nature portfolio

https://doi.org/10.1038/s41586-025-09316-0

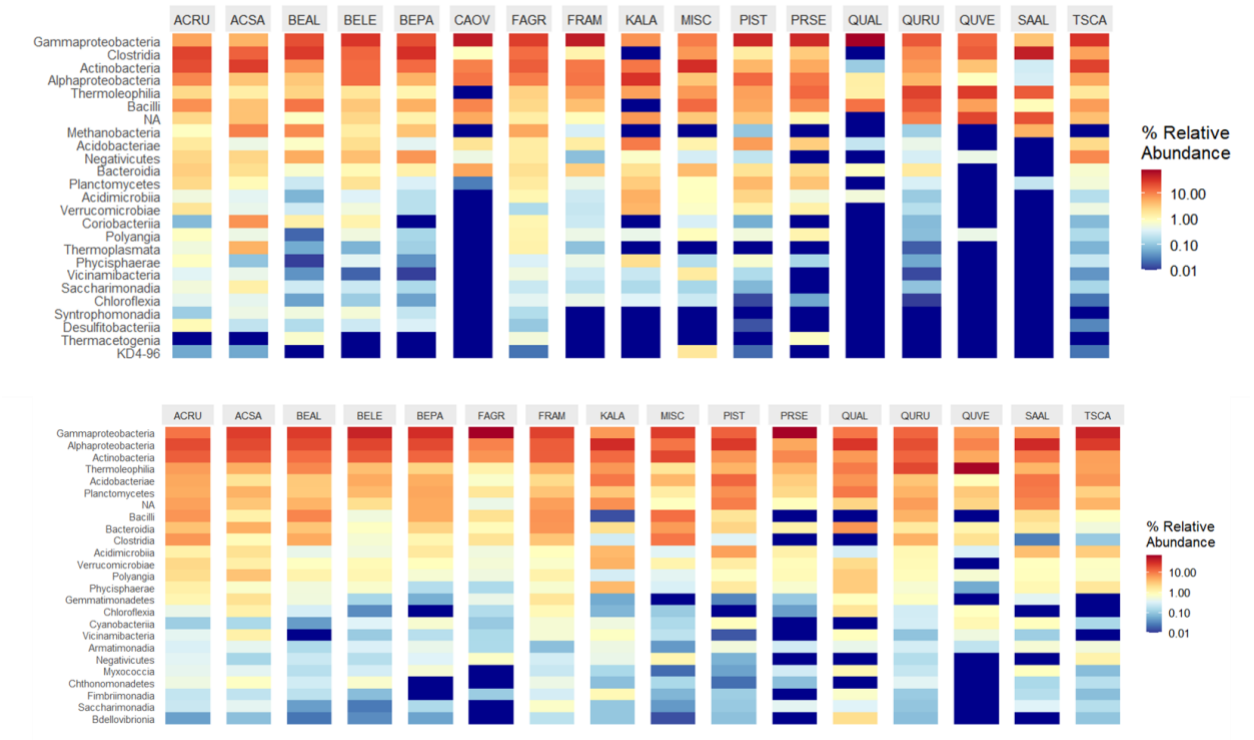
Supplementary information

A diverse and distinct microbiome inside living trees

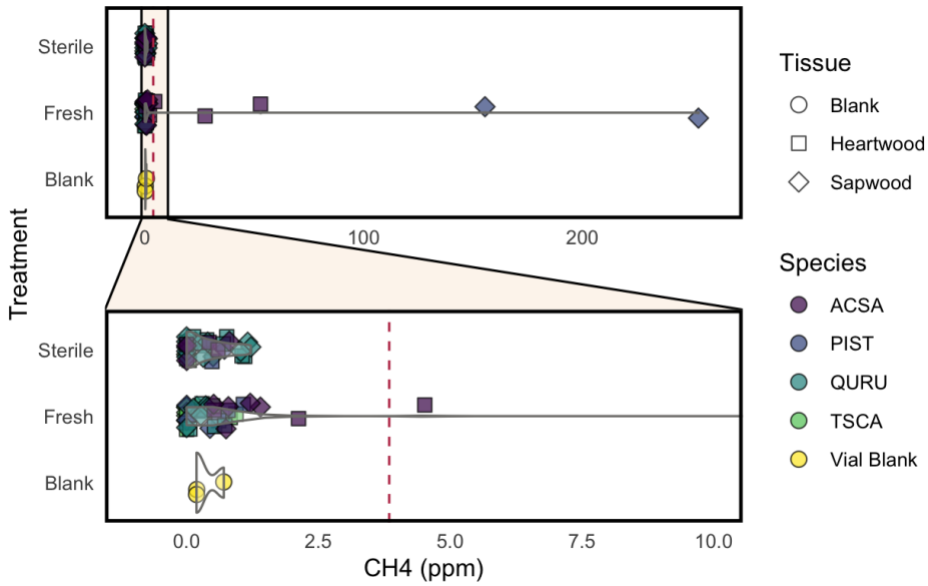
In the format provided by the authors and unedited

SI Table 1: Species codes, scientific names, and common names for species in this study.

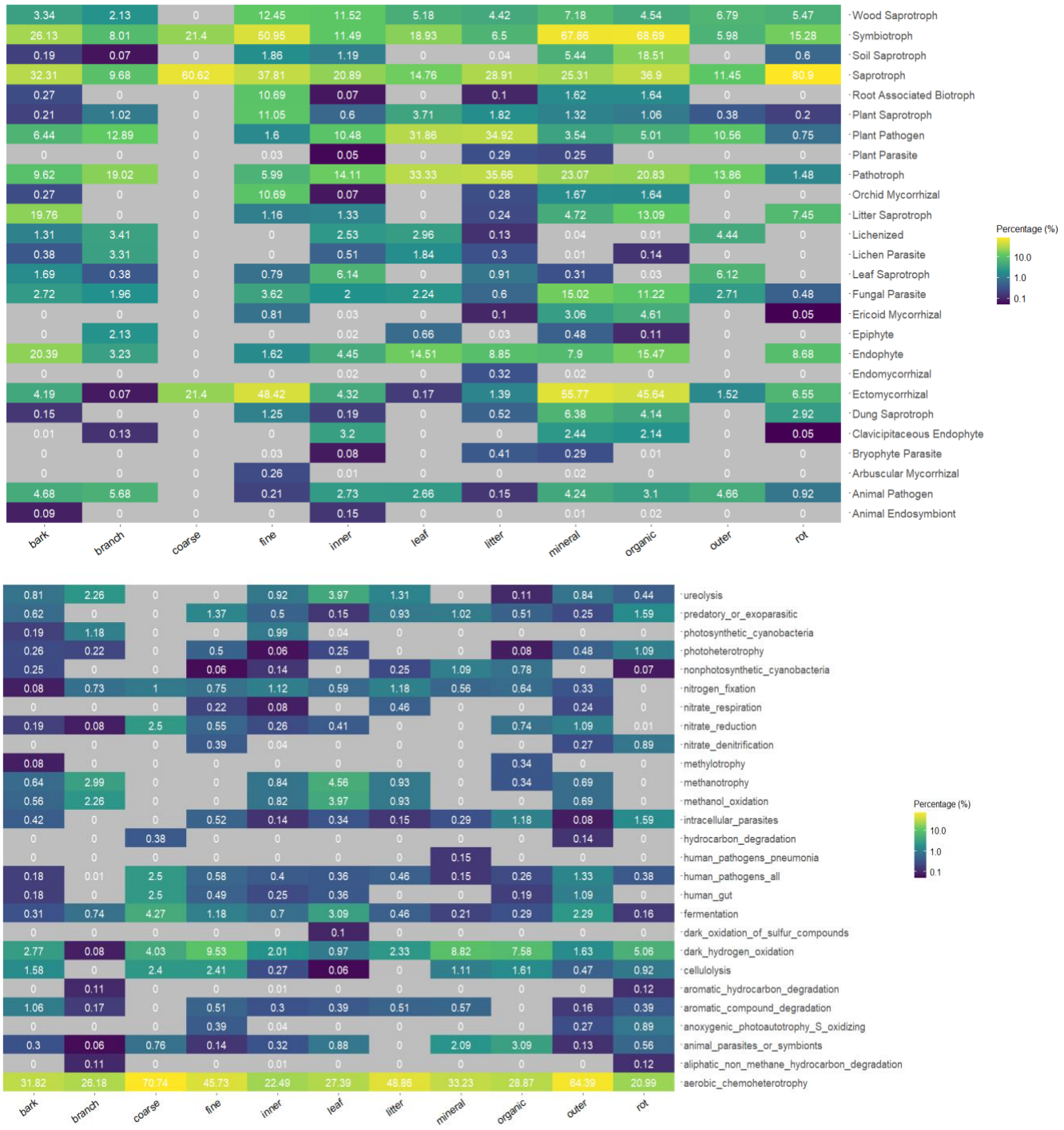
Species Code	Latin name	Common name
ACRU	*Acer rubrum*	Red maple
ACSA	*Acer saccharum*	Sugar maple
BEAL	*Betula alleghaniensis*	Yellow birch
BELE	*Betula lenta*	Black birch
BEPA	*Betula papyrifera*	Paper birch
CAOV	*Carya ovata*	Shagbark hickory
FAGR	*Fagus grandifolia*	American beech
FRAM	*Fraxinus americana*	White ash
KALA	*Kalmia latifolia*	Mountain laurel
PIST	*Pinus strobus*	Eastern white pine
PRSE	*Prunus serotina*	Black cherry
QUAL	*Quercus alba*	White oak
QURU	*Quercus rubra*	Red oak
QUVE	*Quercus velutina*	Black oak
SAAL	*Sassafras albidum*	Sassafras
TSCA	*Tsuga canadensis*	Eastern hemlock



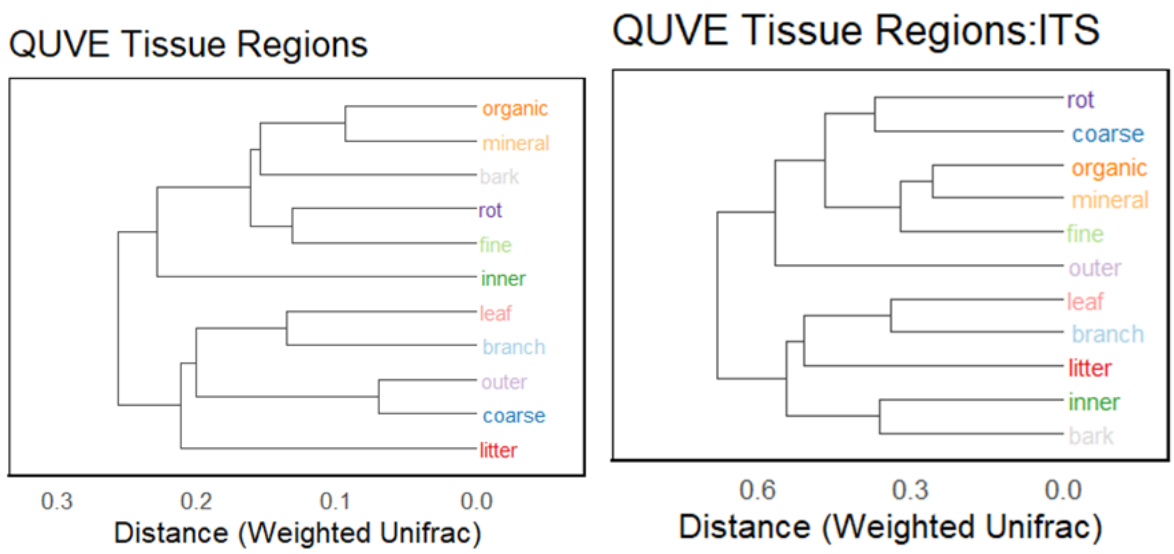
SI Figure 1: Prokaryotic class-level composition across tree species in wood tissues. Relative abundances of 16S taxa at the class level for both **a.)** heartwood and **b.)** sapwood samples, split by tree species (see SI Table 1 for codes).



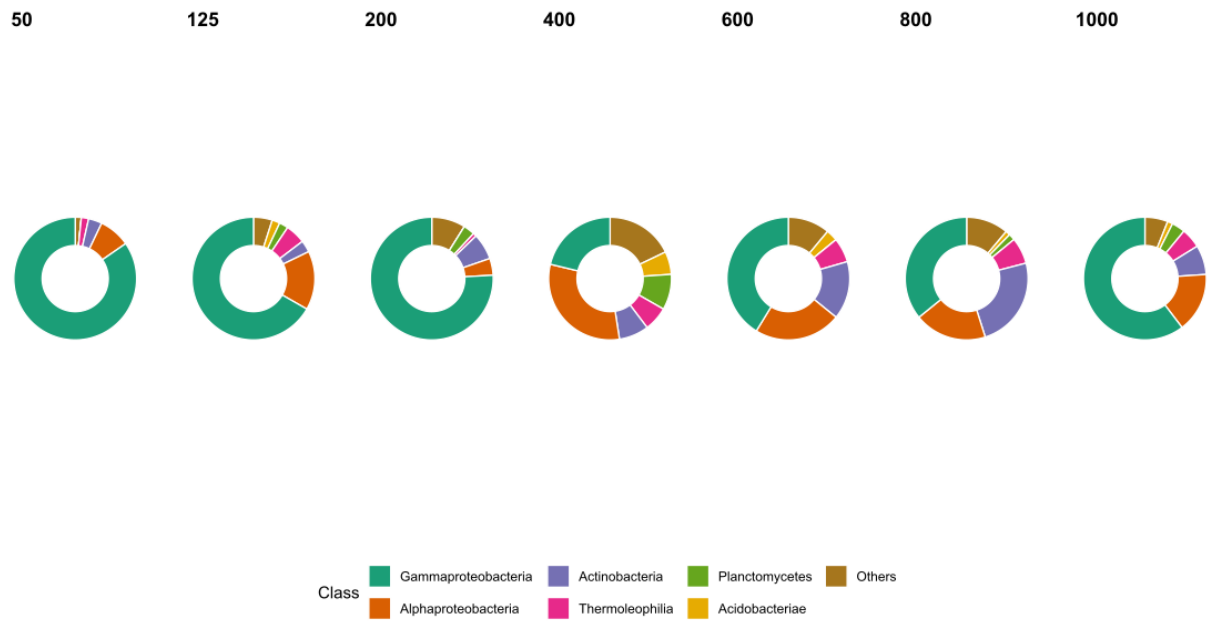
SI Figure 2: Methane production by fresh versus sterilized tree cores under anaerobic conditions. Final concentrations of methane in serum vial headspaces after one-month incubation of tree cores, with a zoomed-in view of the 0–10 ppm CH_4 range. Tree cores were incubated in serum bottles under anaerobic conditions in an atmosphere of 20% CO_2 and 80% H_2, with 4 mL of degassed, sterilized water added to maintain wood saturation. Anaerobic conditions were maintained using butyl rubber stoppers. Tree cores were either transferred directly into serum vials ("fresh") or sterilized using one of three methods: autoclaving prior to sealing the vials, microwaving prior to sealing the vials, or autoclaving after sealing the vials. Three vials without tree cores ("Blank") served as controls. The red dotted line indicates twice the atmospheric concentration of methane (NOAA, 2024), serving as a conservative threshold, beyond which methane production was inferred, to rule out atmospheric contamination. Five fresh cores, and zero sterilized cores or blanks, produced methane in the assay (two-proportion z test: $p=0.0326$).



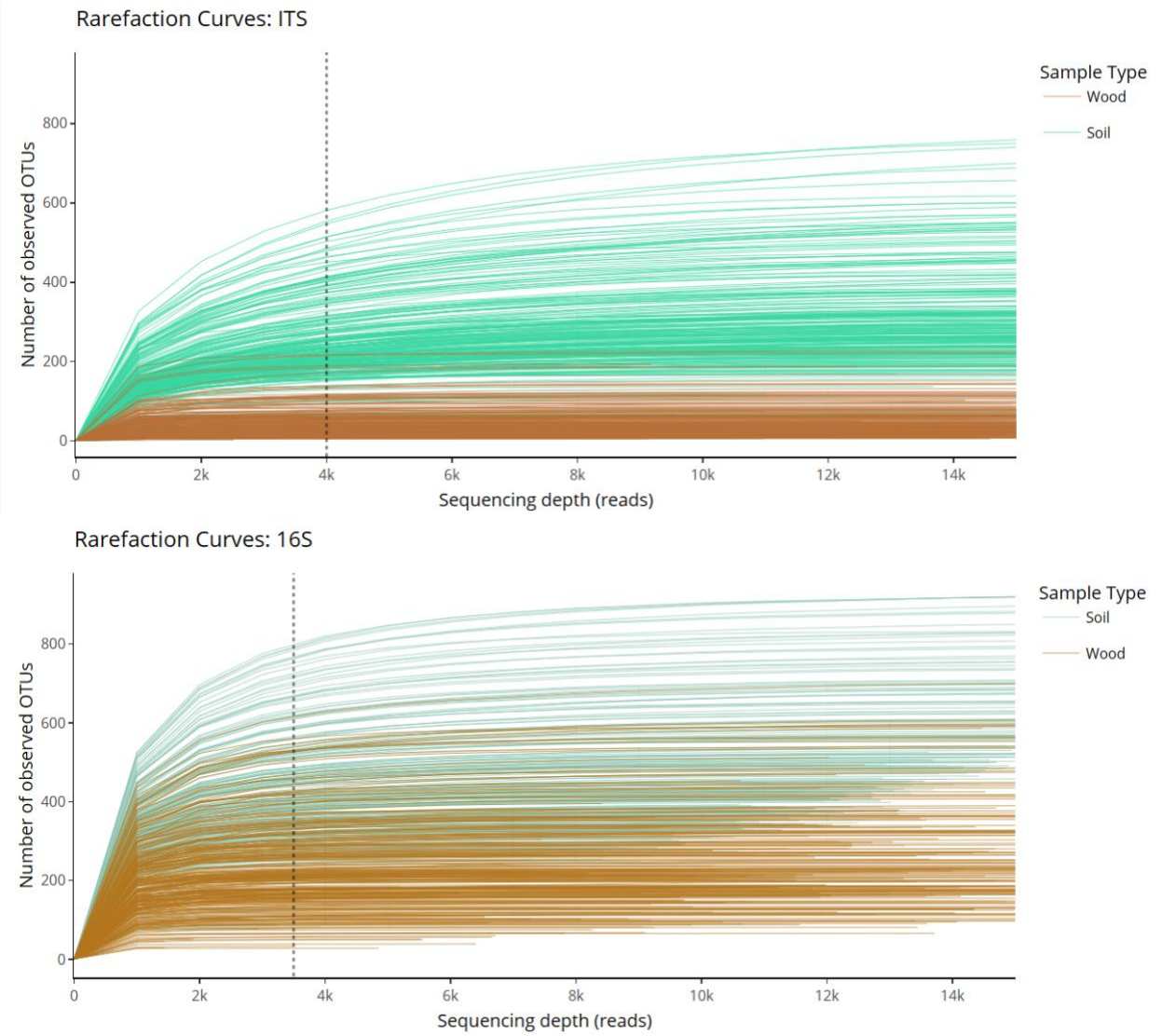
SI Figure 3: Metabolic functions and fungal lifestyles in black oak tissue compartments. Inferred a.) 16S metabolisms using FAPROTAX and b.) fungal lifestyles using FungalTraits for black oak samples, split by sample type.



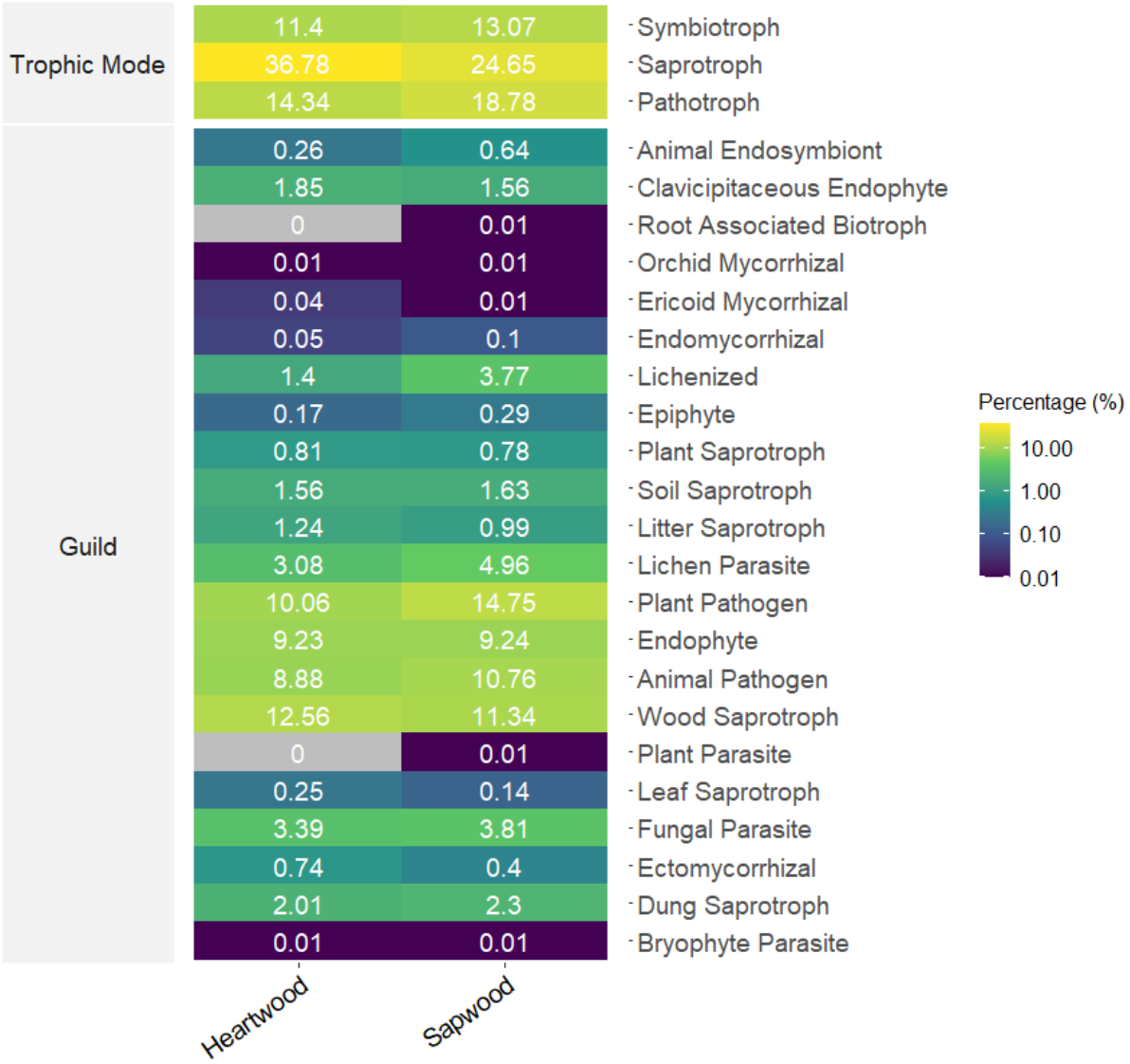
SI Figure 4: Beta diversity relationships among black oak tissue and environmental samples. Relatedness of black oak samples to one another based on weighted UniFrac distance (coarse and fine refer to roots, rot refers to heart-rot; inner refers to heartwood; outer refers to sapwood); 16S (left); ITS (right).



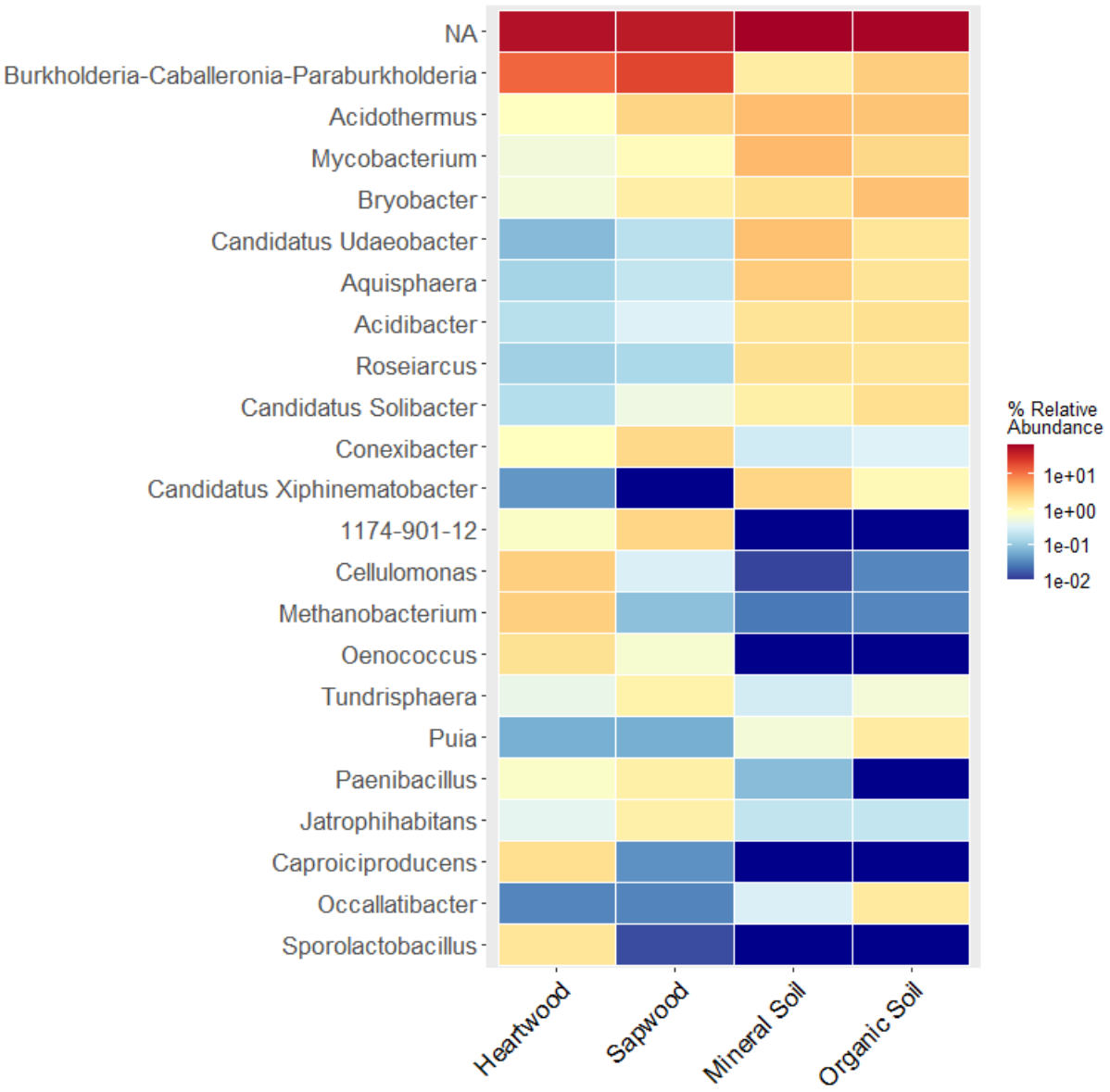
SI Figure 5: Sapwood microbiome stability with tree height in black oak. Variation in the prokaryotic sapwood microbiome with tree height in the black oak (numbers represent distance from ground in cm).



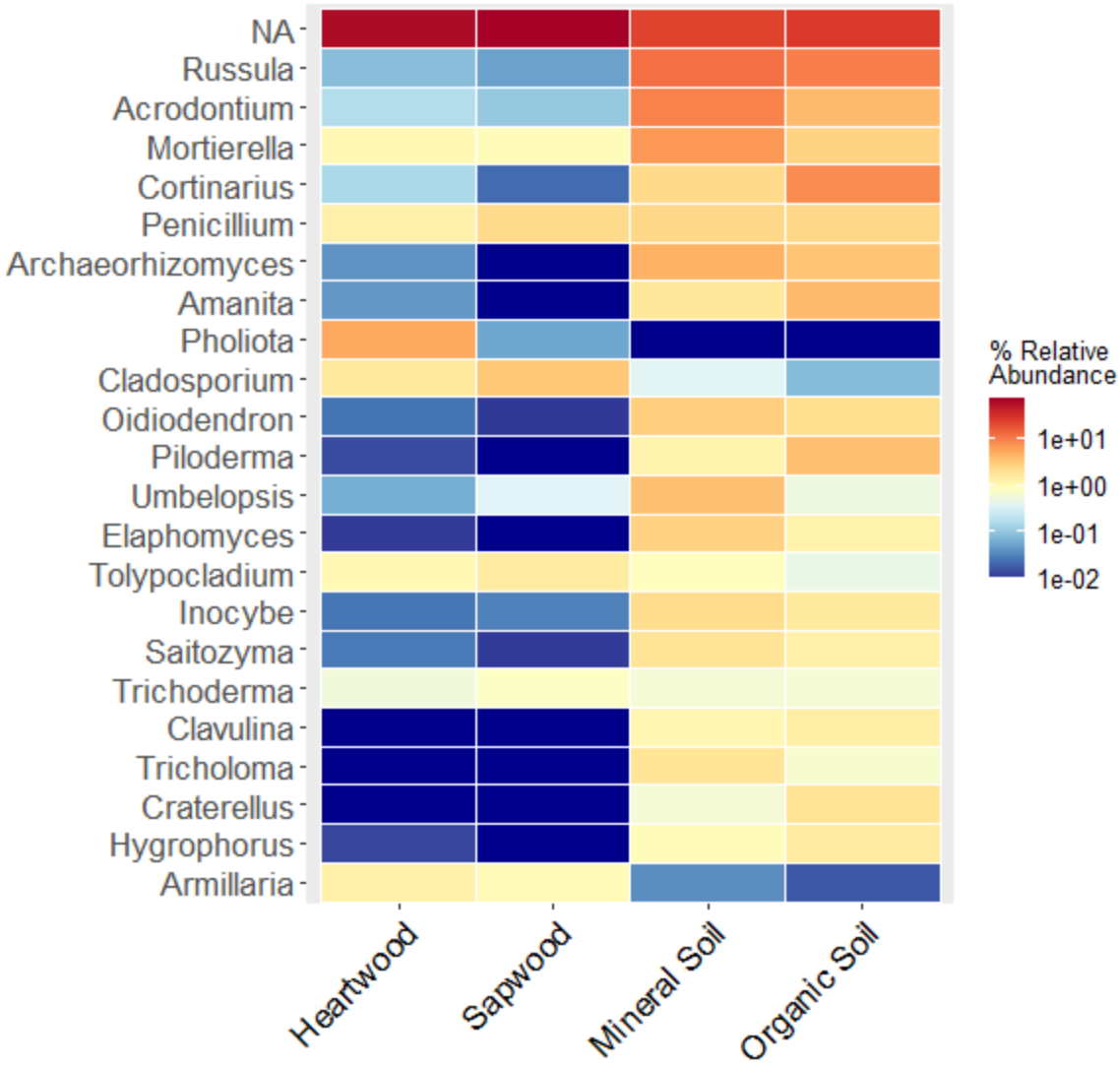
SI Figure 6: Rarefaction curves for wood and soil microbial sequencing data. Rarefaction curves for a.) ITS and b.) 16S sequencing data. Wood samples displayed in brown, with soil samples displayed in teal. For ITS data, samples were rarefied to 4,000 reads per sample (dotted line), and for 16S data, samples were rarefied to 3,500 reads per sample (dotted line) after filtering out remnant mitochondrial and chloroplast sequences.



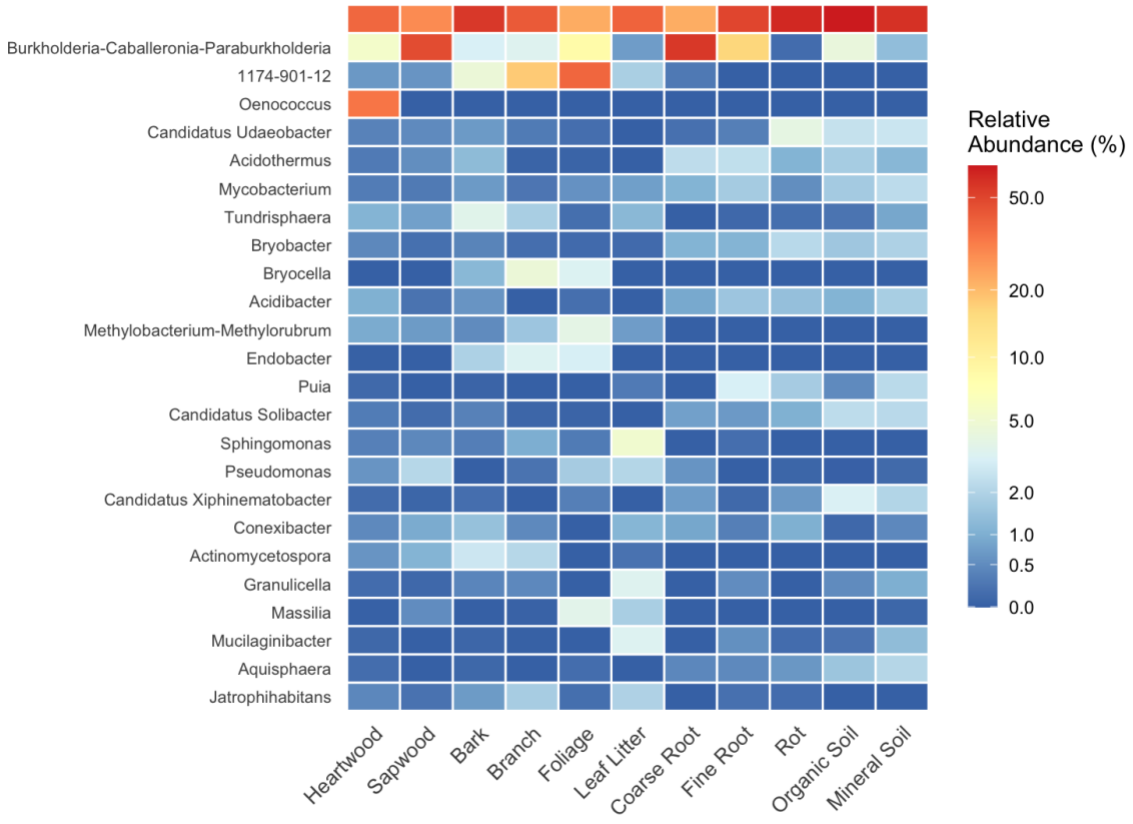
SI Figure 7: Fungal trophic modes and guilds in heartwood and sapwood. Percent abundance (out of 100) of fungal trophic modes and guilds within heartwood and sapwood samples from forest-wide study, as inferred by FUNGuild.



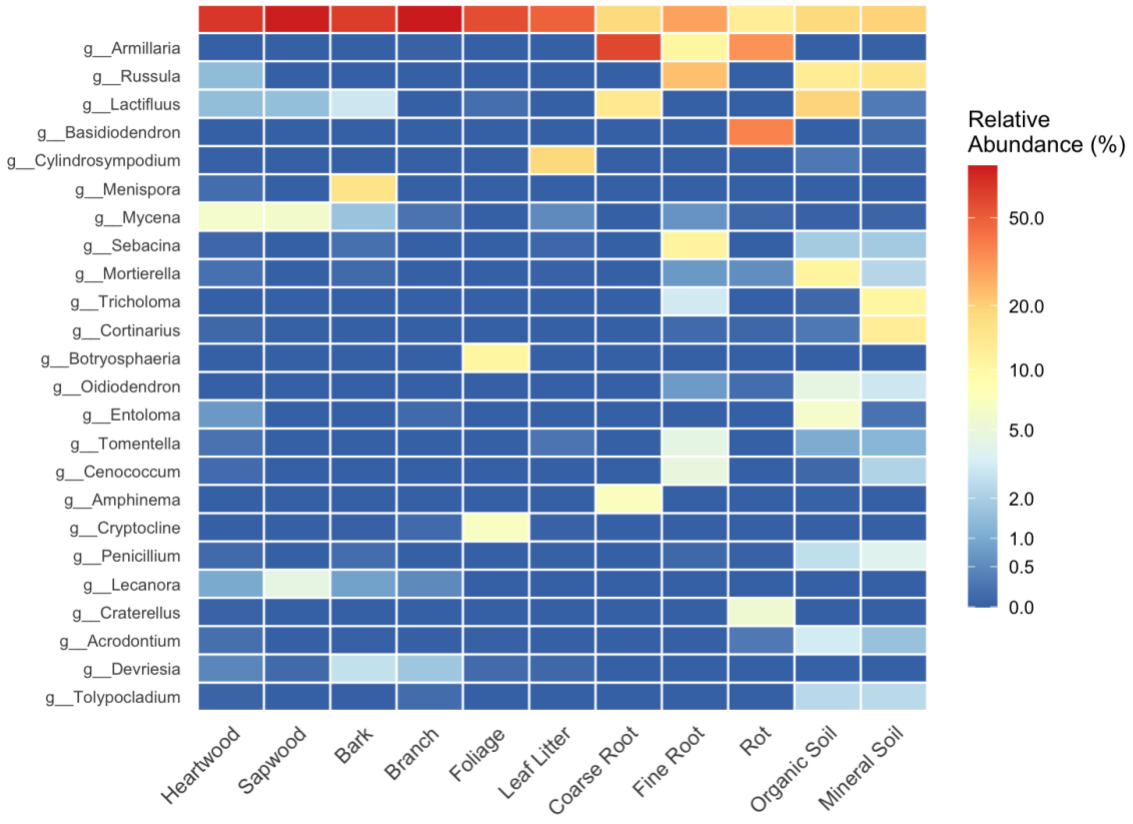
SI Figure 8: Prokaryotic genus-level composition across sample types (forest-wide study). Heatmap of relative abundances of prokaryotic taxa at the genus level across the four sample types from forest-wide study (top 23 taxa by abundance; "NA" refers to taxa unresolved at the taxonomic level). Figure contains same data as heatmap in main text Figure 1, but displayed at different taxonomic level.



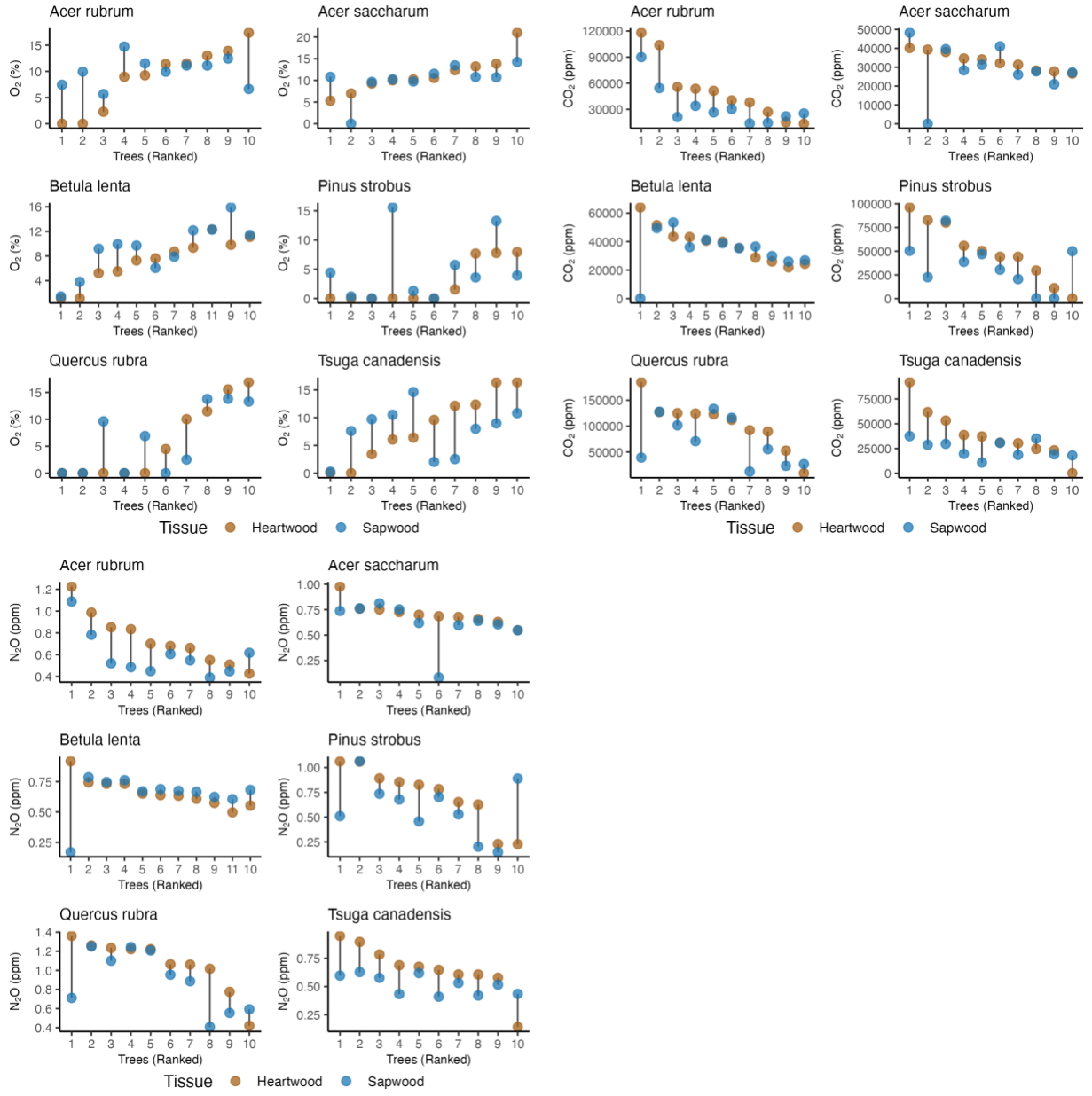
SI Figure 9: Fungal genus-level composition across sample types (forest-wide study). Heatmap of relative abundances of fungal taxa at the genus level across the four sample types (top 23 taxa by abundance; "NA" refers to taxa unresolved at the taxonomic level). Figure contains same data as heatmap in main text Figure 2, but displayed at different taxonomic level.



SI Figure 10: Prokaryotic genus-level composition across black oak compartments. Heatmap showing the relative abundance of prokaryotic genera across different compartments of Black Oak (Quercus velutina). The plot displays the top 25 most abundant genera, with relative abundance shown on a log scale. All samples were rarefied to 3,500 reads prior to analysis. Color intensity indicates relative abundance (%), with darker red representing higher abundance. Genera are ordered by total abundance across all compartments. Figure contains same data as Extended Data Figure 9, but displayed at different taxonomic level.



SI Figure 11: Fungal genus-level composition across black oak compartments. Heatmap showing the relative abundance of fungal genera across different compartments of Black Oak (Quercus velutina). The plot displays the top 25 most abundant genera, with relative abundance shown on a log scale. All samples were rarefied to 4,000 reads prior to analysis. Color intensity indicates relative abundance (%), with darker red representing higher abundance. Genera are ordered by total abundance across all compartments. Figure contains same data as Extended Data Figure 9, but displayed at different taxonomic level.



SI Figure 12: Gas concentration patterns in heartwood and sapwood across tree species. Gas concentrations in tree heartwood and sapwood tissues. Dumbbell plots showing O_2 (top left), CO_2 (top right), and N_2O (bottom) concentrations in paired sapwood and heartwood samples from individual trees across six tree species. Trees within each species are ranked by heartwood concentration (ascending for O_2, descending for CO_2 and N_2O). Lines connect paired measurements from the same individual tree, with brown points representing heartwood and blue points representing sapwood. O_2 concentrations are displayed as percentages, while CO_2 and N_2O are shown in parts per million (ppm).