Microbiome Scale Theta Estimate Results

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Statement regarding the number of features per biological replicate/ pipeline Statement about MA plot

Consistent deviation across pipeline and titration from the expected θ values indicates our assumptions regarding the composition of the mixtures are not valid (Fig. 2). Our assumptions are that the DNA from the pre- and post-treatment samples were titrated following a log2 dilution series and that the pre- and post-treatment samples contained similar proportions of bacterial DNA. The qPCR spike-in results indicate that the unmixed pre- and post-treatment samples were mixed volumetrically according to our expectations. Based on the qPCR bacterial DNA concentration assays, the pre-treatment E01JH0038 sample had the lowest concentration of bacterial DNA between the five biological replicates and the bacterial DNA concentration was higher in the pre-treatment sample compared to the post-treatment sample for only E01JH0017 (Fig **qPCR Bacterial Concentration**).

Table 1: Number of features used to estimate theta by biological replicate and pipeline.

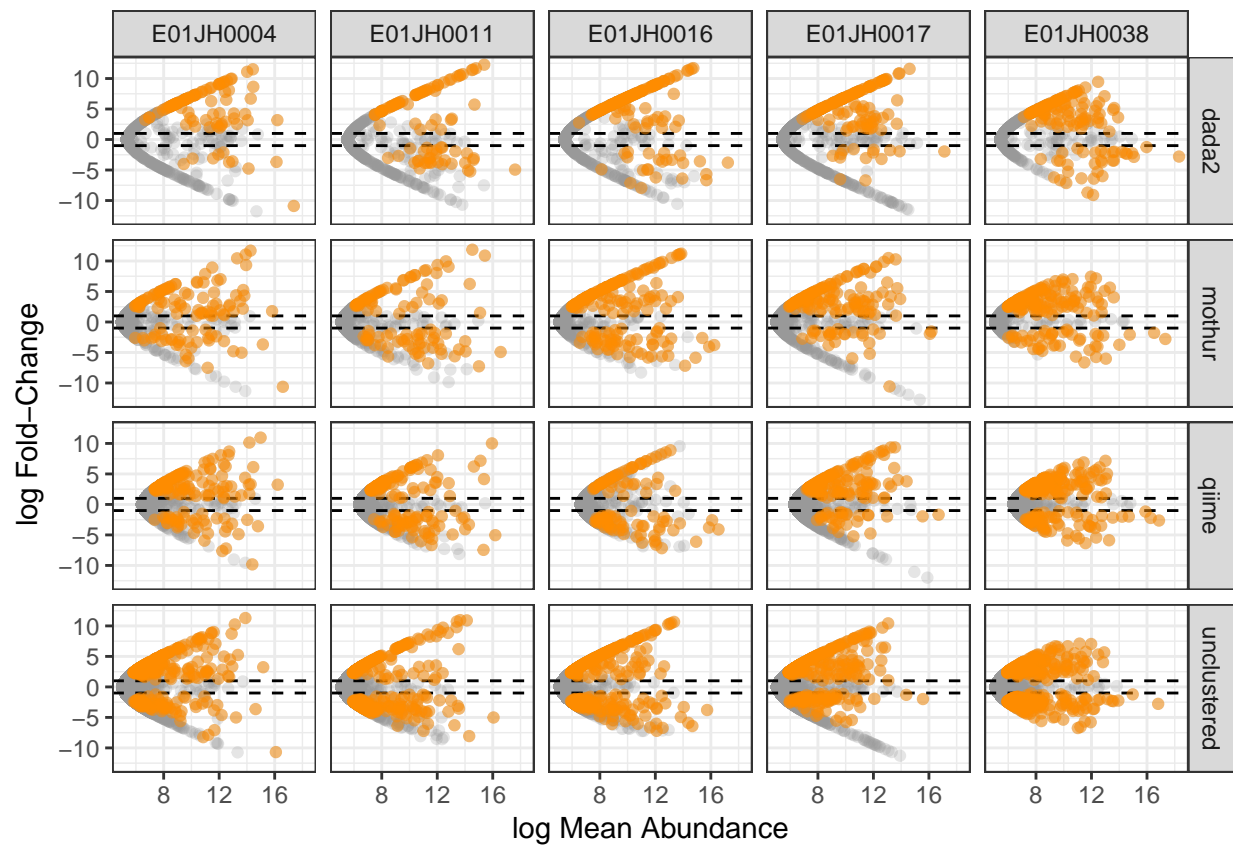| pipe	E01JH0004	E01JH0011	E01JH0016	E01JH0017	E01JH0038
dada2	90	90	144	136	130
mothur	114	104	178	149	177
qiime	145	146	106	155	204
unclustered	346	396	466	343	472

Figure 1: MA plot (x-axis mean abundance, y-axis log2 fold change between unmixed pre- and post-exposure samples. Orange points indicate features used to estimate theta. Dashed lines indicate the +/- 1 log2 fold change filter used when to pick features.
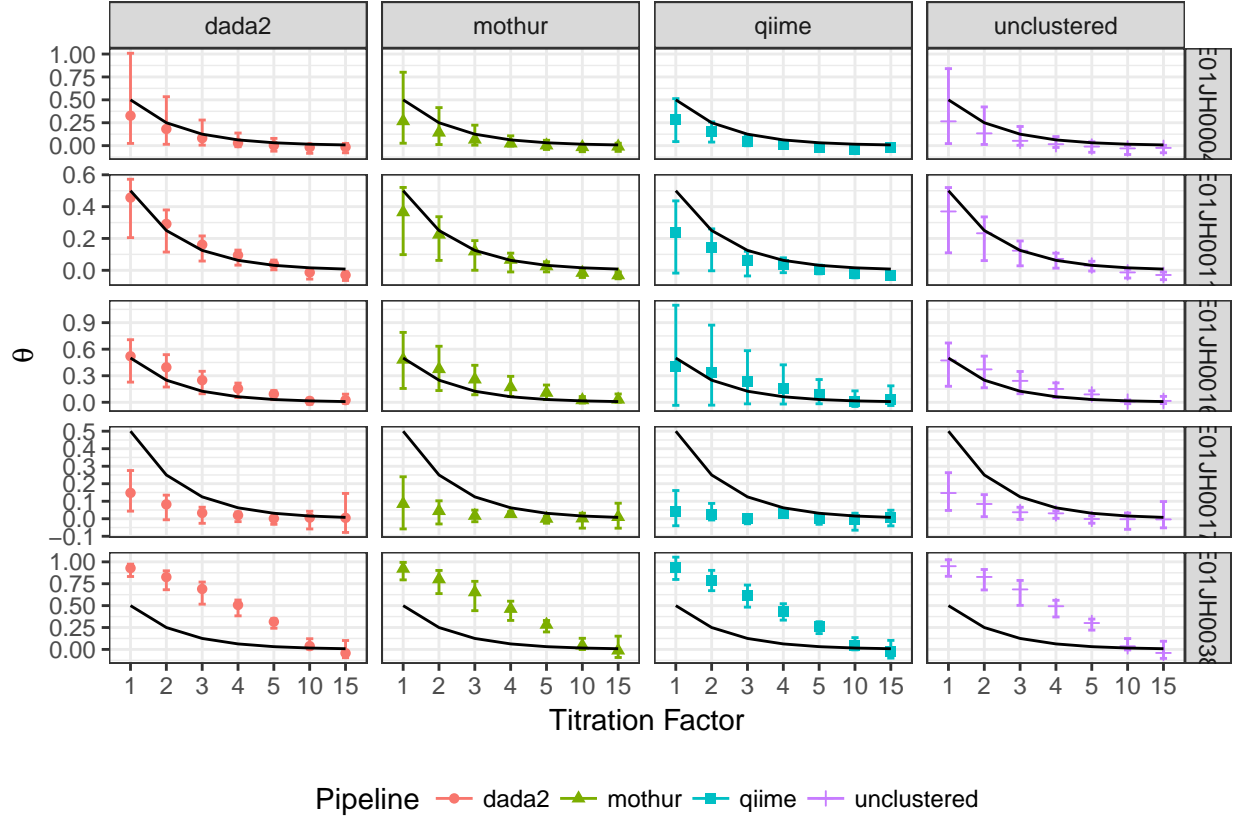
Figure 2: Theta estimates by titration, biological replicate, and bioinformatic pipeline. The points indicate mean estimate of 1000 bootstrap theta estimates and errorbars 95% confidence interval. The black line indicates the expected theta values. Theta estimates below the expected theta indicate that the titrations contains less than expected bacterial DNA from the post-treatment sample. Theta estimates greater than the expected theta indicate the titration contains more bacterial DNA from the pre-treatment sample than expected.