Pharmacologically induced weight loss is associated with distinct gut microbiome changes in obese rats

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Research

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Abstract

Background: Obesity, metabolic disease and some psychiatric conditions are associated with changes to relative abundance of bacterial species and specific genes in the fecal microbiome. Little is known about the impact of pharmacologically induced weight loss on distinct gut microbiome species and their respective gene programs in obese individuals.

Results: Using shotgun metagenomics, the composition of the microbiome was obtained for two cohorts of obese female Wistar rats (n=10-12, total of 82) maintained on a high fat diet before and after a 42-day treatment with a panel of four investigatory or approved anti-obesity drugs (tacrolimus/FK506, bupropion, naltrexone and sibutramine), alone or in combination. We found that sibutramine treatment induced consistent weight loss through reducing food intake. Weight loss in sibutramine-treated rats was associated with changes to the gut microbiome that included increased beta-diversity, increased Bacteroides/Firmicutes ratio and increased relative abundance of multiple Bacteroides species. In addition, the relative abundance of multiple genes was found to be differentially abundant, including significant reductions in components of flagellum and genes involved in flagellum assembly.

Conclusions: This study provides a large resource comprising complete shotgun metagenomics datasets of the fecal microbiome coupled with weight change and food intake at day 3, day 15 and day 42 from 82 obese rats treated with a range of compounds used for weight loss, which are available to the community for detailed analysis. Furthermore, by conducting a detailed analysis of the microbiome associated with sibutramine-induced weight loss, we have identified multiple weight-loss associated microbial taxa and pathways. These include a reduction in components of flagellum and the flagellum assembly pathway that points to a potential role of sibutramine-induced weight-loss on regulating bacterially driven anti-inflammatory responses.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

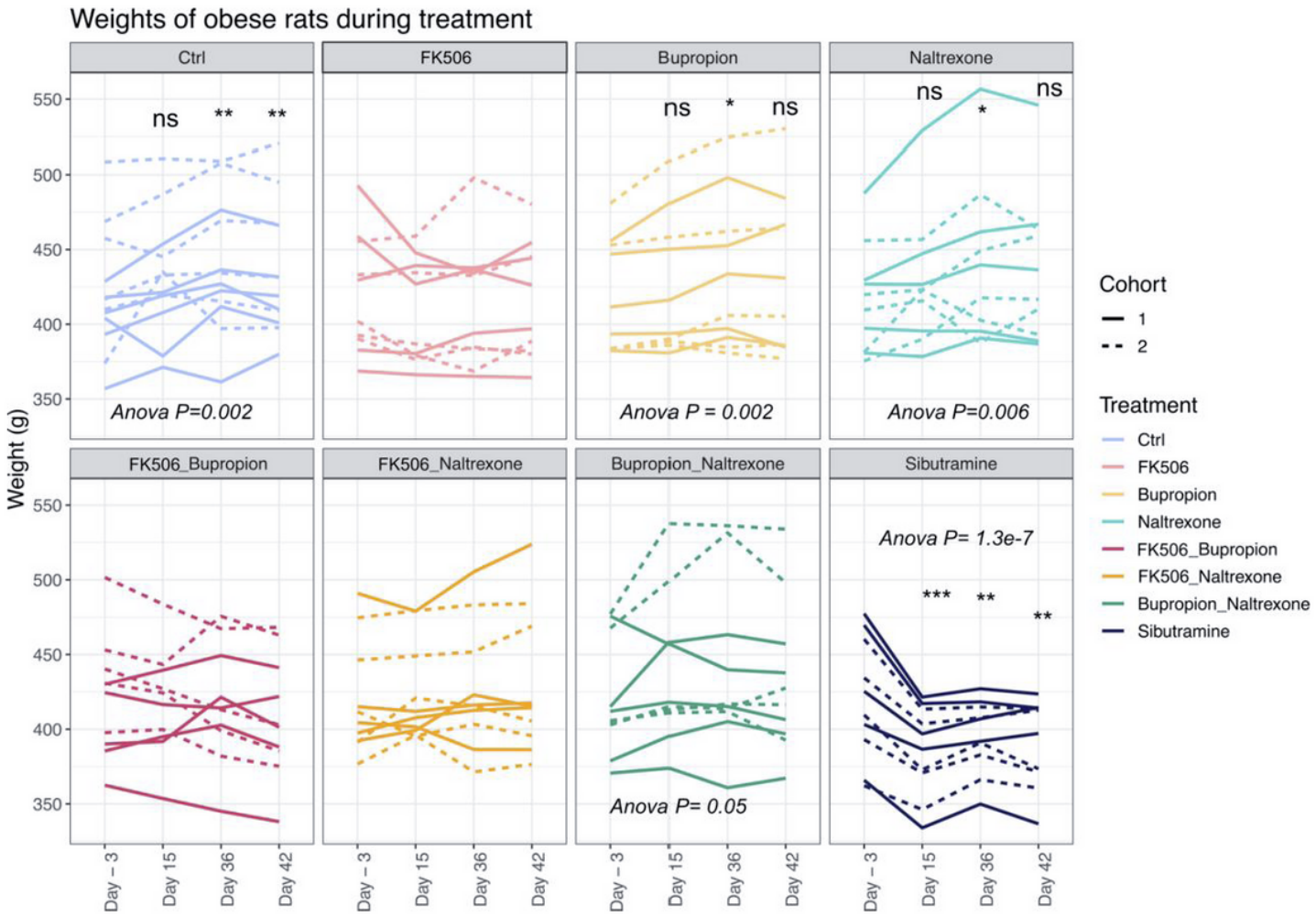

Figure 1

Weights of rats treated with anti-obesity drugs. Each panel shows the weights of rats treated with the respective weight loss drug at specific time points over the course of the study, starting three days before the first administration of the drug, then at Day 15, 36 and 42, the final day of the treatment. Statistical significance has been assessed first by repeated measures Anova, using the formula: weight ~ Timepoint + Rat number + Cohort, then significant timepoints were calculated with a series of paired t-tests, comparing Day – 3 weights with those at Day 15, 36 or 42. Paired T-test analysis within rats from the same treatment group showed only sibutramine treatment significantly reduced weight by the end of the treatment (Day -3 vs Day 42,Benjamini-Hochberg corrected p.values: Ctrl = 0.005 (T-statistic = -4.31, final weight significantly higher), FK506 = 0.52, bupropion = 0.06, naltrexone = 0.04 (T-statistic = -2.9, final weight significantly higher), FK506 and bupropion = 0.14, FK506 and naltrexone = 0.17 , bupropion and naltrexone = 0.22, sibutramine = 0.005(T-statistic = 4.06, final weight significantly lower). Statistical significance levels: ns P > 0.05; * P < 0.05; ** P < 0.01; *** P < 0.00 (Control: N=12; sibutramine: N=10 rats per group).

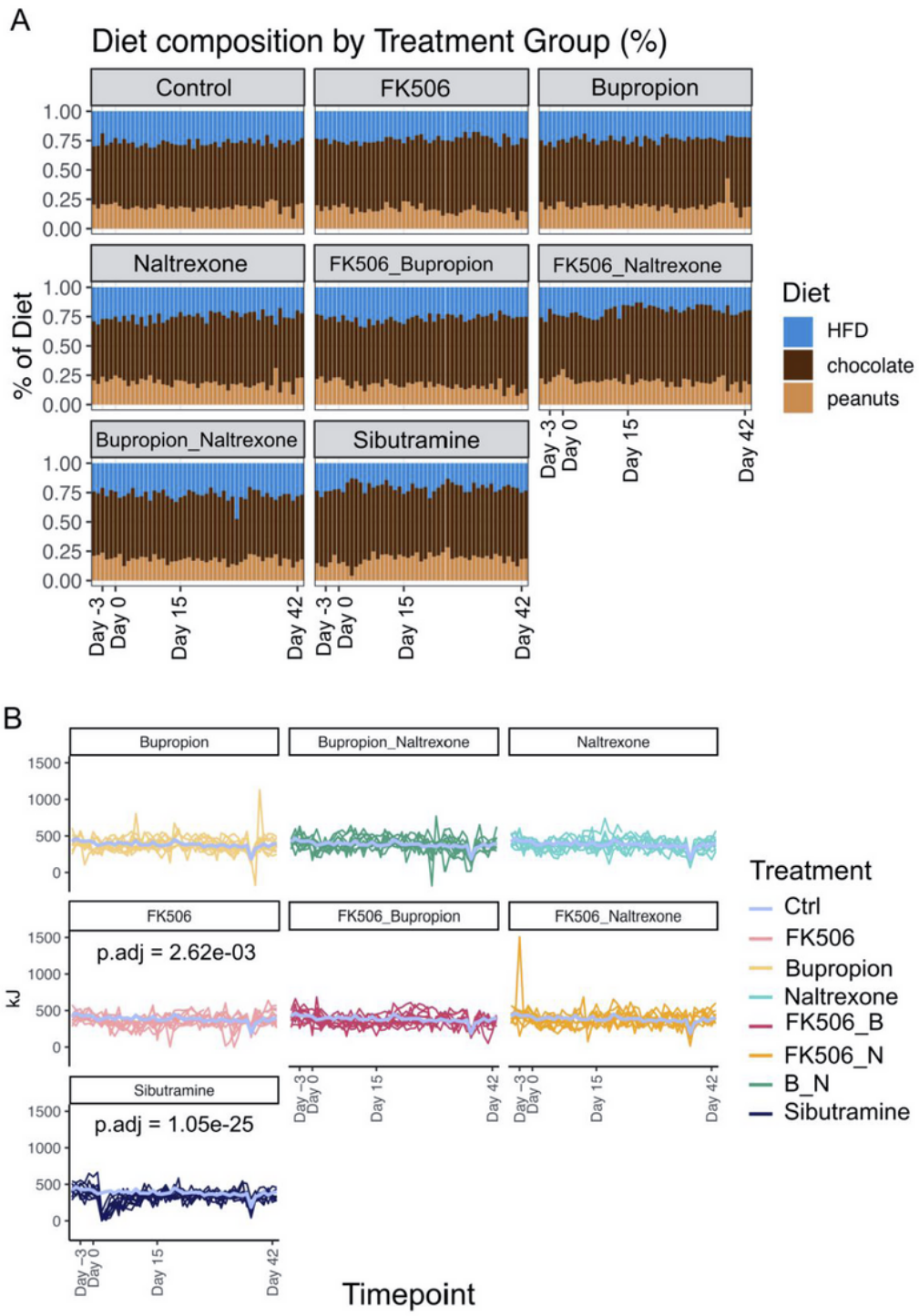

Figure 2

Food intake in obese rats treated with anti-obesity drugs. A) Bar plot representing the percentage of high fat diet (HFD), chocolate or peanuts consumed by each rat throughout the experiment. B) Combined food intake (kJ) for each rat throughout the duration of the treatment. A violet line represents the average food intake for rats in the control group. Statistical significance was assessed by ANOVA with the model: food intake value ~ Day + Treatment Group + Day*Treatment Group + sample, (function anova_test within the

rstatix R package, parameters: wid = sample, within = Day, between = treatment, dv = food intake value) [46], and significant adjusted p-values for the day by group statistic have been reported on the appropriate panels.

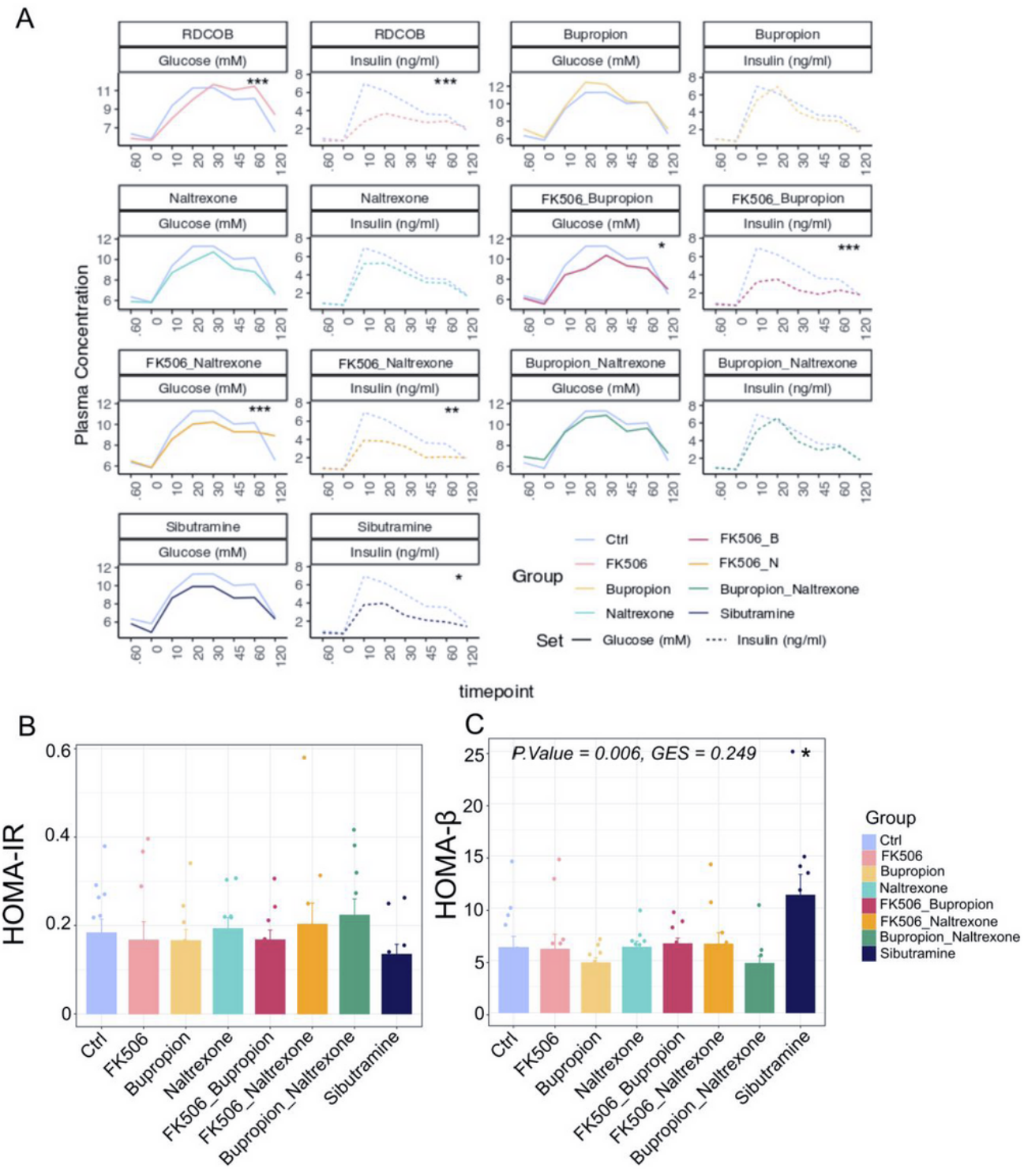

Figure 3

Assessment of glucose homeostasis in obese rats treated with anti-obesity drugs. A) Line plot of the treatment group average values for plasmatic Glucose and Insulin throughout the oGTT. Each treatment

is plotted against a violet line, representing Glucose and Insulin levels in the controls. Statistical significance was assessed by ANOVA with the formula: value ~ Treatment Group + timepoint + timepoint * Treatment Group + sample, (function anova_test within the rstatix R package, parameters: wid = sample, within = Day, between = treatment, dv = value) [46]. P.values were corrected using the Benjamini-Hochberg method (Adjusted p.values: FK506, Glucose = 9.69e-04, Insulin = 1.98e-04; FK506_Buproprion, Glucose = 0.022, Insulin =4.17e-04; FK506_naltrexone, Glucose = 4.46e-04, Insulin = 7.41e-03; sibutramine, Insulin = 0.0256). Statistically significant results have been printed on the corresponding panels. Statistical significance levels = ns P > 0.05; * P < 0.05; ** P < 0.01; *** P < 0.001 (Control: N=12; Treatment Groups: N=10 rats per group). B) Average HOMA-IR values for each treatment group. Fasting insulin and blood glucose measurements were taken from the 0 timepoint of the oGTT test, then HOMA-IR values were calculated according to the formula: (fasting insulin [ng/mL] × fasting blood glucose [mg/dL])/405 [47]. Data represented as mean ± SEM. Statistical significance was assessed by one-way ANOVA test using the model: HOMA-IR ~ Group + Cohort (P.value = 0.685, GES = 0.062, n.s). C) Bar plot representing the values of HOMA-□, as function of insulin secretion and □ cells activity, calculated with the formula: HOMA-□ =(20× fasting insulin [μU/ml])/(fasting glucose [mmol/L] −3.5)[48] . Data was taken from the 0 timepoint in the oGTT and is represented as mean ± SEM. Statistical significance was assessed by one-way ANOVA test using the model: HOMA-□ ~ Group + Cohort (Adjusted P.value = 0.006, GES = 0.249, **), followed by Tukey's post-hoc test, which indicated the Ctrl vs. sibutramine comparison as statistically significant (Adjusted P.value = 0.0243, *). Statistical significance levels = nsP > 0.05; * P < 0.05; ** P < 0.01; *** P < 0.001 (Control: N=12; Treatment Groups: N=10 rats per group).

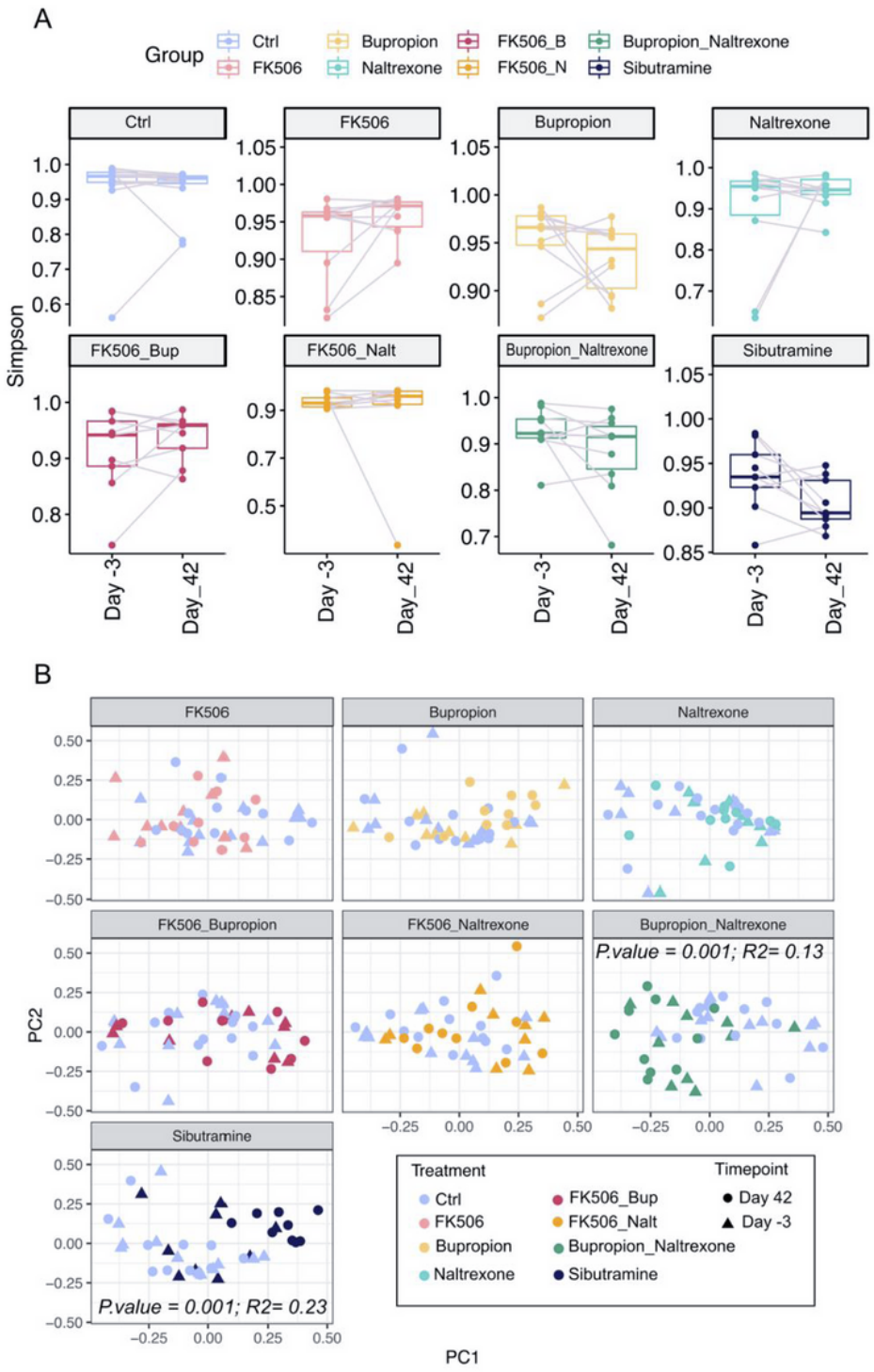

Figure 4

Diversity analyses on all treatment groups reveal no clear alteration of alpha diversity but some differences in beta diversity. A) Boxplots of Simpson's index to estimate diversity changes in each treatment group between the start (Day -3) and the end of the treatment (Day 42). Diversity analysis was carried out using Phyloseq's estimate richness function. Statistical significance was calculated by Student's paired t-test and resulting P.values were further corrected by the Benjamini-Hochberg method,

but none of the comparisons were statistically significant. Notably, the higher the alpha diversity, the lower the Simpson index value will be. B) PCA plots showing β diversity amongst samples, calculated on relative abundances by Bray-Curtis distance between each sample, using the phyloseq R/Bioconductor package [38]. Statistical significance was assessed by the adonis test (Treatment * Group + cohort). The combination of bupropion and naltrexone, as well as sibutramine, were statistically significant. bupropion and naltrexone: P.value = 0.001, R2= 0.13, sibutramine: P.value = 0.001, R2= 0.23.

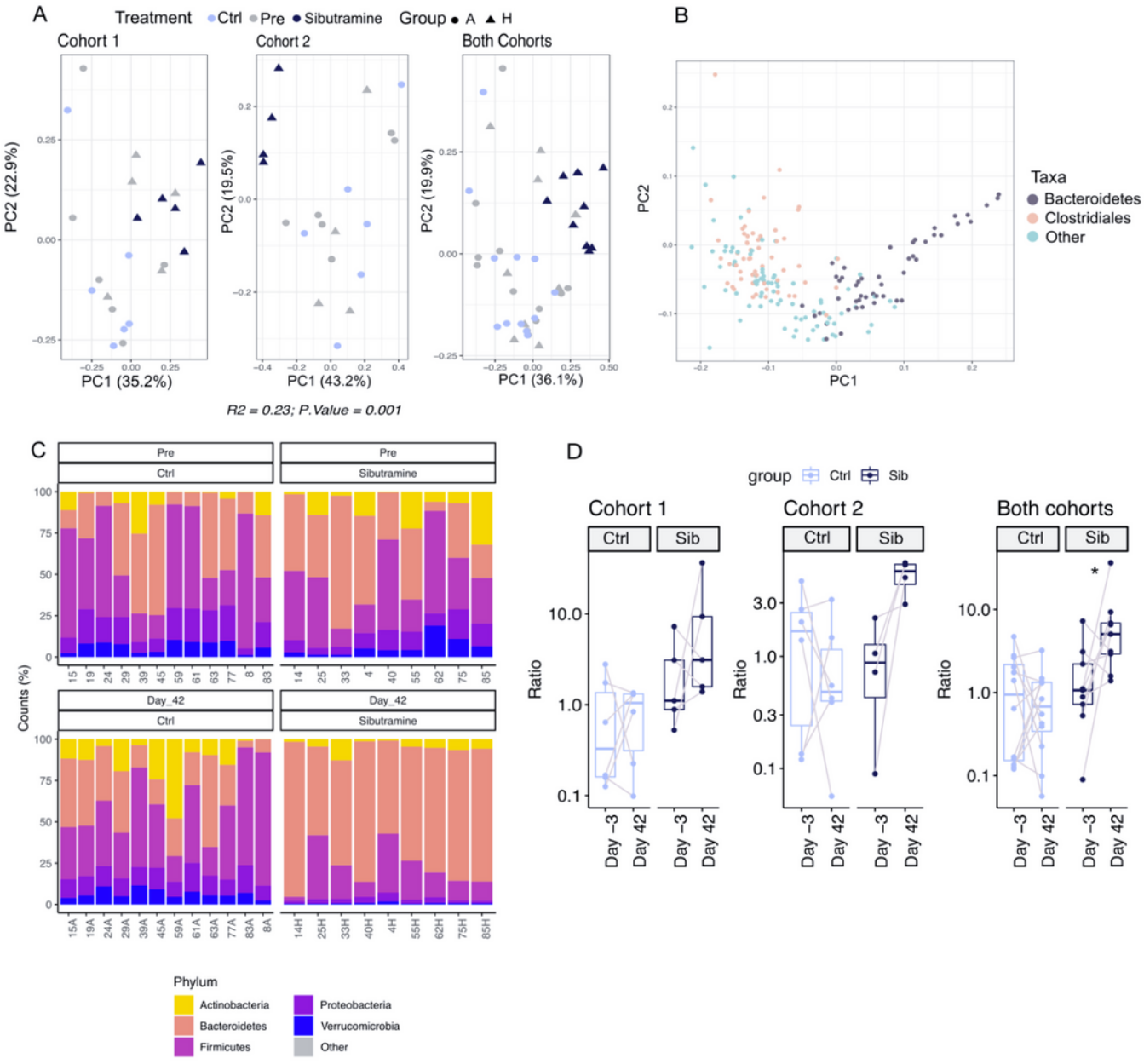

Figure 5

Sibutramine treatment impact on the microbiome. A) PCA of β diversity amongst samples, calculated on relative abundances by Bray-Curtis distance between each sample, using the phyloseq R/Bioconductor

package [38]. Statistical significance was assessed by adonis test (Treatment * Group + cohort) and resulting variance explained (R2) and p.value are printed at the bottom of the plot. Plot shapes describe the different treatment groups, with circles representing the Control group (A) and triangles the sibutramine-treated samples (H). B) PCA of diversity amongst taxa, calculated on relative abundances by Bray-Curtis distance between each sample, using the phyloseq R/Bioconductor package [38]. C) Bar plot summarizing the percent composition of the top 5 phyla across control (left panel) or sibutramine (right panel) samples, at the start (top) or the end (bottom) of the treatment. D) Boxplots of the comparison of Ratio Bacteroidetes/Firmicutes between Day -3 and Day 42 of Ctrl and sibutramine-treated group. Statistical significance was assessed by T-test. Statistical significance levels: ns P > 0.05; * P < 0.05; ** P < 0.01; *** P < 0.00 (Control: N=12; sibutramine: N=10 rats per group).

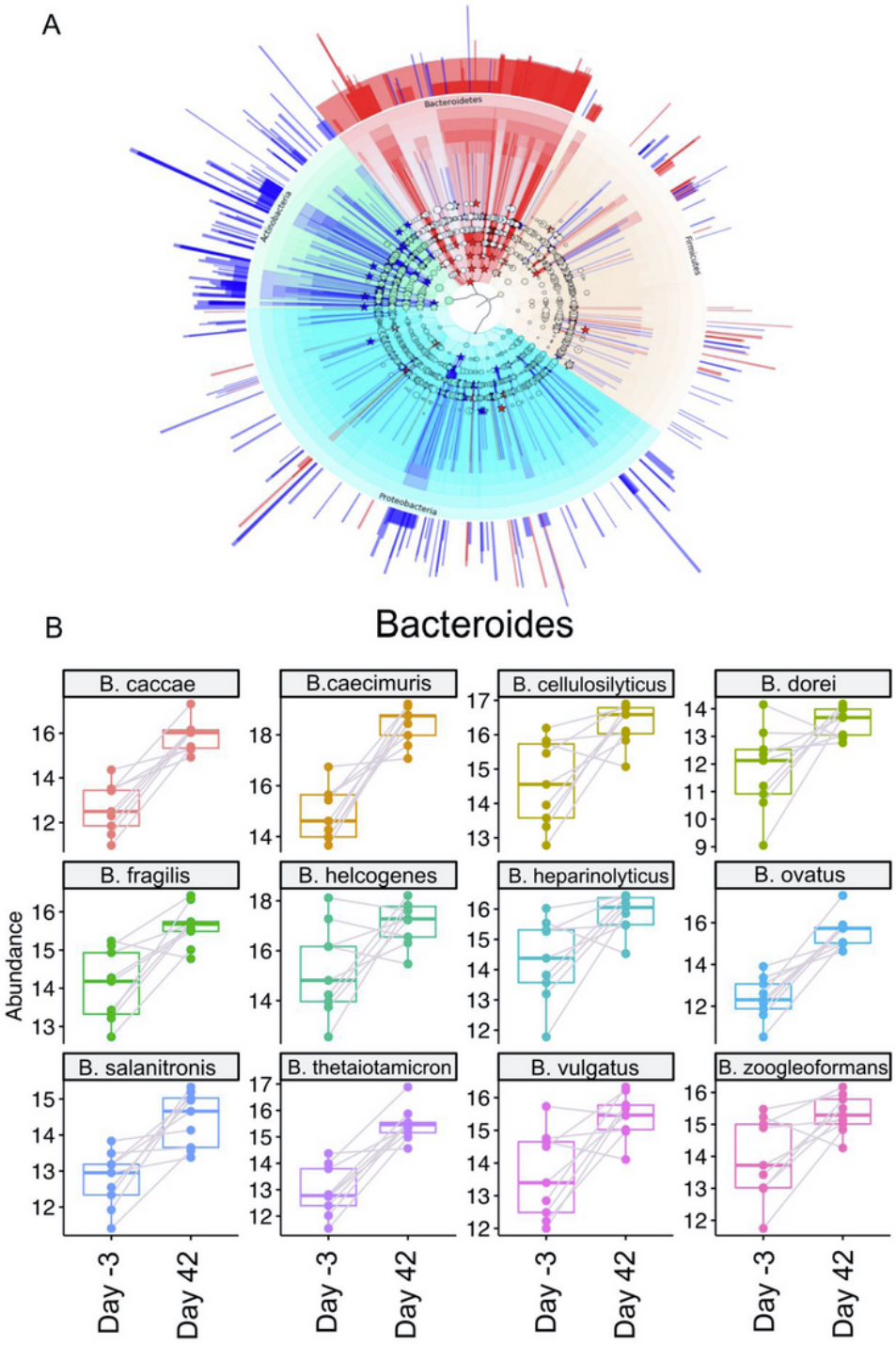

Figure 6

Differentially enriched species between rats before (Day -3) and after (Day 42) sibutramine treatment. A) Phylogenetic tree representation of differentially expressed taxa between samples at the start and end of the treatment. Differential enrichment at every classification level (Phylum, Class, Order, Family, Genus and Species) was calculated with R package DESeq2 using a linear model (~ Day + rat number). P values were adjusted by Benjamini-Hochberg correction and only species with P.adj <= 0.05 were

considered significantly different, then tables with log2 and library size normalized counts (rlog) for every taxon were extracted [41,52]. These were fed into GraPhlAn for phylogenetic tree visualization. External annotation corresponds to Log2 fold-change values for every differentially expressed species. Species of interest were indicated in the circular tree by stars and coloured according to their Log2 Fold change (Log2 fold-change < 0, blue; Log2 fold-change > 0, red). Concentric circles each represent a taxonomical level, starting from Phylum (Proteobacteria, light blue; Firmicutes, yellow; Bacteroidetes, red; Actinobacteria, green), then proceeding outwards to Class, Order, Family, Genus and Species. Notably, Bacteroidetes as a whole are significantly increased by the end of the treatment (see red star in the innermost circle). B) Boxplots showing levels of differentially enriched Bacteroides species, before and after sibutramine treatment. Grey lines connect dots corresponding to the same rat. Counts used were log2 transformed and normalized by library size [52].

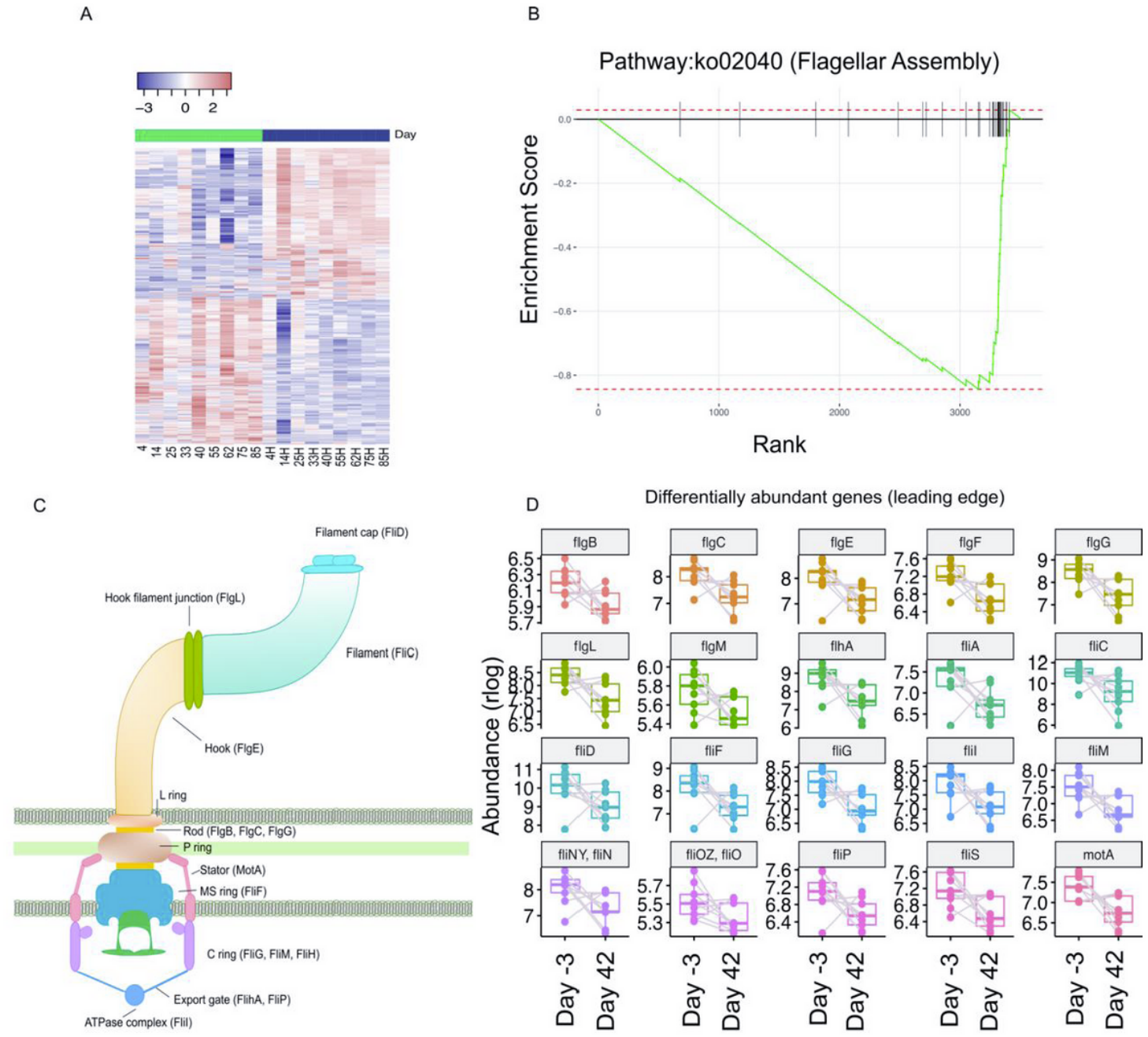

Figure 7

Gene Set Enrichment Analysis highlights decrease of genes related to flagellar assembly. A) Differential gene abundance between sibutramine-treated rats at the start and end of the treatment. Heatmap representing log2-transformed and normalized read counts for each of the 1047 differentially abundant genes. As with species, significant changes in gene levels were calculated using DESeq2 with the model ~ rat number + Day [52]. P values were corrected with the Benjamini-Hochberg method, and only genes with adjusted P value <= 0. 05 were considered statistically significant. Top bar shows which columns correspond to Day -3 (green) or Day 42 (blue) samples. B) Enrichment plot for the pathway “Flagellar Assembly” (ko:02040). Briefly, genes have been ranked by log2 fold change multiplied by the -log10(p.adjusted), then gsea was performed with the fgsea R/Bioconductor package [55]. “Flagellar Assembly” was the only statistically significant result (normalized Enrichment Score: -1.7, p.adjusted = 0.03). C) Schematic representation of the bacterial flagellum and its protein components. Genes detected in our analysis are indicated on the figure. [56]. D) Boxplots of leading edge genes belonging to this pathway, all of which are differentially abundant in sibutramine samples at Day 42 compared to Day -3. Boxplots were obtained by plotting the log2-normalized expression values for each gene.

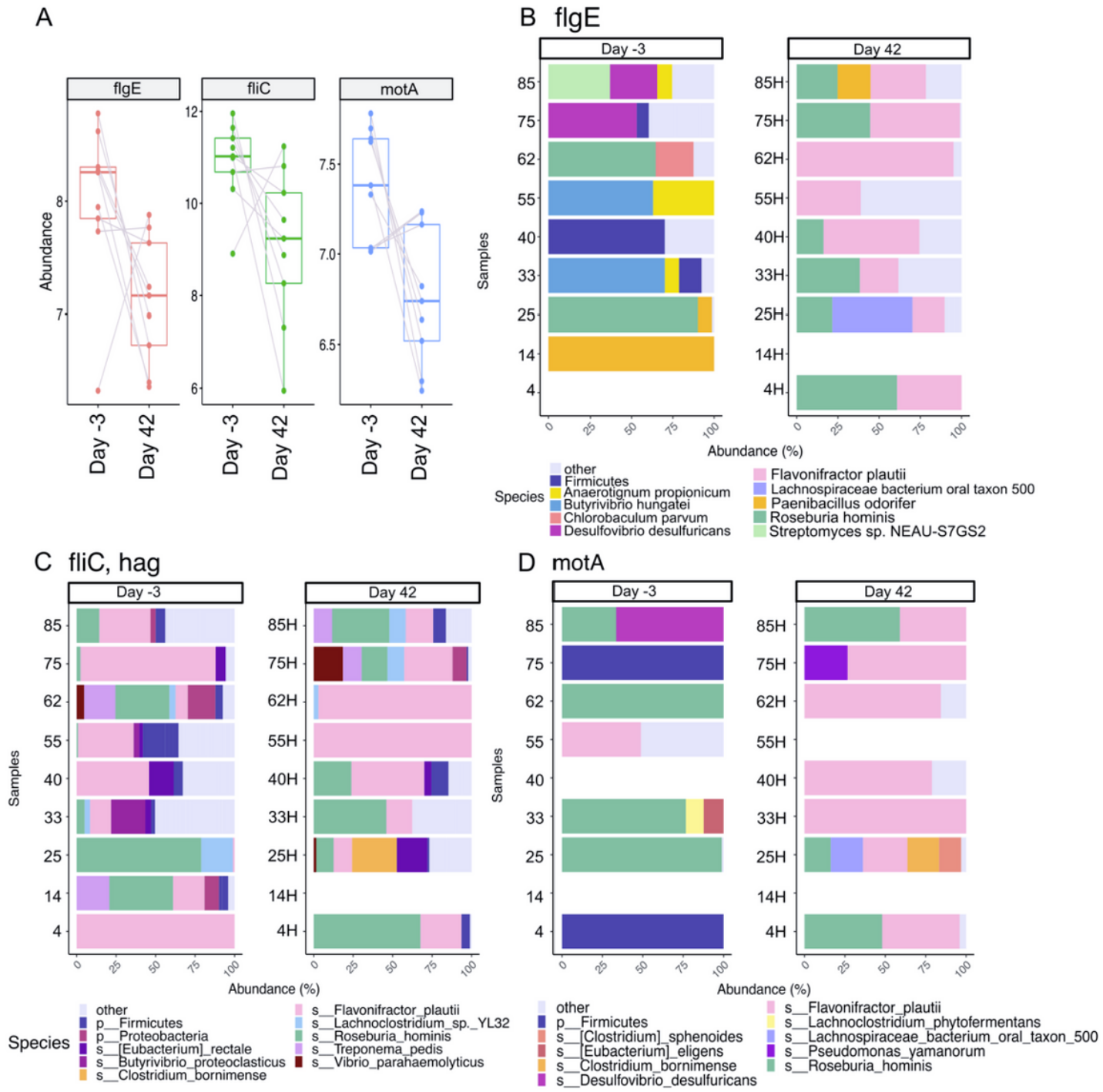

Figure 8

Species composition of three representative genes from the “Flagellar Assembly” pathway. A) Boxplots showing expression levels of three representative members of the “Flagellar Assembly” pathway. Expression values have been log2 normalized as in Figure 7. B-D) Species contribution to the levels of flgE (B),fliC (C) and motA (D). Gene counts for each species are expressed as percentage and only the top 10 species for each gene are represented, whilst the remainder have been collapsed under the “other” category. Empty rows correspond to samples that either had no reads annotated to the gene (motA, 55H

and 14H; flgE 4 and 14H) or whose reads were entirely annotated to “k_unassigned” (fliC, 14H; motA, 14 and 40). Notably, every sample had a certain proportion of reads annotated to “k_unassigned” (Kingdom unassigned), and thus lacked a reliable annotation. These reads have been excluded from the dataset prior to plotting of fliC, flgE and motA. The percentage of unassigned reads for each gene, within every sample, is described in Table S2.

Supplementary Files

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- FigS1.png
- FigS2.png
- FigS3.png
- FigS4.png