Online Data Supplement

Measures of Cystic Fibrosis Airway Microbiota during Periods of Clinical Stability

Lindsay J. Caverly M.D., Junnan Lu Ph.D., Lisa A. Carmody M.A., Linda M. Kalikin Ph.D., Kerby Shedden Ph.D. , Kristopher Opron Ph.D., Michelle Azar B.S., Shannon Cahalan B.S., Bridget Foster M.S., Donald R. VanDevanter, Ph.D., Richard H. Simon M.D., John J. LiPuma M.D.

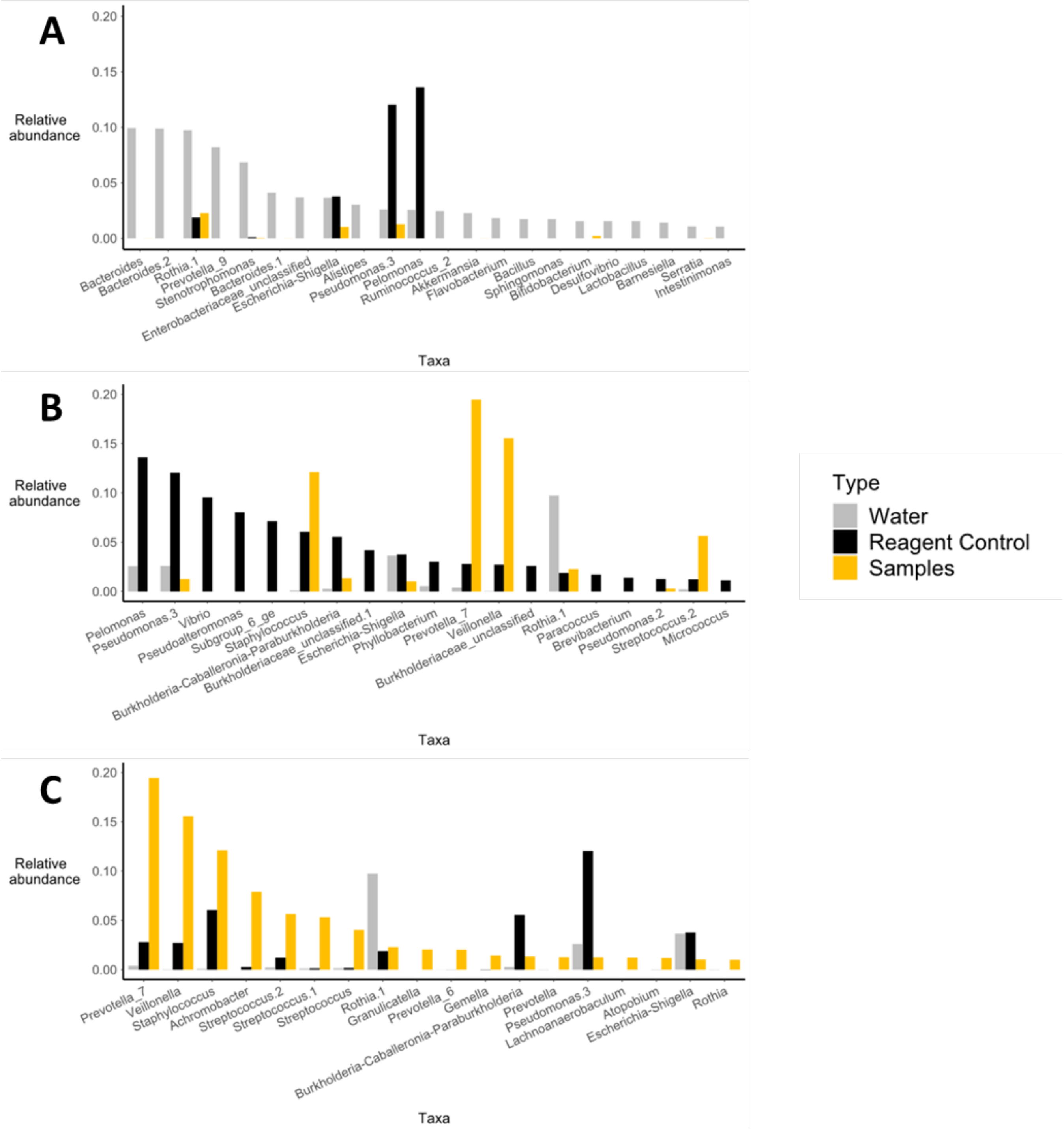
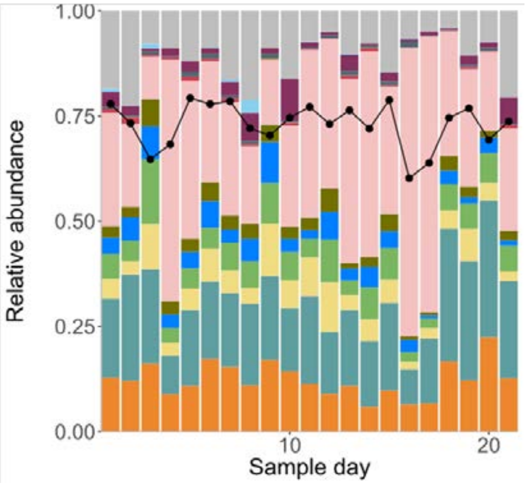
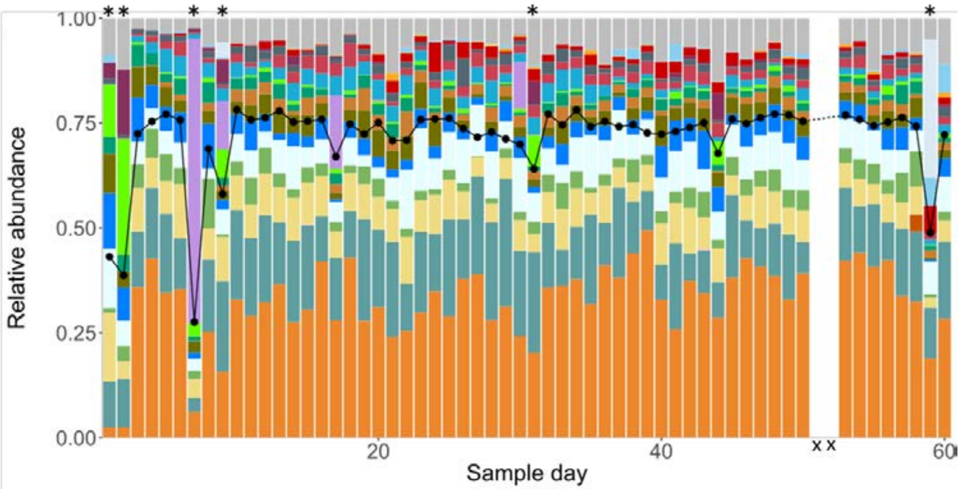


Figure E1. Rank abundance plots of mean relative abundance of OTUs. (**A**) water controls; (**B**) reagent control; and (**C**) sputum samples. All OTUs with >1% mean relative abundance for each of the ranked types are shown.

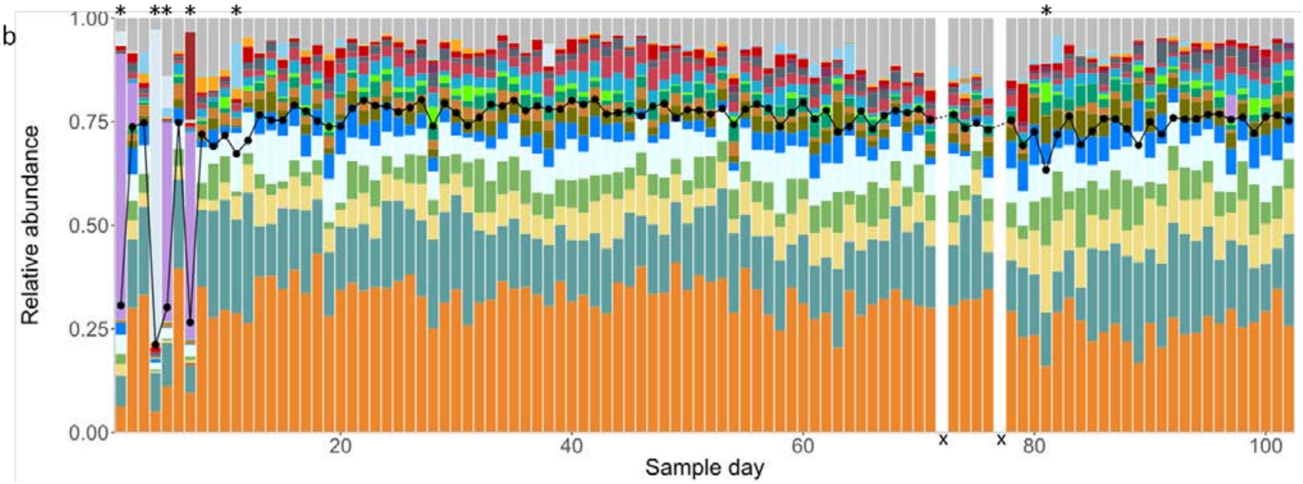
Baseline 1



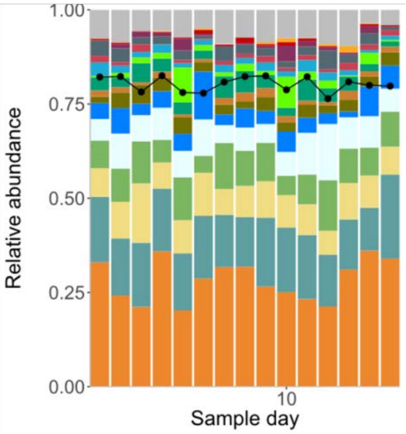
Baseline 2a



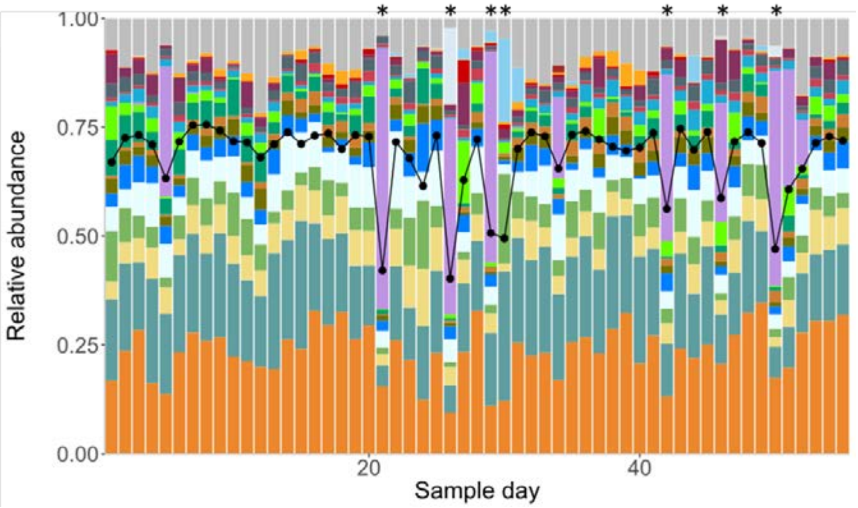
Baseline 2b



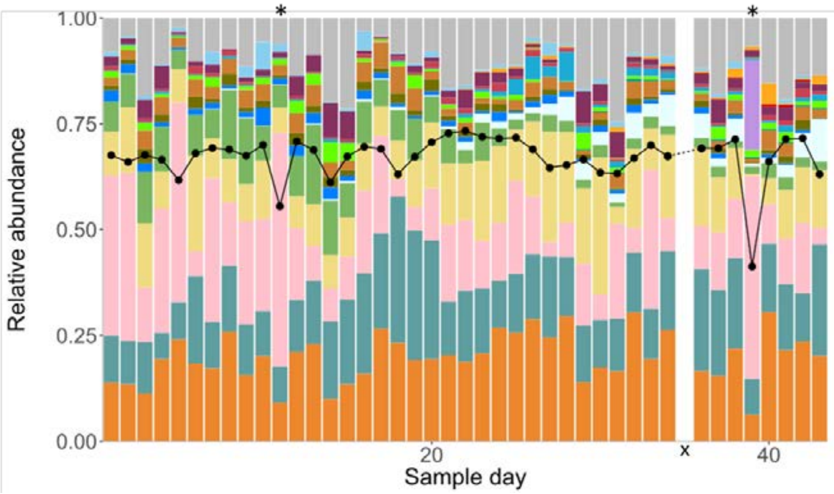
Baseline 2c



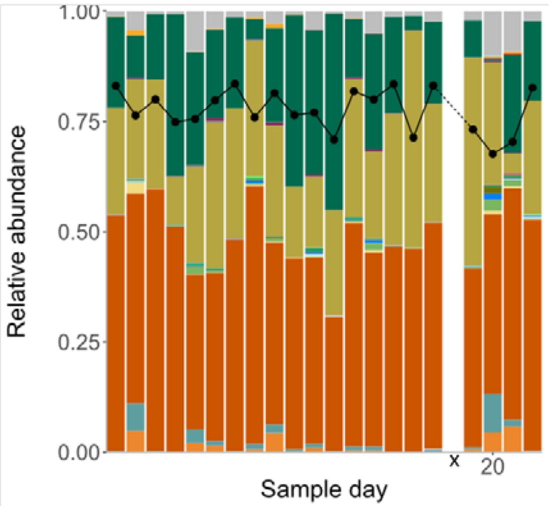
Baseline 2d



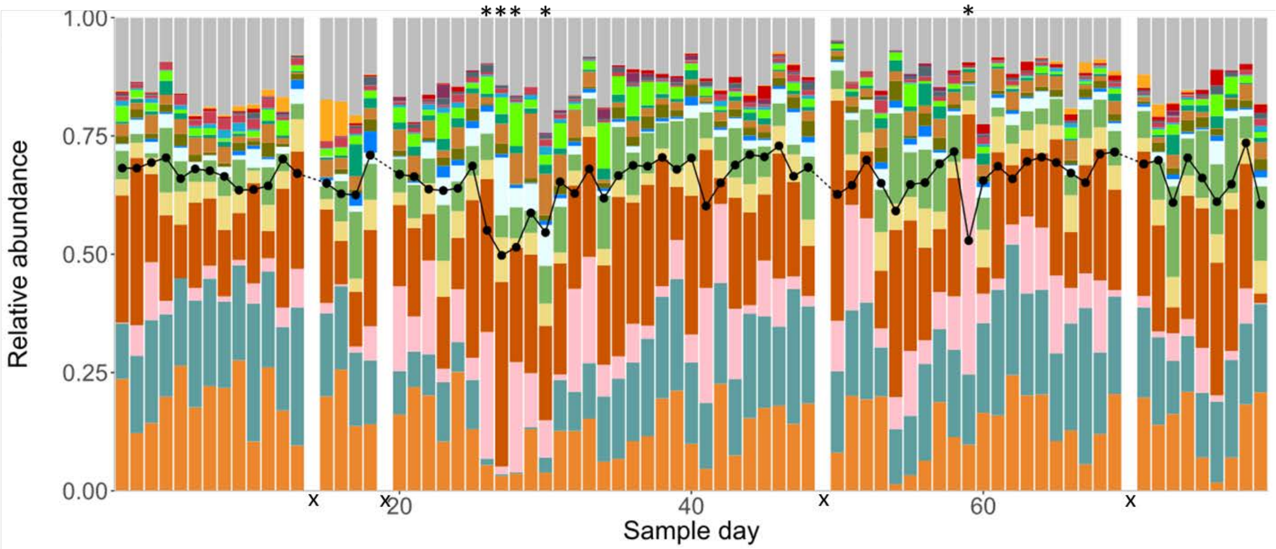
Baseline 3



Baseline 4



Baseline 5a



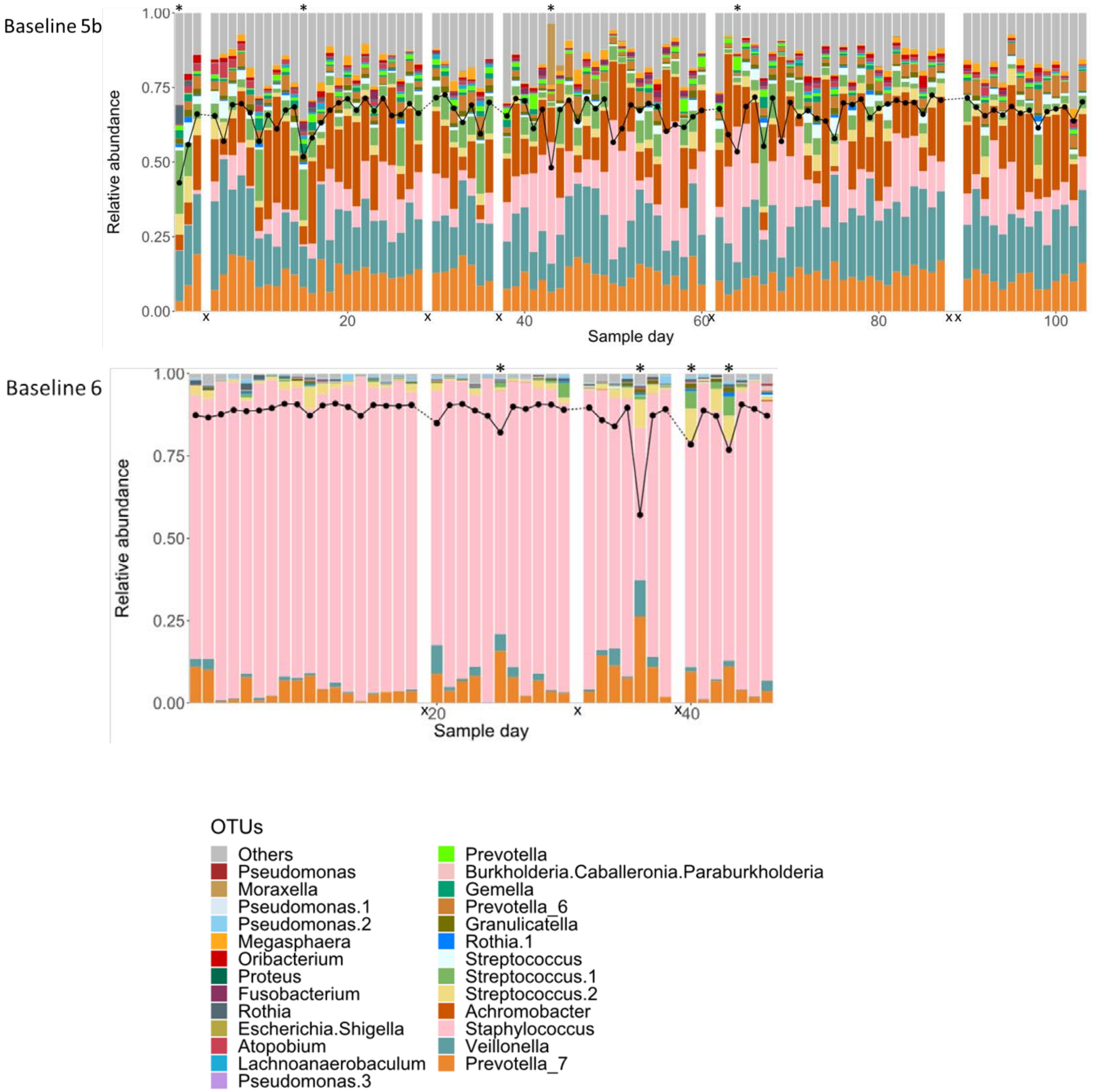

Baseline 5b
Relative abundance
1.00
0.75
0.50
0.25
0.00
*
*
*
*
x
20
x
x
40
60
x
80
x x
100
Sample day
Baseline 6
Relative abundance
1.00
0.75
0.50
0.25
0.00
*
*
*
*
x
20
x
x
40
Sample day
OTUs
Others
Pseudomonas
Moraxella
Pseudomonas.1
Pseudomonas.2
Megasphaera
Oribacterium
Proteus
Fusobacterium
Rothia
Escherichia.Shigella
Atopobium
Lachnoanaerobaculum
Pseudomonas.3
Prevotella
Burkholderia.Caballeronia.Paraburkholderia
Gemella
Prevotella_6
Granulicatella
Rothia.1
Streptococcus
Streptococcus.1
Streptococcus.2
Achromobacter
Staphylococcus
Veillonella
Prevotella_7

Figure E2. Stacked bar graphs of OTU relative abundances for samples within each baseline period. All days within each baseline period are depicted. OTUs listed in the color key are each either >0.5% mean relative abundance in the entire sample set, or >15% relative abundance in at least one sample. Black dots represent the mean of the Bray-Curtis pairwise-similarities of that sample to each other sample within the same baseline period. Dashed lines indicate non- continuous day linkage. ‘X’ below the graph indicates no available sample on that day. Asterisks above the graph indicate an outlier sample in beta diversity.

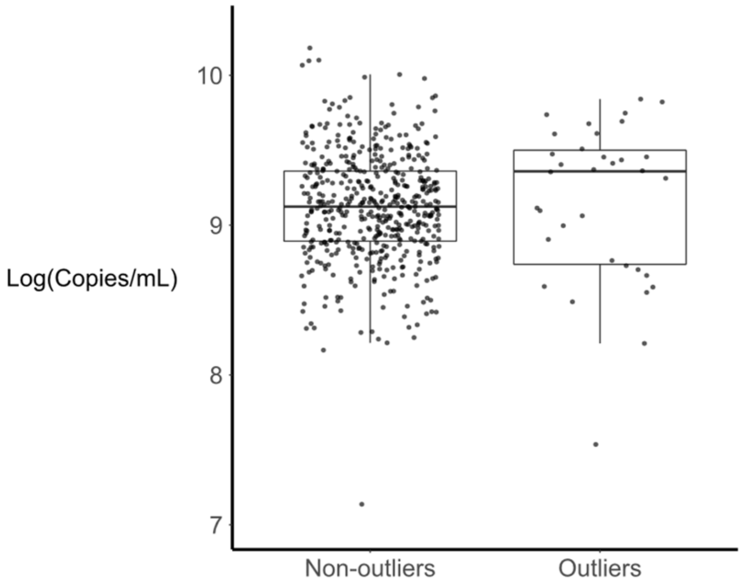


Figure E3. Comparisons of total bacterial load between outliers of beta diversity and non-outlier samples across all subjects and samples. Outliers of beta diversity are defined as samples outside of 1.5 times the interquartile range of the within-baseline period average pairwise Bray-Curtis similarity. Median and interquartile ranges are shown. Total bacterial load did not significantly differ between outliers and non-outliers (*P*=0.69, 95% CI [-0.09, 0.14], linear mixed model).

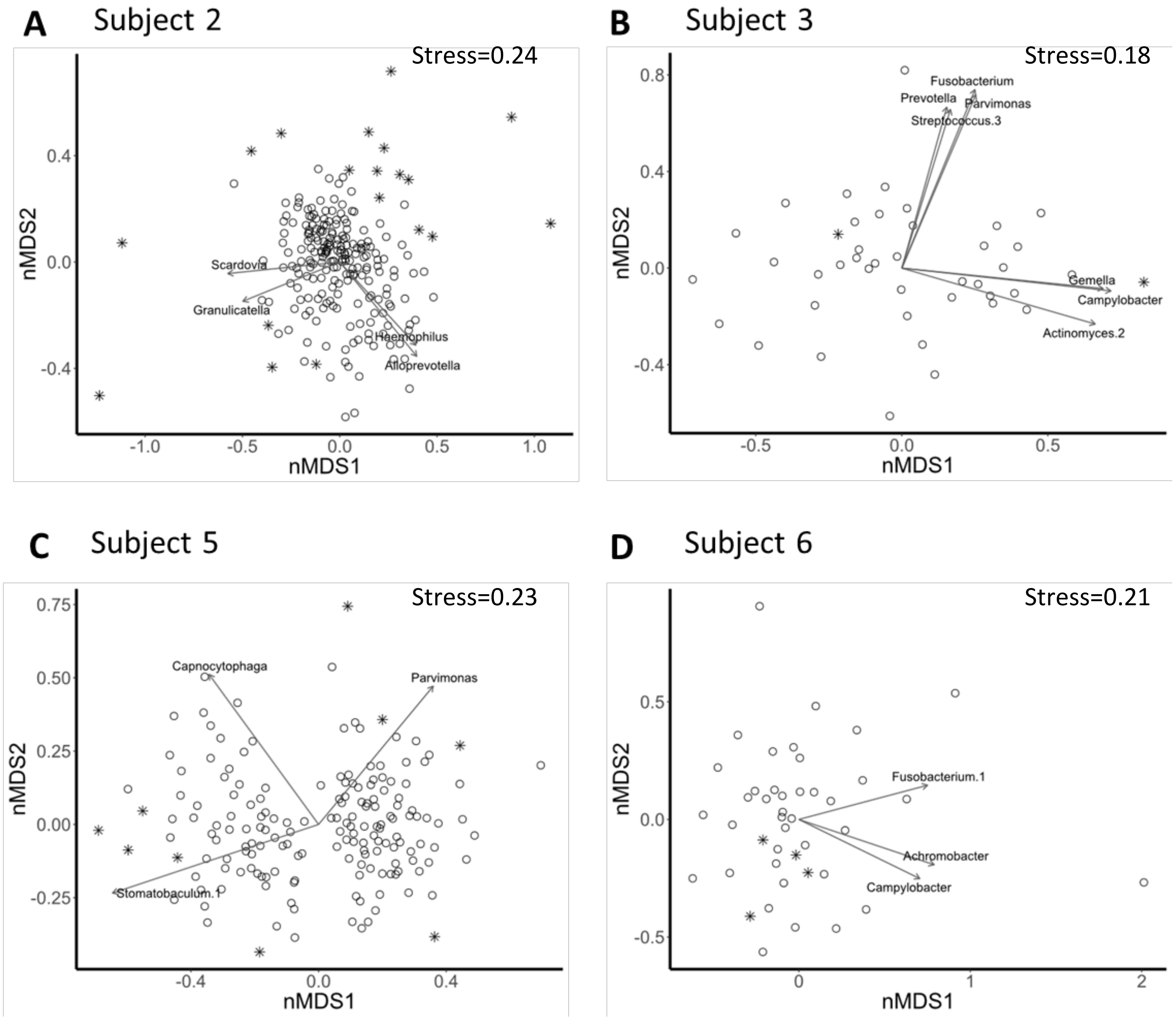


Figure E4. Within-subject comparison of community structure of outlier to non-outlier samples. Bray-Curtis based nMDS ordination showing all non-outlier (open circles) and outlier (asterisk) samples from subjects in whom outlier samples were identified. For each subject, arrows of the biplot show the top influential OTUs on the position of the samples in the ordination space. Within each subject, outlier samples do not consistently cluster together with respect to non-outlier samples. Across subjects, the arrows of the biplots do not indicate consistent OTUs driving the position of outliers.

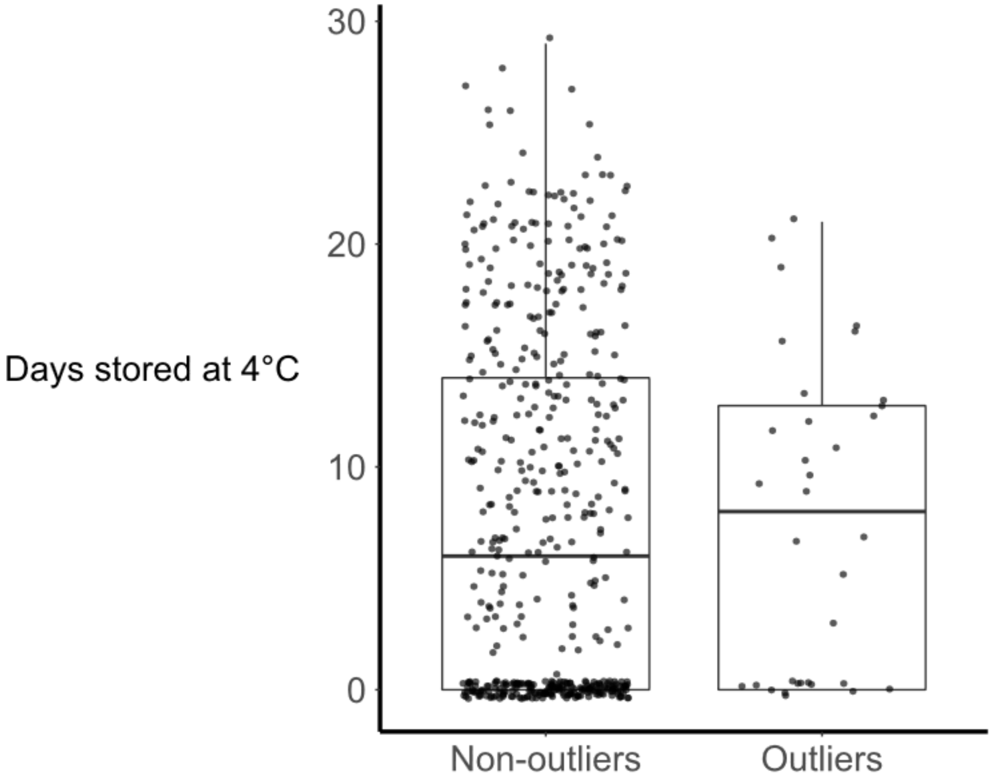


Figure E5. Comparison of time that each sample was stored at 4ºC between outliers of beta diversity and non-outlier samples, across all samples and subjects. Median and interquartile ranges are shown. Days stored at 4ºC did not significantly differ between outliers and non-outliers (*P*=0.59, 95% CI [-2.13, 1.21], linear mixed model).

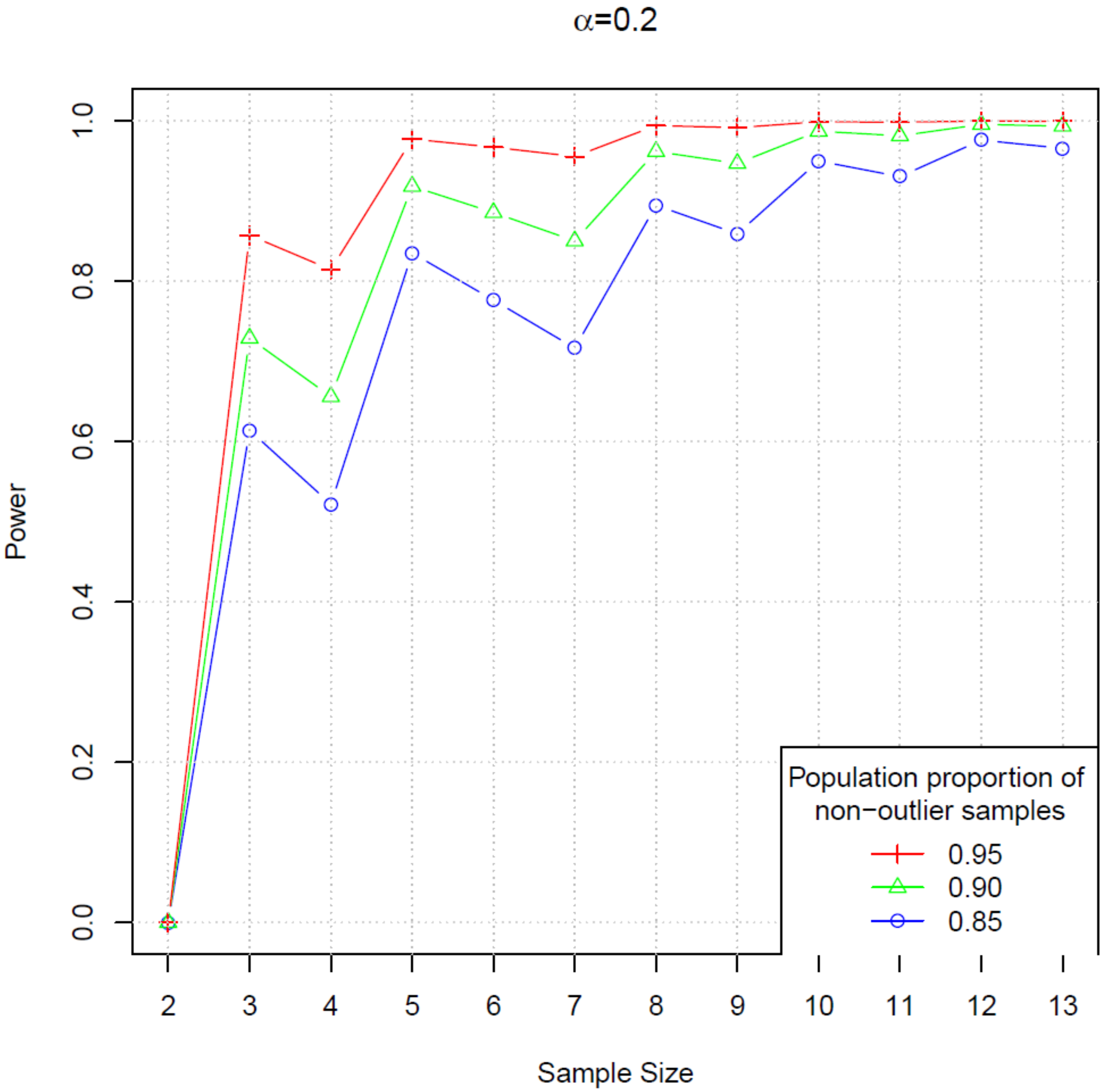


Figure E6. Power to determine whether the proportion of non-outlier samples is significantly greater than 0.5 across increasing sample sizes. Colors and symbols depict various proportions of non-outlier samples in a baseline period (e.g., red hashed line indicates that 95% of samples are non-outliers; 5% are outliers). Calculations performed with a one-sided binomial exact test, with α=0.2.

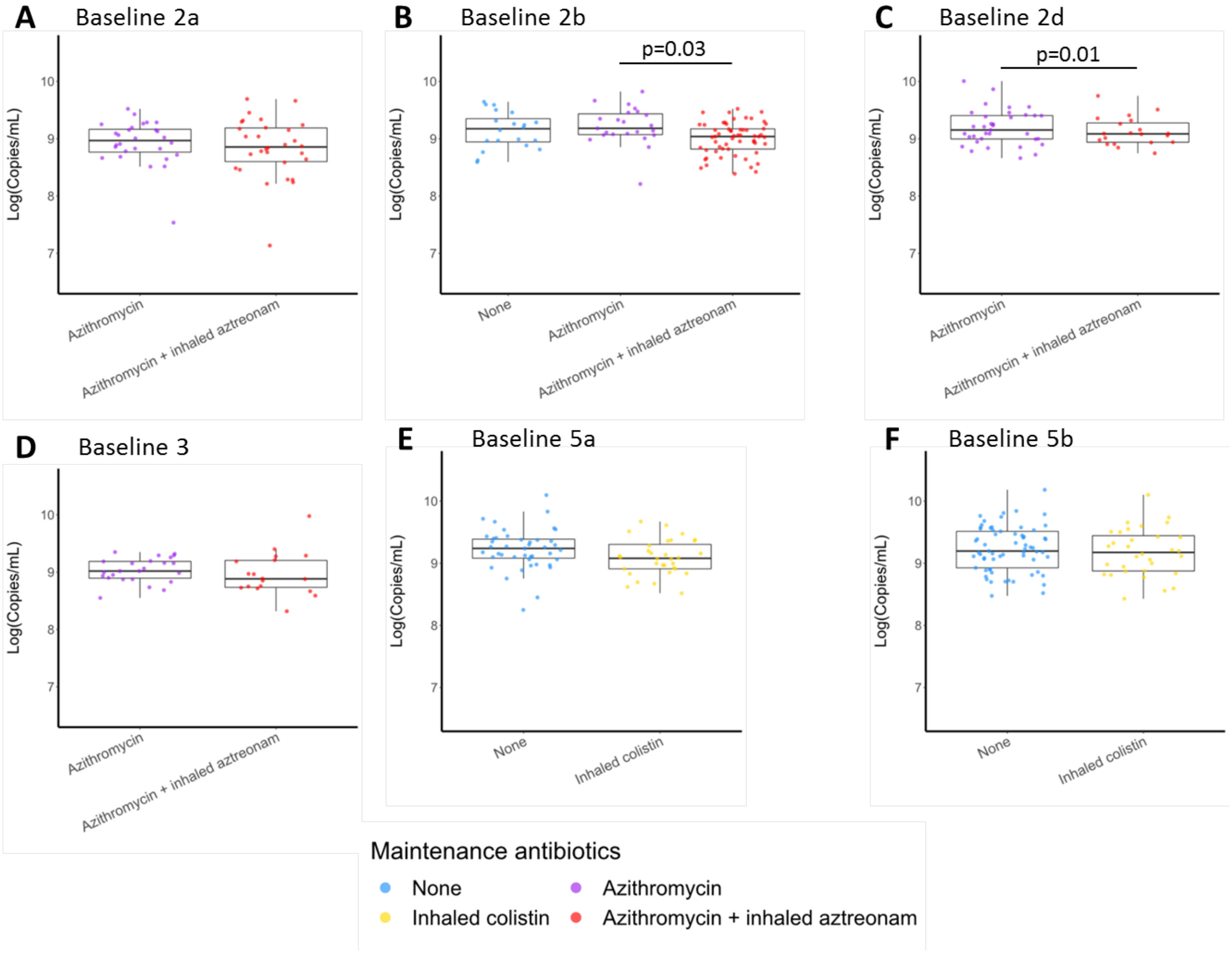


Figure E7. Total bacterial load of samples collected during different maintenance antibiotic regimens. Log_{10} transformed total bacterial load is compared between samples collected on different maintenance antibiotic regimens for the baseline period during which the subject had a change in maintenance antibiotic use. Median and interquartile ranges are shown. Total bacterial load did not significantly differ between maintenance antibiotic regimens in the majority of baseline periods ((A,D,E,F) *P* >0.05, (B) *P* =0.03, 95% CI [-0.41, -0.05], (C) *P*=0.01, 95% CI [-0.71, -0.09], generalized least-squares autoregressive model).

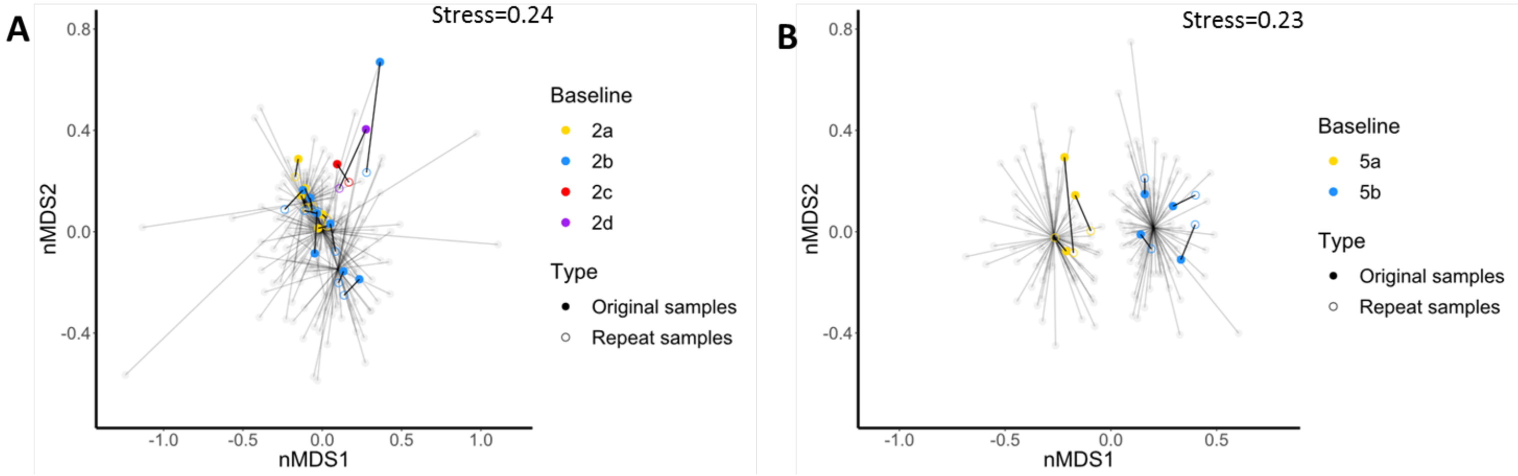


Figure E8. Comparison of sequencing run variation to within-subject changes in bacterial community structure over time. Bray-Curtis-based nMDS plots showing (A) subject 2 and (B) subject 5 with multiple baseline periods. Samples shown in Figure 4 are now shaded gray, except for those randomly selected for repeat 16S rRNA gene sequencing (colored closed circles). Repeat sequenced samples (colored open circles) cluster with the original baseline period, and do not consistently follow the direction of the movement of the baseline periods over time.

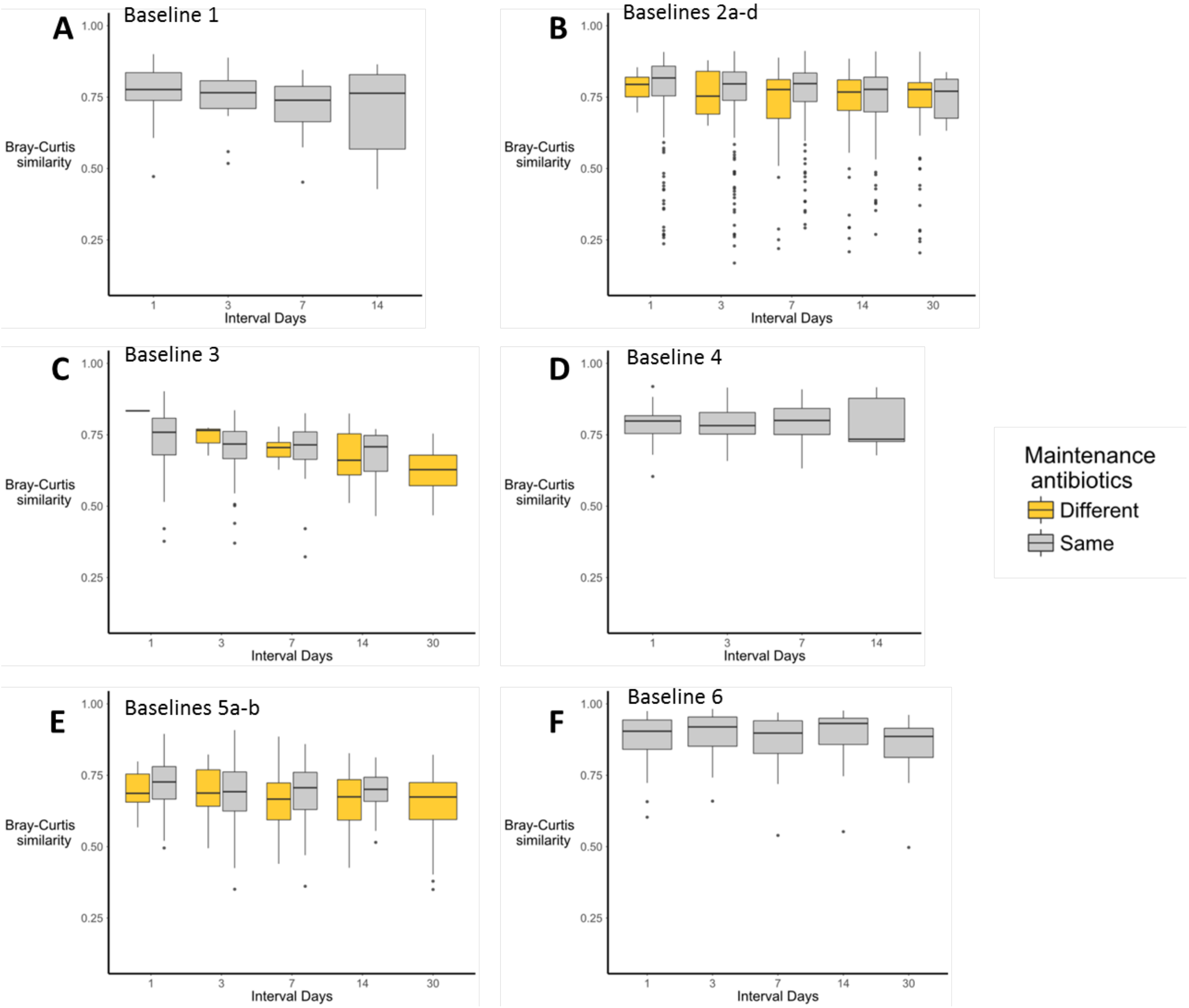


Figure E9. Sample-to-sample Bray-Curtis similarity at increasing sampling intervals for each subject. For subjects with changes in maintenance antibiotic regimens during the baseline period (B, C, E), pairwise comparisons of samples from different maintenance antibiotic regimens are shown separately from comparisons of samples from the same maintenance antibiotics regimens. Median and interquartile ranges are shown.