Power estimation for the multivariate microbiome data is complicated, especially when there is no standard/ consensus on how to estimate the effect size between groups. In this study, we assessed the variation in the community composition mainly by the pairwise distance metrics **Bray-Curtis dissimilarities**. The differences in microbiome between groups were then tested by PERMANOVA statistics, a permutation-based extension of the multivariate analysis of variance (ANOVA). Kelly et al. 2015[[1]](#footnote-1) developed a framework for the simulation and bootstrapping test to estimate the power of PERMANOVA test for microbiome study (R package “*micropower*”).

**Details about the statistical parameters are quoted from the reference as below:**

“The power of PERMANOVA, like the power of traditional analysis of variance, depends on the number of exposure or intervention groups (degrees of freedom), the number of subjects per group (residual degrees of freedom), the within-group distances (within-group sum of squares) and the size of the effect (the difference between the between-group sum of squares and within-group sum of squares). Type II error increases and statistical power decreases with more groups, fewer subjects, greater within-group distance and lesser effects (Zar, 1999). However, the pseudo-F ratio is not distributed like Fisher’s F-ratio under the null hypothesis, so standard methods of power estimation for parametric ANOVA do not apply to the studies analyzed by PERMANOVA (Anderson, 2001)…. As with the conventional ANOVA, the effect size of the pseudo F-ratio can be quantified as the coefficient of determination (R2). However, the R2, which is the proportion of distance accounted for by the grouping factor, is biased because it depends solely on the sums of squares of the sample, without adjustment to estimate the effect size in the general population. Omega-squared (**ω2**) provides a less biased measure of effect size for ANOVA-type analyses by accounting for the mean-squared error of the observed samples,”

The effect size Omega-squared parameter was calculated using the function *calcOmega2*. Power of the PERMANOVA test was retrospectively evaluated from our data using the *bootDM* and *calcPERMANOVp* functions in the micropower R package. During which we randomly selected bootstrap samples with replacement from our Bray-Curtis distance matrix at the specified number of subjects and groupings and repeated the selection procedure for 100 times. PERMANOVA tests (1000 permutation) were then performed on each bootstrap distance matrix. Power was then calculated as the proportion of bootstrap statistical tests for *p*-values that are less than alpha (type I error 0.05), the results are summarized in the table below.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **PERMANOVA (α =0.05)** | **DF** | **pseudo-F** | **R2** | ***p*** | **ω2** | **power** |
| **All\_Control\_vs\_Test** | **1** | **2.3809** | **0.0471** | **0.00108** | **0.033** | **1** |
| Baseline\_Control\_vs\_Test | 1 | 1.0373 | 0.124 | 0.0558 | 0.0657 | 0.61 |
| Wk2\_Control\_vs\_Test | 1 | 0.9586 | 0.113 | 0.0462 | 0.0543 | 0.71 |
| **Wk4\_Control\_vs\_Test** | **1** | **1.818** | **0.159** | **0.00419** | **0.101** | **0.99** |
| Wk14\_Control\_vs\_Test | 1 | 0.90287 | 0.113 | 0.0445 | 0.0547 | 0.7 |
| Control\_Wk2\_vs\_Wk4 | 1 | 0.35733 | 0.0812 | 0.26 | 0.0242 | 0.1 |
| **Test\_Wk2\_vs\_Wk4** | **1** | **1.4356** | **0.138** | **0.0205** | **0.0798** | **0.88** |

Note1- the PERMANOVA test statistics (based on 1000 permutation) reported here is slightly different from the results from the manuscript (supplementary table) due to randomness involved in the UniFrac distance calculation and the PERMANOVA permutation.

Note2- The extrapolation estimation of power with increased sample size may not be accurate, as we did not account for the addition of new taxa with increase sampling.

1. Kelly, B.J., Gross, R., Bittinger, K., Sherrill-Mix, S., Lewis, J.D., Collman, R.G., Bushman, F.D. and Li, H., 2015. Power and sample-size estimation for microbiome studies using pairwise distances and PERMANOVA. Bioinformatics, 31(15), pp.2461-2468. [↑](#footnote-ref-1)