```
plot_microbiome <- function(
  feature_table,
  taxonomy_file,
  metadata_file,
  tree_file = NULL,
  output_dir = "microbiome_outputs",
  plot_types = c("abundance","rarefaction","alpha","beta"),
  tax_ranks = c("Phylum","Class","Order","Family","Genus","Species"),
  dpi = 300,
  width = 10,
  height = 6,
  group_by = NULL,
  alpha_show_p = TRUE
)
```

Parameters (what each file/option does)

- **feature_table:** Tab-delimited counts per ASV/OTU by sample. Sample IDs must match the metadata.
- **taxonomy_file:** Taxonomic annotations for features. Required for abundance plots; not strictly needed for alpha, but safe to include.
- **metadata_file:** Tab-delimited sample metadata. Must contain the column named in group_by (e.g., “Group”).
- **tree_file:** Newick tree. Used for phylogenetic metrics if enabled; optional for the current alpha run.
- **output_dir:** Folder where figures, logs, and Excel workbooks are written.
- **plot_types:** Which analyses to produce. You’re using "alpha" here.
- **group_by:** Metadata column that defines groups for plotting and statistics.

Expected outputs (for plot_types = "alpha")

- Figures (TIFF)
- alpha_shannon_observed_p001.tiff, alpha_simpson_chao1_p001.tiff, alpha_chao1_overall_box_p001.tiff
- The same three files with _p05.tiff suffix
- Boxplots use translucent fills, only statistically significant pairwise differences are annotated (Wilcoxon, BH-adjusted), brackets are evenly spaced, and extreme outliers are removed using a 3×IQR rule. A right-hand note on each figure states these criteria.
- Excel workbook
- alpha_diversity_combined.xlsx containing:
    - Alpha_All_Indices: one row per sample with the following columns, in order:
    - observed, chao1, diversity_inverse_simpson, diversity_gini_simpson, diversity_shannon, diversity_fisher, diversity_coverage, evenness_camargo, evenness_pielou, evenness_simpson, evenness_evar, evenness_bulla, dominance_dbp, dominance_dmn, dominance_absolute, dominance_relative,

dominance_simpson, dominance_core_abundance, dominance_gini, rarity_log_modulo_skewness, rarity_low_abundance, rarity_rare_abundance.
    - When the microbiome R package is available these are computed directly; otherwise, everything feasible is filled, and the rest are marked NA.
    - Alpha_Long and Alpha_Wide data tables.
    - Observed_Wilcoxon_BH, Shannon_Wilcoxon_BH, Simpson_Wilcoxon_BH, Chao1_Wilcoxon_BH: BH-adjusted pairwise Wilcoxon p-value matrices, computed on the trimmed data to match the plots.
    - Significant_Pairs_p001 and Significant_Pairs_p05: tidy tables of the significant comparisons retained in the figures for each alpha threshold.

If something needs a fix

- If anything errors, looks cramped, or a sheet is missing, please email me:
- The exact console message,
- A screenshot or listing of the files written to output_dir,
- The first ~10 lines of your metadata1.txt (including headers).

Quick checks that solve most issues:

- The group_by column exists in the metadata and is spelled exactly the same.
- Sample IDs in the metadata match those in the feature table.
- Files are tab-delimited without stray quotes.
- If the microbiome package is not installed, some indices in Alpha_All_Indices will be NA, which is expected.