.03	0.05±0.00	–	–	0.04±0.01	0.01±0.00	0.56

*The amount of each compound is expressed as mg/mg of dry fungal comb weight (Values are expressed as the means determined for two replicates ± SE).

†Acid-soluble lignin.

‡Sum of the values calculated based on all determined lignocellulose components for each sample.

§Not detected.

Table S2. Assignment of lignin unit correlations in $^{1}H–^{13}C$ in HSQC spectra of *Populus tomentosa* Carr.

Labels	δ_C/δ_H (ppm)	Assignment
Lignin units		
Aromatic and double-bond region		
$S_{2,6}$	104.3/6.71	C_2-H_2 and C_6-H_6 in syringyl
$S'_{2,6}$	106.5/7.29 and 7.17	C_2-H_2 and C_6-H_6 in syringyl (Oxidized α-ketone)
G_2	111.8/6.98	C_2-H_2 in guaiacyl
G_5	115.3/6.75 and 6.93	C_5-H_5 in guaiacyl
G_6	119.3/6.74	C_6-H_6 in guaiacyl
$H_{2,6}$	128.3/7.27	C_2-H_2 and C_6-H_6 in *p*-hydroxyphenyl
$H_{3,5}$	114.2/6.68	C_3-H_3 and C_5-H_5 in *p*-hydroxyphenyl
$PB_{2,6}$	131.8/7.67	C_2-H_2 and C_6-H_6 in *p*-hydroxybenzoates
$PB_{3,5}$	114.0/6.67	C_3-H_3 and C_5-H_5 in *p*-hydroxybenzoates
$X_{1\alpha}$	129.1/6.45	C_α-H_α in cinnamyl alcohol end-groups
$X_{1\beta}$	129.1/6.25	C_β-H_β in cinnamyl alcohol end-groups
Aliphatic Region		
B_β	53.7/3.12	C_β-H_β in phenylcoumaran substructures
C_β	53.5/3.06	C_β-H_β in resinol substructures
D_β	60.3/2.75	C_β-H_β in spirodienone
A_γ	59.5-59.7/3.40-3.63	C_γ-H_γ in β-O-4' substructures
$X_{1\gamma}$	61.4/4.12	C_γ-H_γ in cinnamyl alcohol end groups
A_α	71.8/4.86	C_α-H_α in β-O-4' substructures and γ-acetylated β-O-4' substructures
C_γ	71.0/3.82 and 4.20	C_γ-H_γ in resinol substructures
A_β (G/H)	83.9/4.28	C_β-H_β in β-O-4' substructures linked to G and H units
C_α	84.8/4.67	C_α-H_α in resinol substructures
A_β (S)	85.9/4.12	C_β-H_β in β-O-4' substructures linked to S units
B_α	86.8/5.46	C_α-H_α in phenylcoumaran substructures
D_α	81.7/5.09	C_α-H_α in spirodienone substructures

Table S3. Assignment of the polysaccharide $^{1}H–^{13}C$ correlations in HSQC spectra of *Populus tomentosa* Carr.

Labels	δ_C/δ_H (ppm)	Assignment
X_5	62.6/3.25	C_5-H_5 in β-D-xylopyranosyl units
X_2	72.3/3.09	C_2-H_2 in β-D-xylopyranosyl units
2-O-Ac-Xyl*p*	73.2/4.50	C_2-H_2 in 2-*O*-acetyl-β-D-xylopyranosyl units
X_3	73.7/3.28	C_3-H_3 in β-D-xylopyranosyl units
3-O-Ac-Xyl*p*	74.7/4.82	C_3-H_3 in 3-*O*-acetyl-β-D-xylopyranosyl units
X_4	75.4/3.60	C_4-H_4 in β-D-xylopyranosyl units
2,3-O-Ac-Xyl*p*	70.6/4.65	C_2-H_2 in 2,3-di-*O*-acetyl-β-D-xylopyranosyl units
2-O-Ac-Man*p*	70.5/5.30	C_2-H_2 in 2-*O*-acetyl-β-D-mannopyranosyl units
3-O-Ac-Man*p*	72.5/5.00	C_3-H_3 in 3-*O*-acetyl-β-D-mannopyranosyl units
U_4	81.7/3.10	C_4-H_4 in 4-*O*-methyl-α-D-glucuronopyranosyl units

Table S4. Assignment of the polysaccharide anomeric $^{1}H–^{13}C$ correlations in HSQC spectra of *Populus tomentosa* Carr.

Labels	δ_C/δ_H (ppm)	Assignment
α-D-Xyl*p*(R)	92.7/4.88	C_1-H_1 in (1→4) α-D-xylopyranosyl units (R)
α-D-Man*p*(R)	94.5/4.90	C_1-H_1 in (1→4) α-D-mannopyranosyl units (R)
U_1	97.2/5.08	C_1-H_1 in 4-*O*-methyl-α-D-glucuronopyranosyl units
(1→2)-α-L-Rha*p*	99.3/5.00	C_1-H_1 in (1→2) α-L-rhamnopyranosyl units (R)
2,3-di-O-Ac-β-D-Xyl*p*	99.5/4.71	C_1-H_1 in 2,3-*O*-acetyl-β-D-xylopyranosyl units
β-D-Xyl*p*(R)	99.4/4.28	C_1-H_1 in (1→4) β-D-xylopyranosyl units (R)
2-O-Ac-β-D-Xyl*p*	99.8/4.52	C_1-H_1 in 2-*O*-acetyl-β-D-xylopyranosyl units
(1→4)-β-D-Man*p*	100.8/4.58	C_1-H_1 in (1→4) β-D-mannopyranosyl units
3-O-Ac-β-D-Xyl*p*	101.8/4.40	C_1-H_1 in 3-*O*-acetyl-β-D-xylopyranosyl units
α-D-Man*p*	102.2/4.76	C_1-H_1 in Related mannan structures
(1→4)-β-D-Xyl*p*	102.1/4.28	C_1-H_1 in (1→4) β-D-xylopyranosyl units
(1→4)-β-D-Glc*p*	103.1/4.38	C_1-H_1 in (1→4) β-D-glucopyranosyl units
(1→4)-β-D-Glc*p* (NR)	103.7/4.18	C_1-H_1 in (1→4) β-D-glucopyranosyl units (NR)
α-L-Ara*f*	108.3/4.78	C_1-H_1 in α-L-arabinofuranosyl units

Table S5. Relative molar areas of the phenolic compounds derived peaks identified in the Py-GC-MS of the original poplar wood, fresh fungus comb, middle-aged fungus comb and mature fungus comb. Peak molar areas were calculated for the lignin degradation products, the summed areas were normalized, and the amount of each compound is expressed as percentages (Values are expressed as the means determined for two replicates ± SE).

No.	Compound	Origin*	Original poplar wood	Fresh fungus comb	Middle-aged fungus comb	Mature fungus comb
1	phenol†	H/C	1.1±0.1	3.8±1.0	4.6±0.9	6.3±0.1
2	methylphenol	H	0.7±0.3	1.9±0.2	2.7±0.5	4.7±0.3
3	methylphenol	H	0.8±0.3	1.9±0.2	3.0±0.3	4.9±0.3
4	guaiacol	G	2.2±0.3	3.4±0.5	6.9±1.0	11.1±0.0
5	ethylphenol	H	0.0±0.0	0.7±0.0	1.1±0.0	1.5±0.0
6	ethylphenol	H	0.0±0.0	0.6±0.0	1.3±0.0	3.9±0.6
7	2,3-dihydroxybenzaldehyde		0.9±0.0	0.7±0.3	0.0±0.0	1.2±0.6
8	methylguaiacol	G	0.5±0.0	0.0±0.0	0.0±0.0	0.0±0.0
9	methylguaiacol	G	1.8±0.1	5.9±1.1	13.8±1.7	0.0±0.0
10	catechol		0.7±0.2	0.0±0.0	0.0±0.0	0.0±0.0
11	3-methoxycatechol		1.8±0.2	3.8±0.6	4.8±0.7	6.2±0.6
12	4-ethylguaiacol	G	1.7±0.5	0.6±0.0	0.6±0.2	0.0±0.0
13	4-vinylguaicol	G	5.1±0.1	3.6±0.9	4.3±0.1	5.3±0.9
14	syringol	S	5.5±0.8	7.1±1.4	11.4±1.7	14.2±0.7
15	eugenol	G	1.9±0.5	1.4±0.1	1.0±0.2	0.0±0.0
16	3,4-dimethoxyphenol		1.7±0.4	2.1±0.1	3.4±1.0	2.3±0.3
17	vanillin	G	1.0±0.2	2.2±0.1	3.3±0.8	4.5±0.0
18	*trans*-isoeugenol	G	1.4±0.7	0.9±0.0	0.0±0.0	0.0±0.0
19	4-methylsyringol	S	8.4±0.8	7.1±1.9	3.6±1.2	3.4±0.4
20	4-propylguaiacol	G	1.4±0.2	0.0±0.0	0.0±0.0	0.0±0.0

I.S.	3,5-dimethoxyphenol		2.2±0.1	3.8±0.0	7.6±0.1	9.2±0.8
21	acetoguaiacone	G	1.0±0.1	2.1±0.0	2.7±0.0	1.9±0.0
22	vanillic acid methyl ester	G	0.0±0.0	1.1±0.3	2.0±0.0	3.4±0.5
23	4-propylguaiacol	G	0.9±0.1	1.4±0.4	3.0±0.7	1.4±0.0
24	guaiacylacetone	G	0.9±0.1	0.0±0.0	0.0±0.0	0.0±0.0
25	4-vinylsyringol	S	13.8±0.2	10.3±2.0	14.6±3.4	17.1±2.4
26	4-hydroxybenzoic acid	PB	2.4±0.0	0.0±0.0	0.0±0.0	0.0±0.0
27	*tans*-coniferyl alcohol	G	2.7±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28	vanillic acid	G	0.0±0.0	8.3±0.4	2.0±0.1	0.0±0.0
29	4-allylsyringol	S	6.2±1.1	6.4±0.3	0.9±0.0	0.0±0.0
30	*cis*-4-propenylsyringol	S	7.1±0.5	0.0±0.0	0.7±0.0	0.0±0.0
31	syringaldehyde	S	15.8±2.9	18.6±3.0	3.6±1.2	4.9±0.3
32	propynesyringol	S	1.4±0.1	1.5±0.3	0.0±0.0	0.0±0.0
33	*trans*-4-propenylsyringol	S	7.3±0.3	6.6±1.2	4.5±0.4	6.2±1.4
34	acetosyringone	S	2.9±0.1	2.8±0.2	4.5±0.4	4.4±1.0
35	syringylacetone	S	1.8±0.2	3.6±0.2	8.4±2.6	7.1±0.3
36	syringyl vinyl ketone	S	0.3±0.1	0.0±0.0	0.0±0.0	0.0±0.0
37	syringic acid	S	0.9±0.1	0.0±0.0	0.0±0.0	0.0±0.0
38	dihydrosinapyl alcohol	S	1.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0
39	*trans*-sinapaldehyde	S	3.5±0.1	0.0±0.0	0.0±0.0	0.0±0.0
	H:G:S:PB molar ratio[‡]		2: 23: 76: 2	5: 31: 64: 0	8: 40: 50: 0	15: 28: 57: 0
	Total lignin monomers from 1 mg sample[§]		0.23	0.13	0.07	0.05

[*]H: H-lignin units, G: G-lignin units, S: S-lignin units, PB: *p*-hydroxybenzoates, C: carbohydrates.
[†]Phenol content has not been calculated into %H because it could also be derived from carbohydrates under pyrolysis conditions.

‡The compositional data were calculated based on H+G+S=100%.
§The total lignin content of 1 mg sample is calculated based on percentage of Internal standard (I.S.) in all lignin degradation products.

Table S6. Relative molar areas (%) of the phenolic compounds derived peaks identified in the TMAH-Py-GC-MS of the original poplar wood, fresh fungus comb, middle-aged fungus comb and mature fungus comb. Peak molar areas were calculated for the lignin degradation products, the summed areas were normalized, and the amount of each compound is expressed as percentages (Values are expressed as the means determined for two replicates ± SE).

No.	Compound	Original poplar wood	Fresh fungus comb	Middle-aged fungus comb	Mature fungus comb
1	methoxybenzene	1.3±0.2	0.4±0.4	0.0±0.0	0.0±0.0
2	benzaldehyde	0.5±0.0	0.5±0.0	0.5±0.0	0.4±0.0
3	phenol	1.8±0.1	2.0±0.8	1.6±0.3	1.4±0.2
4	4-methoxytoluene	0.6±0.0	0.6±0.0	1.0±0.0	1.2±0.1
5	2-methylphenol	1.5±0.1	1.2±0.6	0.7±0.1	0.9±0.3
6	3-methylphenol	1.2±0.0	1.9±0.1	2.5±0.2	2.7±0.0
7	2-methoxyphenol	3.0±0.2	4.6±0.2	4.6±0.6	6.0±0.1
8	benzoic acid methyl ester	0.8±0.0	1.2±0.0	1.2±0.0	1.2±0.0
9	1,2-dimethoxybenzene	0.6±0.0	1.4±0.2	2.2±0.1	4.0±0.0
10	2-methoxy-4-methylphenol	1.7±0.2	1.3±0.0	0.8±0.1	0.0±0.0
11	4-methoxybenzaldehyde	0.3±0.0	0.6±0.0	0.5±0.0	0.0±0.0
12	3,4-dimethoxytoluene	1.5±0.1	0.0±0.0	0.0±0.0	0.0±0.0
13	4-ethyl-2-methoxyphenol	0.6±0.1	0.5±0.2	0.0±0.0	0.0±0.0
14	1,2,3-trimethoxybenzene	1.2±0.1	2.4±0.3	3.0±0.7	4.6±0.3
15	2-methoxy-4-vinylphenol	1.2±0.3	0.7±0.0	0.0±0.0	0.0±0.0
16	1,2-dimethoxy-4-ethylbenzene	0.9±0.0	0.0±0.0	0.0±0.0	0.0±0.0
17	2,6-dimethoxyphenol	5.0±0.5	4.3±0.7	3.6±0.8	4.3±0.3
18	3,4-dimethoxystyrene	1.3±0.2	1.3±0.1	1.0±0.1	1.3±0.1
19	1,2,4-trimethoxybenzene	0.5±0.0	1.4±0.8	2.2±0.1	2.8±0.4
20	3,4-dimethoxybenzaldehyde	17.4±0.5	13.5±1.7	12.7±0.5	9.5±0.2

21	3,4,5-trimethoxytoluene	4.3±0.8	6.1±0.4	8.6±0.5	7.5±0.8
22	4-methoxybenzoic acid	2.1±0.2	0.0±0.0	0.0±0.0	0.0±0.0
23	4-hydroxy-3-methoxybenzoic acid	3.0±0.3	2.6±0.0	1.1±0.0	0.0±0.0
24	4-methoxybenzoic acid methyl ester	2.2±0.4	1.8±0.7	1.8±0.4	2.8±0.2
25	1-(3,4-dimethoxyphenyl)-1-propene	0.6±0.0	0.7±0.1	0.0±0.0	0.0±0.0
26	1,2-dimethoxy-4-n-propylbenzene	1.3±0.4	0.0±0.0	0.0±0.0	0.0±0.0
27	*cis*-1-(3,4-dimethoxyphenyl)-2-methoxyethylene	2.0±0.0	1.8±0.1	1.5±0.0	2.2±0.6
28	4-hydroxy-3-methoxy-benzoic acid methyl ester	0.8±0.1	2.4±0.8	2.0±0.5	3.8±0.6
29	1-(3,4,5-trimethoxyphenyl)-1-propene	0.8±0.0	0.8±0.1	0.0±0.0	0.0±0.0
30	3,4-dimethoxyacetophenone	3.9±0.6	3.6±1.0	2.4±0.0	0.0±0.0
31	1-(3,4-dimethoxyphenyl)-2-propanone	0.0±0.0	0.8±0.1	1.0±0.1	1.5±0.4
32	3,4-dimethoxybenzoic acid methyl ester	2.1±0.1	8.6±0.8	13.8±0.2	17.9±0.2
33	3,4,5-trimethoxybenzaldehyde	4.9±0.3	3.2±0.7	2.8±0.5	1.7±0.1
34	3-(3,4-dimethoxyphenyl)-propionic acid	1.0±0.1	0.9±0.1	0.6±0.1	0.6±0.1
35	*trans*-1-(3,4-dimethoxyphenyl)-2-methoxyethylene	1.2±0.0	1.6±0.1	1.6±0.2	0.0±0.0
36	1-(3,4,5-trimethoxyphenyl)-1-propene	1.3±0.0	1.7±0.4	1.9±0.0	1.2±0.6
37	4-hydroxy-3,5-dimethoxybenzaldehyde	1.4±0.1	0.0±0.0	0.0±0.0	0.0±0.0
38	1,2,3-trimethoxy-5-(1-propenyl)-benzene	0.7±0.1	0.0±0.0	0.0±0.0	0.0±0.0
39	3,4,5-trimethoxyacetophenone	2.9±0.1	2.3±0.1	1.8±0.0	1.2±0.1
40	3,4,5-trimethoxybenzoic acid	0.0±0.0	0.9±0.1	0.9±0.0	1.4±0.5
41	2,6-dimethoxy-4-(2-propenyl)-phenol	1.6±0.4	0.0±0.0	0.8±0.0	0.0±0.0
42	3,4,5-trimethoxybenzoic acid methyl ester	8.0±0.5	14.4±1.0	19.2±0.6	17.9±0.1
43	1,2-dimethoxy-4-(3-methoxy-1-propenyl)benzene	2.0±0.1	0.6±0.0	0.0±0.0	0.0±0.0
44	3,5-dimethoxy-4-hydroxyphenylacetic acid	2.3±0.3	4.0±0.6	0.0±0.0	0.0±0.0

45	2,3,4,6-tetramethoxystyrene	2.9±0.2	1.1±0.0	0.0±0.0	0.0±0.0
46	3-(3,4,5-trimethoxyphenyl)-2-propenoic acid	1.6±0.2	0.0±0.0	0.0±0.0	0.0±0.0
47	1,2,3,4-tetramethoxy-5-(2-propenyl)-benzene	1.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0
48	3-(3,4-dimethoxyphenyl)-2-propenoic acid methyl ester	0.9±0.1	0.0±0.0	0.0±0.0	0.0±0.0
	Peak 32/Peak 20 Ratio*	0.12	0.64	1.08	1.88
	Peak 42/ Peak 33 Ratio†	1.62	4.53	6.87	10.50

*Peak 20: 3,4-dimethoxybenzaldehyde, Peak 32: 3,4-dimethoxybenzoic acid methyl ester.
†Peak 33: 3,4,5-trimethoxybenzaldehyde, Peak 42: 3,4,5-trimethoxybenzoic acid methyl ester.

Table S7. Carbohydrates in extractives and extractive-free fractions from poplar fungus comb of different ages.

Comb sample	Fraction	Concentration (nmol mg^{-1})[§]						
		Glucose	Xylose	Glucuronic acid	Arabinose	Mannose	Galactose	Total
Fresh	**Extractives**[*]	9±0	3±0	–[¶]	–	–	–	12
	Extractive-free[†]	2037±57	820±45	–	19±1	31±6	96±2	3003
	Total fraction[‡]	2046±57	823±45	–	19±0	31±6	96±2	3103
Middle-aged	**Extractives**	21±9	6±2	–	–	1±1	–	29
	Extractive-free	1631±71	560±31	–	11±1	23±4	130±1	2355
	Total fraction	1651±75	566±32	–	12±1	25±5	130±1	2465
Mature	**Extractives**	48±9	9±2	–	–	2±1	–	61
	Extractive-free	517±180	96±21	–	2±0	5±2	118±38	739
	Total fraction	564±178	106±22	–	3±0	8±3	119±38	930

[*]Water-soluble extractives subjected to HPAEC analysis without pre-acid hydrolysis.
[†]Water-insoluble extractive-free samples subjected to HPAEC analysis after acid hydrolysis.
[‡]Sum of the values determined for the individual fractions.
[§]The amount of each compound is expressed as nmol/mg of dry fungal comb weight (Values are expressed as mean determined for three replicates ± SE).
[¶]Not detected.

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