

Comparative 16S rRNA metabarcoding analysis of gut bacterial diversity across seven termite species from Senegal: ecological roles and community structure

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Supplementary data

Supplementary data Table S1: Primers and overhang adapters used for 16S rRNA V3-V4 amplification

The adapters enable library preparation for Illumina sequencing, while the primers targeting the hypervariable V3-V4 regions of the bacterial 16S rRNA gene allow reliable taxonomic identification of a wide range of bacteria, offering an optimal balance between coverage and phylogenetic resolution.

Primer name	Sequence (5' → 3')	Target region
FwOvAd_341F	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGC CTACGGGNGGCWGCAG	V3 (forward)
RevOvAd_785R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGGGTATCTAATC	V4 (reverse)

Supplementary data Table 2: Metadata for Senegalese termites: from sample collection (site mapping, species–caste information, and glyphosate status of plots treated vs. untreated), through gut microbiome DNA sequencing (*Illumina MiSeq*), to molecular identification of termites (16S/GenBank assignments).

Sequencing code	ID samples	Closest GenBank match [Accession number]/ identity %	Sampling date	Castes	GB accession number	Sites of collection	Coordinates	Molecular identification	Type of treatment of the field
M11597	dt02b	<i>Ancistrotermes cavithorax</i> [AY127715] / 98.9%–99.2%	08.12.2020	worker	OR782858	Dandé, Kédougou	N 12° 21' 38.301" O 12° 20' 5.179"	<i>Ancistrotermes cavithorax</i>	Not treated with gluphosate
M11617	dt03	<i>Macrotermes bellicosus</i> [MT154684] / 99.8%–100%	08.12.2020	worker	OR782862	Dandé, Kédougou	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Macrotermes bellicosus</i>	Not treated with gluphosate
M11601	DT04	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	09.11.2020	soldier	OR782841	Dindéfel o, Kédougou	N 12° 22' 44.354" O 12° 19' 25.743"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11602	DT06	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	09.11.2020	worker	OR782842	Dindéfel o, Kédougou	N 12° 22' 44.354" O 12° 19' 25.743"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11603	DT08			soldier	OR782843				

		<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	09.11.2020			Dindéfel o, Kédougou u	N 12° 22' 44.354" O 12° 19' 25.743"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11604	DT09	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	09.11.2020	worker	OR782848	Dindéfel o, Kédougou u	N 12° 22' 44.354" O 12° 19' 25.743"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11590	DT10	<i>Trinervitermes geminatus</i> / [JF923317] / 96.4%–96.8%	09.11.2020	soldier	OR782881	Dindéfel o, Kédougou u	N 12° 22' 44.354" O 12° 19' 25.743"	<i>Trinervitermes geminatus</i>	Not treated with gluphosate
M11591	DT12	<i>Trinervitermes trinervius</i> / [MZ029087] / 100%	10.11.2020	soldier	OR782886	Ségou, Kédougou u	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Trinervitermes occidentalis</i>	Not treated with gluphosate
M11605	DT14	<i>Macrotermes bellicosus</i> [OQ703624] / 99.5%	10.11.2020	soldier	OR782861	Ségou, Kédougou u	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Macrotermes bellicosus</i>	Not treated with gluphosate
M11575	DT15	<i>Trinervitermes geminatus</i> / [OQ555367] / 94.8%–95.66%	10.11.2020	soldier	OR782880	Ségou, Kédougou u	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Trinervitermes occidentalis</i>	treated with gluphosate
M11606	DT16	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	11.11.2020	worker	OR782844	Kondoji, Kédougou u	N 12° 22' 30.59" E 12° 21' 16.386"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11607	DT18	<i>Macrotermes subhyalinus</i>	11.11.2020	worker	OR782849	Kondoji, Kédougou u	N 12° 22' 30.59" E 12° 21' 16.386"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate

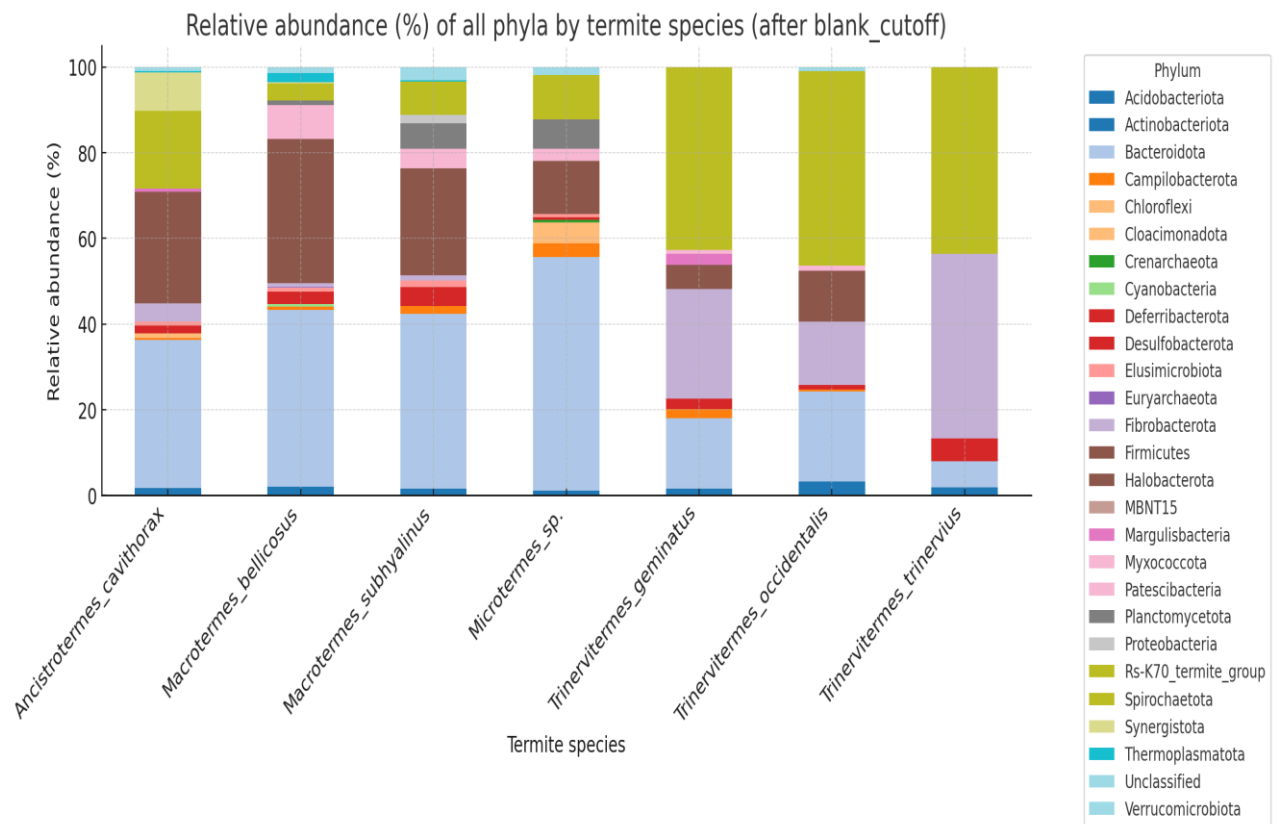
		[MZ029067] / 99.7%–100%							
M11608	DT19	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	11.11.2020	worker	OR782837	Kondouji, Kédougou	N 12° 22' 30.59" E 12° 21' 16.386"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11609	DT20	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	12.11.2020	soldier	OR782838	Nandou mary, Kédougou	N 12° 20' 7.937" O 12° 21' 29.224"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11610	DT21	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	12.11.2020	worker	OR782839	Nandou mary, Kédougou	N 12° 20' 7.937" O 12° 21' 29.224"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11576	DT22	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	12.11.2020	worker	OR782840	Nandou mary, Kédougou	N 12° 20' 7.937" O 12° 21' 29.224"	<i>Macrotermes subhyalinus</i>	treated with gluphosate
M11577	DT23	<i>Microtermes</i> sp. DKA-2002-2 [AY127713] / 99.5%	13.11.2020	worker	OR782893	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Microtermes</i> sp.	treated with gluphosate
M11578	DT24	<i>Trinervitermes occidentalis</i> [JF923351] / 99.2%–99.3%	13.11.2020	soldier	OR782889	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Trinervitermes occidentalis</i>	treated with gluphosate
M11579	DT25	<i>Trinervitermes geminatus</i> [OQ555367] / 94.8%–95.66%	13.11.2020	soldier	OR782879	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Trinervitermes geminatus</i>	treated with gluphosate

M11580	DT26	<i>Microtermes</i> sp. DKA-2002-2 [AY127713] / 99.5%	13.11.2020	worker	OR782894	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Microtermes</i> sp.	treated with gluphosate
M11581	DT27	<i>Ancistrotermes cavithorax</i> [AY127715] / 98.9%–99.2%	13.11.2020	worker	OR782859	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Ancistrotermes cavithorax</i>	treated with gluphosate
M11582	DT28	<i>Microtermes</i> sp. DKA-2002-2 [AY127713] / 99.5%	13.11.2020	worker	OR782895	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Ancistrotermes cavithorax</i>	treated with gluphosate
M11583	DT29	<i>Microtermes</i> sp. 2 BH-2011 [JF923357] / 93.8%	13.11.2020	worker	OR782891	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Microtermes</i> sp.	treated with gluphosate
M11584	DT30	<i>Trinervitermes geminatus</i> [JF923317] / 96.4%–96.8%	13.11.2020	worker	OR782882	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Trinervitermes geminatus</i>	treated with gluphosate
M11585	DT31	<i>Trinervitermes occidentalis</i> [JF923351] / 99.2%–99.3%	13.11.2020	worker	OR782888	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Trinervitermes occidentalis</i>	treated with gluphosate
M11586	DT32	<i>Microtermes</i> sp. 1 BH-2011 [JF923373] / 93.1%	10.11.2020	worker	OR782892	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Microtermes</i> sp.	treated with gluphosate
M11587	DT33	<i>Macrotermes bellicosus</i> [MT154684] / 99.8%–100%	13.11.2020	young soldier	OR782867	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Macrotermes bellicosus</i>	treated with gluphosate
M11588	DT34	<i>Macrotermes bellicosus</i> [MT154684] / 99.8%–100%	13.11.2020	soldier	OR782868	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Macrotermes bellicosus</i>	treated with gluphosate
M11589	DT35	<i>Microtermes</i> sp. DKA-2002-2 [AY127713] / 99.5%	13.11.2020	worker	OR782896	Ségou, Kédougou	N 12° 23' 51.226" O 12° 16' 56.143"	<i>Microtermes</i> sp.	treated with gluphosate

M11611	TC01	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100%	03.12.2020	worker	OR782846	Ziguinch or, Ziguinch or	N 12° 21' 38.301" O 12° 20' 5.179"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11592	TC02	<i>Ancistrotermes cavithorax</i> [JF302843] / 100%	03.12.2020	worker	OR782860	Ziguinch or, Ziguinch or	N 12° 22' 44.354" O 12° 19' 25.743"	<i>Ancistrotermes cavithorax</i>	Not treated with gluphosate
M11612	TC03	<i>Macrotermes bellicosus</i> [MT154684] / 99.8%–100%	03.12.2020	worker	OR782863	Ziguinch or, Ziguinch or	N 12° 34' 1.463" O 16° 16' 28.518"	<i>Macrotermes bellicosus</i>	Not treated with gluphosate
M11613	TC05	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%–100% <i>Trinervitermes</i>	05.12.2020	worker	OR782847	Oussouye, Ziguinch or	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Macrotermes bellicosus</i>	Not treated with gluphosate
M11593	TC07	<i>occidentalis</i> [JF923351] / 99.2%–99.3%	05.12.2020	soldier	OR782887	Oussouye, Ziguinch or	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Trinervitermes occidentalis</i>	Not treated with gluphosate
M11614	TC08	<i>Macrotermes bellicosus</i> [MT154684] / 99.8%–100%	05.12.2020	soldier	OR782864	Oussouye, Ziguinch or	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Macrotermes bellicosus</i>	Not treated with gluphosate

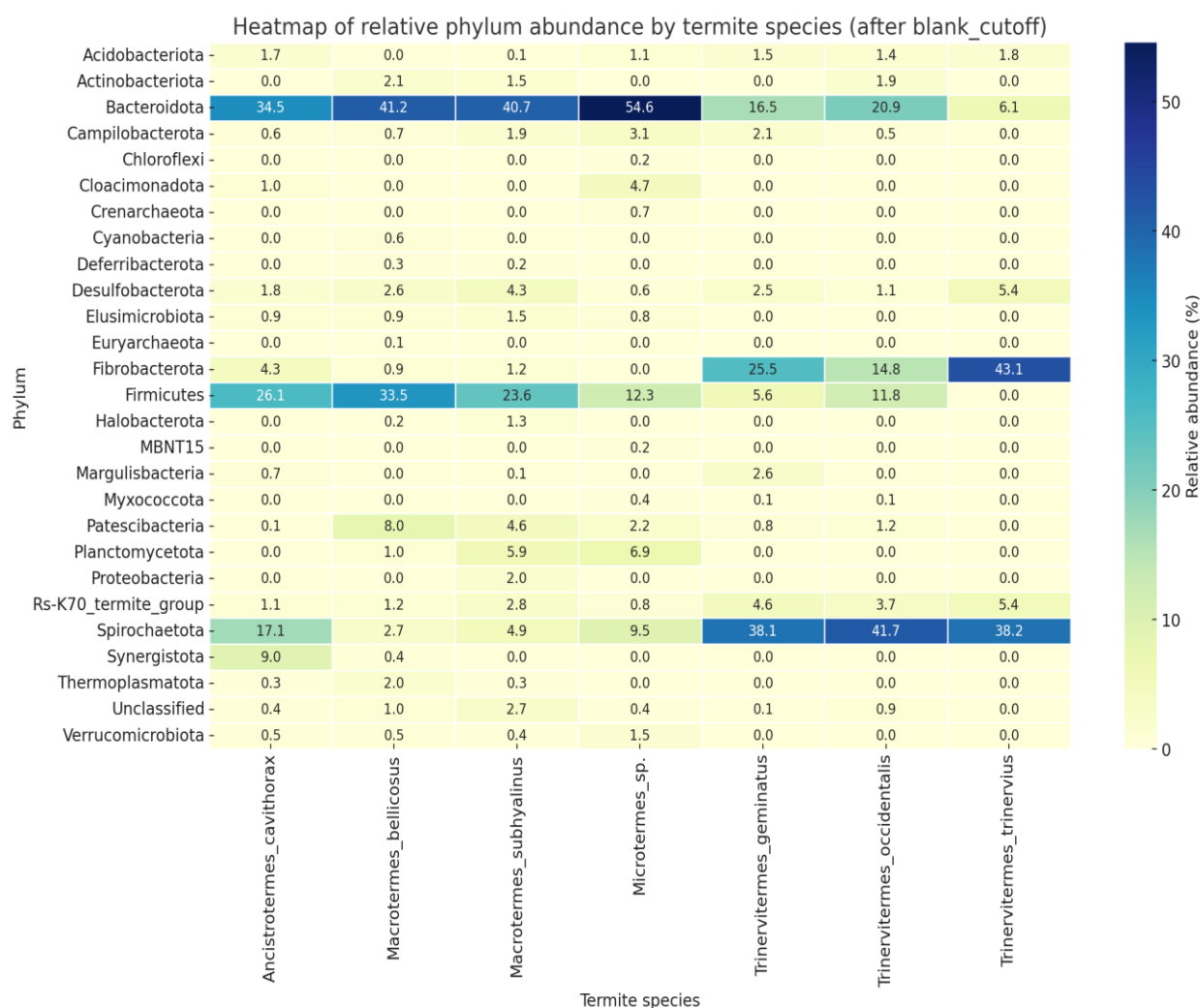
M11594	TC09	<i>Trinervitermes geminatus</i> / [JF923317] / 96.4%–96.8%	05.12.202 0	worker	OR782883	Oussouye, Ziguinchor	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Trinervitermes geminatus</i>	Not treated with gluphosate
M11615	TC10	<i>Macrotermes bellicosus</i> [MT154684] / 99.8%– 100%	05.12.202 0	soldier	OR782865	Oussouye, Ziguinchor	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Macrotermes bellicosus</i>	Not treated with gluphosate
M11595	TC12	<i>Trinervitermes occidentalis</i> / [MZ029083] / 99.5%– 99.6%	05.12.202 0	soldier	OR782884	Oussouye, Ziguinchor	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Trinervitermes occidentalis</i>	Not treated with gluphosate
M11616	TC13	<i>Macrotermes bellicosus</i> [MT154684] / 99.8%– 100%	05.12.202 0	soldier	OR782866	Oussouye, Ziguinchor	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Macrotermes bellicosus</i>	Not treated with gluphosate
M11596	TC14	<i>Trinervitermes occidentalis</i> / [MZ029083] / 99.5%– 99.6%	06.12.202 0	worker	OR782885	Oussouye, Ziguinchor	N 12° 29' 14.117" O 16° 33' 42.807"	<i>Trinervitermes occidentalis</i>	Not treated with gluphosate
M11598	TS01	<i>Macrotermes subhyalinus</i> [MZ029067] / 99.7%– 100%	01.11.202 0	soldier	OR782835	« Km 50 », Thiès	N 14° 45' 50.839" O 17° 6' 10.806"	<i>Macrotermes subhyalinus</i>	Not treated with gluphosate
M11599	TS02			soldier	OR782836				

<i>Macrotermes</i> <i>subhyalinus</i> [MZ029067] / 99.7%– 100%	01.11.202 0	« Km 50 », Thiès	N 14° 45' 50.839" O 17° 6' 10.806"	<i>Macrotermes</i> <i>subhyalinus</i>	Not treated with gluphosate
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Supplementary data Figure S1: Relative abundance of microbial phyla across seven Senegalese termite species (after *blank_cutoff*)

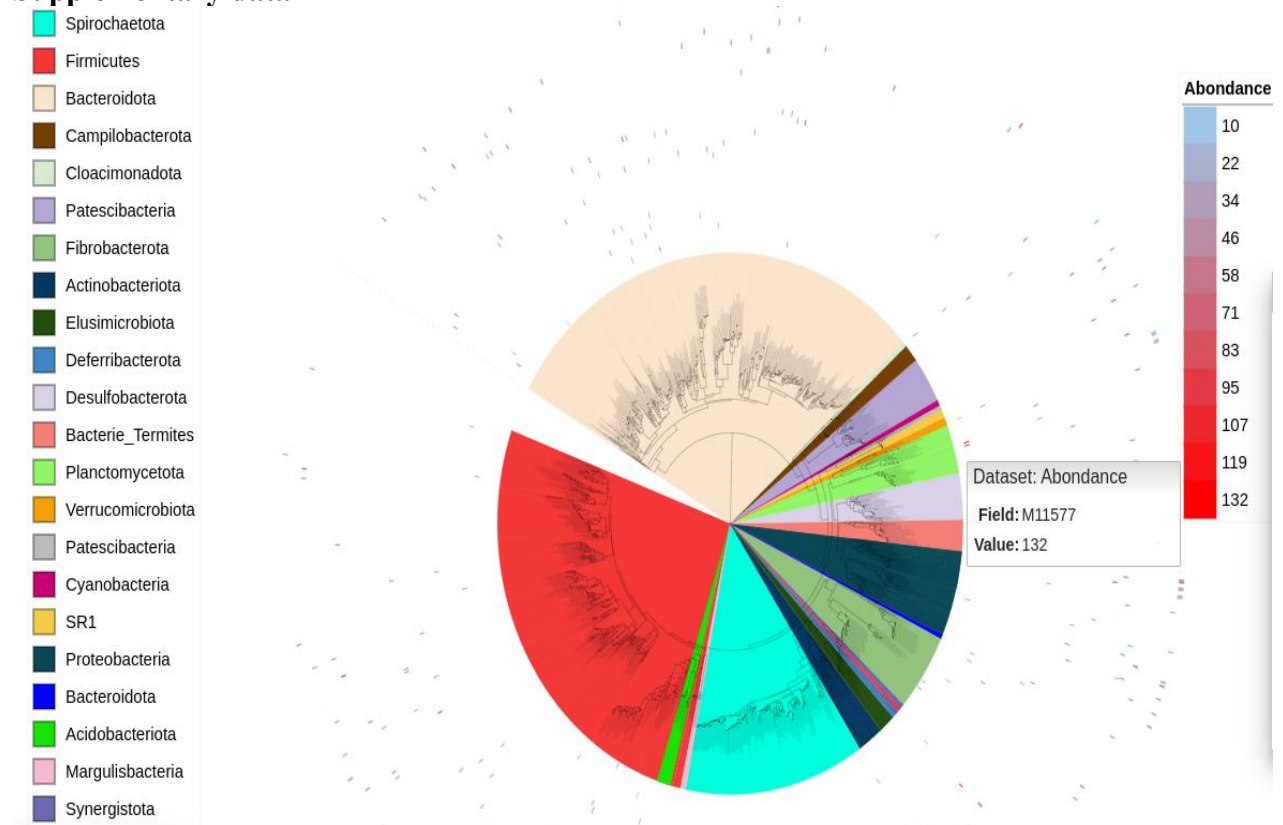
Stacked bar chart showing the relative abundance (%) of microbiome phyla per species after subtracting the blank control (*blank_cutoff*) to reduce bias. Each species displays a distinct microbial signature, with a pronounced enrichment of the phylum Spirochaetota in *Trinervitermes* compared with *Macrotermes* and *Ancistrotermes*.



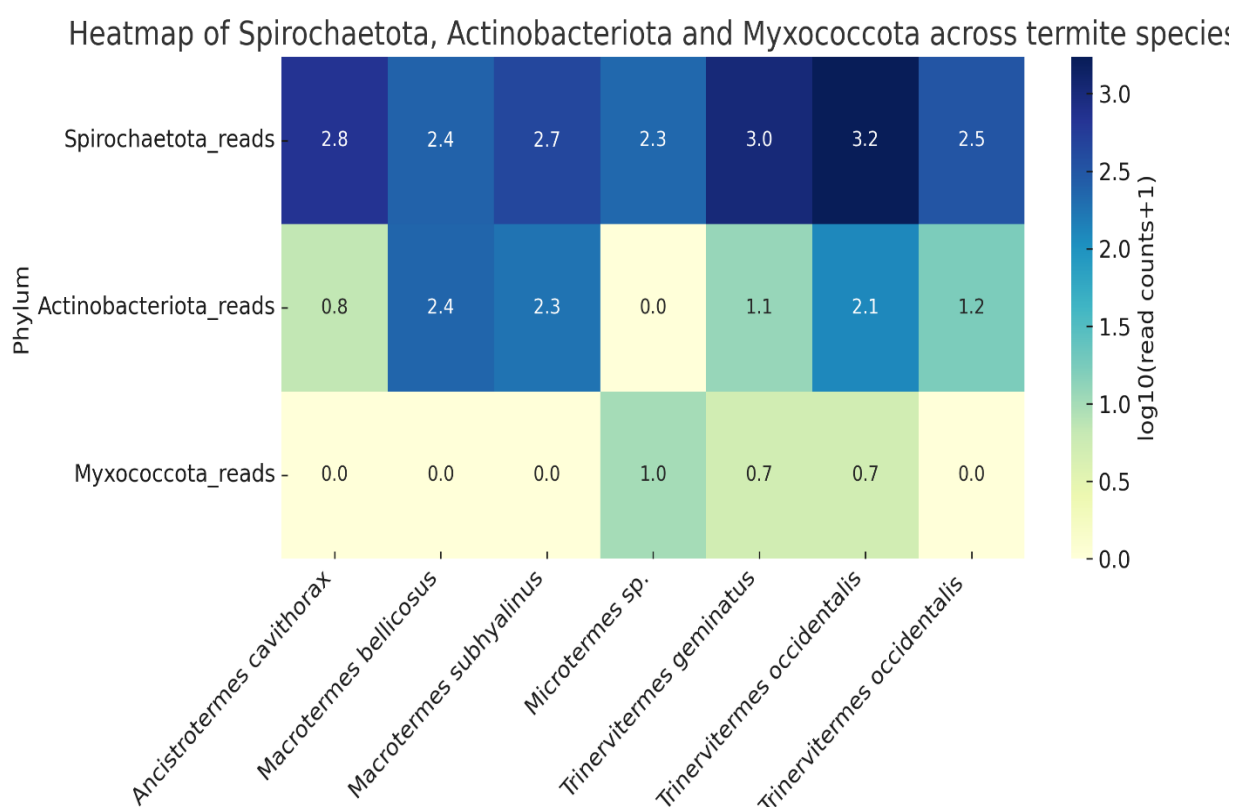
Supplementary data Figure S2. Heatmap of phylum-level relative abundances across termite species.

Gut microbiomes of seven termite species were profiled by 16S rRNA metabarcoding (Illumina); after quality control, ASV inference (DADA2), decontamination using a blank cutoff, normalization to relative abundance, and aggregation at the phylum rank, percentages were visualized as a heatmap. Each cell shows the percentage of a phylum per species according to the right-hand scale. High levels of Bacteroidota and Firmicutes are observed in Macrotermes, whereas Spirochaetota (and Fibrobacterota) are enriched in Trinervitermes. The heatmap was generated in Python using pandas for data aggregation and seaborn/matplotlib for visualization.

Supplementary data

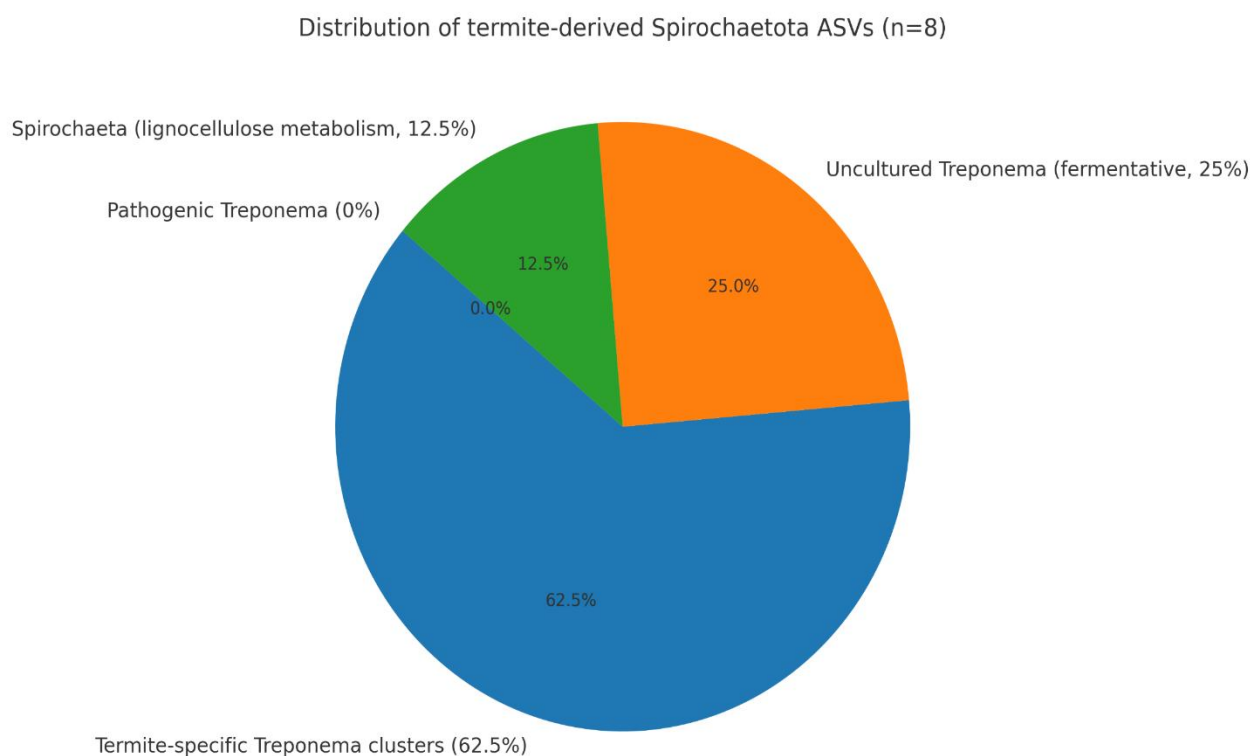


Supplementary data Figure S3: Circular phylogenetic tree of bacterial phyla with abundance. Tree grouping ASVs by phylum (sector colors). It is surrounded by small bars with a blue-to-red gradient blue indicates abundance, while red indicates no abundance or absence of ASVs in the corresponding phylum showing each taxon's relative abundance.

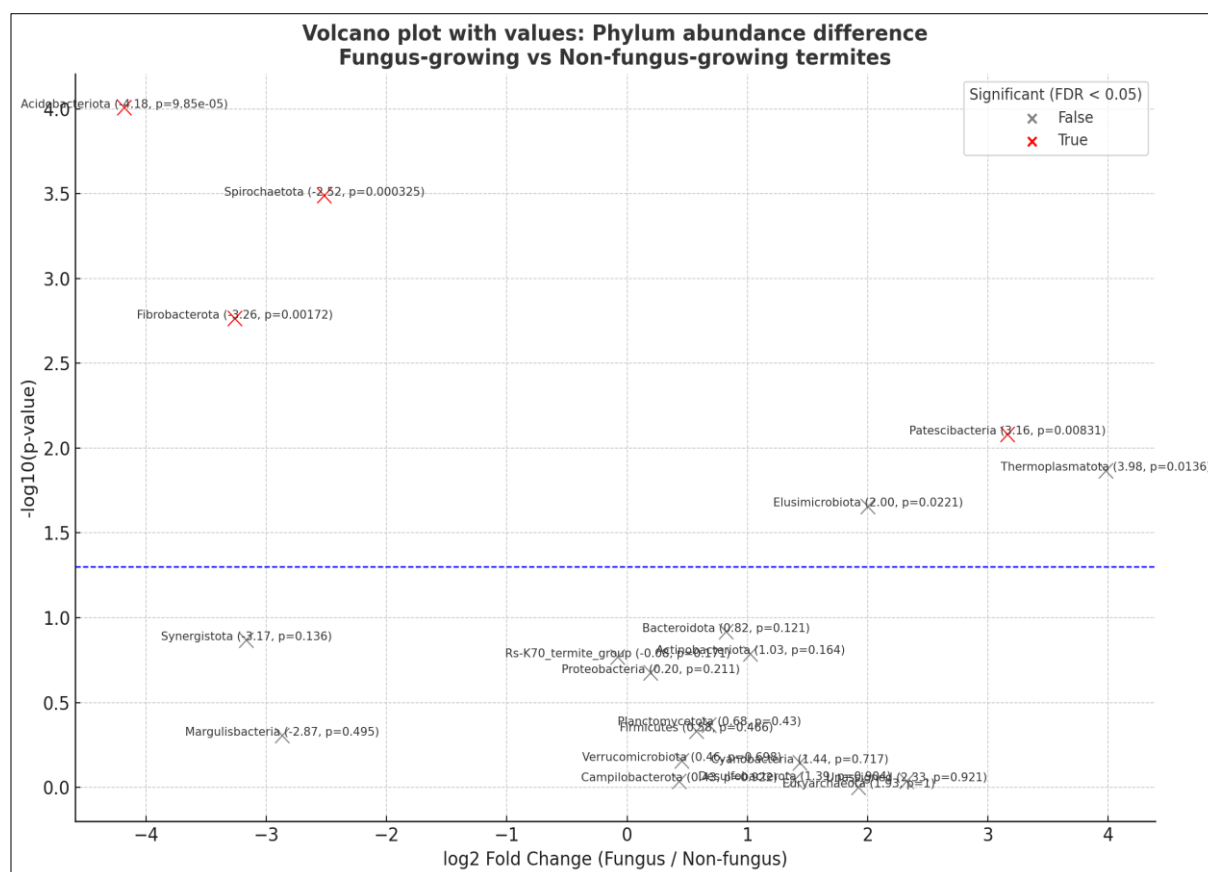


Supplementary data Figure S4. Heatmap of *Spirochaetota*, *Actinobacteriota*, and

***Myxococcota* across termite species.** The heatmap represents log₁₀-transformed read counts for each phylum. Spirochaetota were consistently abundant across species, Actinobacteriota were present at lower levels, and Myxococcota were detected sporadically, highlighting their minor but non-negligible contribution to termite gut community composition. Among the phyla analyzed, Spirochaetota were the most abundant, represented by 12 ASVs and totaling 4,698 reads across nearly all termite species. Actinobacteriota accounted for 15 ASVs with a cumulative abundance of 617 reads, with higher read counts in fungus-growing termites (*Macrotermes* spp.) compared to non-fungus-growing species. Myxococcota were detected at low levels, represented by 3 ASVs and 17 reads in total, and were only present in a few samples. The barplot thus indicates the dominance of Spirochaetota, followed by Actinobacteriota at lower abundance, and sporadic detection of Myxococcota.

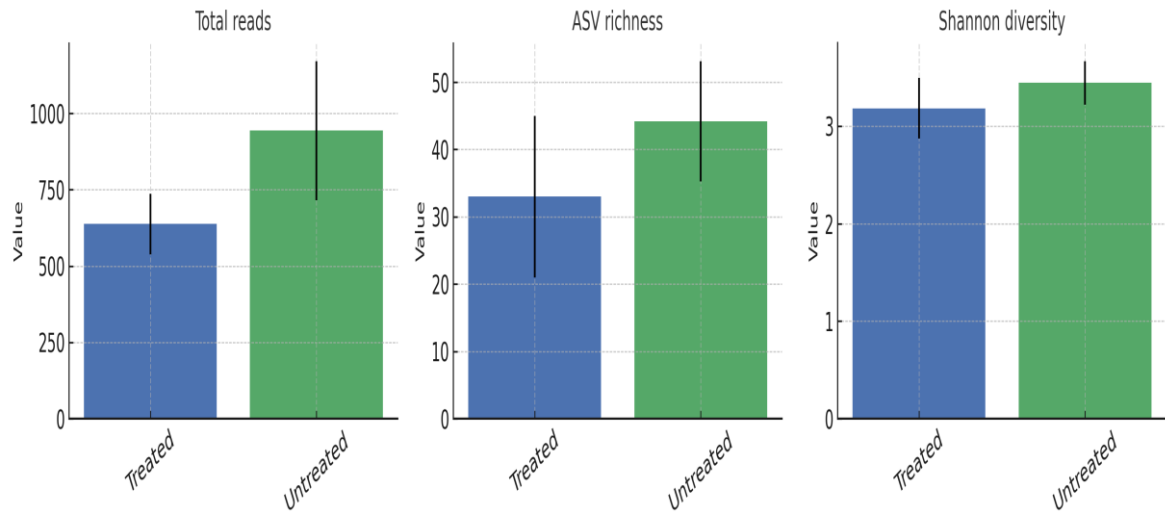


Supplementary data Figure S5. Phylogenetic distribution of termite-derived *Spirochaetota* ASVs (n = 8). The majority of sequences (62.5%) clustered with termite-specific *Treponema* lineages, 25% with uncultured fermentative *Treponema*, and 12.5% with *Spirochaeta*. No ASVs clustered with pathogenic *Treponema* species such as *T. pallidum*.



Supplementary data Figure S6. Phylum-level differential abundance between fungus-growing and non-fungus-growing termites. Volcano plot displaying log2 fold change (fungus vs. non-fungus) against $-\log_{10}(\text{p-value})$. Significant phyla (FDR < 0.05, red crosses) include Acidobacteriota, Spirochaetota, and Fibrobacterota enriched in non-fungus-growing termites, while Patescibacteria, Thermoplasmatota, and Elusimicrobiota were enriched in fungus-growing termites. Non-significant phyla are shown in grey, highlighting the specificity of microbial shifts associated with termite feeding strategy.

Alpha diversity metrics - *Trinervitermes* (Cutoff blanc_mixte)



Supplementary data Figure S7. Alpha diversity metrics of *Trinervitermes* from glyphosate-treated and untreated fields. Bar plots comparing (left) total sequencing reads, (middle) ASV richness, and (right) Shannon diversity index between treated (blue) and untreated (green) groups. Untreated termites showed slightly higher values across all metrics, suggesting a trend toward greater diversity, but none of the differences reached statistical significance. Error bars indicate standard deviations.

Abbreviation and definitions

Abbreviation	Definition
ASV	Amplicon Sequence Variant
bp	Base Pair
COI	Cytochrome c oxidase subunit I gene
CPR	Candidate Phyla Radiation
DADA2	Divisive Amplicon Denoising Algorithm 2
DNA	Deoxyribonucleic Acid
FDR	False Discovery Rate
GBH	Glyphosate-Based Herbicide
NCBI	National Center for Biotechnology Information
OTU	Operational Taxonomic Unit
PBS	Phosphate-Buffered Saline
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational Multivariate Analysis of Variance
QIIME 2	Quantitative Insights Into Microbial Ecology 2
rRNA	Ribosomal RNA
RNA	Ribonucleic Acid
SRA	Sequence Read Archive
SD	Standard Deviation