

Supporting Information

The termite fungal cultivar *Termitomyces* combines diverse enzymes and oxidative reactions for plant biomass conversion

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1. Cultivation conditions

Termitomyces spp. were cultivated on PDA (25 mL per plate, standard 15x90 mm) for a maximum of four weeks at room temperature. Sub-culturing was done by scraping mycelium from half a plate, mixing with 10 mL sterile PBS and spreading 500 µL suspension per plate or used as inoculum of liquid culture broth.

Table S1. Composition of cultivation media (for solid agar: 20.0 g/L agar-agar was added)

	Chemical	Company	Amount
MA	Malt extract	Carl Roth	30.0 g/L
	Peptone	Carl Roth	5.0 g/L
PDB		Carl Roth	26.5 g/L
YM	Dextrose	Carl Roth	10.0 g/L
	Peptone	Carl Roth	5.0 g/L
	Yeast extract	Carl Roth	3.0 g/L
	Malt extract	Carl Roth	3.0 g/L
Minimal medium	MgSO ₄ x 7 H ₂ O	Carl Roth	0.5 g/L
	L-Asparagine x H ₂ O	Carbolution	1.5 g/L
	Solution A	See below	1.0 mL/L
	FeCl ₃ x 6 H ₂ O	Alfa Aesar	3.0 mg/L
	Solution C	See below	1 mL/l
	Solution D	See below	2.5 mL/l
	Agar Agar	Carl Roth	20 g/L
	Urea	Carl Roth	20 mM
	Carbon source	See below	
Solution A	Thiamine hydrochloride	Carl Roth	0.012 g/ 100 mL
Solution C	Na ₂ B ₄ O ₇ x 10 H ₂ O	Carl Roth	0.009 g/L
	(NH ₄)Mo ₇ O ₂₄ x 4 H ₂ O		0.04 g/L
	CuSO ₄ x 5 H ₂ O	Carl Roth	0.2 g/L
	ZnSO ₄ x 7 H ₂ O	Merck	2.0 g/L
	MnSO ₄ x 4 H ₂ O		0.1 g/L
	CoCl ₂ x 6 H ₂ O	Carl Roth	0.4 g/L
	Ca(NO ₃) ₂ x 4 H ₂ O	Carl Roth	1.2 g/L
Solution D	K ₂ HPO ₄	Merck	400 g/L
	KH ₂ PO ₄	Rearal	184 g/L
Soil	Mn-162		100 g/L
Carbon source	PDB	Carl Roth	26.5 g/L
	Cellulose	Alfa Aesar	30 g/L
	Fungus comb		30 g/L

2. Sequencing and annotation

Genome sequencing: DNA was extracted from laboratory-grown heterokaryotic *Termitomyces* strains T112 and T153. In short, mycelium from 2-3 week old cultures grown on PDA plates was scraped from the surface. Mycelium was ground under liquid nitrogen with subsequent DNA extraction by CTAB method. DNA was separated from proteins by chloroform-isoamylalcohol (24:1) extraction and purified using alcohol precipitation. Genome sequences were produced at LGC Genomics (Berlin, Germany) using Illumina MiSeq V3 platform with 300 bp paired-end reads and approx. 12 million read pairs per sequencing.

Table S2. Genome sequencing statistics of *Termitomyces* spp.

Strain	Appr. size [Mb]	GC-content	Scaffold count	Scaffold N50	Annotated genes
P5 ¹	83.7	46.36%	11,244	262,000	> 11,000
T112	79.8	46.78%	9,274	33,341	13,651
T153	84.1	46.62%	11,237	23,880	13,728

Data pre-processing: All library groups were demultiplexed using the Illumina bcl2fastq 2.17.1.14 software (folder 'RAW', 'Group' subfolders). Up to two mismatches or Ns were allowed in the barcode read when the barcode distances between all libraries on the lane allowed for it. Sequencing adapters were clipped from all raw reads and reads with final length < 20 bases were discarded. Afterwards reads were quality trimmed by removal of reads containing more than one N, deleting reads with sequencing errors, trimming of reads at 3'-end to get a minimum average Phred quality score of 10 over a window of ten bases and discarding reads with final length of less than 20 bp. From the final set of reads, FastQC reports were created for all FASTQ files. Genomes were assembled *de novo* using SPAdes version 3.13.0.

Genome annotation: Prior to annotation, the genomes were soft masked with RepeatMasker 4.0.9 ². RNAseq data was mapped to the genomes with STAR 2.7.3a ³ and used to train the Augustus gene predictor with Braker 2.1.5. ⁴ Finally, the genomes T112 and T153 were annotated with Augustus 3.3.3. ⁵ Protein and mRNA sequences were used for the annotation. Protein hits were generated from Spaln 2.4.0 ⁶ alignment of J132 proteome against the genome. mRNA sequences were generated from a Blat v36 ⁷ alignment of the T112 and T153 transcriptomes against the genome. The transcriptomes were previously assembled with Trinity 2.8.5 ⁸ after quality trimming the raw reads with Sickle 1.33 ⁹ using the quality score threshold of 20, and mapping the trimmed reads to the genome with STAR 2.7.3a. ³ The number of reads per assembled transcript was counted with Salmon 0.15.0 ¹⁰ and only the transcripts with at least 100 reads were used in the generation of annotations. Unannotated genomes recovered from GenBank were also annotated with Augustus 3.3.3 ⁵ trained on the genome T112, as described above but only with protein hits.

Table S3. List of *Termitomyces* spp. and selected fungi used for identification of redox active enzymes.¹¹

Organism	Strain	Abbreviation	Data accession (GenBank, RefSeq)	Termite species ^a
<i>Laccaria bicolor</i>		lbc	PRJNA550851	-
<i>Moniliophthora roreri</i>		mrr	LATX00000000.1	-
<i>Moniliophthora perniciosa</i>		mpr	GCA_000183025.1	-
<i>Coprinopsis cinerea</i>		cci	GCA_000182895.1	-
<i>Schizophyllum commune</i>		scm	GCA_000143185.1	-
<i>Agaricus bisporus</i> var. <i>Burnettii</i>	JB137-S8	abp	GCA_000300555.1	-
<i>Agaricus bisporus</i> var. <i>Bisporus</i>	H97	abv	GCA_000300575.2	-
<i>Fomitopsis pinicola</i>		fp	FP-58527 SS1	-
<i>Trametes versicolor</i>		tv	FP-101664 SS1	-
<i>Pleurotus ostreatus</i>		po	ASM1446616v1	-
<i>Irpea lactea</i>		il	CCTCC AF 2014020 MQVO000000000	-
<i>Termitomyces</i> sp.	T112		JACKQM000000000	MN
	T153		JACKQL000000000	MN
	J132		GCA_001263195.1	MN, ref ¹
	JCM 13351		GCA_001972325	MG, ref. ¹²
	MG145		GCA_003313055	n.d., ref. ¹³
	MG16		GCA_003313075	n.d., ref.
	MG15		GCA_003313675	n.d., ref.
	MG148		GCA_003313785	n.d., ref.
	MG13		GCA_003316525	n.d., ref.

a) MA = *Macrotermes natalensis*, MG. = *Macrotermes gilvus*, n.d. = not defined

3. Transcriptome analysis

RNA sequencing: RNA was obtained from *Termitomyces* strains T153 and T112 cultivated on PDA, fungus comb agar (Table S1) and soil agar (50 g/L South African soil, 8.8 g/L PDB, 20 g/L agar agar) for 10 days at room temperature. Mycelium was harvested by scraping it from agar plates with a scalpel, freezing it in liquid nitrogen and storing it at -80 °C until RNA extraction.

RNA was extracted by grinding frozen mycelium to a fine powder under liquid nitrogen and using the Isolate II RNA Plant Kit (Bioline) according to the manufacturer's protocol. RNA extracts underwent 100 bp paired-end BGISEq-500 sequencing with BGI (Hong Kong).

RNAseq data acquisition and processing: RNAseq data for fresh comb (SRR5944783), old comb (SRR5944781) and nodules (SRR5944782) of *Termitomyces* strains from *Macrotermes* colony Mn156 were downloaded from the European Nucleotide Archive.¹⁴ The raw RNAseq data were mapped to the annotated genes of T153 using HiSat2 with spliced alignments disabled (Version 4.8.2).¹⁵ Transcript abundance was then estimated using HTSeq-count (Version 0.11.2).¹⁶

RNAseq analysis: Count data from HTSeq were imported into R (R Core Team, 2018) using the “DESeq2” package (Version 1.22.2).¹⁷ Genes with low transcript abundance (<10) were filtered out and the remaining genes log10 transformed.¹⁸ A heatmap for the identified redox enzymes was generated using the “pheatmap” package (Version 1.0.12)¹⁹ in R (R Core Team, 2018)²⁰ with color schemes generated by “viridis” (Version 0.5.1).²¹ Visualized expression levels (log10) are summarized in Table S25 (Supporting Information Part 2).

4. CAZY analysis

Identification of CAZymes in the predicted proteomes of *Termitomyces* and other basidiomycete strains listed in Table S3 was performed using a local installation of the dbCAN2 server²² and all three included tools (HMMER, DIAMOND, and Hotpep searches against the databases included in dbCAN2). Only matches identified by at least two of these tools were kept for further analysis, a strategy aimed at improving the accuracy of the annotation and recommended by the authors of the software. The number of proteins belonging to individual CAZy families in each predicted proteome were counted and visualized in R using the functions 'cor()' and 'prcomp()' and the libraries 'pheatmap' and 'ggbioplot'.²³ For data, see Table_S25A and Table_S25B.

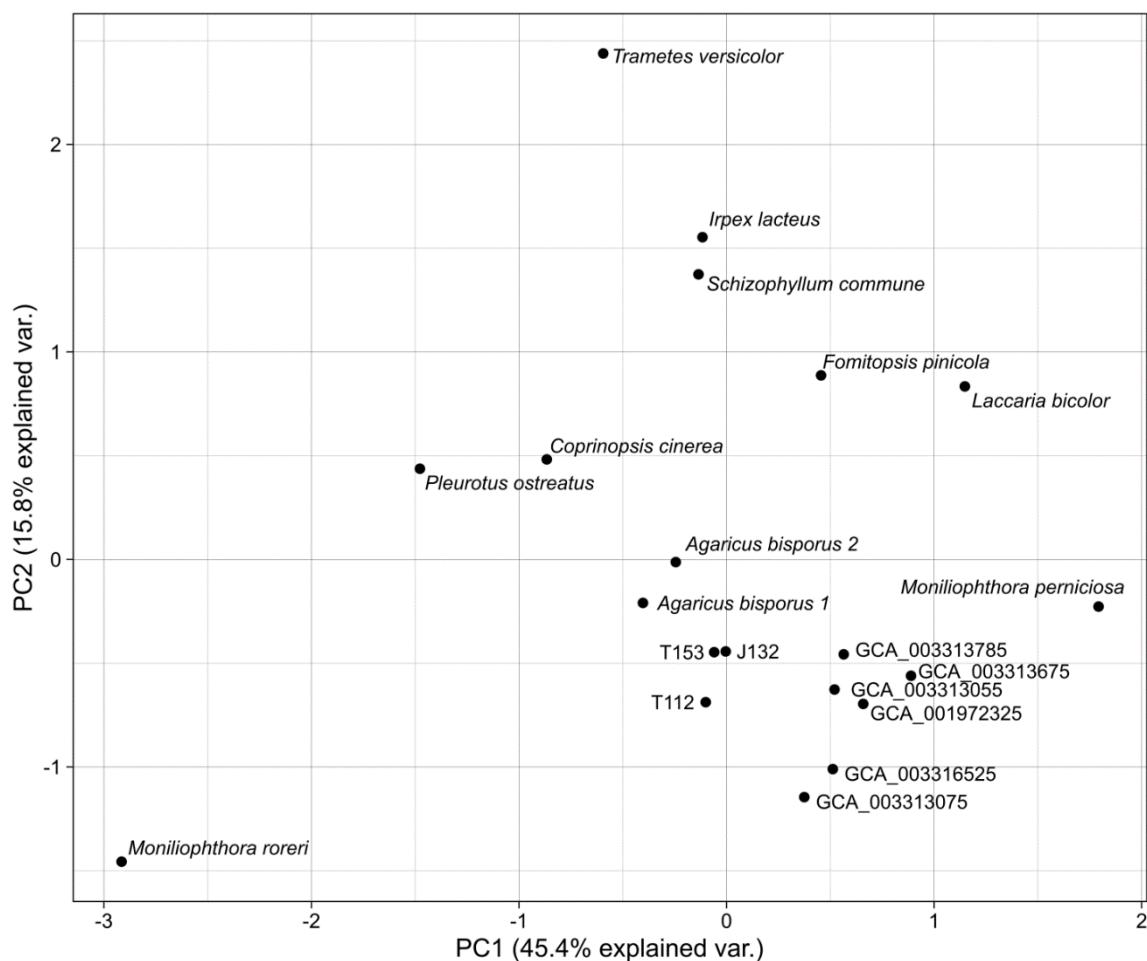


Figure S1. Principle component analysis of the numbers of representatives of different CAZy families in the predicted proteomes of *Termitomyces* spp. and other selected basidiomycetous fungi. Strains of *Termitomyces* spp. are named with their strain numbers (T112, T153, J132) or GenBank accession numbers, other basidiomycetes are named with their three-letter abbreviations as used in the Kyoto Encyclopedia of Genes and Genomes and listed in Table S3.

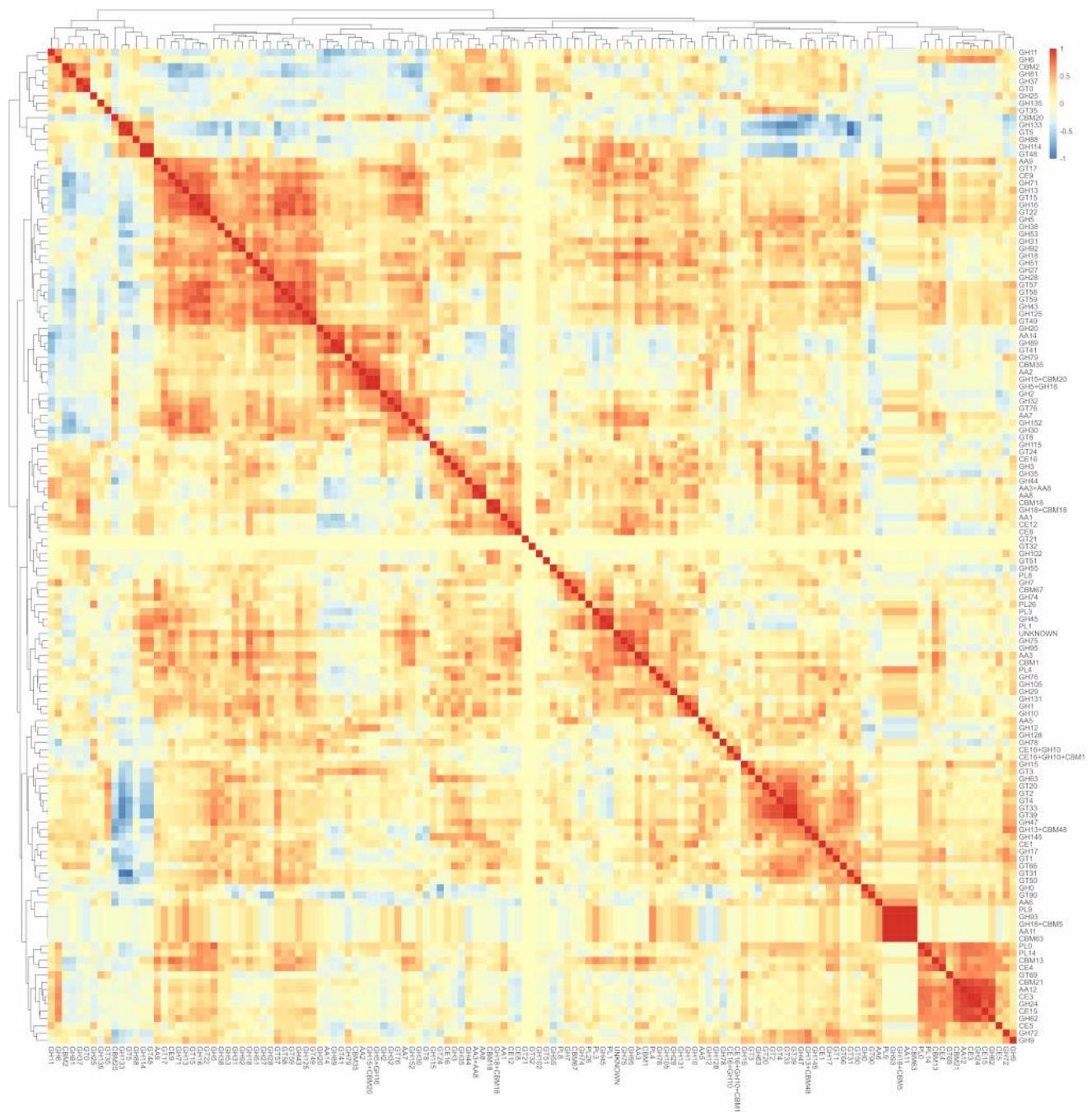


Figure S2. Heatmap of the correlations between the numbers of representatives of different CAZy families in the predicted proteomes of *Termitomyces* spp. and basidiomycetous fungi of Table S3. Two CAZy families were positively correlated (orange to red) if the increase in the numbers of representatives of one family in different species was accompanied by an increase in the number of representatives of the other family. Similarly, the families were negatively correlated (blue) if the increase of one family was accompanied by a decrease in the other family.

5. Analysis of redox-active enzymes

Redox-active enzymes were identified in the predicted proteomes of *Termitomyces* spp. genomes as described by Gostinčar and Gunde-Cimerman.²⁴ In short, protein sequences of redox-active genes of selected fungi were downloaded from the Kyoto Encyclopedia of Genes and Genomes (KEGG)²⁵ or GenBank²⁶ databases. These sequences were used as queries in a BLAST search against the predicted proteomes of *Termitomyces* spp. and reference genomes using standalone BLAST+ version 2.8.1²⁷ and an E-value threshold of 1e⁻²⁰.

Table S4. Numbers of selected putatively assigned redox active enzymes involved in biomass degradation identified in *Termitomyces* spp. and reference strains.

Fungal Strain	lbc	mpn	cci	scm	abp	abv	T112	T153	J132	JCM 13351 ^a	MG 145 ^a	MG 16 ^a	MG 15 ^a	MG 148 ^a	MG 13 ^a	tv	po	il	fp
Benzoquinone Reductase	nd	nd	nd	nd	nd	nd	2	2	2	2	2	2	2	2	2	1	2	3	1
Catalase	1	2	4	2	3	3	3	3	2	2	2	3	2	2	2	3	2	3	3
Glutathione Peroxidase	1	0	1	1	1	1	1	1	1	1	0	0	1	3	1	1	3	1	1
Hydroxy Acid Oxidase	nd	nd	nd	nd	nd	nd	3	3	3	1	2	0	3	2	1	5	5	6	9
Laccase	nd	nd	nd	nd	nd	nd	20	16	15	12	16	19	16	14	19	10	11	1	7
MnP/LiP/VP	1	0	1	0	2	2	1	1	1	1	1	1	1	1	1	nd	nd	nd	nd
Peroxiredoxin	4	3	4	5	4	4	7	7	6	4	3	3	4	7	5	7	7	8	6
Superoxide Dismutase	5	2	4	3	2	2	5	4	4	4	4	5	5	5	6	6	4	4	4
DyP	2	2	3	0	0	0	2	2	2	1	1	1	1	1	1	2	4	5	0
UPO	1	5	5	0	21	21	1	1	1	1	0	1	0	5	1	0	0	0	0

a) Deposited genomes were annotated without RNAseq data using the same Augustus settings as *Termitomyces*. nd. = no KEGG entry available.

Table S5. Amino acid sequence alignment of dye-decolorization peroxidases (DyP, EC 1.11.1.19) identified in *Termitomyces* species (highlighted in red). Conserved structural motif GxxDG is highlighted in yellow.

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>Irpe_x_lacteus_MH120197 YTLDAAARPGEAGHEHFGFLDGISNPAVTGFD-----S-PSPGQSLVPAGVILTGH
>Irpe_x_lacteus_MH120198 LTIASAARPGEAGHEHFGYLDGISNPTITGFG-----T-ALPGQSVDPGIILAGR
>Irpe_x_lacteus_MH120199 YTLNSAARPGAQAGHEHFGFLDGISNPAVIGFN-----D-PLPGQTLALPGIILTGR
>Irpe_x_lacteus_MG209114 YDLDSEAARPNEKGHEHFGYLDGISNPTIPGFG-----T-PHPGQAVVDPGIIFTGR
>Irpe_x_lacteus_MH120196 LDLSAARPGEAKGHEHFGYLDGISNPTIPGFG-----T-PFPGQAVVDSGVIFAGR
>GCA001972325_2235 ITVRGDVRPGDESGHEHFGFLDGISNPSVIGFD-----KNPLPGPKPVRAVLVTGH
>GCA003313055_4831 ITVRGDVRPGEESGHEHFGFLDGISNPSVIGFD-----KNPHPGPKPVQAGVLVTGH
>GCA003313075_6349 TTIRGDVRPGDQSAHEHFGFLDGISNPSVIGFD-----NDPNPGPRPVRAVLVTGH
>GCA003313675_8212 TTIRGDVRPGEVSGHEHFGFLDGISNPSVIDFD-----NNPTPGPTRVRAVLVTGY
>GCA003313785_16209 TTILGDVRPGKVSGHEHFGFLDGISNPSVIGFD-----KNPNPGPKPVAGVLLTGH
>GCA003316525_13886 TTIRGDVRPGDQSAHEHFGFLDGISNPSVIGFD-----NNPNPGPRPVRAVLVTGH
>J132_KNZ72538.1 LLIKGRRRPGHQASSNHFGYRDGISNPEVRGVTFDVQEQSDPRYPGSPIVPLGAIVMGY
>J132_KNZ82347.1 TTIRGDVRPGEESGHEHFGFLDGISNPAVIGFD-----KNPNPGPKPVRAVLVTGH
>po_KAF7416349 THVRGDVRPGDVHAHEHFGFLDGISNPAVDQFD-----QNPFPGQDSIRPGFILAKE
>po_KAF7421291 HSNRGAVRPGEAGKEHFGWLDGFIQPAVAGFA-----TSTYPGQQLLLPGTLLTKE
>po_KAF7422184 HRLKGAHRTGAFAGHEHFGYLDGISQPAIGGFA-----TSILPGQSLVLPGTILTGE
>po_KAF7433658 HRLKGAHRTGAWEGHEHFGFLDGISQPAVAGFA-----TSIFPGQSLILPGTILTGE
>T112_g10505 LLIKGRRRPGHQASSNHFGYRDGISNPEVRGVTFDVQEQSDPRYPGSPIVPLGAIVMGY
>T112_g5833 TTIRGDVRPGEESGHEHFGFLDGISNPAVIGFD-----KNPNPGPKPVRAVLVTGH
>T153_g12592 TTIRGDVRPGEESGHEHFGFLDGISNPAVIGFD-----KNPNPGPKPVRAVLVTGH
>T153_g15327 LLIKGRRRPGHQASSNHFGYRDGISNPEVRGVTFDVQEQSDPRYPGSPIVPLGAIVMGY
>tv_48870 TTLIGKVRPGLEDGHEHFGFLDGISQPAIEGID-----TNPNPGQETVRQGIILLGR
>tv_48874 ITLSGSVRPGDQKGHEHFGFMDGISQPAVQGVD-----TSPNPGQDTVHQGVILCKR

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Table S6. Amino acid sequence alignment of unspecific peroxygenase (UPOs, EC 1.11.2.1) identified in *Termitomyces* species (highlighted in red). Conserved structural motif GxxDG is highlighted in yellow.

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>lbc:LACBIDRAFT_295571 K21820 VNDKNHPWKPLG-YGDIRGPCPGL-NTLASHG
>mpr:MPER_12173 K21820 IDWSKHQYQAPQ-EGDARGPPGGL-NTYATFH
>cci:CC1G_08427 K21820 VNDRAHPWRPLR-RGDVRGPPGGL-NTLASHG
>cci:CC1G_08975 K21820 VNDEDPFMPPR-KGDARGPPGGL-NTLASHG
>cci:CC1G_08981 K21820 VNDKAHPFKPLK-KGDVRGPPGGL-NTLASHG
>cci:CC1G_10471 K21820 VNDAAHPYQAPRPHLDHRGPPGGL-NTLANHG
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>abp:AGABI1DRAFT115049 K21820 VNDAEHPWMPLR-DGDKRGPPGGL-NTLASHG
>abp:AGABI1DRAFT115479 K21820 VNDAEHPFMDPK-DTDIRGPPCAL-NTLANHG
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>abp:AGABI1DRAFT88344 K21820 -----
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>abv:AGABI2DRAFT139842 K21820 VNDAEHPFMDPK-DTDIRGPPCAL-NTLANHG
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>abv:AGABI2DRAFT190985 K21820 VNDAEHPFIAPG-EGDIRGPPCAL-NTLANHG
>abv:AGABI2DRAFT193563 K21820 VNDAHPWKPLR-RGDQRGPPGGL-NTLASHG
>abv:AGABI2DRAFT195432 K21820 VNDAKHPWKPLR-DGDKRGPPGGL-NTLASHG
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>Term_GCA003313785_14571 IPDSSHPFIAPTSR-DLRGPPGGL-NTLANHG
>Term_GCA003313785_4985 IPDRSHPFNAPKPK-DLRGPPGGL-NTLANHG
>Term_GCA003313785_7910 IPDSSHPFIAPKPE-DLRGPPGGL-NTLANHG
>Term_GCA003313785_8032 IPDNSHPFVAPKPK-DLRGPPC-----NHG
>Term_GCA003316525_13640 IPDSSHPFVAPKNR-DLRGPPGGL-NTLANHG
>Term_J132_KNZ76967_1 IPEPSHPFVAPGHK-DLRGPPGGL-NTLANHG
>Term_T112_g13225 IPEPSHPFVAPGHK-DLRGPPGGL-NTLANHG
>Term_T153_g10402 IPEPSHPFVAPGHK-DLRGPPGGL-NTLANHG
>Term_GCA001972325_6111 IFAWLXHVVVRVRAHILVRKSILSERVAEIG
>Term_GCA003313075_3589 -----

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Table S7. List of identified benzoquinone reductase protein sequences from *Termitomyces* spp. and reference genomes.

Fungal Strain	Identifier ^a
T153	mRNA10221; mRNA14670
J132	KNZ73246.1; KNZ73630.1
JCM 13351	mRNA5297; mRNA5586
MG 145	mRNA3673; mRNA816
MG 16	mRNA14653; mRNA17346
MG 15	mRNA2510; mRNA5582
MG 148	mRNA151; mRNA2080
MG 13	mRNA13369; mRNA4318
T112	mRNA19825; mRNA3131

a) ORF, Accession Nr or Protein ID

Table S8. List of identified hydroxy-acid-oxidase protein sequences from *Termitomyces* spp. and reference sequences.

Strain	KEGG	ORF, Accession Nr or Protein ID
T112		mRNA1666; mRNA18178; mRNA8739
T153		mRNA342; mRNA4446; mRNA8062
J132		KNZ71854.1; KNZ73304.1; KNZ74924.1
JCM 13351		mRNA73
MG 145		mRNA11400; mRNA4784
MG 16		
MG 15		mRNA3861; mRNA8882; mRNA8884
MG 148		mRNA15881; mRNA8170
MG 13		mRNA4102
cne	K11517	CND02080
cgi	K11517	CGB_D4500W
wse	K11517	WALSEDRAFT_59627

Table S9. List of identified laccase protein sequences from *Termitomyces* spp. and their respective reference sequences.

Strain	Identifier
T112	E4T56_mRNA10318; E4T56_mRNA11108; E4T56_mRNA13191; E4T56_mRNA13888; E4T56_mRNA14239; E4T56_mRNA15590; E4T56_mRNA15882; E4T56_mRNA17379; E4T56_mRNA19467; E4T56_mRNA20968; E4T56_mRNA2484; E4T56_mRNA3140; E4T56_mRNA4546; E4T56_mRNA5043; E4T56_mRNA6177; E4T56_mRNA6693; E4T56_mRNA6717; E4T56_mRNA6724; E4T56_mRNA7694; E4T56_mRNA9207;
T153	E4T57_mRNA10169; E4T57_mRNA10230; E4T57_mRNA1355; E4T57_mRNA14501; E4T57_mRNA1490; E4T57_mRNA15490; E4T57_mRNA16760; E4T57_mRNA20666; E4T57_mRNA20825; E4T57_mRNA4783; E4T57_mRNA5100; E4T57_mRNA513; E4T57_mRNA6918; E4T57_mRNA7708; E4T57_mRNA7786; E4T57_mRNA9738
J132	KNZ73628.1; KNZ73729.1; KNZ75261.1; KNZ75754.1; KNZ76529.1; KNZ76784.1; KNZ76840.1; KNZ77039.1; KNZ77620.1; KNZ77623.1; KNZ77629.1; KNZ80289.1; KNZ80821.1; KNZ81594.1; KNZ81880.1
JCM 13351	mRNA160; mRNA1956; mRNA28; mRNA3283; mRNA3360; mRNA3472; mRNA5610; mRNA6731; mRNA7480; mRNA7494; mRNA7539; mRNA9865
MG 145	mRNA10224; mRNA12281; mRNA13255; mRNA13536; mRNA1477; mRNA1876; mRNA2625; mRNA2631; mRNA373; mRNA4397; mRNA4545; mRNA5066; mRNA5952; mRNA686; mRNA8125; mRNA9106
MG 16	mRNA11364; mRNA11379; mRNA11960; mRNA12066; mRNA1350; mRNA1351; mRNA14886; mRNA16049; mRNA16052; mRNA16294; mRNA17135; mRNA19814; mRNA2526; mRNA3019; mRNA4136; mRNA478; mRNA567; mRNA8693; mRNA9688
MG 15	mRNA1571; mRNA174; mRNA2848; mRNA2849; mRNA3299; mRNA4106; mRNA4323; mRNA4373; mRNA493; mRNA5133; mRNA5715, mRNA6585; mRNA6869; mRNA7022; mRNA7634; mRNA7994
MG 148	mRNA10262; mRNA11349; mRNA12315; mRNA12522; mRNA13729; mRNA14672; mRNA15762; mRNA15983; mRNA18244; mRNA2016, mRNA3361; mRNA7161; mRNA828; mRNA9744
MG 13	mRNA10079; mRNA11271; mRNA11406; mRNA12907; mRNA13353; mRNA13563; mRNA1528; mRNA15453; mRNA1789; mRNA2302; mRNA2349; mRNA2518; mRNA4095; mRNA4325; mRNA5749; mRNA5832; mRNA6198; mRNA6585; mRNA7162;
mpn	ESK88328.1
cci	ABP81837.1
<i>Agaricus bisporus</i>	AAC18877.1
<i>Pleurotus ostreatus</i>	AGO64760.1
<i>Flammulina velutipes</i>	AIW01083.1

Table S10. List of identified peroxidase protein sequences from *Termitomyces* spp. and reference sequences. Identified hits were assigned to an enzyme group consisting of versatile peroxidase (ViP), manganese peroxidase (MnP) or lignin peroxidase (LiP).

Strain	KEGG	Identifier ^a
T112		mRNA12954
T153		mRNA2365
J132		KNZ77776.1
JCM 13351		mRNA6424
MG 145		mRNA2323
MG 16		mRNA19383
MG 15		mRNA3566
MG 148		mRNA12997
MG 13		mRNA2177
lbc	K20205	LACBIDRAFT_191903
cci	K20205	CC1G_02104
abp	K20205	AGABI1DRAFT71350; AGABI1DRAFT80178
abv	K20205	AGABI2DRAFT188334; AGABI2DRAFT221245
<i>Trametes versicolor</i>		AAA34049.1
<i>Phanerochaete chrysosporium</i>		AAA33739.1
<i>Pleurotus eryngii</i>		Q9UVP6.1
<i>Arthromyces ramosus</i>		pdb_2E39

a) ORF, Accession Nr or Protein ID

Table S11. List of identified catalase protein sequences from *Termitomyces* spp. and their respective reference sequences.

Strain	KEGG	Identifier ^a
T112		mRNA1051; mRNA20006; mRNA9981
T153		mRNA10779; mRNA11870; mRNA3741
J132		KNZ73766.1; KNZ79517.1
JCM 13351		mRNA5152; mRNA7807
MG 145		mRNA4443; mRNA8401
MG 16		mRNA12084; mRNA16747; mRNA527
MG 15		mRNA1934; mRNA7520
MG 148		mRNA16504; mRNA5248
MG 13		mRNA15551; mRNA2412
lbc	K03781	LACBIDRAFT_123238
mpr	K03781	MPER_05026; MPER_11214
cci	K03781	CC1G_03890; CC1G_05908; CC1G_09926; CC1G_11919
scm	K03781	SCHCODRAFT_46891; SCHCODRAFT_66245
abp	K03781	AGABI1DRAFT116622; AGABI1DRAFT125561; AGABI1DRAFT53795
abv	K03781	AGABI2DRAFT115586; AGABI2DRAFT200291; AGABI2DRAFT239410

a) ORF, Accession Nr. or Protein ID

Table S12. List of identified peroxiredoxin protein sequences from *Termitomyces* spp. and reference sequences.

Strain	KEGG	Identifier ^a
T112		mRNA12774; mRNA16120; mRNA16321; mRNA16835; mRNA8115; mRNA9106
T153		mRNA11039; mRNA12645; mRNA1456; mRNA403; mRNA8672; mRNA8719
J132		KNZ78734.1; KNZ79888.1; KNZ80305.1; KNZ80395.1; KNZ80927.1; KNZ82361.1
JCM 13351		mRNA1334; mRNA4283; mRNA4811; mRNA7322
MG 145		mRNA10442; mRNA13835; mRNA5277
MG 16		mRNA15719; mRNA7856; mRNA9896
MG 15		mRNA5186; mRNA7851; mRNA8779; mRNA8837
MG 148		mRNA10850; mRNA13035; mRNA15726; mRNA3678; mRNA5969; mRNA7539; mRNA9001
MG 13		mRNA10333; mRNA10449; mRNA15591; mRNA6728; mRNA6797
lbc	K03386	LACBIDRAFT_174328
	K03564	LACBIDRAFT_178653
	K11187	LACBIDRAFT_241727
	K14171	LACBIDRAFT_318118
mpr	K03386	MPER_10926
	K03564	MPER_12474

	K14171	MPER_06313
cci	K03386	CC1G_04730
	K03564	CC1G_07744
	K11187	CC1G_02672
	K14171	CC1G_07749
scm	K03386	SCHCODRAFT_38664; SCHCODRAFT_82385
	K03564	SCHCODRAFT_34254
	K11187	SCHCODRAFT_56996
	K14171	SCHCODRAFT_67750
abp	K03386	AGABI1DRAFT102439
	K03564	AGABI1DRAFT114007; AGABI1DRAFT115050
	K11187	
	K14171	AGABI1DRAFT93140
abv	K03386	AGABI2DRAFT77750
	K03564	AGABI2DRAFT193844; AGABI2DRAFT195437
	K11187	
	K14171	AGABI2DRAFT122146

a) ORF, Accession Nr or Protein ID

Table S13. List of identified superoxide dismutase protein sequences from *Termitomyces* spp. and reference sequences.

Strain	KEGG	Identifier ^a
T112		mRNA16928; mRNA19264; mRNA3009; mRNA7891
T153		mRNA1423; mRNA19342; mRNA3689
J132		KNZ71712.1; KNZ74977.1; KNZ76168.1; KNZ79105.1
JCM 13351		mRNA5428; mRNA613; mRNA7230; mRNA7447
MG 145		mRNA1274; mRNA13769; mRNA278; mRNA3207
MG 16		mRNA10461; mRNA12238; mRNA18893; mRNA8556; mRNA950
MG 15		mRNA1968; mRNA4950; mRNA5142; mRNA538; mRNA970
MG 148		mRNA11307; mRNA1237; mRNA1975; mRNA3081; mRNA840
MG 13		mRNA14196; mRNA14314; mRNA3191; mRNA4093; mRNA5512; mRNA9242
lbc	K04564	LACBIDRAFT_192586; LACBIDRAFT_291347; LACBIDRAFT_295682; LACBIDRAFT_312019
	K04565	LACBIDRAFT_303799
mpr	K04564	MPER_04451; MPER_06163
cci	K04564	CC1G_00459; CC1G_03559; CC1G_06963
	K04565	CC1G_07167
scm	K04564	SCHCODRAFT_43770; SCHCODRAFT_84612; SCHCODRAFT_85386
abp	K04564	AGABI1DRAFT114765
	K04565	AGABI1DRAFT74557
abv	K04564	AGABI2DRAFT194955
	K04565	AGABI2DRAFT207393

a) ORF, Accession Nr or Protein ID

Table S14. List of identified glutathione peroxidase protein sequences from *Termitomyces* spp. and their respective reference sequences.

Strain	KEGG	Identifier ^a
T112		mRNA20869
T153		mRNA20037
J132		KNZ81839.1
JCM 13351		mRNA9648
MG 145		mRNA2723
MG 16		
MG 15		
MG 148		mRNA16648
MG 13		mRNA2202; mRNA4376; mRNA5290
lbc	K00432	LACBIDRAFT_184771
cci	K00432	CC1G_07055
scm	K00432	SCHCODRAFT_55478
abp	K00432	AGABI1DRAFT85542
abv	K00432	AGABI2DRAFT134722

a) ORF, Accession Nr or Protein ID

6. Elemental analysis of comb, gut and soil

Measurement of pH and conductivity: The pH value was determined in aqueous solution. The amount of each sample used was adjusted to 2 g of sifted soil, mixed with 10 mL of dd H₂O (except for soil sample 12: 1.8 g soil in 9 mL water.) The soil suspensions were thoroughly mixed by shaking every 10 min over the course of 1 h and left standing at room temperature for 24 h. The suspension was mixed once again before measurement with a pH electrode (WTW, Weilheim; pH 330) and conductivity meter (Sensortechnik Meinsberg GmbH, Waldheim; LF 39, 030071); graphite-electrode (Sensortechnik Meinsberg GmbH, Waldheim; LTC 1/21).

Elemental analysis (CHNS): Samples (30 soil samples, 4 fungus comb samples) were sifted to a grain size below 2 mm and ground into a fine powder. 10 mg of each sample were incinerated in the oxygen stream of the combustion cell of an elemental analysis device at 1100 °C. Resulting products of total combustion were separated by column chromatography with He as carrier gas. Detection and quantification of analytes was achieved with a thermal conductivity detector and by integration of corresponding peak shapes using a calibration curve.

Element analysis of soil samples: Soil and comb samples were homogenized to a grain size < 0.1 mm utilizing an agate-mortar. Termite guts were obtained by dissection of roughly 150 and 210 guts for *Macrotermes* sp. and *Odontotermes* sp. respectively. The guts were dried at 30 °C for one week and were homogenized to a grain size < 0.1 mm utilizing an agate-mortar.

In general, 100 – 150 mg of dried sample was transferred into a pressure container and resuspended in a mixture containing 2 mL 65% HNO₃, 3 mL 40% HF and 3 mL 70% HClO₄. Over the course of 6 hours the mixture was heated to 180 °C and incubated for 12 h. Samples were allowed to cool down and afterwards reheated to 180 °C over the course of approximately 4 to 5 h. Removal of the acids was achieved by incubation for 14 h at 180 °C. Residues were dissolved in a mixture containing 2.0 mL HNO₃, 0.6 mL HCl and 7.0 mL H₂O at 150 °C over a time period of 8 h. Samples were cooled down to room temperature and transferred into 25 mL glass bottles. The final volume was adjusted to 25 mL with H₂O. Sample compositions were analyzed using ICP-MS (Thermo Fisher Scientific, Schwerte Xseries II) and ICP-OES (Agilent, Waldbronn, 725 ES) (MS: As, Ba, Cd, Co, Cr, Cu, Li, Mn, Mo, Ni, Pb, V, Zn, Sc, Y, Cs, La, Ce, Pr, Nd, Sm, Eu, Tb, Gd, Dy, Ho, Er, Tm, Yb, Lu, Th, U; OES: Al, Ca, Fe, K, Mg, Na, P, Sr, Ti).

Effective cation-exchange capacity (CEC): The CEC of soil samples was determined by exchange of cations against an unbuffered HCl solution following DIN ISO 11260 1996-05 (1996) guidelines adjusted by Buhler and Lorenz (2009, Institute of Geosciences, FSU-Jena). 30 mL of a BaCl₂ solution (0.1 M) was added to a sample of 2.5 g air dried soil, sifted to a grain size below 2 mm and mixed in an overhead shaker for 1 h at 20 rpm. Samples were centrifuged for 10 min at 3000 rpm and the

supernatant was discarded. This process was repeated two times. The resulting soil deposit was resuspended in 30 mL BaCl₂ solution (2.5 mM) and mixed for 18 h at 20 rpm in an overhead shaker.

The soil sample was centrifuged again at 3000 rpm for 10 min and supernatant was removed followed by resuspension in 30 mL MgSO₄ (0.02 M) and shaking at 20 rpm in an overhead shaker for 18 h. The final sample was received after centrifugation at 3000 rpm for 10 min and filtration of supernatant utilizing a 0.45 µm cellulose-acetate filter. Two droplets of 65% HNO₃ were added to avoid undesired interactions with possible organic contaminants.²⁸ Samples were stored at 4 °C in the dark until measurement with flame atomic absorption spectroscopy (FAAS).

Table S15. Termite mound sampling sites for soil collection

Year	Sample	Location	Sample Nr	Latitude	Longitude
2015	OD-145	out	1	S25 45.118	E28 15.525
		out	2		
		out	3		
	OD-146	out	4	S25 45.121	E28 15.544
		out	5		
		out	6		
	Mn-160 (1)	out	7	n.b.	n.b.
		out	8		
		out	9		
		out	10		
	Mn-161	out	11	S24 39.668	E28 47.555
	Mn-162	out	12	S24 39.693	E28 47.559
2016	OD-152	out	13	S25 43.715	E28 14.198
		rc	14		
		in	15		
	OD-167	out	16	S25 43.777	E28 14.423
		rc	17		
		in	18		
		fungus	A		
		gut	B		
		out	19		
	Mn-154	rc	20	S25 44.581	E28 15.659
		in	21		
		fungus	C		
		gut	D		
	Mn-160 (2)	out	22	S25 44.578	E28 15.645
		rc	23		
		In	24		
	Mn-164	out	25	S25 44.762	E28 15.434
		rc	26		
		in	27		
	Mn-173	fungus	E	S24 39.694	E28 47.588
		out	29		
		rc	29		
		in	30		
		fungus	F		
		gut	G		

n.b. = not determined; OD = *Odontotermes*, Mn = *Macrotermes*, out = outside of the termite mound,

in = inside the termite mound, rc = royal chamber, fungus = fungus comb, gut = gut fluids

7. GC-MS analysis

The fungal isolates *Termitomyces* sp. P5 and T153 were cultivated on PDA (25 mL per plate, standard 15x90 mm) for a maximum of four weeks at room temperature as an inoculum for the minimal media. Sub-culturing was done by scraping mycelium from half a plate, mixing with 10 mL sterile PBS and spreading 500 µL liquid per plate. Three minimal media containing different carbon sources were prepared according to Table S1, inoculated with mycelium and cultivated at room temperature until the first measurement.

GC-MS analyses were carried out with an Agilent (Santa Clara, USA) HP 7890B gas chromatograph fitted with a HP5-MS silica capillary column (30 m, 0.25 mm i. d., 0.50 µm film) connected to a HP 5977A inert mass detector. Used MS parameters were 1) transfer line: 250 °C, and 2) electron energy: 70 eV. The GC parameters were 1) inlet pressure: 77.1 kPa, He 23.3 mL min⁻¹, 2) temperature program: 5 min at 50 °C increasing at 5 °C min⁻¹ to 320 °C, 3) injection volume: 1 µL, 4) split ratio 10:1, 60 s valve time and 5) carrier gas: He at 1 mL min⁻¹. Retention indices (*I*) were determined from a homologous series of *n*-alkanes (C₈-C₄₀).

Table S16. Detection of putative redox active metabolites from *Termitomyces* sp. P5 grown on PDA by GC-MS (see Table S24).

	RT[min]	I	I(lit)	#	PRN1242
2-Methoxyphenol	15.03	1087	1087	46	x
Methyl benzoate	15.23	1093	1088	47	x
1,2-Dimethoxybenzene	16.87	1144	1141	54	x
1,4-Dimethoxybenzene	17.42	1161	1161	55	x
1,3-Dimethoxybenzene	17.53	1165	1165	56	x
2-Methoxy-1,4-benzoquinone	19.69	1236		65	x
3,5-Dimethoxytoluene	20.52	1265	1260	66	x
1,2,4-Trimethoxybenzene	23.40	1368	1368	72	x
2-Methoxy-1,4-dihydroxybenzene	24.05	1392		73	x
Vanillin	24.13	1395	1393	74	x

Table S17. Detection of putative redox active metabolites from *Termitomyces* sp. T153, grown on different media, by GC-MS (see Figure S19, Figure S20).

Compound	RT [min]	I	I (Lit.)	no.	PDA		Cellulose		FCA	
					IBN 1116	IBN 1122	IBN 1117	IBN 1132	IBN 1118	IBN 1131
1,2,4-Trimethoxybenzene	24.07	1368	1368	18	xx	x	xx	xx	xx	xx

8. Redox activity studies on *Termitomyces* sp. T153

Table S18. Composition of general reagents used for redox assays

Name	Composition	Company	Molarity, pH
Acetate buffer A	NaOAc, HCl	Carl Roth	0.1 M, pH 4
FeCl ₃ solution	FeCl ₃	Alfa Aesar	1 mM
FeSO ₄	FeSO ₄ *7 H ₂ O	Carl Roth	0 – 1000 µM
Ferrozin solution	3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt	Sigma-Aldrich	1% w/v
Oxalic acid solution	Na ₂ C ₂ O ₄ , HCl	Carl Roth	0 - 10 mM, pH 4
Fumaric acid solution	C ₄ H ₄ O ₄ , HCl	Carl Roth	0 – 10 mM, pH 4
L-(-)-Maleic acid solution	C ₄ H ₆ O ₅ , HCl	Alpha Aesar	0 – 10 mM, pH 4
Succinic acid solution	C ₄ H ₆ O ₄ , HCl	Carl Roth	0 – 10 mM, pH 4
L-(+)-Tartaric acid solution	C ₄ H ₆ O ₆ , HCl	Carl Roth	0 – 10 mM, pH 4

8.1 Fungal alteration of pH in surrounding area

The pH indicator dye bromocresol green (0.0015% (w/v)) was added to PDA medium before autoclaving. *Termitomyces* T153 was cultured as described above for up to 4 weeks at room temperature.

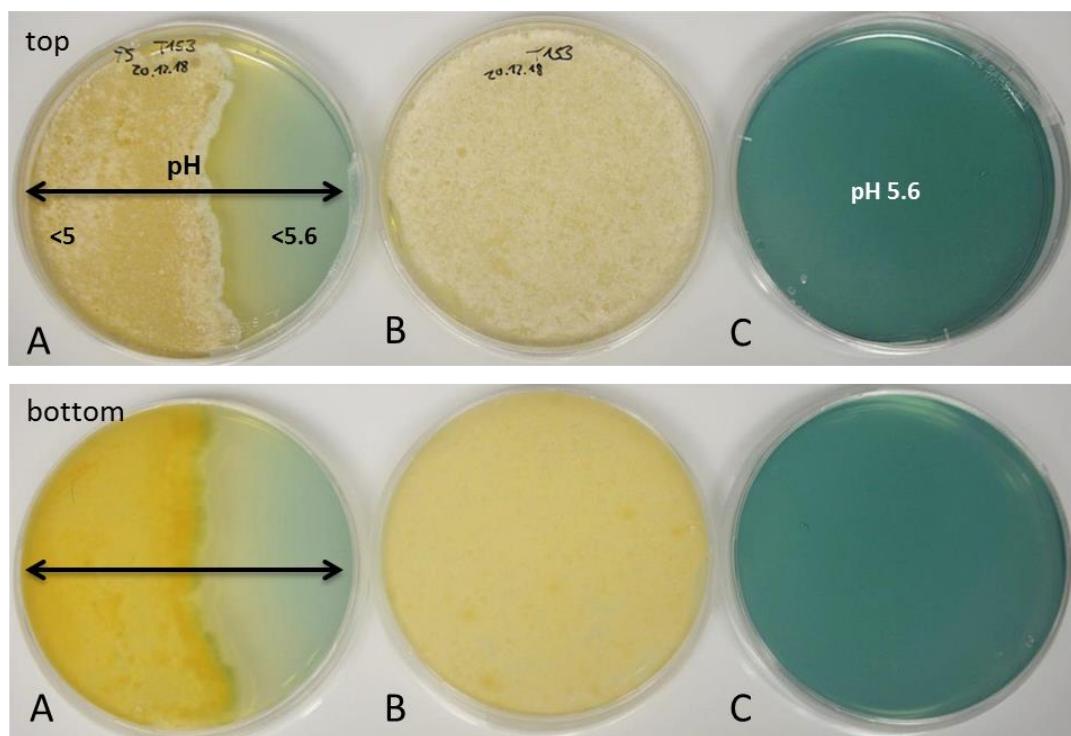


Figure S3. **A)** *Termitomyces* sp. T153 growing on the left half of a PDA plate stained with bromocresol green; **B)** *Termitomyces* sp. T153 fully grown on PDA without indicator); **C)** empty PDA plate stained with bromocresol green (pH 5.6 ± 0.2).

8.2 Detection and quantification of H₂O₂ in culture medium of *Termitomyces* sp. T153

A fluorimetric hydrogen peroxide assay kit (Sigma-Aldrich) was used to detect H₂O₂. The kit contained the following ingredients: red peroxidase substrate stock solution (DMSO), horseradish peroxidase stock solution (20 units/mL), FeCl₃ (1.0 mM), H₂O₂ (20.0 mM) and standard solutions (10.0 µM; 1.0 µM; 0.1 µM; 0.01 µM; 0.0 µM). Sample preparation: Wet mycelium (350 mg) was scraped off a fully grown plate of *Termitomyces* sp. T153 (21 d old, PDA). The mycelium was suspended in 1.5 mL ddH₂O. The suspension was thoroughly mixed and ultrasonicated for 30 s. The mycelium was pelleted by centrifugation for 15 min at 12000 rpm. 700 µL of the clear supernatant was collected and stored in a separate tube. Assay samples were prepared according to Table S17.

Table S19. Sample composition for H₂O₂ detection assay

Sample	V _{Sample} [µL]	V _{H₂O} [µL]	V _{FeCl₃} [µL]	Sample Type
1	100	0	0	Sample T153
3	90	0	10	Sample T153 + FeCl ₃
2	10	90	0	Sample T153 1:10 diluted
4	10	80	10	Sample T153 1:10 + FeCl ₃
5	10	89	1	Sample T153 1:10 + FeCl ₃ 1:10
6	100	0	0	DMHQ 100 µM
7	90	0	10	DMHQ 100 µM + FeCl ₃

Preparation of reaction mixture (1.0 mL): 950 µL of assay buffer was mixed with 10 µL red peroxidase substrate stock solution and 40 µL horseradish peroxidase stock solution (20 units/mL) on ice. 50 µL of each sample was transferred to a 96 well plate (measurements were done in duplicates) and the reaction mixture was allowed to reach room temperature. Then, 50 µL of the reaction mixture was added to each sample and the reaction was incubated for 20 min in the dark at room temperature. The fluorescence was analyzed at λ_{Exi} = 540 nm and λ_{Emi} = 590 nm.

Table S20. Dilution factor and total concentration of H₂O₂ in samples

Sample	Sample Dilution	Conc. H ₂ O ₂ [µM]	Total conc. H ₂ O ₂ [µM]
1	100%	23.66	23.66
2	10%	3.53	35.27
3	90%	21.46	23.85
4	10%	3.09	30.87
5	10%	3.83	38.35
6	100%	11.78	11.78
7	90%	10.37	11.53

8.3 Detection of hydroxyl radicals

The active generation of hydroxyl radicals was measured using a fluorometric assay based on the reaction with terephthalic acid (TPA) yielding the fluorescent oxidation product hydroxy-terephthalic acid (hTPA).

A reaction mixture consisting of 80 μ L TPA (100 μ M), 80 μ L FeCl_3 (30 μ M) dissolved in 0.1 M acetate buffer (pH 4) and 40 μ L H_2O_2 (3 μ M) was prepared and quickly distributed in 200 μ L aliquots on a 96 well plate. The reaction was initiated by addition of 4 μ L redox active sample (1 mM in ACN) and incubated in the dark for continuous measurements. Negative controls were prepared in ACN. Changes in fluorescence intensity were measured for up to 3 hours with an excitation wavelength of $\lambda_{\text{ex}} = 315$ nm and an emission wavelength of $\lambda_{\text{emi}} = 425$ nm. A standard curve of hTPA formation was prepared in the range of 0 to 100 nM. Samples were analyzed in triplicates, $n = 3$, and results averaged.

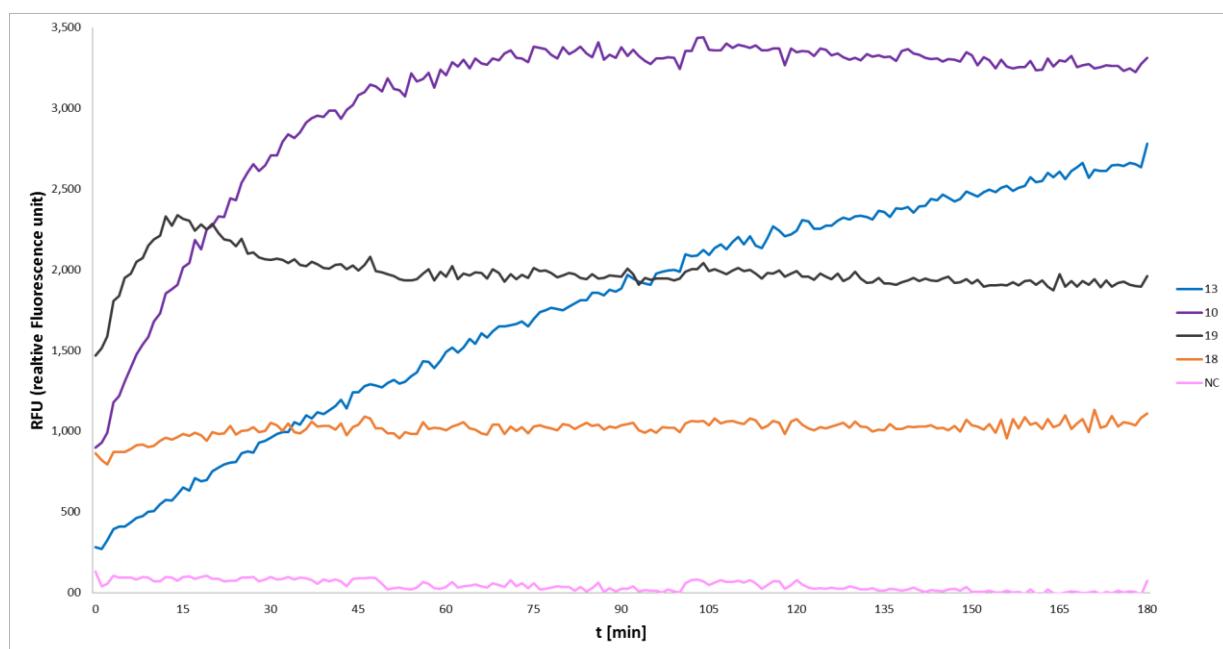


Figure S4. Hydroxy radical detection assay during Fenton reaction initiated by selected redox active compounds (**13** blue, **10** purple, **19** black, **18** orange and negative control grey) showing changes in fluorescence at 425 nm over time caused by hTPA. Average values from triplicates ($n=3$).

8.4 *In vivo* Ferrozin reaction on *Termitomyces* sp. T153 mycelium

A reaction mixture containing acetate buffer A (2.0 mL), FeCl_3 solution (200 μL) and Ferrozin solution (200 μL) (for concentrations, see Table S18) was placed as 100 or 200 μL droplets on the mycelium surface of a PDA culture plate of *Termitomyces* sp. T153 (18 d old). The hydrophobic mycelium surface below the droplets was disturbed slightly using pipette tips. Pictures were taken after 5 and 30 min of incubation. Droplets incubated on sterile PDA plates were compared after 5, 10 and 20 min as negative controls.

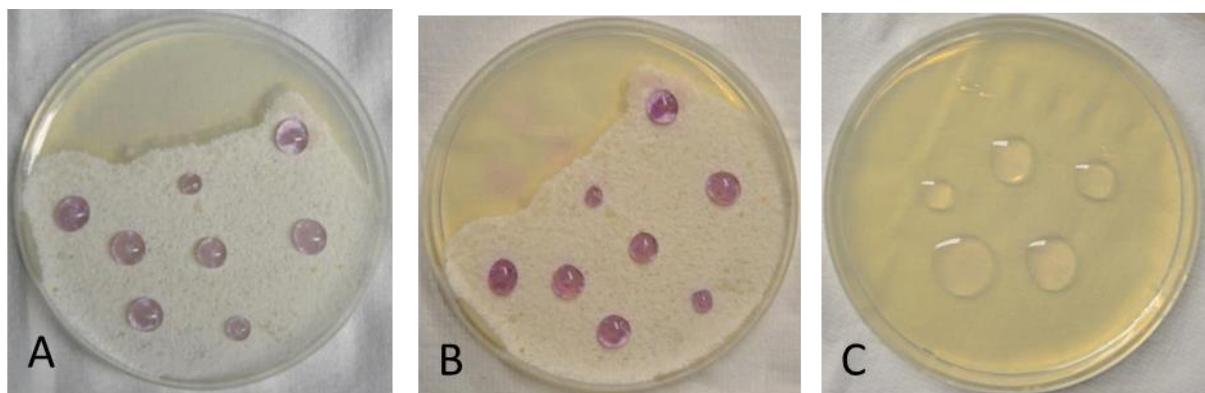


Figure S5. Ferrozin assay *in vivo* after **A**) 5 min and **B**) 30 min incubation and **C**) Ferrozin assay on empty PDA plate (negative control). Red color indicates active iron reduction.

8.5 *In vitro* Ferrozin assay with fungal metabolites

For each test reaction, a mixture of acetate buffer A (196 μL), freshly prepared FeCl_3 solution (20 μL) and Ferrozin solution (20 μL) was mix per well. The reaction was initiated by addition of 24 μL sample solution (1.0 mM of compound) and incubated in the dark at room temperature. The experiment was performed in triplicates. Absorbance was measured at $\lambda = 562 \text{ nm}$ after 5, 30, 60, 120, 180 and 240 min of incubation. A standard curve was prepared using FeSO_4 standards with molarities ranging from 0-1000 μM .

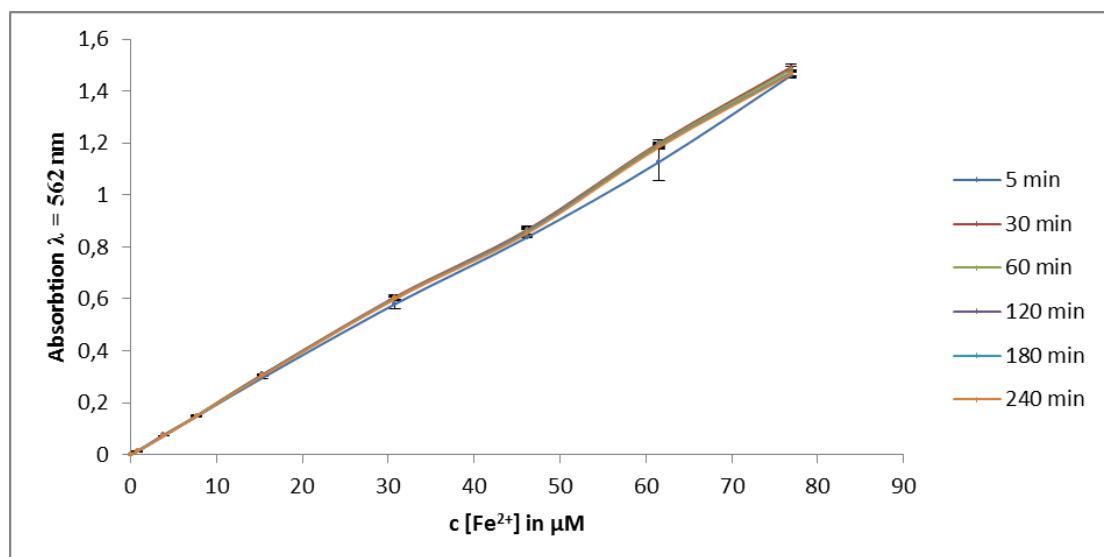


Figure S6. Standard curve used for *in vitro* Ferrozin assays (5 – 240 min).

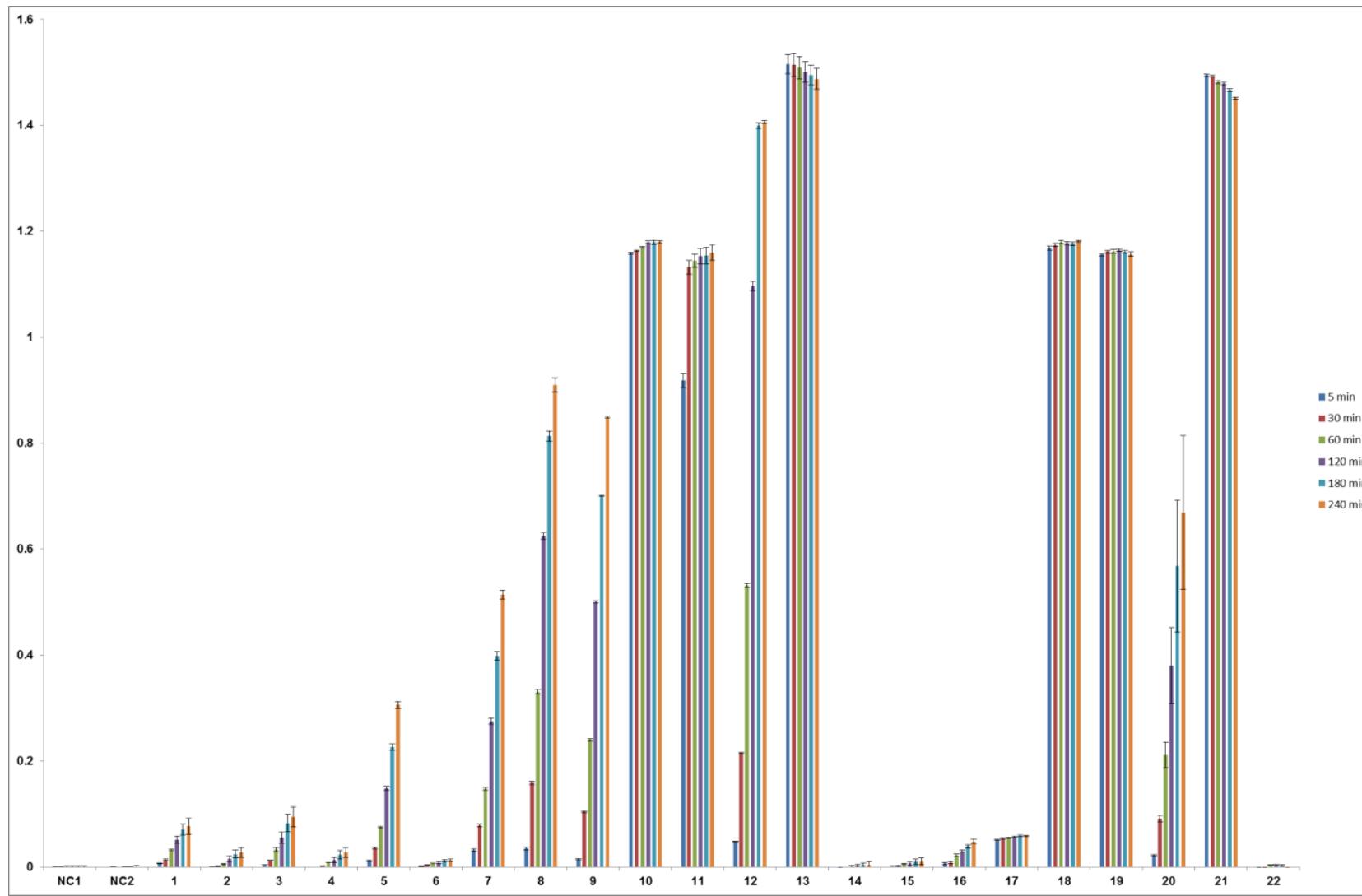


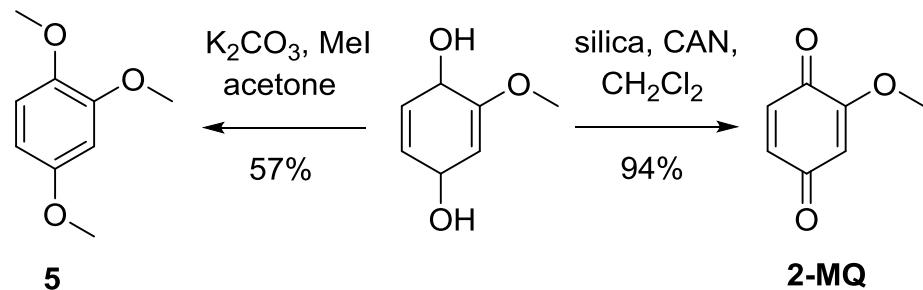
Figure S7. *In vitro* screening of iron reduction activity of detected fungal metabolites in Ferrozin assay over 5 – 240 min. Positive control: #18. Error bars indicate ± 0.5 standard deviation, $n = 3$.

Table S21. List of fungal metabolites and related commercial compounds used for redox assays

Number		Name
1		1,4-Dimethoxybenzene
2		1,3-Dimethoxybenzene
3		1,2-Dimethoxybenzene
10		1,2-Dihydroxybenzene
4		1,2,3-Trimethoxybenzene
6		1,3,5-Trimethoxybenzene
8		1,3,5-Trihydroxybenzene
5		1,2,4-Trimethoxybenzene
19 (2-MH ₂ Q)		Methoxyhydroquinone
18 (2,6-DMH ₂ Q)		1,4-Dihydroxy-2,6-dimethoxybenzene
13		Syringic acid
20		3,4-Dimethoxybenzyl alcohol

9		2',4',6'-Trihydroxyacetophenone
12		Vanillin
14		4-Hydroxybenzaldehyde
22		p -Toluic acid
15		4-Hydroxybenzoic acid
16		3-Hydroxybenzoic acid
17		2-Hydroxybenzoic acid
11		2,3-Dihydroxybenzoic acid
7		2,4-Dihydroxy-6-methylbenzoic acid
21		3,5-Dimethoxy-4-hydroxycinnamic acid

8.6 Synthesis of standards



Synthesis 1,2,4-trimethoxybenzene (**5**)

2-Methoxy-1,4-hydroquinone (280 mg, 2.00 mmol, 1.0 eq.) was placed in a 100 mL round bottomed flask equipped with a reflux condenser. The alcohol was dissolved in acetone (20 ml, 0.1 m) followed by addition of K₂CO₃ (1.38 g, 10.00 mmol, 5.0 eq.) and MeI (738 mg, 5.20 mmol, 2.6 eq.). The reaction mixture was stirred under reflux for 10 h. The reaction was quenched by addition of H₂O (10 mL/mmol) and the mixture was extracted three times with Et₂O (3 × 20 mL). The combined organic layers were dried with MgSO₄ and concentrated under reduced pressure. The compound was purified by column chromatography on silica to yield the product 1,2,4-trimethoxybenzene (**5**, 57%, 196 mg, 1.14 mmol) as pale yellow oil.

¹H-NMR (500 MHz, CDCl₃): δ = 6.78 (d, 1H, ³J_{H,H} = 8.8 Hz, CH), 6.51 (d, 1H, ⁴J_{H,H} = 2.8 Hz, CH), 6.39 (dd, 1H, ³J_{H,H} = 8.8 Hz, ⁴J_{H,H} = 2.8 Hz CH), 3.85 (s, 3H), 3.83 (s, 3H), 3.76 (s, 3H ppm. ¹³C-NMR (125 MHz, CDCl₃): δ = 154.3 (C_q), 149.9 (C_q), 143.6 (C_q), 111.9 (CH), 102.9 (CH), 100.4 (CH), 56.5 (CH₃), 55.8 (CH₃), 55.7 (CH₃) ppm.

2-methoxy-1,4-benzoquinone (**2-MQ**)

2-Methoxy-1,4-benzoquinone was prepared as reported previously.²⁹ In a 100 mL round bottomed flask equipped with a septum and a stirring bar, silica gel (6.0 g) was placed and stirred, followed by a dropwise addition of cerium ammonium nitrate (CAN, 2.75 g, 5.02 mmol, 2.51 eq. dissolved in water (3 mL)). The CAN containing silica was stirred for 5 min followed by addition of CH₂Cl₂ (25 mL). 2-Methoxy-1,4-hydroquinone (280 mg, 2.00 mmol, 1.0 eq. suspended in CH₂Cl₂ (5 mL)) was added and the suspension was stirred for 30 minutes at room temperature. The reaction mixture was filtered through a glass column with a frit followed by an additional wash with CH₂Cl₂ (3 × 50 mL). The solvent was removed under reduced pressure yielding 2-methoxy-1,4-benzoquinone (**2-MQ**, 94%, 260 mg, 1.88 mmol) as pale yellow powder.

TLC (cyclohexane/ethyl acetate 3:1): R_f = 0.17. ¹H-NMR (700 MHz, CDCl₃): δ = 6.74 (m, 2H), 5.97 (s, 1H), 3.86 (m, 3H) ppm. ¹³C-NMR (175 MHz, CDCl₃): δ = 187.3 (C_q), 181.6 (C_q), 158.7 (C_q), 137.3 (CH), 134.5 (CH), 107.7 (CH), 56.2 (CH₃) ppm. These data are in accord with previously reported data.

8.7 Kinetic measurements

Acetate Buffer: A master reaction mixture consisting of 2.0 mL acetate buffer A, 200 μ L FeCl₃ solution and 200 μ L Ferrozin solution was freshly prepared. Using a 96 well plate set-up, 20 μ L of each candidate compound (1.0 mM, dissolved in ACN) was added to 200 μ L reaction mixture. The reaction was incubated in the dark at room temperature and absorbance was measured at $\lambda_{\text{Abs}} = 562$ nm after 5, 30, 60, 120, 180, 240 min. Each reaction was tested at least in duplicates.

Kinetic measurements: A master reaction mixture consisting of 2.0 mL acetate buffer A, 200 μ L FeCl₃ solution and 200 μ L Ferrozin solution was freshly prepared. Using a 96 well plate set-up, 20 μ L of each candidate compound (0.1 mM) dissolved in ACN was added to 200 μ L reaction mixture. The reaction was incubated in the dark at room temperature and absorbance was measured at $\lambda_{\text{Abs}} = 562$ nm every 7 s for up to 30 min. Mixtures of candidates used to evaluate synergistic effects were created by mixing 10 μ L of two compounds (0.1 mM) dissolved in ACN. Each reaction was tested at least in duplicates.

Synergistic combination assays: A reaction mixture consisting of 2.0 mL acetate buffer A, 200 μ L FeCl₃ solution and 200 μ L Ferrozin solution were freshly prepared. 20 μ L of each sample combination (0.1 mM) were added per well in a 96 well plate. The reaction was initiated by addition of 200 μ L reaction mixture to each sample and incubated in the dark at room temperature. Absorbance was measured at $\lambda = 562$ nm after 5, 10, 30 and 60 min. Each reaction was tested at least in duplicates.

Test of other additives: A 10 mM stock solution of (oxalic acid, fumaric acid, tartaric acid, succinic acid, malic acid) in dd H₂O was prepared and the solution was adjusted to pH 4 using HCl or NaOH. The stock solution was further diluted to yield working buffers of the concentrations X = 10 mM; 1.0 mM; 0.5 mM; 0.1 mM; 0.05 mM and 0 mM (additionally 2.5 mM in case of oxalic acid). For each resulting buffer working solution a reaction mixture was prepared containing 1.5 mL diluted buffer (0 – 10 mM), 150 μ L FeCl₃ solution and 150 μ L Ferrozin solution.

20 μ L of each sample (1.0 mM) was prepared on a 96 well plate. The reaction was initiated by the addition of 200 μ L buffer working solution to each sample and incubated in the dark at room temperature. Absorbance was measured after incubation for 5, 30, 60 and 120 min at $\lambda_{\text{Abs}} = 562$ nm. Each buffer reaction was tested at least in duplicates.

5 min	13	21	10	11	18	19		10 min	13	21	10	11	18	19
13	0.644	0.677	0.935	0.702	0.477	0.531		13	0.698	0.719	1.02	0.781	0.505	0.564
21	0.668	0.627	1.021	0.744	0.464	0.515		21	0.72	0.654	1.057	0.799	0.48	0.531
10	0.971	1.004	1.247	0.882	0.794	0.931		10	1.059	1.052	1.346	0.988	0.835	0.991
11	0.641	0.739	0.882	0.475	0.403	0.425		11	0.711	0.787	0.99	0.605	0.468	0.49
18	0.472	0.452	0.781	0.385	0.296	0.337		18	0.524	0.471	0.814	0.451	0.296	0.341
19	0.494	0.499	0.935	0.436	0.336	0.372		19	0.529	0.518	1.001	0.51	0.336	0.371
NC	0.056	0.049	0.052					NC	0.057	0.05	0.053			
30 min	13	21	10	11	18	19		60 min	13	21	10	11	18	19
13	0.728	0.733	1.172	0.846	0.519	0.58		13	0.731	0.761	1.338	0.858	0.522	0.582
21	0.758	0.708	1.194	0.818	0.514	0.562		21	0.788	0.77	1.386	0.86	0.553	0.597
10	1.228	1.19	1.46	1.251	0.934	1.107		10	1.384	1.38	1.492	1.431	1.114	1.307
11	0.761	0.836	1.249	0.805	0.568	0.583		11	0.773	0.884	1.43	0.845	0.587	0.601
18	0.524	0.505	0.908	0.543	0.3	0.343		18	0.528	0.542	1.081	0.564	0.305	0.346
19	0.544	0.549	1.122	0.615	0.339	0.374		19	0.547	0.584	1.325	0.633	0.343	0.378
								NC	0.066	0.056	0.059			
NC	0.061	0.052	0.056											

Figure S8. Cross-plot for combination assay of selected standard compounds with strong Fe³⁺ reducing activity (**13, 21, 10, 11, 18, 19**) showing no clear signs of synergy (n = 2).

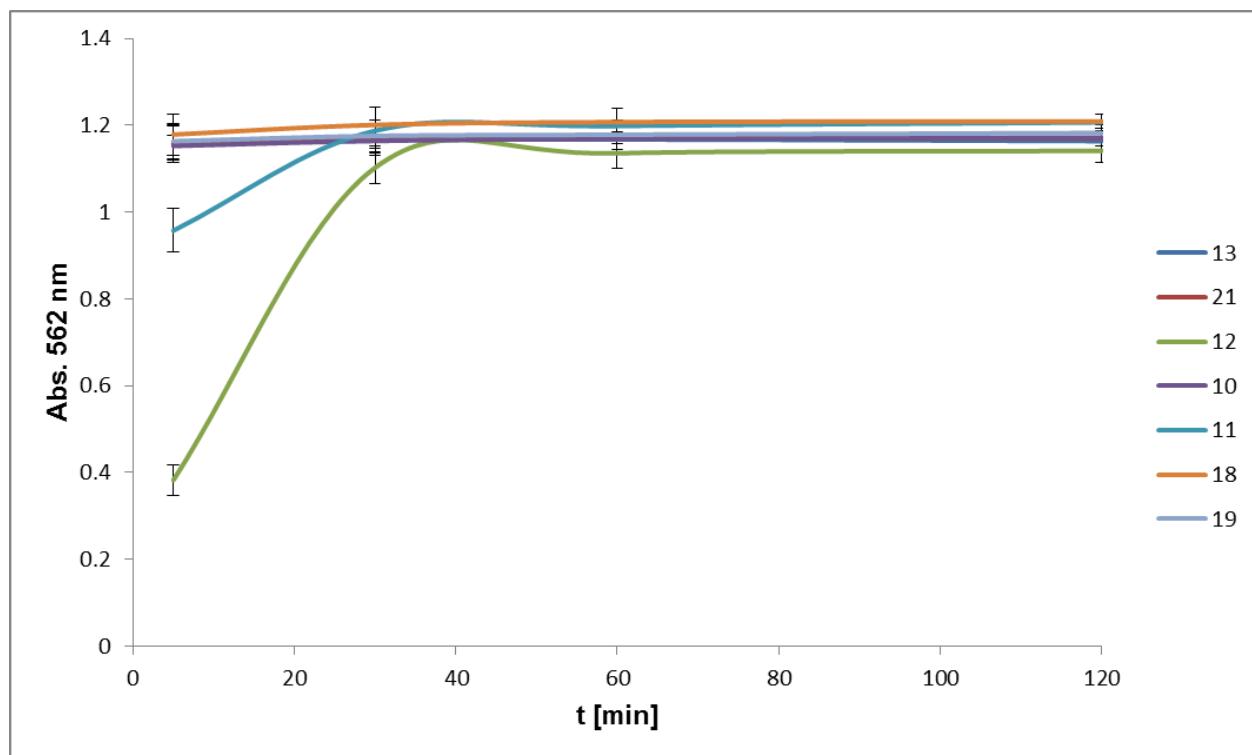


Figure S9. Ferrozin kinetic assay testing standard compounds # (**13** blue, **21** red, **12** green, **10** purple, **11** light blue, **18** orange, **19** grey) based on the formation of Fe^{2+} -Ferrozin complex over time in 0 mM oxalic acid buffer. Error bars indicate ± 0.5 standard deviation ($n = 2$).

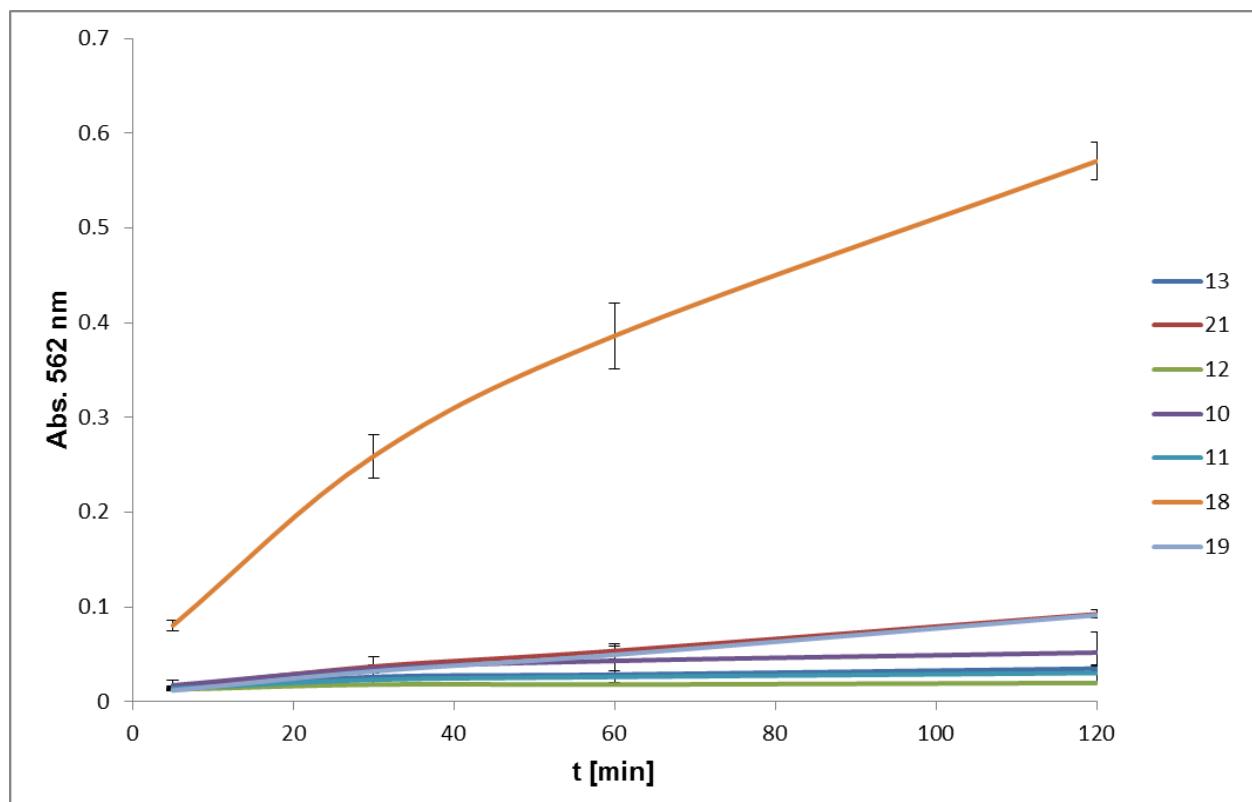


Figure S10. Ferrozin kinetic assay testing standard compounds # (**13** blue, **21** red, **12** green, **10** purple, **11** light blue, **18** orange, **19** grey) based on the formation of Fe^{2+} -Ferrozin complex over time in 1 mM oxalic acid buffer. Error bars indicate ± 0.5 standard deviation ($n = 2$).

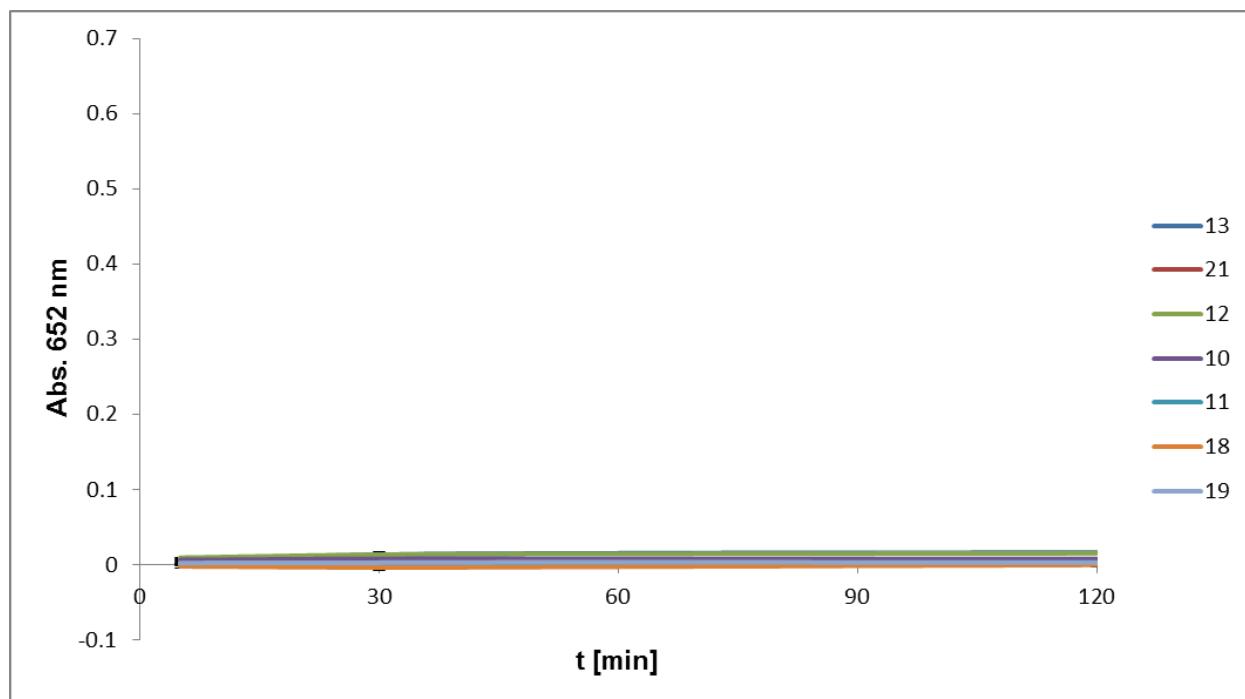


Figure S11. Ferrozin kinetic assay testing standard compounds # (13 blue, 21 red, 12 green, 10 purple, 11 light blue, 18 orange, 19 grey) based on the formation of Fe^{2+} -Ferrozin complex over time in 10 mM oxalic acid buffer. Error bars indicate ± 0.5 standard deviation ($n = 2$).

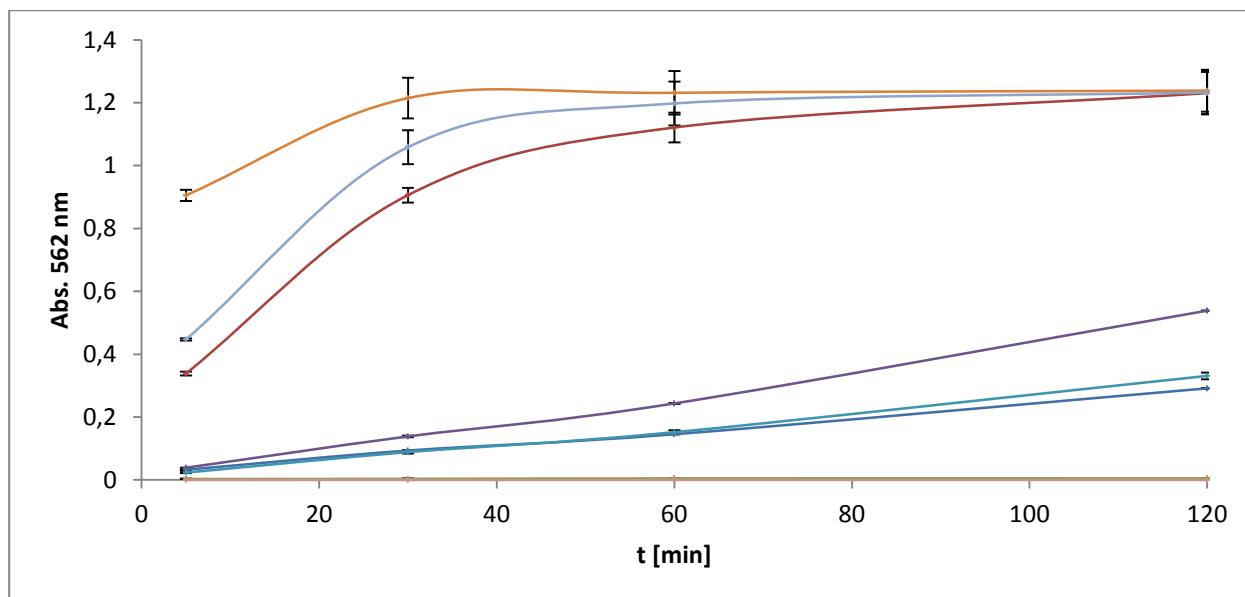


Figure S12. Ferrozin kinetic assay testing standard compounds # (13 blue, 21 red, 12 green, 10 purple, 11 light blue, 18 orange, 19 grey) based on the formation of Fe^{2+} -Ferrozin complex over time in 10 mM L-(-)-malic acid buffer. Error bars indicate ± 0.5 standard deviation ($n = 3$).

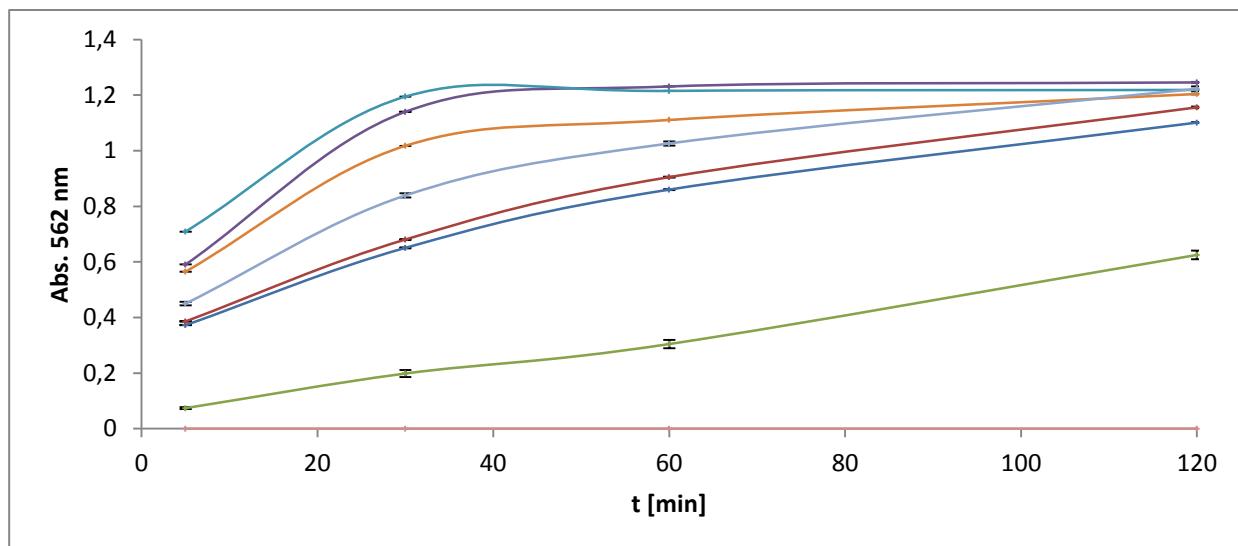


Figure S13. Ferrozin kinetic assay testing standard compounds # (13 blue, 21 red, 12 green, 10 purple, 11 light blue, 18 orange, 19 grey) based on the formation of Fe^{2+} -Ferrozin complex over time in 10 mM fumaric acid buffer. Error bars indicate ± 0.5 standard deviation ($n = 3$).

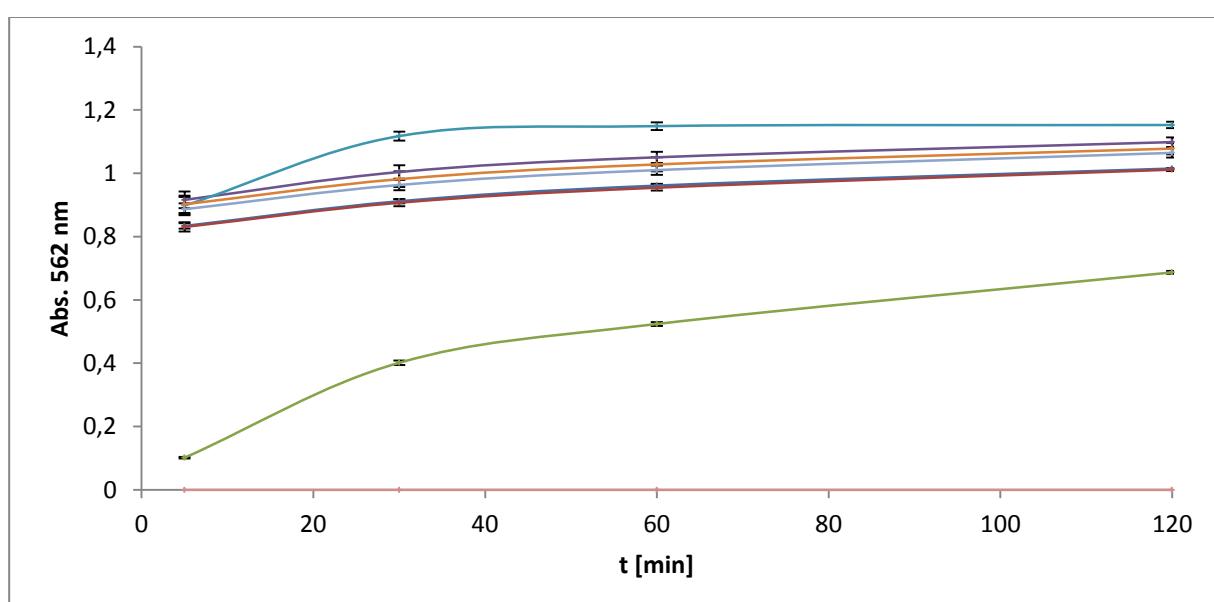


Figure S14. Ferrozin kinetic assay testing standard compounds # (13 blue, 21 red, 12 green, 10 purple, 11 light blue, 18 orange, 19 grey) based on the formation of Fe^{2+} -Ferrozin complex over time in 10 mM succinic acid buffer. Error bars indicate ± 0.5 standard deviation ($n = 3$).

8.8 Test of ligninolytic activity by degradation of model compound Azure B

Azure B dye degradation *in vivo*:³⁰ *Termitomyces* sp. T153 was inoculated on agar plates (PDA) containing 30 mg/L Azure B (3-(Dimethylamino)-7-(methylamino)phenothiazin-5-i um chloride) and grown for 6 weeks. Agar plates were regularly checked for decolorization against a sterile control plate. First decolorization zones started appearing after 7 days around the mycelium and indicate ligninolytic activity.

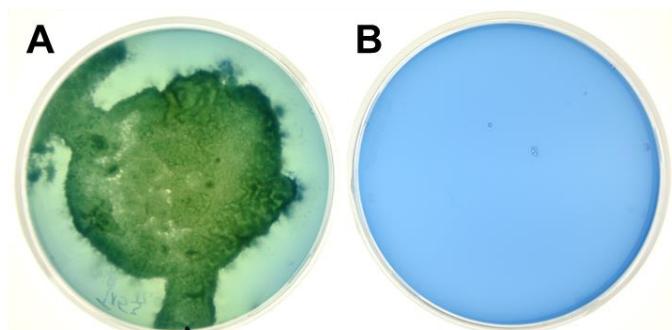


Figure S15. Azure B dye decolorization assay showing A) *In vivo* halo formation and decolorization gradient around *Termitomyces* sp. T153 mycelium (41 d, PDA + Azure B) indicating ligninolytic potential B) Sterile control plate (41 d, PDA + Azure B) without signs of decolorization.

Azure B dye degradation *in vitro* based on Fenton chemistry: 190 µL aliquots of reaction mix (700 µL Azure B dye (0.2 mM), 490 µL NaOAc Buffer (50 mM), 70 µL freshly prepared FeCl₃ (2 mM), 70 µL H₂O₂ (20 mM)) were distributed on a 96 well-plate in duplicates. Iron reduction was initiated with the addition of 10 µL hydroquinone **18** or **19** (2 mM). The reaction was incubated in the dark and absorbance at $\lambda_{Abs} = 651$ nm was measured in 30 s intervals for 30 min. H₂O was used instead of hydroquinone as a negative control. Addition of purified secreted fungal enzymes to the reaction mixture did not increase the degradation process.

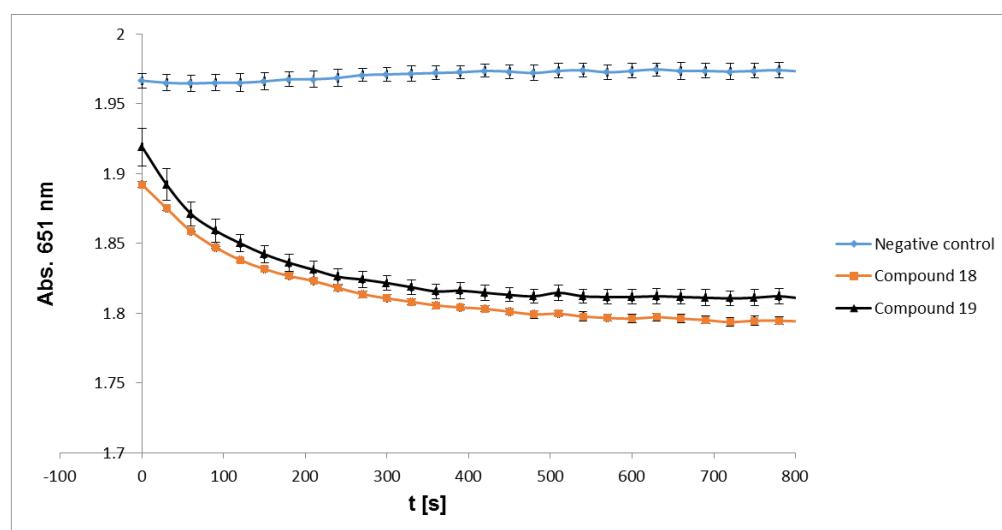


Figure S16. Fenton based Azure B degradation assay initiated by Fe³⁺ reducing hydroquinones **18** (orange squares) or **19** (black triangles) compared to negative H₂O control (blue circles). Decrease in absorption indicates degradation of Azure B dye. Error bars showing ± 0.5 standard deviation, n = 2.

9. Proteomic analysis

Collection and analysis of secreted proteins: 25 mL of PDB medium (Potato-Dextrose-Broth) were inoculated with a suspension of 1.0 mL *Termitomyces* sp. T153 mycelium in PBS buffer and the resulting culture was grown for 12 days (20 °C, 150 rpm). The culture was transferred on ice and mycelium was removed by centrifugation (4.000 rpm, 10 min, 4 °C) without cell disruption. The supernatant containing the secreted fungal enzymes was collected in a pre-cooled tube. Enzymes were transferred into KH₂PO₄ (100 mM, pH 6.5) or NaOAc (50 mM, pH 4.5) buffered solution and media components, salts were removed with a PD-10 column (GE Healthcare) according to manufacturer's manual with constant cooling. Protein concentration was determined according to the method of Bradford. The pH of the enzyme solution was adjusted to 2-3 by addition of TFA (approx. 0.1 %).

Protein digestion: Protein content was extracted with an SPE-C4 cartridge (Macherey-Nagel, 4 mL, 500 mg), washed with 4.0 mL 5 % MeOH 0.1 % TFA and eluted with 4.0 mL 80% ACN 0.1 % TFA. Samples were dried *in vacuo*. Samples were resolubilized by thorough pipetting, vortexing and ultrasonication for 15 min in 100 µL denaturation buffer consisting of 50 mM triethylammonium bicarbonate (TEAB) in a 50/50 mixture of 2,2,2-trifluoroethanol (TFE) and H₂O. 2.0 µL of reduction buffer (500 mM tris(2-carboxyethyl)phosphine solution in TEAB (100 mM)) was added and the samples were mixed. Subsequently, 2.0 µL of freshly prepared alkylation buffer (2-chloroacetamide (625 mM) in TEAB (100 mM)) was added and the solution was mixed followed by incubation at 70 °C for 30 min, 500 rpm in the dark. Samples were frozen at -20 °C for 15 min and proteins were prepared for precipitation by addition of 400 µL chilled MeOH (-20 °C) followed by 400 µL CHCl₃ (-20 °C). After mixing proteins were forced to precipitate by addition of 300 µL ice cold H₂O. After mixing, phase separation was achieved by incubation at -20 °C for 5 min followed by centrifugation (5 min, 14.000 rpm, <4 °C). The top aqueous layer was removed and 400 µL MeOH (-20 °C) was added and samples thoroughly mixed. After another centrifugation step the MeOH supernatant was removed and samples were concentrated close to dryness *in vacuo*. The protein precipitate was resolubilized and thoroughly mixed in 100 µL TEAB (100 mM) in 5 % (v/v) TFE/H₂O by pipetting, vortexing and ultrasonication for 15 min. Total protein concentration was determined with a Merck Milipore Direct Detect System according to manufacturer's manual. Tryptic digestion was achieved by addition of 2.0 µL of a 2 µg/µl Trypsin/Lys C protease mixture (Promega #V5072). Samples were mixed gently and incubated for 18 h at 37 °C in the dark. Samples were dried *in vacuo* and resolubilized in 30 µL 0.05% TFA and 2 % ACN in H₂O by pipetting, vortexing and sonication for 15 min. After homogenization samples were filtered through a 0.2 µm spin filter (Merck-Milipore Ultrafree-MC hydrophilic PTFE membrane) at 14.000 rpm, 4 °C, 15 min. the filtrate was transferred into HPLC vials and subjected to LC-MS/MS analysis.

LC-MS/MS analysis: LC-MS/MS analysis was performed on an Ultimate 3000 nano RSLC system connected to a QExactive Plus mass spectrometer (both Thermo Fisher Scientific, Waltham, MA, USA). Peptide trapping for 5 min on an Acclaim Pep Map 100 column (2 cm x 75 µm, 3 µm) at 5 µL/min was followed by separation on an analytical Acclaim Pep Map RSLC nano column (50 cm x 75 µm, 2µm). Mobile phase gradient elution of eluent A (0.1% (v/v) formic acid in water) mixed with eluent B (0.1% (v/v) formic acid in 90/10 acetonitrile/water) was performed as follows: 0-5 min at 4% B, 30 min at 7% B, 60 min at 10% B, 100 min at 15% B, 140 min at 25% B, 180 min at 45% B, 200 min at 65 %B, 210-215 min at 96% B, 215.1-240 min at 4% B.

Positively charged ions were generated at spray voltage of 2.2 kV using a stainless steel emitter attached to the Nanospray Flex Ion Source (Thermo Fisher Scientific). The quadrupole/orbitrap instrument was operated in Full MS / data-dependent MS2 (Top10) mode. Precursor ions were monitored at m/z 300-1500 at a resolution of 70,000 FWHM (full width at half maximum) using a maximum injection time (ITmax) of 120 ms and an AGC (automatic gain control) target of 3e6. Precursor ions with a charge state of z=2-5 were filtered at an isolation width of m/z 1.6 amu for further HCD fragmentation at 30% normalized collision energy (NCE). MS2 ions were scanned at 17,500 FWHM (ITmax=120 ms, AGC= 2e5) using a fixed first mass of m/z 120 amu. Dynamic exclusion of precursor ions was set to 30 s and the underfill ratio was set to 1.0%. The LC-MS/MS instrument was controlled by DCMS Link 2.14, QExactive Tune 2.9 and Xcalibur 3.0 software.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE³¹ partner repository with the dataset identifier PXD025936 under the project name: The termite fungal cultivar *Termitomyces* combines diverse enzymes and oxidative reactions for plant biomass conversion.

Protein database search

Tandem mass spectra were searched against the UniProt database of *Termitomyces* sp. J132 (<https://www.uniprot.org/proteomes/UP000053712>; 2019/11/04) using Proteome Discoverer (PD) 2.4 (Thermo) and the algorithms of Mascot 2.4, Sequest HT (version of PD2.2), and MS Amanda 2.0. Two missed cleavages were allowed for the tryptic digestion. The precursor mass tolerance was set to 10 ppm and the fragment mass tolerance was set to 0.02 Da. Modifications were defined as dynamic Met oxidation and protein N-term acetylation as well as static Cys carbamidomethylation. A strict false discovery rate (FDR) < 1% (peptide and protein level) and at least a search engine threshold >30 (Mascot), >4 (Sequest HT) or >300 (MS Amanda) were required for positive protein hits. The Percolator node of PD2.4 and a reverse decoy database was used for qvalue validation of spectral matches. Only rank 1 proteins and peptides of the top scored proteins were counted.

Ranking of proteins is summarized in **Table S28-29** (Supporting Information Part2): Protein and peptide data for acetate (NaOAc) buffered samples can be found in excel sheets **Table_S28_A_B**; protein and peptide data for phosphate (KH₂PO₄) buffered samples can be found in **Table_S29_A_B**.

10. Protein analysis and activity tests

Laccase activity tests: 20 µL of a syringaldazine solution (0.216 mM, MeOH) were added to an ice cold solution containing 146.6 µL reaction buffer (100 mM K₂HPO₄, pH 6.5) and 33 µL freshly prepared and rebuffered fungal enzymes. The reaction mixture was incubated at 30 °C and absorption was monitored at λ_{Abs} 530 nm. Laccase from *Trametes versicolor* was used as positive control.

MnP activity using plate cultures: 100 ml PDA medium were prepared and sterilized by autoclaving. Subsequently, sterile-filtered leukoberbelin blue I dye (4 mg in 5 mL) and MnCl₂ (final concentration 500 µM, in ddH₂O) were added. The medium was thoroughly mixed and poured into plates. *Termitomyces* T153 was cultivated for up to 2 weeks on the plates.

MnP activity using liquid cultures: *Termitomyces* sp. T153 was inoculated in PDB medium containing elevated MnCl₂ concentrations (200-500 µM). Leukoberbelin blue dye was added (4 mg / 100 mL) and strong blue color was observed after two days. A similar but weaker blue color shift was also observed in cell free culture supernatants containing only media components and secreted enzymes or metabolites when amended with MnCl₂, leukoberbelin blue dye and incubated at 30 °C.

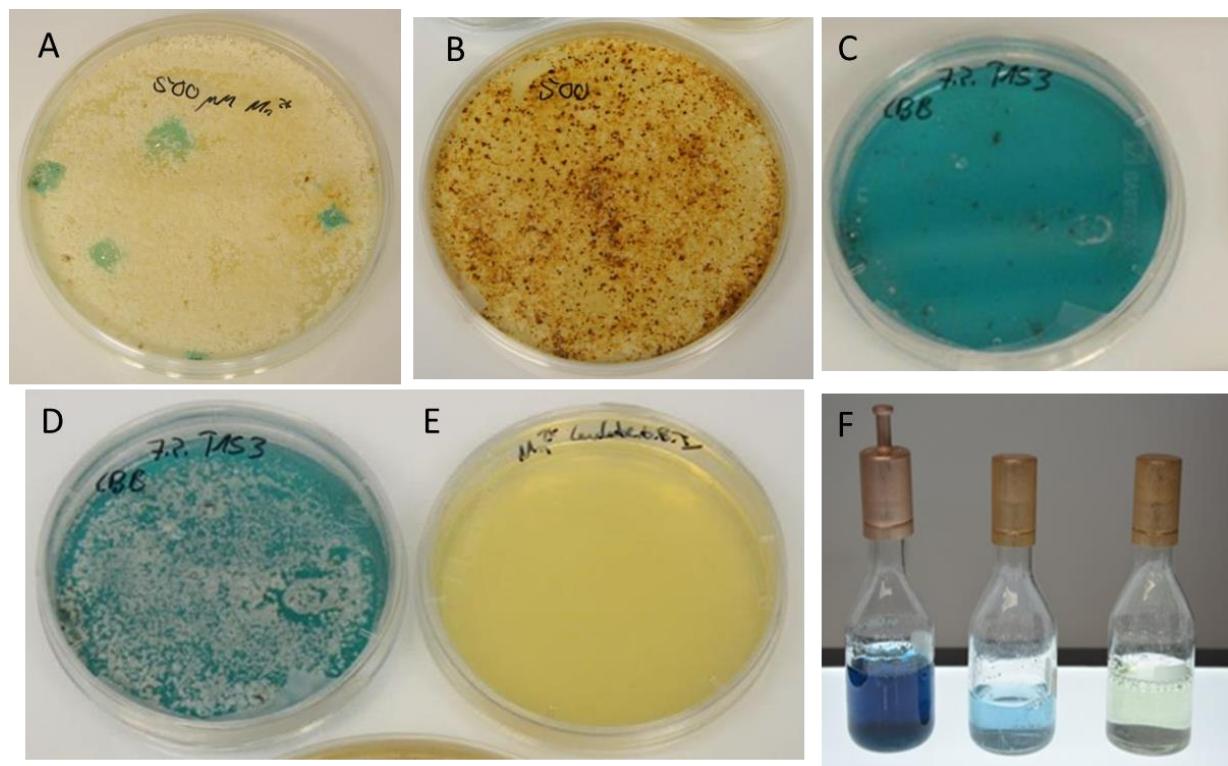


Figure S17. **A)** *Termitomyces* sp. T153 grown on PDA containing 500 µM MnCl₂ for 21 d and addition of leukoberbelin blue I dye on four random mycelium spots; **B)** *Termitomyces* sp. T153 grown on PDA for 24 d containing 500 µM MnCl₂ (without leukoberbelin blue I dye) showing brown Mn-oxide formation; **C)** *Termitomyces* sp. T153 grown on PDA containing 500 µM MnCl₂ and leukoberbelin blue I dye after 4 d of inoculation; **D)** *Termitomyces* sp. T153 grown on PDA containing 500 µM MnCl₂ and leukoberbelin blue I dye after 7 d of inoculation; **E)** Empty PDA plate containing leukoberbelin blue I dye (control) and 500 µM MnCl₂. **F)** Leukoberbelin blue I assay: liquid culture of *Termitomyces* sp. 153, cell-free culture supernatant of *Termitomyces* sp. T153 and PDB (from left to right)

MnP enzyme activity test: 100 µL of freshly prepared and rebuffered enzyme solution were mixed with 100 µL of a solution containing 500 µM MnCl₂ and 0.004 % (w/v) leukoberberlin blue I dye. The mixture was put to room temperature and enzymatic reaction was initiated by addition of 10 µL H₂O₂ (20 mM) solution. The reaction progress was monitored via absorption at λ_{Abs} 620 nm.

Expression studies of MnP: RNA extraction and cDNA preparation: *Termitomyces* sp. T153 was grown on PDA (Potato-Dextrose-Agar) or FCA (Fungus-Comb-Agar) medium at room temperature. After 10 days fungal mycelium was harvested and stored at -80 °C. For RNA extraction, mycelium was ground to a fine powder with liquid nitrogen and extracted using ISOLATE II RNA plant kit (Bioline). The cDNA synthesis was performed using Maxima H Minus Reverse Transcriptase (Thermo) with 2 µg of extracted RNA as a template. RNA was mixed with Oligo d(T)₂₃VN primers (3.33 µM final concentration, NEB), dNTP mix (25 mM each, final concentration 0.83 mM final concentration; Biozym) and adjusted with RNase free water to a volume of 18 µL. Samples were incubated at 65°C for 5 min. Afterwards 5x RT buffer, RNaseOUT Ribonuclease Inhibitor (Invitrogen, 40U per 30 µL reaction), reverse transcriptase (200 U) and RNase free water up to 30 µL were mixed and incubated at 46°C for 2 hours. The enzyme was inactivated for 5 min at 85 °C.

For cDNA purification, 0.5 reaction volumes NaOH (1 M) were added and incubated for 10 min at 70°C. Afterwards the same amount of HCl was added to neutralize the solution. cDNA precipitation was performed by adding linear acrylamide (30 µg/mL final concentration, Invitrogen), NaOAc (18 µM) and 2.5 vol of ice cold EtOH. Product was precipitated for 30 min at -20°C and pelleted by centrifugation for 15 min, 13000 rpm, 4 °C. Pellets were washed three times with ice cold 70% EtOH. Samples were dried *in vacuo* and resuspended in 20 µL pure water.

PCR: Amplification of target manganese peroxidase gene was carried out using S7 Phusion polymerase (Biozym): 98 °C 30 s, 35 cycles of 98 °C/60 s, 61.5 °C/60 s and 72 °C/60 s followed by a final extension time at 72 °C for 5 min. The size of the expected PCR product was confirmed via agarose gel electrophoresis (1% agarose, 25 min, 120 V), purified using a Nucleospin Gel and PCR clean up kit (Macherey Nagel) and identity of target gene was confirmed by sequencing (Eurofins).

Table S22. Primer sequence used for RT-PCR detection of MnP

Name primer	Sequence	Direction
MnP_FW1	GAGTGTGGCGAAGAGGGTATC	Forward
MnP_FW2	AAATGCCCTGACGGTGTTC	Forward
MnP_RV1	GTGACTCGGCTTCTCCTTG	Reverse
MnP_RV2	GACTCGGCTTCTCCTTGATTG	Reverse

Table S23. Composition for 25 µL PCR reaction

Component	Amount
cDNA mixture / Template	2 µL (500 ng/µL)
dNTP mix	0.5 µL (10 mM)
HF buffer (5x)	5 µL
1:1 Primer mix (FW+RV)	10 µL (2.5 µM)
S7 Phusion polymerase	0.25 µL
H ₂ O	7.25 µL

Manganese peroxidase cDNA sequence from *Termitomyces* sp. T153 (based on prediction from *Termitomyces* sp. P5 RNA):

ATGGCTTCAAATTCTGCTTGCTTCCTGTTTGAGTGTGTTCATACCTCTCAAGCC
 TCTAACATCAAGCGGGTAAATGCCCTGACGGTGTTCACAGTGCCTCGAACGCTGCGTGT
 GCGCATTGTACCCCGTTATGGAAGACCTCCAGAAAAACCTATTGGCGGGGGAGAGTGTG
 GCGAAGAGGTATCGGTGCACGAGTCTCTCAGATTGACTTCCATGATGCCATCGGTTTCC
 CCGACAAAAGGTGGTGGCGCGCGGATGGTTCAATCATTATTTCAATGATAACGGAGCTGG
 CATTCCATGCTAATATTGGCTTGGATAAGATCATCAACAAACAGAACGCCCTCATTGCTAG
 ACACACCTAACCGCTGGCGATT CGTCCAATCGCCGGCACTCTGGTGTGAGTAATTGTC
 CGGGAGCACCAAGCTCAAGTTCTCCTGGACGTCCAATGCCACGGCTCCCTCCCCGA
 TCTCCTGGTCCCTGAACCTTCGAATGGGAGATGCCGGCTCACTCCAGATGAAGTTGTTG
 CTCTTCTTGCATCGTAAGCAAGCGTCTGTCGTCAACATTCACTCTGTCGGCATCCGACCA
 TATCGATCCA ACTAAGTTCAGCTTGGGGCACCCCTTCCCAGGGTCAGTTAATGTGTCGA
 GCTGATTCCGAATTACTGACAATGCATACAACAAACAGTACCGGCGGAAATCAAGGAG
 AAGCCGAGTCACCCTTGA

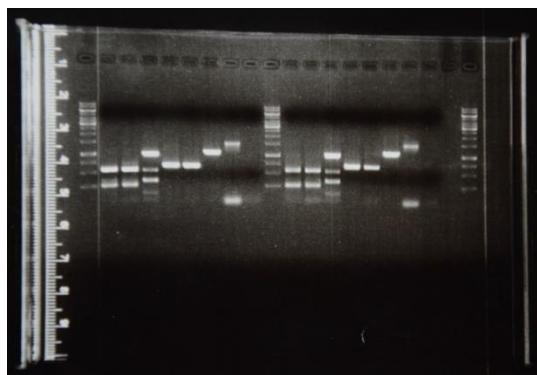


Figure S18. RT-PCR of *Termitomyces* sp. T153, from left to right: Ladder; cDNA MnP primer pair 1; cDNA MnP primer pair 1; gDNA MnP primer pair 1 (control); cDNA MnP primer pair 2; cDNA MnP primer pair 2; gDNA MnP primer pair 2 (control); terpene synthase (positive control); water (negative control); ladder.

10. Appendix

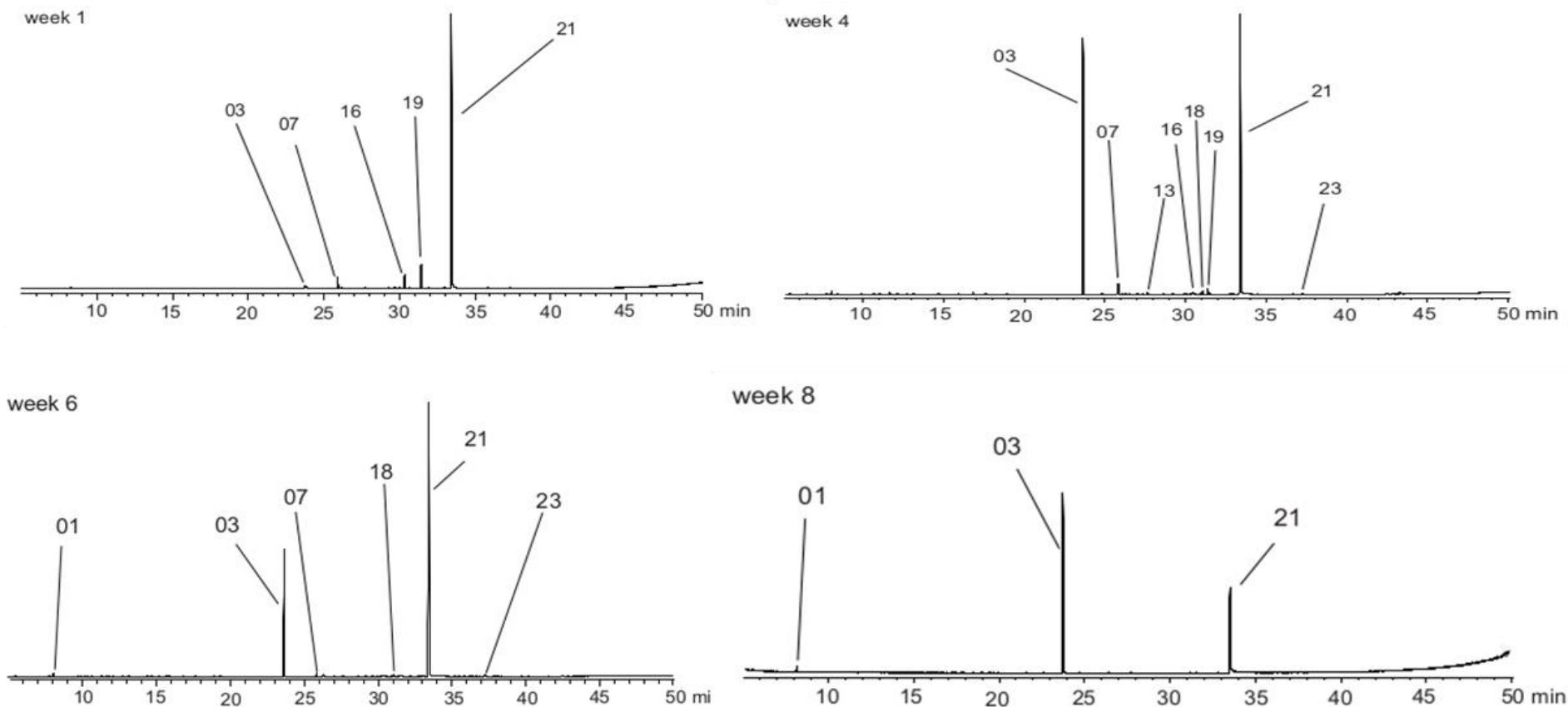


Figure S19. GC-MS chromatogram of volatiles from *Termitomyces* sp. T153 grown on PDA agar over a period of 8 weeks (Peak label **03**: 1,2,4-trimethoxybenzene (**5**))

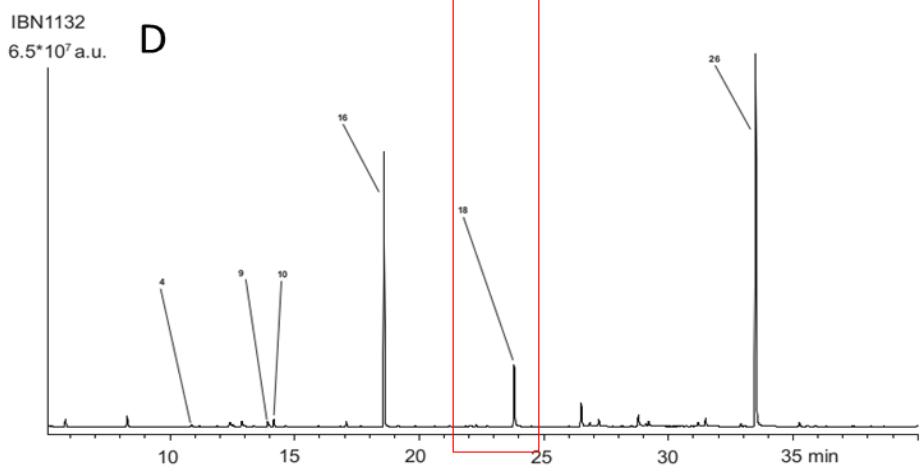
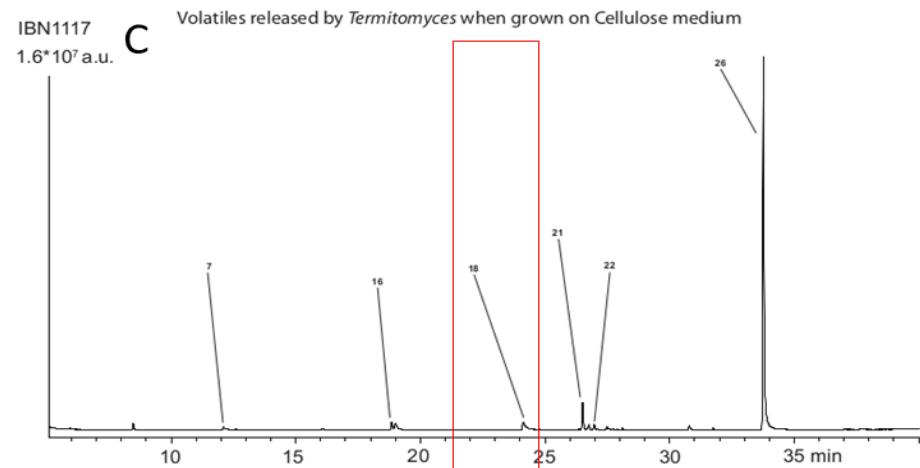
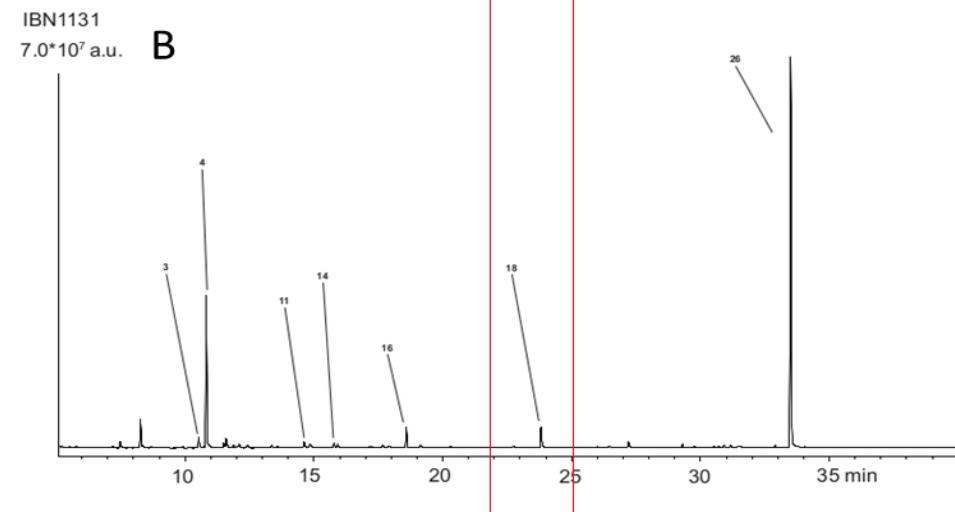
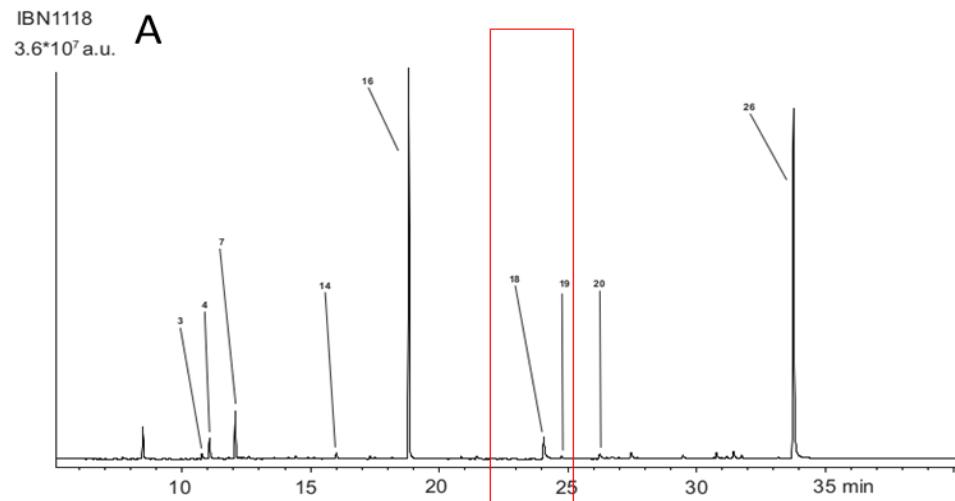
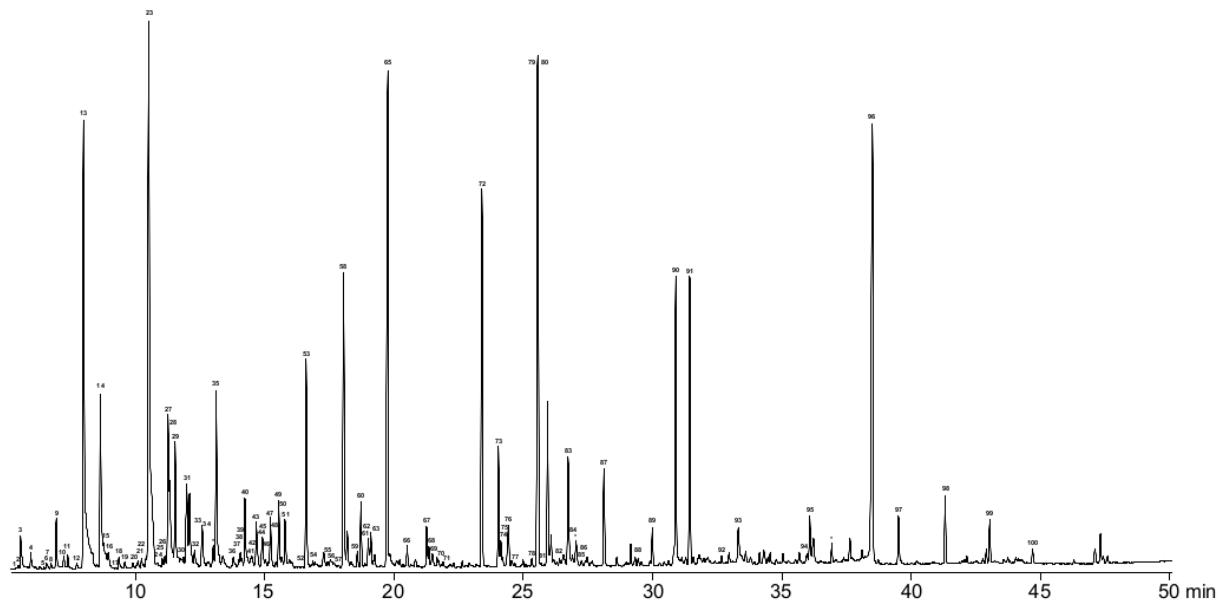


Figure S20. GC-MS chromatogram of volatiles from *Termitomyces* sp. T153 grown on A-B) fungus comb agar and C-D) cellulose agar measured two weeks after inoculation (Peak label **18**: 1,2,4-trimethoxybenzene (**5**))

Table S24. GC-MS chromatogram and compound list of volatiles from *Termitomyces* sp. P5 on PDA agar after 3 weeks (highlighted in red are compounds discussed within this study).



Identity		C	H	N	O	S	RT[min]	I	I(lit)	PRN1242
1	Butyl acetate	6	12		2		5.40	822		x
2	4-Hydroxypentan-2-one	5	10		2		5.47	824		x
3	Methylpyrazine	5	6	2			5.55	826	819	x
4	Furfural	5	4		2		5.95	838	828	x
5	N-Formylpyrrole	5	5	1	1		6.46	852		x
6	3-Hydroxy-4-methylpentan-2-on	6	12		2		6.56	855	859	x
7	Ethyl 2-methylbutyrate	7	14		2		6.63	857	850	x
8	2-Furanmethanol	5	6		2		6.74	860	852	x
9	Ethylbenzene	8	10				6.95	866	860	x
10	1,3-Dimethylbenzene	8	10				7.23	874	868	x
11	4-Heptanone	7	14		1		7.39	879	877	x
12	Cyclohexanol	6	12		1		7.73	889	882	x
13	Styrene	8	8				8.00	896		x
14	2,5-Dimethylpyrazine	6	8	2			8.63	913	912	x
15	But-2-en-4-olide	4	4		2		8.77	916	916	x
16	2,3-Dimethylpyrazine	6	8	2			8.95	921	914	x
17	Isopropylbenzene	9	12				9.25	929	924	x
18	2,5-Hexanedione	6	10		2		9.36	931	919	x
19	α -Pinene	10	16				9.58	937	932	x
20	Allylbenzene (?)	9	10				10.02	948	947	x

21	Pentan-4-oxide	5	8		2		10.23	954	951	x
22	Propylbenzene	9	12				10.32	956	969	x
23	Benzaldehyde	7	6		1		10.50	961	959	x
24	Dimethyl trisulfide	2	6			3	10.83	969	968	x
25	Methyl 4-methyl-2-oxopentanoate	7	12			3	11.04	974		x
26	2-Methylbut-2-en-4-oxide	5	6		2		11.13	977	977	x
27	Oct-1-en-3-ol	8	16		1		11.25	980	974	x
28	Phenol	6	6		1		11.32	982	980	x
29	3-Octanone	8	16		1		11.54	987	979	x
30	2-Ethyl-5-methylpyrazine	7	10	2			11.88	996	998	x
31	Trimethylpyrazine	7	10	2			11.99	999	1002	x
32	Pentyl propanoate	8	16		2		12.27	1006	1005	x
33	2-Acetylthiazole	5	5	1	1	1	12.54	1014	1014	x
34	2-Methylenebornane	11	18				12.60	1016	1015	x
*	2-Ethylhexan-1-ol	8	18		1		13.00	1028		x
35	Benzyl alcohol	7	8		1		13.11	1031	1026	x
36	4-Hexanolide	6	10		2		13.78	1051	1042	x
37	(E)-Oct-2-enal	8	14		1		13.98	1056	1049	x
38	1-Phenylethanol	8	10		1		14.05	1058	1057	x
39	2-Cyanopyridine	6	4	2			14.13	1061	1069	x
40	Acetophenone	8	8		1		14.22	1063	1059	x
41	Diethyl malonate	7	12		4		14.45	1070	1067	x
42	3-Methylphenol	7	8		1		14.55	1073	1072	x
43	2-Ethyl-3,6-dimethylpyrazine	8	12	2			14.68	1077	1077	x
44	2-Phenyl-2-propanol	9	12		1		14.88	1082	1080	x
45	Tetramethylpyrazine	8	12	2			14.94	1084	1087	x
46	2-Methoxyphenol	7	8		2		15.03	1087	1087	x
47	Methyl benzoate	8	8		2		15.23	1093	1088	x
48	Undecane	11	24				15.42	1098	1100	x
49	Nonanal	9	18		1		15.54	1102	1100	x
50	2,3-Dimethylbut-2-en-4-oxide	6	8		2		15.65	1105		x
51	2-Phenylethanol	8	10		1		15.79	1110	1106	x
52	1-Phenyl-2-propanol	9	12		1		16.45	1131	1135	x
53	Benzyl nitrile	8	7	1			16.57	1134	1134	x
54	1,2-Dimethoxybenzene	8	10		2		16.87	1144	1141	x
55	1,4-Dimethoxybenzene	8	10		2		17.42	1161	1161	x

56	1,3-Dimethoxybenzene	8	10	2		17.53	1165	1165	x
57	Methyl phenylacetate	9	10	2		17.87	1176	1175	x
58	2-Methylisoborneol	11	20	1		18.02	1180	1178	x
59	Dodecane	12	26			18.58	1198	1200	x
60	Decanal	10	20	1		18.72	1203	1201	x
61	2-Aminobenzaldehyde	7	7	1	1	18.99	1212	1222	x
62	Dimethyl tetrasulfide	2	6		4	19.04	1214	1218	x
63	2-Phenoxyethanol	8	10	2		19.11	1216	1221	x
64	Benzothiazole	7	5	1	1	19.27	1222	1227	x
65	2-Methoxy-1,4-benzoquinone	7	6	3		19.69	1236		x
66	3,5-Dimethoxytoluene	9	12	2		20.52	1265	1260	x
67	Indole	8	7	1		21.26	1290	1290	x
68	S-Methyl thiobenzoate	8	8	1	1	21.35	1293	1295	x
69	Tridecane	13	28			21.48	1298	1300	x
70	Undecanal	11	22	1		21.66	1304	1305	x
71	2-Isopentyl-3,6-dimethylpyrazin	11	18	2		21.90	1313	1321	x
72	1,2,4-Trimethoxybenzene	9	12	3		23.40	1368	1368	x
73	2-Methoxy-1,4-dihydroxybenzene	7	8	3		24.05	1392		x
74	Vanillin	8	8	3		24.13	1395	1393	x
75	Tetradecane	14	30			24.20	1398	1400	x
76	Geosmin	12	22	1		24.44	1406	1403	x
77	α -Barbatene	15	24			24.67	1416	1414	x
78	Isobazzanene	15	24			25.33	1442	1436	x
79	β -Barbatene	15	24			25.52	1449	1445	x
80	Geranylacetone	13	22	1		25.57	1451	1453	x
81	(E)- β -Farnesene	15	24			25.69	1456	1454	x
82	β -Chamigrene	15	24			26.40	1484	1476	x
83	Sulfur (S ₆)			6		26.74	1497		x
84	α -Murolene	15	24			26.91	1504	1500	x
*	2,4-Di-tert-butylphenol	14	22	1		27.03	1509		x
85	Hypodoratoxid	15	26	1		27.24	1518	1512	x
86	7- <i>epi</i> - α -Selinene	15	24			27.38	1523	1520	x
87	unidentified					28.13	1555		x
88	Tetradecanal	14	28	1		29.44	1609	1611	x
89	1- <i>epi</i> -Cubenol	15	26	1		30.00	1634	1627	x

90	1-Tetradecanol	14	30		1		30.89	1673	1671	x
91	Heptadecane	17	36				31.43	1697	1700	x
92	Diphenylcyclobutane Isomer 1 (putative)	16	16				32.68	1754		x
93	Sulfur (S ₇)				7		33.32	1784		x
94	Diphenylcyclobutane Isomer 2 (putative)	16	16				35.97	1912		x
95	Farnesylacetone	18	30		1		36.07	1917	1913	x
*	Dibutyl phthalate	16	22		4		36.92	1960		x
96	Sulfur (S ₈)				8		38.49	2042		x
97	Heneicosane	21	44				39.51	2096	2100	x
98	Docosane	22	46				41.30	2195	2200	x
99	Tricosane	23	48				43.04	2296	2300	x
100	Tetracosane	24	50				44.69	2395	2400	x

Table S25. Determination of pH and electrical conductivity of soil samples from termite colonies.

pH(H_2O)					electrical conductivity [$\mu S/cm$]				pH-classification (Blume <i>et al.</i> , 2011)			
sample		1. value	2. value	mean	SD	1. value	2. value	mean	SD			
2015	OD-145	1	6.13	6.16	6.15	0.02	195.5	197.3	196.4	1.3		
		2	6.16	6.14	6.15	0.01		236.0	236.3	236.2	0.2	
		3	6.16	6.16	6.16	0.00		197.9	197.4	197.7	0.4	
	OD-146	4	N/A				N/A			7.2 – 7.9		
		5	7.30	7.21	7.26	0.06	109.4	109.2	109.3	0.1	6.8 – 7.2	
		6	7.43	7.38	7.41	0.04	64.5	65.0	64.8	0.4	6.1 – 6.8	
	Mn-160 (1)	7	6.17	6.06	6.12	0.08	174.5	176.5	175.5	1.4	5.4 – 6.1	
		8	6.06	5.96	6.01	0.07	48.5	48.3	48.4	0.1		
		9	6.03	5.99	6.01	0.03	46.0	46.2	46.1	0.1		
		10	6.02	5.95	5.99	0.05	48.1	48.2	48.2	0.1		
	Mn-161	11	6.46	6.49	6.48	0.02	75.3	75.3	75.3	0.0		
		12	6.36	6.31	6.34	0.04	76.3	75.8	76.1	0.4		
	OD-152	out	13	5.89	5.90	5.90	0.01	1144.0	1177.0	1160.5	23.3	0-50
		r.c.	14	7.05	6.99	7.02	0.04	89.9	90.4	90.2	0.4	50-100
		in	15	7.07	7.03	7.05	0.03	177.4	177.9	177.7	0.4	100-150
	OD-167	out	16	6.02	6.14	6.08	0.08	32.0	32.4	32.2	0.3	150-200
		r.c.	17	6.86	6.81	6.84	0.04	108.1	107.9	108.0	0.1	> 200
		in	18	6.57	6.56	6.57	0.01	71.5	71.2	71.4	0.2	
	Mn-154	out	19	6.49	6.37	6.43	0.08	64.1	63.7	63.9	0.3	
		r.c.	20	6.30	6.41	6.36	0.08	56.4	56.3	56.4	0.1	
		in	21	6.40	6.39	6.40	0.01	124.0	123.4	123.7	0.4	
	Mn-160 (2)	out	22	6.14	6.16	6.15	0.01	128.6	128.0	128.3	0.4	
		r.c.	23	6.70	6.75	6.73	0.04	63.8	62.8	63.3	0.7	
		in	24	6.07	6.06	6.07	0.01	66.6	65.9	66.3	0.5	
	Mn-164	out	25	7.10	7.12	7.11	0.01	136.5	135.8	136.2	0.5	
		r.c.	26	6.56	6.55	6.56	0.01	59.3	59.0	59.2	0.2	
		in	27	6.32	6.30	6.31	0.01	53.3	53.1	53.2	0.1	
	Mn-173	out	28	6.60	6.59	6.60	0.01	33.1	33.0	33.1	0.1	
		r.c.	29	6.78	6.81	6.80	0.02	39.2	38.7	39.0	0.4	
		in	30	6.78	6.74	6.76	0.03	44.3	44.0	44.2	0.2	

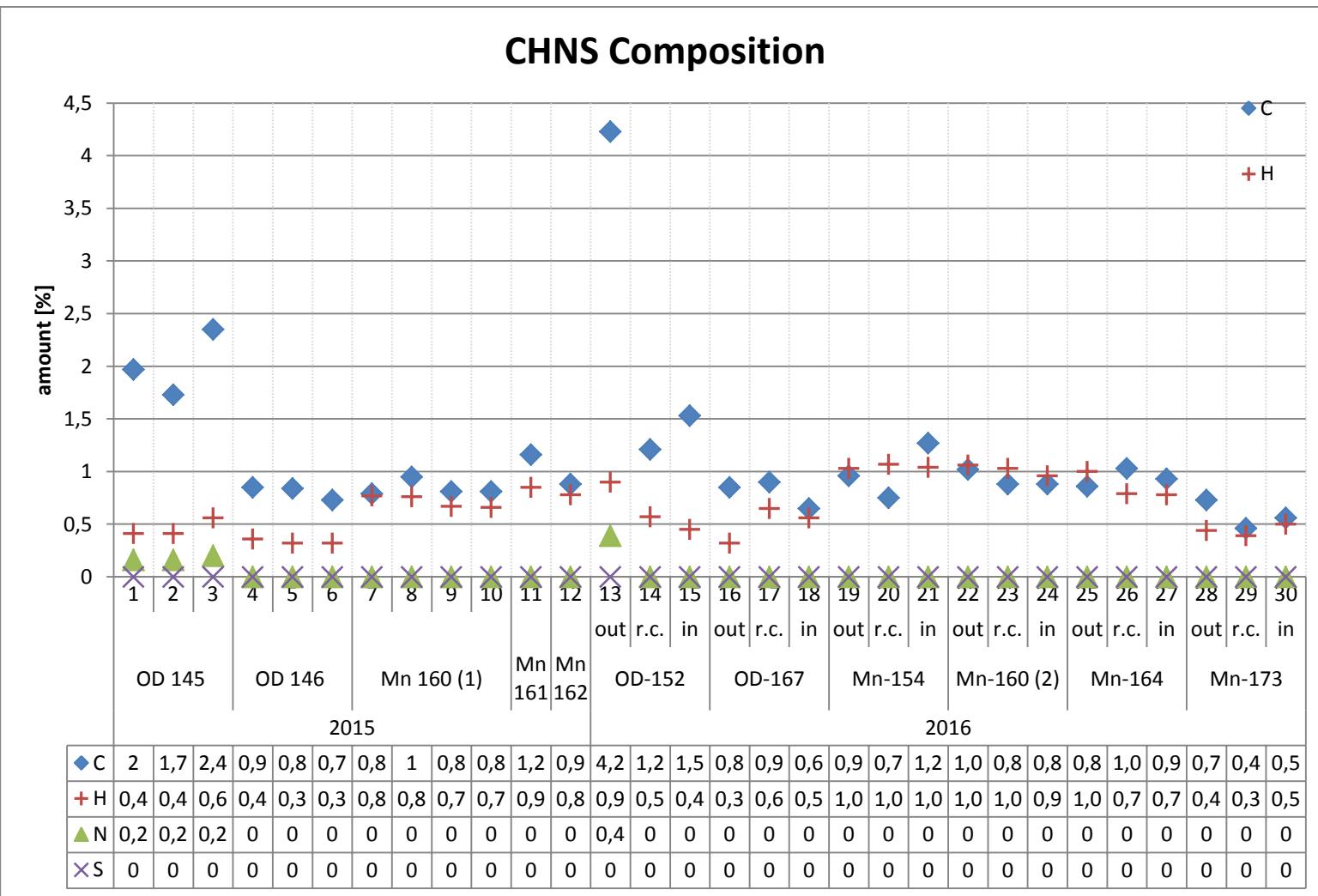


Figure S21. Determination of relative CHNS content of soil samples obtained from *Macrotermes* and *Odontotermes* colonies.

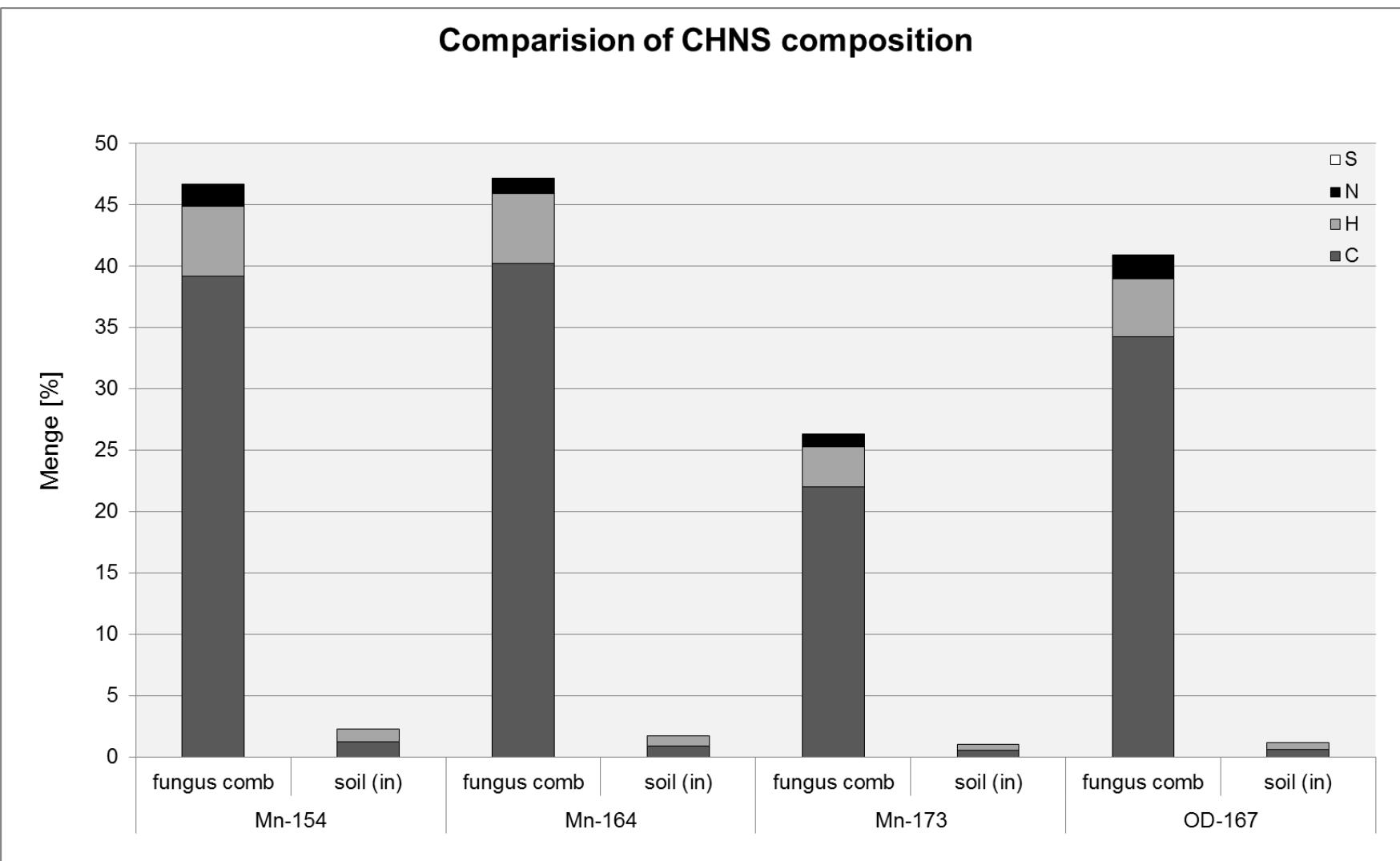


Figure S22. Comparison of relative CHNS content of soil and fungus comb samples obtained from *Macrotermes* and *Odontotermes* colonies.

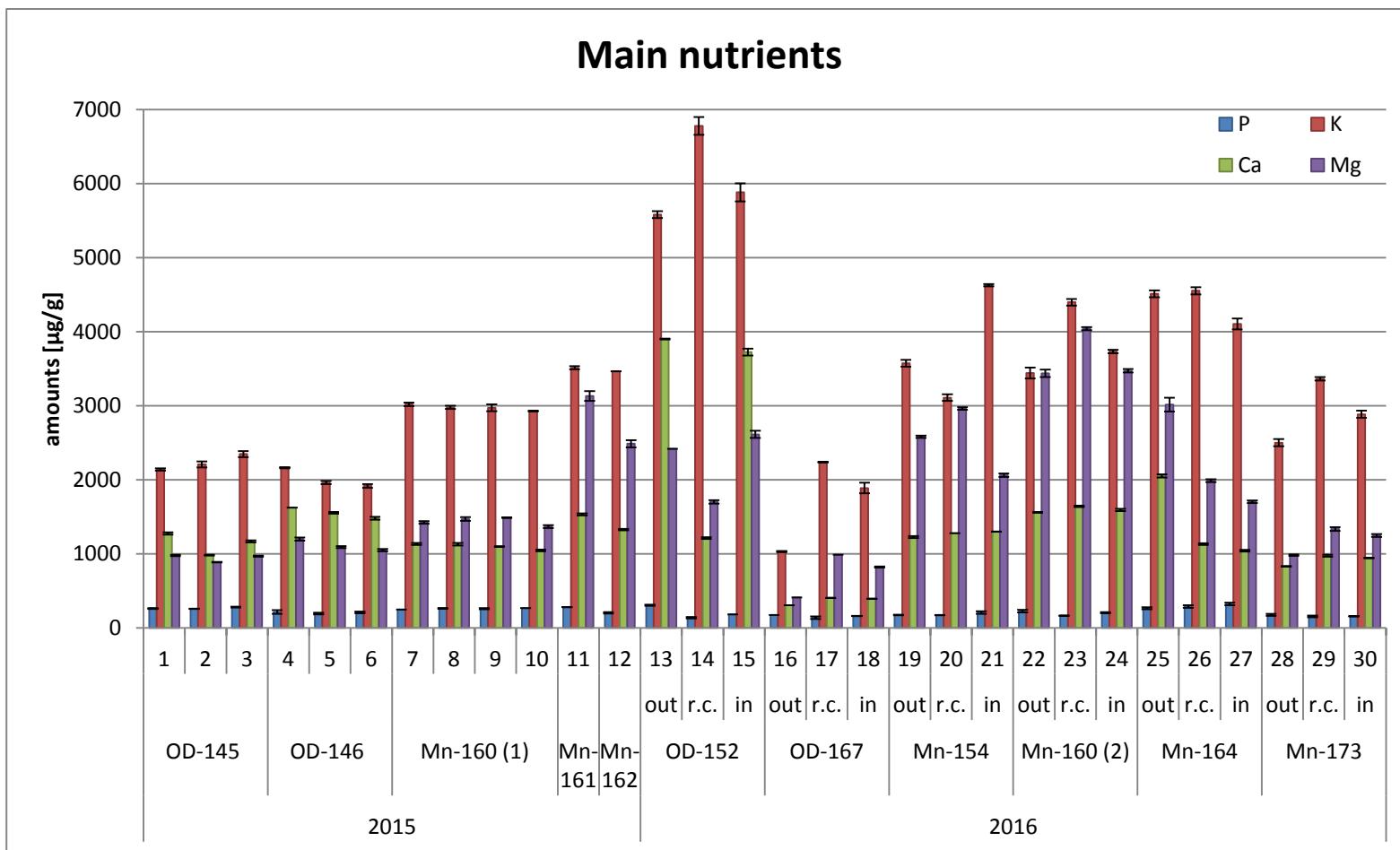


Figure S23. Determination of P, K, Ca and Mg content [µg/g] of soil samples obtained from *Macrotermes* and *Odontotermes* colonies.

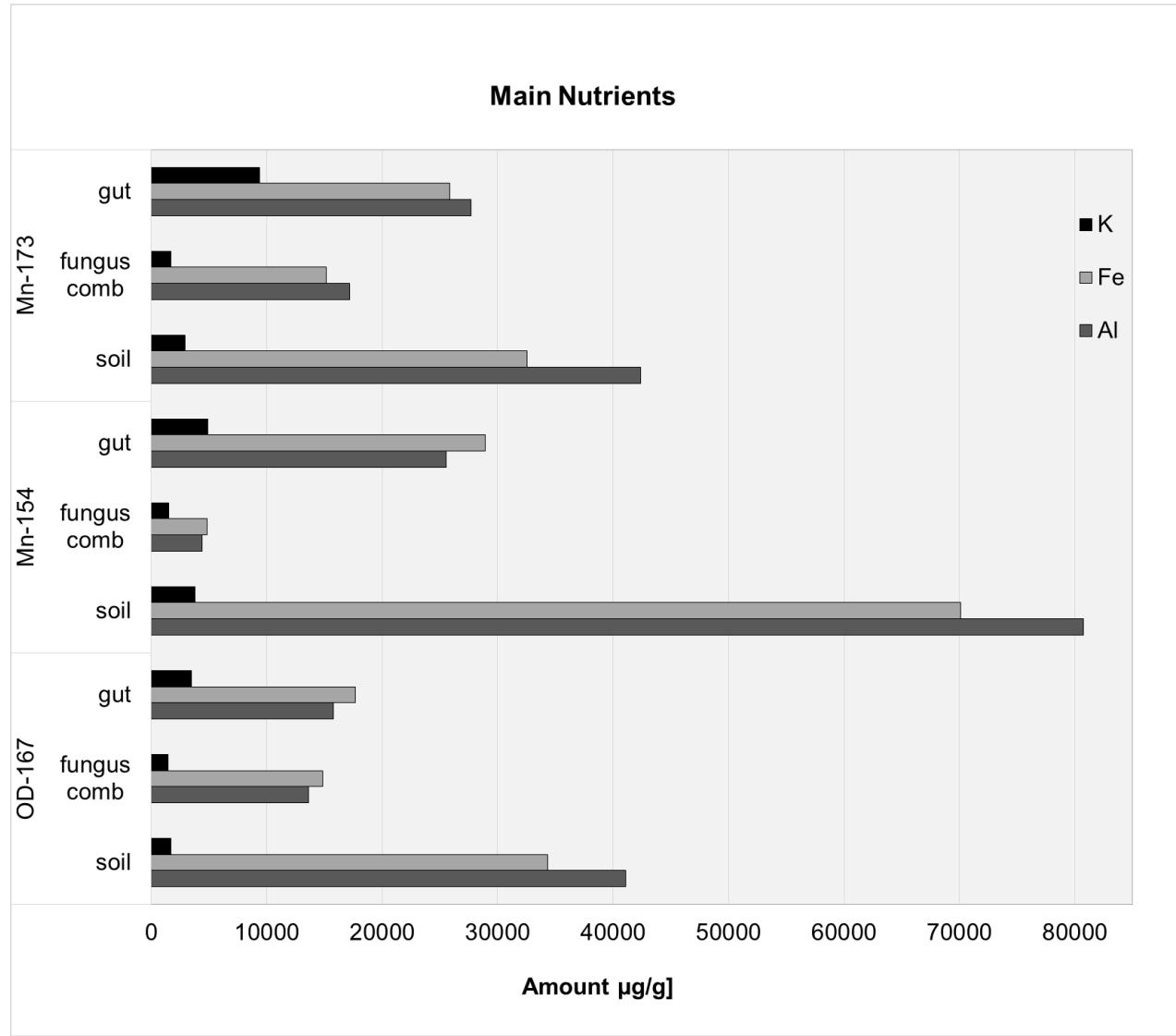


Figure S24. Determination of P, K, Ca and Mg content [$\mu\text{g/g}$] of gut and comb samples obtained from *Macrotermes* and *Odontotermes* colonies.

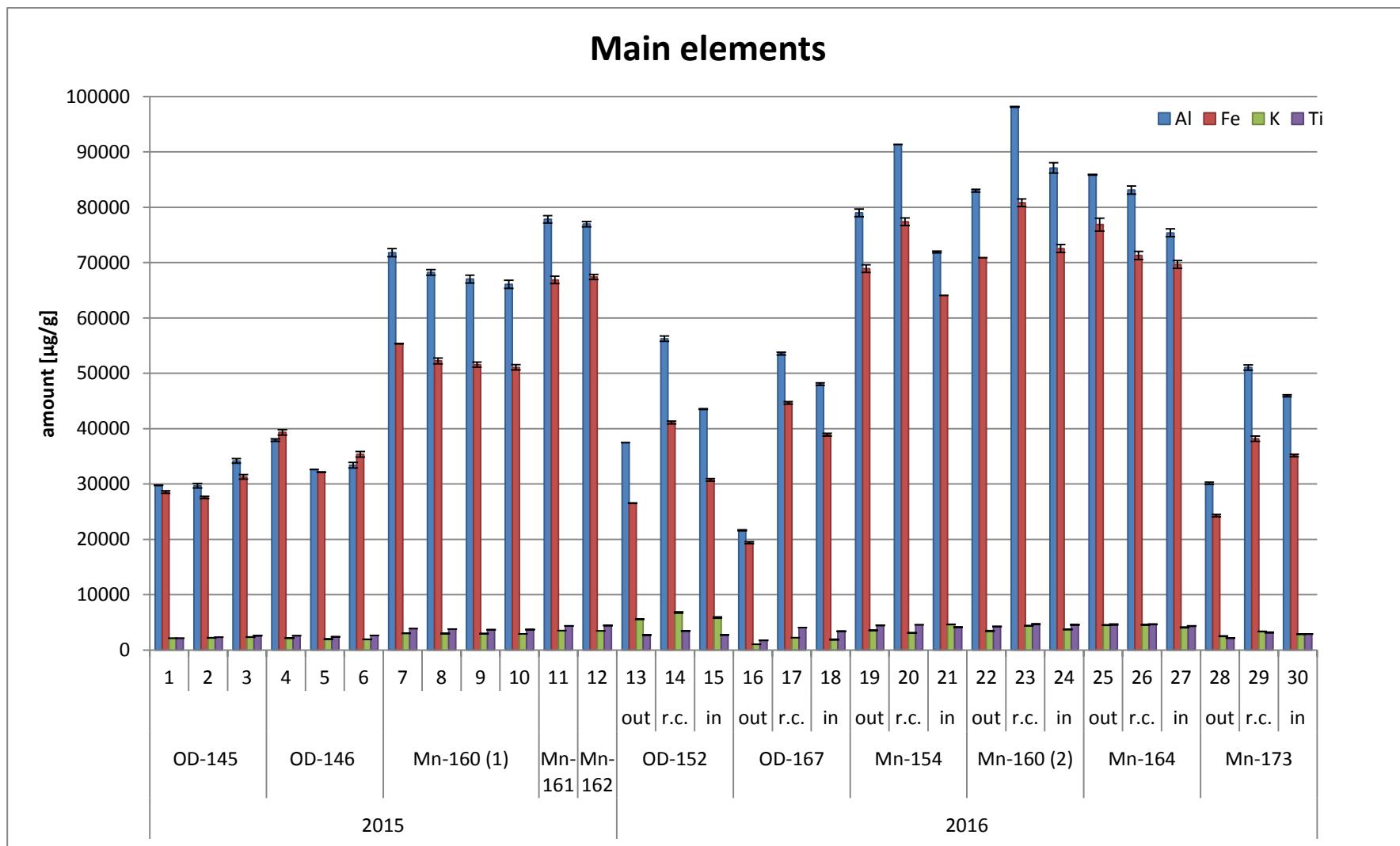


Figure S25. Determination of Al, Fe, K and Ti content [$\mu\text{g/g}$] of soil samples obtained from *Macrotermes* and *Odontotermes* colonies.

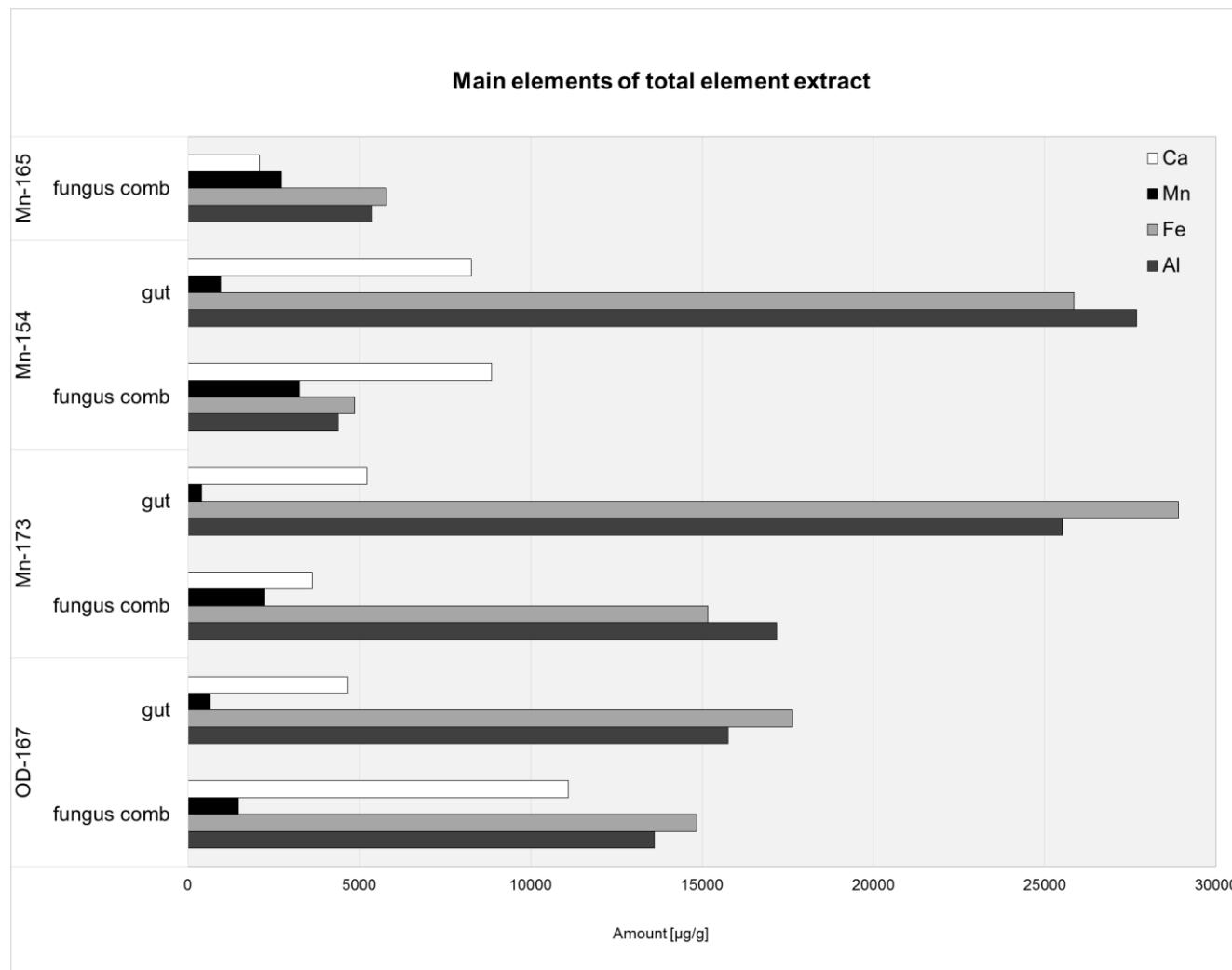


Figure S26. Determination of main element content [µg/g] of gut and fungus comb samples obtained from *Macrotermes* and *Odontotermes* colonies.

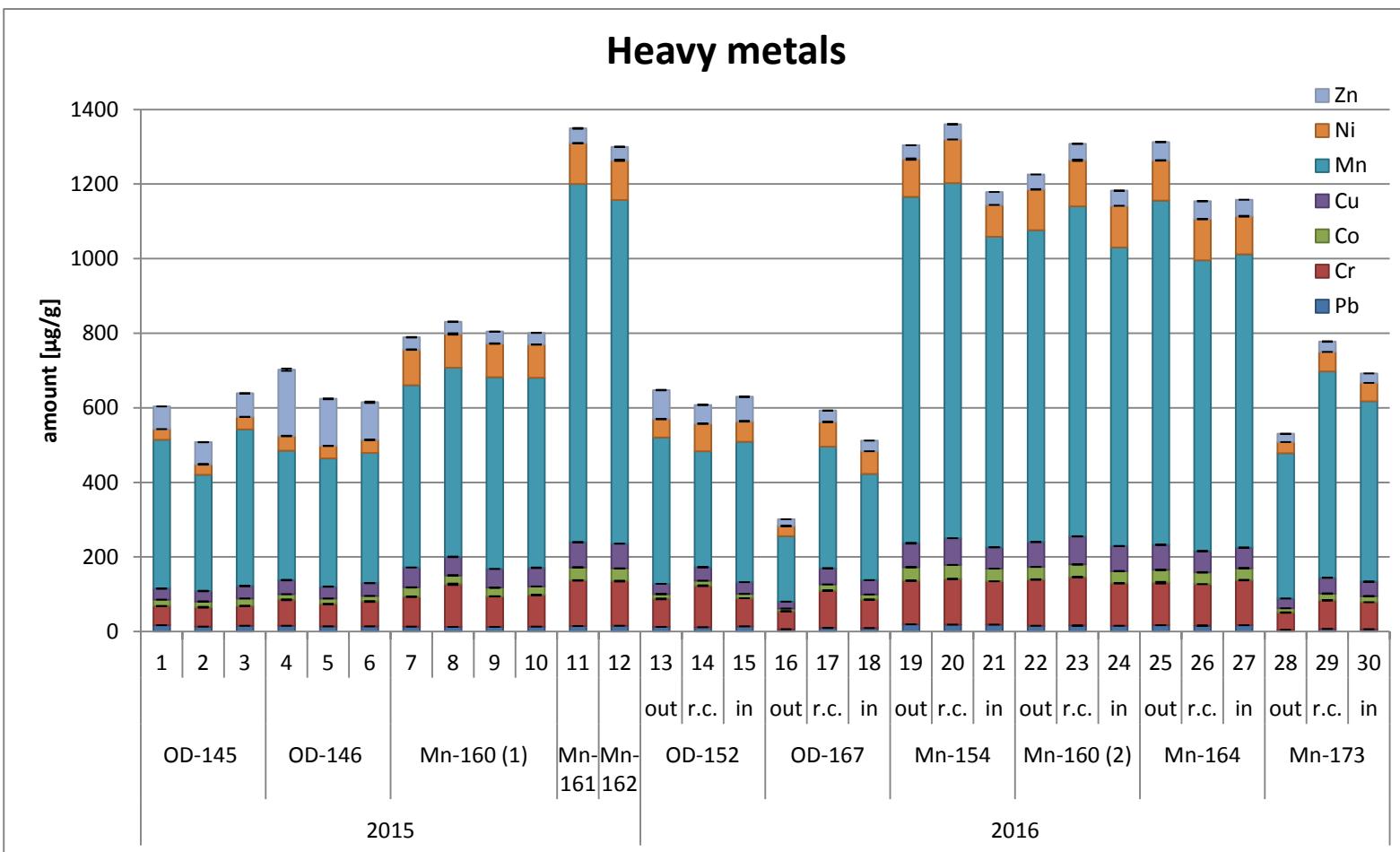


Figure S27. Determination of heavy metal content ($\mu\text{g/g}$) Zn, Ni, Mn, Cu, Co, Cr, Pb) of soil samples obtained from *Macrotermes* and *Odontotermes* colonies.

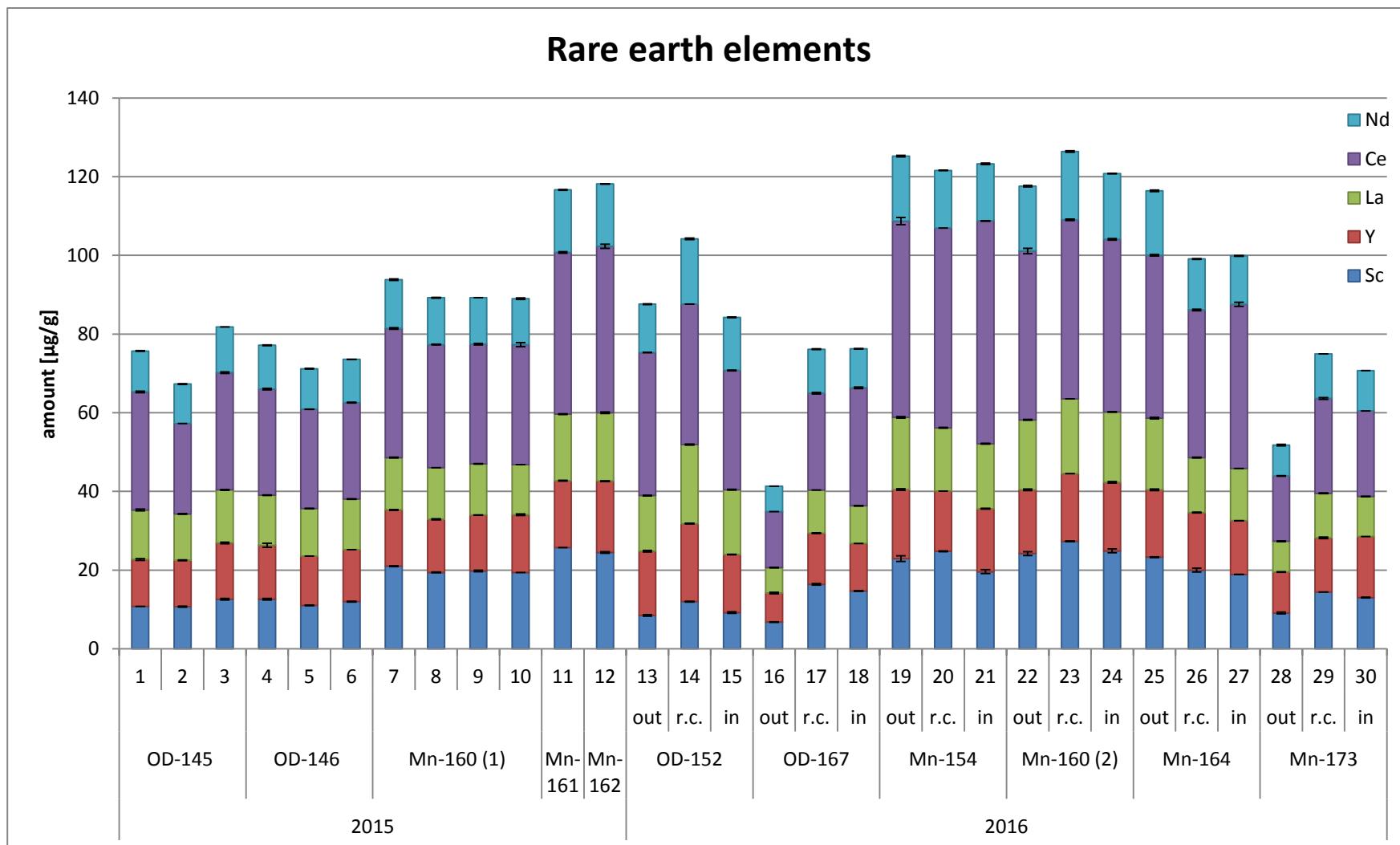


Figure S28. Determination of rare elements [$\mu\text{g/g}$] of soil samples obtained from *Macrotermes* and *Odontotermes* colonies.

Table S26. Determination of element amount [µg/g] per soil sample.

soil elements [µg/g]																	
No.	Al (OES)	As SD (MS)	Ba SD (MS)	Ca SD (OES)	Cd Ca (MS)	Co SD (MS)	Cr SD (MS)	Cu SD (MS)	Fe SD (OES)	K SD (OES)							
1	29748	17	5,00	0,10	119,0	1,0	1274,0	15,0	0,170	0,010	17,50	0,10	51,4	0,6	29,60	0,40	28547
2	29703	413	5,86	0,02	110,0	2,0	982,0	6,0	0,210	0,040	15,60	0,10	52,0	1,0	28,30	0,60	27558
3	34156	412	5,60	0,20	132,0	1,0	1167,0	12,0	0,189	0,004	19,90	0,10	53,3	0,8	33,70	0,40	31276
4	37915	237	7,70	0,10	134,8	0,9	1622,5	0,5	0,450	0,070	14,90	0,20	70,0	1,0	37,70	0,70	39336
5	32609	4	6,70	0,20	133,0	1,0	1553,0	13,0	0,305	0,007	15,16	0,01	60,2	0,4	31,40	0,40	32152
6	33399	491	8,00	1,00	130,0	1,0	1478,0	20,0	0,340	0,020	14,78	0,02	67,0	1,0	34,40	0,20	35363
7	71811	730	8,00	0,50	105,4	0,7	1132,0	15,0	0,122	0,005	25,10	0,50	80,1	0,7	53,08	0,02	55343
8	68205	507	7,48	0,05	100,0	1,0	1128,0	18,0	0,140	0,020	23,60	0,30	114,0	2,0	50,00	1,00	52231
9	67015	713	7,40	0,10	102,4	0,2	1097,0	2,0	0,140	0,010	23,30	0,20	81,8	0,2	50,48	0,02	51568
10	66070	726	7,00	0,50	97,0	1,0	1046,0	12,0	0,150	0,050	23,62	0,07	84,7	0,7	49,60	0,70	51065
11	77818	671	6,65	0,01	139,0	2,0	1532,0	13,0	0,224	0,004	35,10	0,40	122,1	0,7	67,50	0,70	66860
12	76960	478	7,40	0,50	134,0	2,0	1326,0	10,0	0,170	0,020	33,90	0,20	120,0	1,0	66,35	0,00	67400
13	37463	2	2,77	0,00	254,0	2,0	3899,0	5,0	0,71	0,02	12,90	0,20	75,1	0,9	27,27	0,00	26515
14	56250	481	4,18	0,07	305,0	2,0	1212,0	12,0	0,79	0,02	13,60	0,10	111,0	1,0	36,30	0,50	41106
15	43538	73	3,90	0,10	270,0	3,0	3723,0	48,0	0,58	0,05	11,18	0,01	75,6	0,0	32,09	0,00	30736
16	21645	83	2,30	0,20	42,8	0,1	304,6	0,1	0,08	0,01	7,48	0,00	47,7	0,4	18,64	0,08	19387
17	53555	234	5,16	0,01	75,8	0,5	403,4	0,1	0,12	0,02	16,32	0,07	100,0	1,0	43,50	0,50	44668
18	48042	212	3,90	0,10	73,1	0,5	392,0	2,0	0,15	0,02	14,10	0,20	76,2	0,7	38,40	0,20	38915
19	78984	687	7,60	0,20	160,0	3,0	1226,0	14,0	0,13	0,02	36,20	0,70	117,0	1,0	64,30	0,90	68910
20	91329	11	7,70	0,50	133,6	0,2	1278,0	1,0	0,165	0,001	37,60	0,10	122,1	0,9	71,50	0,10	77359
21	71902	169	6,61	0,05	160,0	2,0	1298,0	2,0	0,16	0,02	34,44	0,02	116,3	0,0	56,90	0,20	64063
22	83001	249	6,90	0,20	146,0	1,0	1559,0	7,0	0,12	0,02	33,82	0,05	124,0	1,0	67,00	1,00	70880
23	98147	67	7,10	0,40	155,0	2,0	1641,0	9,0	0,13	0,04	34,20	0,40	130,0	1,0	75,00	0,40	80804
24	87089	939	6,80	0,20	149,5	0,5	1592,0	16,0	0,16	0,02	32,60	0,50	114,0	1,0	67,10	0,20	72535
25	85864	70	8,00	0,20	170,0	1,0	2050,0	21,0	0,12	0,02	34,21	0,09	114,0	2,0	67,10	0,70	76869
26	83092	723	8,40	0,50	141,0	1,0	1130,0	10,0	0,17	0,02	31,80	0,70	111,1	0,1	57,00	1,00	71291
27	75382	716	7,20	0,20	135,0	1,0	1041,0	10,0	0,19	0,02	32,00	0,50	121,0	1,0	55,00	1,00	69656
28	30118	212	1,44	0,09	92,7	0,9	831,0	2,0	0,1017	0,0009	11,60	0,20	45,8	0,5	26,20	0,20	24292
29	51050	477	1,90	0,50	127,6	0,7	972,0	14,0	0,14	0,02	18,10	0,50	76,9	0,5	42,60	0,20	38168
30	45930	170	2,17	0,02	119,0	1,0	942,0	2,0	0,189	0,005	16,20	0,50	72,3	0,1	39,00	0,70	35126

soil elements [µg/g]

No.	Li (MS)	SD	Mg (OES)	SD	Mn (MS)	SD	Mo (MS)	SD	Na (OES)	SD	Ni (MS)	SD	P (OES)	SD	Pb (MS)	SD	Sr (OES)	SD	Ti (OES)	SD
1	6,440	0,060	977	8	399,0	3,0	0,586	0,001	243,0	2,0	28,5	0,2	260,0	4,0	16,880	0,020	10,02	0,00	2139,0	1,0
2	6,500	0,200	886	2	311,7	0,0	0,580	0,020	248,0	2,0	28,0	1,0	256,2	0,4	12,900	0,080	9,30	0,10	2321,0	8,0
3	7,550	0,006	966	8	420,0	4,0	0,710	0,040	257,0	6,0	33,5	0,4	278,0	6,0	15,217	0,008	10,70	0,20	2593,0	41,0
4	9,700	0,200	1198	21	347,3	0,2	0,810	0,050	308,0	2,0	39,3	0,5	213,0	24,0	15,230	0,050	9,81	0,05	2610,0	0,1
5	8,230	0,020	1089	13	344,0	3,0	0,720	0,040	297,9	0,2	33,8	0,4	192,0	11,0	13,600	0,020	8,93	0,04	2384,0	22,0
6	7,900	0,050	1050	15	349,2	0,7	0,740	0,050	287,0	5,0	34,8	0,5	209,0	12,0	13,700	0,100	9,00	0,10	2624,5	0,2
7	27,600	0,500	1423	19	489,0	5,0	1,300	0,100	329,0	5,0	95,9	0,7	247,2	0,2	13,160	0,020	9,63	0,02	3890,0	5,0
8	26,500	0,500	1469	25	507,0	7,0	1,200	0,100	343,0	1,0	91,0	2,0	261,0	3,0	12,710	0,020	9,18	0,08	3773,0	3,0
9	26,000	0,200	1487	5	514,0	33,0	1,290	0,050	342,0	5,0	90,0	0,7	257,0	7,0	12,547	0,002	9,00	0,10	3660,0	24,0
10	26,300	0,500	1366	19	510,0	3,0	1,240	0,050	323,0	1,0	89,0	0,5	265,0	1,0	12,910	0,010	8,68	0,00	3703,0	48,0
11	25,300	0,200	3130	67	961,0	1,0	0,697	0,002	1096,0	11,0	109,1	0,9	278,0	2,0	14,800	0,100	11,02	0,02	4338,0	22,0
12	25,000	0,500	2485	48	922,0	1,0	0,890	0,050	958,0	5,0	106,0	2,0	203,0	10,0	15,300	0,100	12,20	0,20	4422,0	48,0
13	10,800	0,200	2417	1	393,0	4,0	0,510	0,020	755,5	0,7	49,0	0,5	305,0	7,0	12,440	0,020	17,59	0,01	2709,0	23,0
14	13,200	0,100	1700	22	311,0	2,0	0,670	0,070	787,3	0,5	73,9	0,5	137,0	10,0	11,670	0,010	14,40	0,10	3452,0	17,0
15	10,600	0,200	2612	48	376,0	3,0	0,610	0,050	714,0	7,0	55,0	1,0	179,8	0,7	13,880	0,070	14,70	0,20	2735,0	48,0
16	8,100	0,200	411	0	175,0	2,0	0,410	0,040	120,0	4,0	27,7	0,8	174,5	0,0	6,460	0,080	4,87	0,06	1765,0	17,0
17	18,450	0,020	986	1	326,0	1,0	0,810	0,020	181,5	0,2	66,6	0,7	136,0	19,0	9,700	0,050	6,42	0,01	4048,4	0,2
18	16,400	0,200	819	7	284,9	0,9	0,710	0,070	163,9	0,1	60,7	0,1	158,7	0,9	9,000	0,070	5,90	0,05	3382,0	21,0
19	23,950	0,000	2579	16	929,0	19,0	0,900	0,100	1124,0	14,0	101,0	2,0	172,0	5,0	19,340	0,070	12,60	0,20	4464,0	23,0
20	27,700	0,700	2961	18	953,0	5,0	1,000	0,200	985,0	5,0	116,4	0,1	171,0	2,0	18,800	0,100	10,91	0,09	4569,0	14,0
21	21,600	0,100	2060	22	833,0	9,0	0,990	0,050	863,0	1,0	85,3	0,1	206,0	17,0	18,170	0,010	15,08	0,07	4129,0	0,7
22	24,900	0,500	3438	50	836,4	0,7	0,870	0,020	1186,0	7,0	109,1	0,7	224,0	17,0	15,450	0,070	11,83	0,02	4262,0	50,0
23	28,800	0,400	4039	22	885,0	2,0	0,890	0,020	1369,0	2,0	124,0	2,0	164,0	2,0	15,840	0,070	12,23	0,07	4688,0	67,0
24	26,220	0,050	3473	23	801,0	4,0	0,870	0,090	1256,0	14,0	111,7	0,1	204,0	9,0	15,500	0,200	12,20	0,10	4577,0	47,0
25	30,600	0,900	3013	93	923,0	13,0	1,000	0,100	478,7	0,2	108,3	0,9	264,0	12,0	17,000	0,200	15,50	0,10	4626,0	93,0
26	27,440	0,020	1988	19	780,0	11,0	0,900	0,100	269,7	0,7	110,3	0,1	287,0	17,0	15,800	0,100	11,00	0,10	4672,0	48,0
27	25,000	0,000	1702	19	787,0	12,0	1,090	0,020	246,0	5,0	102,0	1,0	322,0	19,0	16,790	0,020	10,30	0,10	4318,0	48,0
28	10,100	0,200	982	9	389,3	0,0	0,330	0,020	1255,0	7,0	30,2	0,1	175,0	12,0	4,970	0,020	12,40	0,10	2156,0	21,0
29	15,600	0,100	1334	24	552,9	0,7	0,550	0,020	1505,0	19,0	52,9	0,0	153,0	12,0	6,700	0,070	15,50	0,20	3173,0	72,0
30	14,800	0,200	1248	19	484,1	0,2	0,460	0,020	1267,0	2,0	49,0	0,0	155,0	1,0	6,160	0,010	14,50	0,10	2907,0	24,0

soil elements [µg/g]

No.	V (MS)	Zn (MS)	Sc (MS)	Y (MS)	Cs (MS)	La (MS)	Ce (MS)	Pr (MS)	Nd (MS)	Sm (MS)										
	V SD	Zn SD	Sc SD	Y SD	Cs SD	La SD	Ce SD	Pr SD	Nd SD	Sm SD										
1	66,02	0,06	60,9	0,2	10,783	0,004	11,900	0,200	1,510	0,040	12,60	0,20	30,00	0,20	2,790	0,020	10,410	0,080	2,040	0,060
2	66,36	0,06	59,4	0,2	10,700	0,100	11,800	0,100	1,530	0,040	11,78	0,06	23,00	0,00	2,708	0,006	10,000	0,100	2,028	0,002
3	72,60	0,10	63,2	0,6	12,600	0,200	14,300	0,200	1,710	0,001	13,50	0,10	29,80	0,20	3,090	0,020	11,600	0,020	2,450	0,060
4	93,50	0,70	178,0	3,0	12,600	0,200	13,700	0,500	2,060	0,020	12,73	0,07	27,00	0,20	2,940	0,020	11,110	0,090	2,370	0,050
5	81,30	0,20	126,0	1,0	11,030	0,070	12,540	0,000	1,870	0,040	12,08	0,07	25,25	0,04	2,720	0,040	10,290	0,090	2,160	0,020
6	89,80	0,20	101,0	2,0	12,000	0,100	13,195	0,007	1,840	0,020	12,87	0,05	24,50	0,10	2,940	0,020	10,980	0,020	2,330	0,050
7	140,00	1,00	33,0	1,0	21,000	0,100	14,300	0,100	2,560	0,050	13,30	0,10	32,80	0,20	3,260	0,020	12,400	0,200	2,580	0,070
8	134,00	3,00	32,2	0,8	19,400	0,100	13,500	0,100	2,430	0,050	13,13	0,02	31,30	0,10	3,170	0,010	11,900	0,100	2,540	0,080
9	132,00	1,00	32,1	0,5	19,800	0,200	14,180	0,020	2,430	0,010	13,05	0,07	30,40	0,20	3,180	0,002	11,832	0,007	2,540	0,050
10	132,00	2,00	31,2	0,5	19,385	0,005	14,700	0,200	2,370	0,050	12,72	0,02	30,50	0,50	3,100	0,070	11,700	0,200	2,540	0,050
11	148,00	0,40	39,8	0,9	25,720	0,020	16,990	0,090	1,990	0,020	16,93	0,09	41,10	0,20	4,173	0,004	15,900	0,100	3,300	0,100
12	152,95	0,02	36,3	0,5	24,500	0,200	18,100	0,100	2,170	0,070	17,40	0,20	42,30	0,50	4,250	0,050	15,840	0,010	3,300	0,050
13	62,20	0,20	77,9	0,9	8,500	0,200	16,300	0,200	2,130	0,050	14,14	0,01	36,35	0,02	3,190	0,020	12,300	0,100	2,553	0,009
14	97,80	0,70	50,3	0,5	12,000	0,100	19,800	0,100	3,090	0,020	20,10	0,10	35,72	0,02	4,400	0,070	16,600	0,200	3,200	0,100
15	72,60	0,70	65,9	0,5	9,200	0,200	14,740	0,070	2,350	0,010	16,50	0,10	30,30	0,07	3,630	0,050	13,500	0,100	2,650	0,020
16	55,30	0,40	18,1	0,2	6,790	0,080	7,400	0,200	0,910	0,020	6,46	0,06	14,20	0,00	1,640	0,020	6,460	0,010	1,470	0,080
17	121,80	0,50	30,5	0,2	16,400	0,200	12,980	0,050	1,940	0,050	10,96	0,01	24,60	0,20	2,840	0,020	11,200	0,100	2,488	0,007
18	109,00	2,00	28,5	0,1	14,670	0,070	12,087	0,005	1,712	0,001	9,58	0,02	30,00	0,20	2,504	0,002	9,930	0,050	2,190	0,050
19	153,00	1,00	37,4	0,0	22,900	0,700	17,600	0,200	2,333	0,000	18,30	0,20	49,90	0,90	4,290	0,010	16,500	0,200	3,420	0,005
20	167,60	0,20	40,9	0,7	24,800	0,100	15,260	0,010	2,360	0,020	16,10	0,10	50,79	0,02	3,830	0,070	14,639	0,009	3,020	0,070
21	142,00	2,00	34,4	0,0	19,600	0,500	16,000	0,100	2,280	0,010	16,50	0,10	56,64	0,02	3,792	0,000	14,500	0,200	2,930	0,050
22	148,80	0,50	39,7	0,0	24,200	0,500	16,200	0,200	2,100	0,010	17,80	0,10	42,90	0,70	4,310	0,020	16,500	0,200	3,400	0,100
23	171,00	3,00	43,8	0,7	27,300	0,100	17,214	0,004	2,470	0,010	19,01	0,01	45,50	0,20	4,440	0,020	17,400	0,200	3,400	0,100
24	152,80	0,90	40,5	0,2	24,900	0,500	17,400	0,200	2,250	0,050	17,89	0,05	43,90	0,20	4,270	0,020	16,690	0,090	3,330	0,050
25	180,00	3,00	49,0	1,0	23,300	0,100	17,100	0,200	2,917	0,002	18,20	0,20	41,40	0,20	4,278	0,002	16,400	0,200	3,200	0,100
26	153,00	1,00	48,0	1,0	20,000	0,500	14,600	0,100	3,027	0,007	14,00	0,10	37,50	0,20	3,320	0,050	13,000	0,100	2,680	0,010
27	150,00	3,00	44,2	0,2	18,890	0,020	13,660	0,020	2,720	0,020	13,30	0,01	41,70	0,50	3,100	0,050	12,310	0,070	2,502	0,005
28	60,10	0,70	22,3	0,5	9,100	0,200	10,420	0,070	0,875	0,005	7,80	0,10	16,63	0,05	1,910	0,050	7,800	0,200	1,750	0,020
29	94,70	0,70	27,3	0,7	14,420	0,020	13,800	0,200	1,430	0,050	11,30	0,10	24,10	0,20	2,790	0,020	11,350	0,000	2,430	0,050
30	87,90	0,20	25,3	0,0	13,000	0,100	15,511	0,007	1,257	0,002	10,20	0,07	21,72	0,01	2,540	0,020	10,269	0,005	2,330	0,070

soil elements [µg/g]

No.	Eu (MS)	Tb (MS)	Gd (MS)	Dy (MS)	Ho (MS)	Er (MS)	Tm (MS)	Yb (MS)	Lu (MS)
	SD								
1	0,4898	0,0001	0,3281	0,0008	2,020	0,040	2,120	0,040	0,440
2	0,5000	0,0200	0,3260	0,0020	2,080	0,040	2,210	0,020	0,450
3	0,6000	0,0200	0,4110	0,0010	2,390	0,060	2,630	0,060	0,550
4	0,5760	0,0020	0,3800	0,0200	2,350	0,050	2,460	0,090	0,514
5	0,5190	0,0090	0,3400	0,0200	2,130	0,020	2,315	0,007	0,470
6	0,5679	0,0002	0,3700	0,0100	2,323	0,007	2,460	0,070	0,499
7	0,6290	0,0020	0,4090	0,0050	2,510	0,050	2,690	0,020	0,550
8	0,6030	0,0080	0,4000	0,0200	2,400	0,100	2,540	0,050	0,527
9	0,6100	0,0200	0,3990	0,0050	2,400	0,070	2,640	0,050	0,544
10	0,6100	0,0100	0,4140	0,0050	2,548	0,007	2,769	0,002	0,569
11	0,7500	0,0100	0,4900	0,0100	3,090	0,040	3,260	0,090	0,673
12	0,7800	0,0100	0,5280	0,0070	3,150	0,020	3,370	0,050	0,720
13	0,5350	0,0090	0,4290	0,0070	2,550	0,090	3,010	0,090	0,626
14	0,6680	0,0020	0,5140	0,0020	3,105	0,002	3,560	0,050	0,733
15	0,5500	0,0100	0,4020	0,0070	2,490	0,050	2,690	0,050	0,560
16	0,3210	0,0080	0,2200	0,0100	1,350	0,040	1,517	0,001	0,298
17	0,5920	0,0090	0,3950	0,0002	2,390	0,050	2,640	0,050	0,543
18	0,5300	0,0200	0,3630	0,0070	2,080	0,090	2,480	0,050	0,500
19	0,8080	0,0020	0,5000	0,0100	3,160	0,070	3,470	0,010	0,710
20	0,6800	0,0200	0,4500	0,0200	2,799	0,009	2,900	0,100	0,597
21	0,6500	0,0200	0,4600	0,0100	2,765	0,002	3,050	0,070	0,620
22	0,8000	0,0200	0,5000	0,0200	3,160	0,020	3,258	0,002	0,670
23	0,8300	0,0100	0,5000	0,0100	3,230	0,010	3,390	0,040	0,680
24	0,8100	0,0200	0,5280	0,0090	3,170	0,090	3,430	0,020	0,676
25	0,7900	0,0200	0,4900	0,0100	3,000	0,100	3,270	0,090	0,660
26	0,6300	0,0200	0,4260	0,0050	2,560	0,020	2,870	0,050	0,580
27	0,6210	0,0010	0,4000	0,0100	2,410	0,050	2,593	0,002	0,542
28	0,4270	0,0010	0,2920	0,0050	1,750	0,070	1,930	0,050	0,389
29	0,5840	0,0050	0,3800	0,0200	2,390	0,050	2,600	0,100	0,518
30	0,5500	0,0200	0,3900	0,0070	2,347	0,002	2,830	0,050	0,591

11. References

- ¹ Poulsen, M.; Hu, H.; Li, C.; Chen, Z.; Xu, L.; Otani, S.; Nygaard, S.; Nobre, T.; Klaubauf, S.; Schindler, P. M.; Hauser, F.; Pan, H.; Yang, Z.; Sonnenberg, A. S. M.; de Beer, Z. W.; Zhang, Y.; Wingfield, M. J.; Grimmelikhuijzen, C. J. P.; de Vries, R. P.; Korb, J.; Aanen, D. K.; Wang, J.; Boomsma, J. J.; Zhang, G., Complementary symbiont contributions to plant decomposition in a fungus-farming termite. *Proc. Natl. Acad. Sci.* **2014**, *111*, 14500. doi:10.1073/pnas.1319718111
- ² Smit, A.; Hubley, R.; Green, P. RepeatMasker Open-4.0 Available online: <http://www.repeatmasker.org>.
- ³ Dobin, A.; Davis, C.A.; Schlesinger, F.; Drenkow, J.; Zaleski, C.; Jha, S.; Batut, P.; Chaisson, M.; Gingeras, T.R. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **2013**, *29*, 15–21. doi:10.1093/bioinformatics/bts635.
- ⁴ Hoff, K.J.; Lomsadze, A.; Borodovsky, M.; Stanke, M. Whole-genome annotation with BRAKER. In *Methods in Molecular Biology*; **2019**; pp. 65–95. doi:10.1007/978-1-4939-9173-0_5
- ⁵ Stanke, M.; Morgenstern, B. AUGUSTUS: a web server for gene prediction in eukaryotes that allows user-defined constraints. *Nucleic Acids Res.* **2005**, *33*, 465–467. doi:10.1093/nar/gki458.
- ⁶ Iwata, H.; Gotoh, O. Benchmarking spliced alignment programs including Spaln2, an extended version of Spaln that incorporates additional species-specific features. *Nucleic Acids Res.* **2012**, *40*, e161–e161. doi:10.1093/nar/gks708.
- ⁷ Kent, W.J. BLAT - The BLAST-like alignment tool. *Genome Res.* **2002**, *12*, 656–664. doi:10.1101/Gr.229202.
- ⁸ Grabherr, M.G.; Haas, B.J.; Yassour, M.; Levin, J.Z.; Thompson, D.A.; Amit, I.; Adiconis, X.; Fan, L.; Raychowdhury, R.; Zeng, Q.; et al. Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nat. Biotechnol.* **2011**, *29*, 644–652. doi:10.1038/nbt.1883.
- ⁹ Joshi, N.A.; Fass J.N. (2011). Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33) [Software]. Available at <https://github.com/najoshi/sickle>.
- ¹⁰ Patro, R.; Duggal, G.; Love, M.I.; Irizarry, R.A.; Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods* **2017**, *14*, 417–419. doi:10.1038/nmeth.4197.
- ¹¹ https://www.genome.jp/kegg-bin/show_organism?category=Fungi

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- ¹² Y. Taprab , T. Johjima, Y. Maeda, S. Moriya, S. Trakulnaleamsai, N. Noparatnaraporn, M. Ohkuma, T. Kudo, Symbiotic fungi produce laccases potentially involved in phenol degradation in fungus combs of fungus-growing termites in Thailand, *Appl. Environ. Microbiol.* **2005**, *71*, 7696-704
- ¹³ Li H, Wu S, Ma X, Chen W, Zhang J, Duan S, Gao Y, Kui L, Huang W, Wu P, Shi R, Li Y, Wang Y, Li J, Guo X, Luo X, Li Q, Xiong C, Liu H, Gui M, Sheng J, Dong Y. The Genome Sequences of 90 Mushrooms. *Sci Rep.* **2018**, *8*, 9982.
doi: 10.1038/s41598-018-28303-2. Erratum in: *Sci Rep.* **2020** May 18;10(1):8460.
- ¹⁴ da Costa, R.R.; Hu, H.; Pilgaard, B.; Vreeburg, S.M.; Schückel, J.; Pedersen, K.S.; Kračun, S.K.; Busk, P.K.; Harholt, J.; Sapountzis, P.; Lange, L.; Aanen, D.K.; Poulsen, M. Enzyme activities at different stages of plant biomass decomposition in three species of fungus-growing termites. *Appl. Environ. Microbiol.*, **2018**, *84*:e01815-17.
doi:10.1128/AEM.01815-17.
- ¹⁵ Kim, D.; Langmead, B; Salzberg, S.L.. HISAT: a fast spliced aligner with low memory requirements. *Nature Methods*, **2015**, *12*, 357.
doi:10.1038/nmeth.3317
- ¹⁶ Anders, S.; Pyl, P.T; Huber, W. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics*, **2015**, *31*, 166-169.
doi:10.1093/bioinformatics/btu638
- ¹⁷ Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.*, **2014**, *15*, 550.
doi:10.1186/s13059-014-0550-8
- ¹⁸ Huber, W.; Von Heydebreck, A.; Sueltmann, H.; Poustka, A.; Vingron, M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics*, **2002**, *18*, 96-104.
doi:10.1093/bioinformatics/18.suppl_1.s96
- ¹⁹ Raivo Kolde, **2019**. pheatmap: Pretty Heatmaps. R package version 1.0.12. <https://CRAN.R-project.org/package=pheatmap>
- ²⁰ R Core Team, **2018**. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- ²¹ Simon Garnier, **2018**. viridis: Default color maps from 'matplotlib'. R package version 0.5.1. <https://CRAN.R-project.org/package=viridis>

²² Zhang, H.; Yohe, T.; Huang, L.; Entwistle, S.; Wu, P.; Yang, Z.; Busk, P.L.; Xu, Y.; Yin, Y. dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res.*, **2018**, *46*, 95–101.
doi:10.1093/nar/gky418

²³ a) Raivo Kolde, **2019**. pheatmap: Pretty Heatmaps. R package version 1.0.12. <https://CRAN.R-project.org/package=pheatmap>; b) R Core Team, **2018**. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>. c) Vincent Q. Vu, **2011**. ggbiopt: A ggplot2 based biplot. R package version 0.55.

²⁴ Gostinčar, C.; Gunde-Cimerman, N. Overview of oxidative stress response genes in selected halophilic fungi. *Genes*, **2018**, *9*, 143.
doi:10.3390/genes9030143.

²⁵ Kanehisa, M.; Furumichi, M.; Tanabe, M.; Sato, Y.; Morishima, K. KEGG: New perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res.* **2017**, *45*, 353–361.
doi:10.1093/nar/gkw1092.

²⁶ Sayers, E.W.; Beck, J.; Brister, J.R.; Bolton, E.E.; Canese, K.; Comeau, D.C.; Funk, K.; Ketter, A.; Kim, S.; Kimchi, A. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* **2020**, *48*, 9–16.
doi:10.1093/nar/gkz899.

²⁷ Altschul, S. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **1997**, *25*, 3389–3402.
doi:10.1093/nar/25.17.3389.

²⁸ DIN ISO 11260 1996-05 (1996); oral communication: Buhler und Lorenz, Institute for Geosciences at the Friedrich-Schiller-University Jena!.
²⁹ Plesner, M. et al. Synthesis and properties of unsymmetrical azatrioxa[8]circulenes. *Org. Biomol. Chem.*, **2015**, *13*, 5937–5943.
doi:10.1039/C5OB00676G

³⁰ Falade, A. O.; Eyisi, O. A. L.; Mabinya, L. V.; Nwodo, U. U.; Okoh, A. I. Peroxidase production and ligninolytic potentials of fresh water bacteria *Raoultella ornithinolytica* and *Ensifer adhaerens*. *Appl. Biotechnol. Rep.*, **2017**, *16*, 12–17.
doi:10.1016/j.btre.2017.10.001

³¹ Perez-Riverol Y, Csordas A, Bai J, Bernal-Llinares M, Hewapathirana S, Kundu DJ, Inuganti A, Griss J, Mayer G, Eisenacher M, Pérez E, Uszkoreit J, Pfeuffer J, Sachsenberg T, Yilmaz S, Tiwary S, Cox J, Audain E, Walzer M, Jarnuczak AF, Ternent T, Brazma A, Vizcaíno JA (2019). The PRIDE database and related tools and resources in 2019: improving support for quantification data. Nucleic Acids Res 47(D1):D442-D450 (PubMed ID: 30395289).