Supplemental Material on "Ensemble test for microbiome data"

Deliang Bu, Jingxin Yan, Wanshuo Yang, Xiaoyu Zhang, Qizhai Li

In this Supplemental Material, we provide the following results. Section 1 gives the calculations of distances and similarity matrix. Section 2 gives the theoretical proof for calculating the first three moments of $T_{d,r}$ under null hypothesis. In Section 3, the quantile-quantile plot is used to examine the approximate accuracy of Pearson type III distribution. In Section 4, we provide the simulation result for scenarios of binary phenotypes.

1 Calculation of distances and similarity matrix

In this section, we briefly introduce the distance used in this paper for measuring the similarity between samples in the context of microbiome data and the detailed calculation for the similarity matrix.

When phylogeny tree information is available, tree-based weighted UniFrac distance is the most commonly used distance measure[1]. Let p_{il} and p_{jl} denote the proportion of the OTUs descending from branch $l(l = 1, 2..., L)$ for samples i and $j(i, j = 1, 2, ..., n)$, respectively. The weighted UniFrac distances $d_{ij,1}$ between samples i and j are defined as

$$d_{ij,1} = \frac{\sum_{l=1}^{L} b_l (p_{il} + p_{jl})^{\alpha} |p_{il} - p_{jl}|}{\sum_{l=1}^{L} b_l (p_{il} + p_{jl})^{\alpha}},$$

here $\alpha \in [0, 1]$. We use $\alpha = 0.5$ throughout this paper, because it is considered more robust compare to other value of α[1]. Another commonly used tree based distance is the unweighted UniFrac distance[2], which is defined as

$$d_{ij,2} = \frac{\sum_{l=1}^{L} b_l |I(p_{il} > 0) - I(p_{jl} > 0)|}{\sum_{l=1}^{L} b_l},$$

here $I(.)$ is an indicator function. The unweighted UniFrac distance complete ignore the abundance of OTUs and only compare the presence/absence information.

Bray-Curtis distance[3] is the most commonly used distance without using the phylogeny tree information. Let p_{ik} and p_{jk} denote the abundance of OTU $k(k = 1, 2, ...K)$ in samples i and $j(i, j = 1, 2, ..., n)$, respectively. Bray-Curtis distance between sample j and k, denote as $d_{ij,2}$, is defined as

$$d_{ij,3} = \frac{\sum_{k=1}^{K} |p_{ik} - p_{jk}|}{\sum_{k=1}^{K} (p_{ik} + p_{jk})}.$$

Each distance has its features, and the choice of distance metrics will affect the final power of the association test. Throughout the paper we use the aforementioned three distance because they are the most commonly used. Denote $\boldsymbol{D} = (d_{ij})_{n\times n}$ the distance matrix regarding the K dimensional variable among the n subjects calculated based on the distances mentioned above, where d_{ij} is the distance between the ith and jth subject, $i, j = 1, 2, \cdots n$. Then the similarity matrix denotes as $\boldsymbol{S} = (s_{ij})_{n\times n}$ can be calculated as $s_{ij} = -\frac{1}{2}d_{ij}^2$.

2 P-value calculation of $T_{d,r}$

Normally, the permutation procedure is needed for PERMANOVA-based method, which is computationally expensive and generates different results run multiple times on the same dataset. This variability arises due to their reliance on generating random samples to compute permutation null distributions. E-MANOVA can completely avoid intensive computation procedure and generate the same result with the same data.

Recall that the E-MANOVA test statistics with fix d and r in main text section 2.3 is

$$T_{d,r} = tr\{(\boldsymbol{H}_{\boldsymbol{X}} - \boldsymbol{H}_{\boldsymbol{X}_2})(\boldsymbol{K}^*)^r\}.$$

It can further be rewritten in the form of

$$T_{d,r} = tr(\boldsymbol{A}\boldsymbol{W}),$$

where $\boldsymbol{A} = \boldsymbol{H}_{\boldsymbol{X}} - \boldsymbol{H}_{\boldsymbol{X}_2}$ and $\boldsymbol{W} = \boldsymbol{H}(\boldsymbol{K}^*)^r\boldsymbol{H}$. It is easily to be proven since $\boldsymbol{H}$ is a centering matrix and all the columns of $\boldsymbol{X}$ are already centered, thus $\boldsymbol{H}\boldsymbol{X} = \boldsymbol{X}$ and $\boldsymbol{H}\boldsymbol{X}_2 = \boldsymbol{X}_2$. To avoid the potential computational burden, we adopt an alternative strategy based on the result derived in [4] that can directly calculate the moments of permuted null distribution without generating its null distribution and approximating the empirical null distribution with a known distribution that matches its moments. The following lemma 1 proves the properties of matrix $\boldsymbol{W}$ and Theorem 1 establishes the first three moments of $T_{d,r}$ without using permutation.

Lemma 1: *Denote $\boldsymbol{E}_{ij}$, $i \neq j$ as a $n \times n$ matrix exchanging ith row and jth row of identity matrix $\boldsymbol{I}_n$. Then,*

$$\boldsymbol{W}\big|_{i\leftrightarrow j} = \boldsymbol{E}_{ij}\boldsymbol{W}\boldsymbol{E}_{ij},$$

here $\boldsymbol{W}\big|_{i\leftrightarrow j}$ indicates $\boldsymbol{W}$ switching ith sample and jth samples.

Proof: Since the elements in $\boldsymbol{S}$, denote as s_{ij}, represent the similarity between ith and jth subjects. Thus, it is easy to see that

$$\boldsymbol{S}\big|_{i\leftrightarrow j} = \boldsymbol{E}_{ij}\boldsymbol{S}\boldsymbol{E}_{ij}, i, j = 1, 2, \cdots, n.$$

Since $\boldsymbol{H}$ is a symmetric matrix, we have

$$\boldsymbol{H}\boldsymbol{E}_{ij}\boldsymbol{S}\boldsymbol{E}_{ij}\boldsymbol{H} = \boldsymbol{E}_{ij}\boldsymbol{H}\boldsymbol{S}\boldsymbol{H}\boldsymbol{E}_{ij}.$$

$\boldsymbol{HSH}$ can be decomposed as $\boldsymbol{HSH} = \boldsymbol{Q}\boldsymbol{\Lambda}\boldsymbol{Q}^\top$ where $\boldsymbol{Q}$ is an orthogonal matrix whose columns are the orthogonal eigenvectors of $\boldsymbol{HSH}$, and $\boldsymbol{\Lambda}$ is a diagonal matrix whose entries are the eigenvalues of $\boldsymbol{HSH}$. We have

$$\boldsymbol{E}_{ij}\boldsymbol{H}\boldsymbol{S}\boldsymbol{H}\boldsymbol{E}_{ij} = \boldsymbol{E}_{ij}\boldsymbol{Q}\boldsymbol{\Lambda}\boldsymbol{Q}^\top\boldsymbol{E}_{ij}.$$

The matrix $\boldsymbol{K}^*$ is obtained by $\boldsymbol{K}^* = \boldsymbol{Q}\boldsymbol{\Lambda}^*\boldsymbol{Q}^\top$, with $\boldsymbol{\Lambda}^* = diag(|\lambda_1|, |\lambda_2|, \cdots, |\lambda_n|)$. Thus, we can conclude that

$$\boldsymbol{K}^*_{i\leftrightarrow j} = \boldsymbol{E}_{ij}\boldsymbol{K}^*\boldsymbol{E}_{ij}.$$

Next, since $\boldsymbol{W} = \boldsymbol{H}(\boldsymbol{K}^*)^r\boldsymbol{H}$, we have

$$(\boldsymbol{K}^*_{i\leftrightarrow j})^r = (\boldsymbol{E}_{ij}\boldsymbol{K}^*\boldsymbol{E}_{ij})^r.$$

Then, we can write that

$$(\boldsymbol{E}_{ij}\boldsymbol{K}^*\boldsymbol{E}_{ij})^r = (\boldsymbol{E}_{ij}\boldsymbol{Q}\boldsymbol{\Lambda}^*\boldsymbol{Q}^\top\boldsymbol{E}_{ij})^r = ((\boldsymbol{E}_{ij}\boldsymbol{Q})\boldsymbol{\Lambda}^*(\boldsymbol{E}_{ij}\boldsymbol{Q}^\top))^r,$$

where $\boldsymbol{Q}$ is orthonormal, resulting in that $\boldsymbol{E}_{ij}\boldsymbol{Q}$ is the eigenvectors of $\boldsymbol{E}_{ij}\boldsymbol{K}^*\boldsymbol{E}_{ij}$. Thus, by the definition of the rth power of matrix, we can conclude that

$$(\boldsymbol{E}_{ij}\boldsymbol{K}^*\boldsymbol{E}_{ij})^r = (\boldsymbol{E}_{ij}\boldsymbol{Q}(\boldsymbol{\Lambda}^*)^r\boldsymbol{Q}^\top\boldsymbol{E}_{ij}).$$

Finally, we conclude the proof that

$$\boldsymbol{W}_{i\leftrightarrow j} = \boldsymbol{H}(\boldsymbol{E}_{ij}\boldsymbol{Q}(\boldsymbol{\Lambda}^*)^r\boldsymbol{Q}^\top\boldsymbol{E}_{ij})\boldsymbol{H} = \boldsymbol{E}_{ij}\boldsymbol{W}\boldsymbol{E}_{ij}.$$

The last step is because $\boldsymbol{H}$ is symmetric, thus $\boldsymbol{H}$ and $\boldsymbol{E}_{ij}$ are exchangeable.

Lemma 1 indicates switching ith sample with jth of sample of $\boldsymbol{W}$ is equal to exchanging both rows and columns i and j. With this propriety of $\boldsymbol{W}$, we can directly use the theorem 1 to calculate the first three moments of $T_{d,r}$ without permutation.

Theorem 1: *Let $\boldsymbol{A}$ be a symmetric matrix, with $\boldsymbol{A}\mathbf{1}_n = 0$, $\boldsymbol{W}$ is a symmetric matrix and satisfies the condition that switching ith sample with jth sample is equivalent to exchanging both rows and columns i and j of $\boldsymbol{W}$. Then the first three moments of the test statistics $T = tr(\boldsymbol{AW})$ can be directly calculated in close form. Denote the first three moments of T considering all $n!$ permutations as $E_p(T)$, $Var_p(T)$, and $E_p(T^3)$, we have*

$$E_p(T) = \frac{\mathrm{tr}(\boldsymbol{A})\,\mathrm{tr}(\boldsymbol{W})}{n-1},$$

$$\begin{aligned} Var_p(T) &= \frac{2((n-1)L_2 - L^2)((n-1)\widetilde{L}_2 - \widetilde{L}^2)}{(n-1)^2(n+1)(n-2)} \\ &+ \frac{(n(n+1)M_2 - (n-1)(L^2+2L_2))(n(n+1)\widetilde{M_2} - (n-1)(\widetilde{L}^2+2\widetilde{L}_2))}{(n+1)n(n-1)(n-2)(n-3)}, \end{aligned}$$

$$\begin{aligned} &n(n-1)(n-2)(n-3)(n-4)(n-5)E_p(T^3) = n^2(n+1)(n^2+15n-4)M_3\widetilde{M_3} \\ &+ 4(n^4-8n^3+19n^2-4n-16)U\widetilde{U} + 24(n^2-n-4)(U\widetilde{B}+B\widetilde{U}) + 6(n^4-8n^3+21n^2-6n-24)B\widetilde{B} \\ &+ 12(n^4-n^3-8n^2+36n-48)R\widetilde{R} + 12(n^3-2n^2+9n-12)(LM_2\widetilde{R} + R\widetilde{L}\widetilde{M_2}) \\ &+ 3(n^4-4n^3-2n^2+9n-12)L\widetilde{L}M_2\widetilde{M_2} + 24\{(n^3-3n^2-2n+8)(R\widetilde{U}+\widetilde{U}R) \\ &+ (n^3-2n^2-3n+12)(R\widetilde{B}+B\widetilde{R})\} + 12(n^2-n+4)(LM_2\widetilde{U} + U\widetilde{L}\widetilde{M_2}) \\ &+ 6(2n^3-7n^2-3n+12)(LM_2\widetilde{B} + B\widetilde{L}\widetilde{M_2}) - 2n(n-1)(n^2-n+4)\{(2U+3B)\widetilde{M_3} + (2\widetilde{U}+3\widetilde{B})M_3\} \\ &- 3n(n-1)^2(n+4)\{(LM_2+4R)\widetilde{M_3} + (\widetilde{L}\widetilde{M_2}+4\widetilde{R})M_3\} + 2n(n-1)(n-2)\{(L^3+6LL_2+8L_3)\widetilde{M_3} \\ &+ (\widetilde{L}^3+6\widetilde{L}\widetilde{L_2}+8\widetilde{L_3})M_3\} + L^3((n^3-9n^2+23n-14)\widetilde{L}^3 + 6(n-4)\widetilde{L}\widetilde{L_2} + 8\widetilde{L_3}) + 6LL_2((n-4)\widetilde{L}^3 \\ &+ (n^3-9n^2+24n-14)\widetilde{L}\widetilde{L}_2 + 4(n-3)\widetilde{L_3}) + 8L_3(\widetilde{L}^3 + 3(n-3)\widetilde{L}\widetilde{L}_2 + (n^3-9n^2+26n-22)\widetilde{L_3}) \\ &- 16(L^3\widetilde{U} + U\widetilde{L}^3) - 6(LL_2\widetilde{U} + U\widetilde{L}\widetilde{L_2})(2n^2-10n+16) - 8(L_3\widetilde{U} + U\widetilde{L_3})(3n^2-15n+16) \\ &- (L^3\widetilde{B} + B\widetilde{T^3})(6n^2-30n+24) - 6(LL_2\widetilde{B} + B\widetilde{L}\widetilde{L_2})(4n^2-20n+24) \\ &- 8(L_3\widetilde{B} + B\widetilde{L_3})(3n^2-15n+24) - (n-2)\{24(L^3\widetilde{R} + R\widetilde{L}^3) + 6(LL_2\widetilde{R} + R\widetilde{L}\widetilde{L_2})(2n^2-10n+24) \\ &+ 8(L_3\widetilde{R} + R\widetilde{L_3})(3n^2-15n+24) + (3n^2-15n+6)(L^3\widetilde{L}\widetilde{M_2} + LM_2\widetilde{L}^3) \\ &+ 6(LL_2\widetilde{L}\widetilde{M_2} + LM_2\widetilde{L}\widetilde{L_2})(n^2-5n+6) + 48(L_3\widetilde{L}\widetilde{M_2} + LM_2\widetilde{L_3})\}, \end{aligned}$$

where $L = tr(\boldsymbol{A})$, $L_2 = tr(\boldsymbol{A}^2)$, $L_3 = tr(\boldsymbol{A}^3)$, $\widetilde{L} = tr(\boldsymbol{W})$, $\widetilde{L}_2 = tr(\boldsymbol{W}^2)$, $\widetilde{L}_3 = tr(\boldsymbol{W}^3)$, $M_2 = \sum_{i=1}^n a_{ii}^2$, $M_3 = \sum_{i=1}^n a_{ii}^3$, $\widetilde{M_2} = \sum_{i=1}^n w_{ii}^2$, $\widetilde{M_3} = \sum_{i=1}^n w_{ii}^3$, $U = \sum_{i=1}^n \sum_{i=1}^n a_{ij}^3$, $\widetilde{U} = \sum_{i=1}^n \sum_{i=1}^n w_{ij}^3$, $R = (diag(\boldsymbol{A}))^\top (diag(\boldsymbol{A}^2))$, $B = (diag(\boldsymbol{A}))^\top \boldsymbol{A}(diag(\boldsymbol{A}))$, $\widetilde{R} = (diag(\boldsymbol{W}))^\top (diag(\boldsymbol{W})^2)$, $\widetilde{B} = (diag(\boldsymbol{W}))^\top \boldsymbol{W}(diag(\boldsymbol{W}))$.

The proof of theorem 1 is similar to [4] after rewriting $T_{d,r}$ as $T_{d,r} = tr(\boldsymbol{AW})$, where $\boldsymbol{A}$ and $\boldsymbol{W}$ satisfy the condition of theorem 1. We can then calculate the close from expression of the three moments of $T_{d,r}$ without using any permutation procedure.

3 Approximation accuracy

In this section, we use quantile-quantile plot to demonstrate the approximation of Pearson type III distribution for $T_{d,r}$ with different values of d and r. From the figures, we can see that the approximation performs well enough with different d and r.

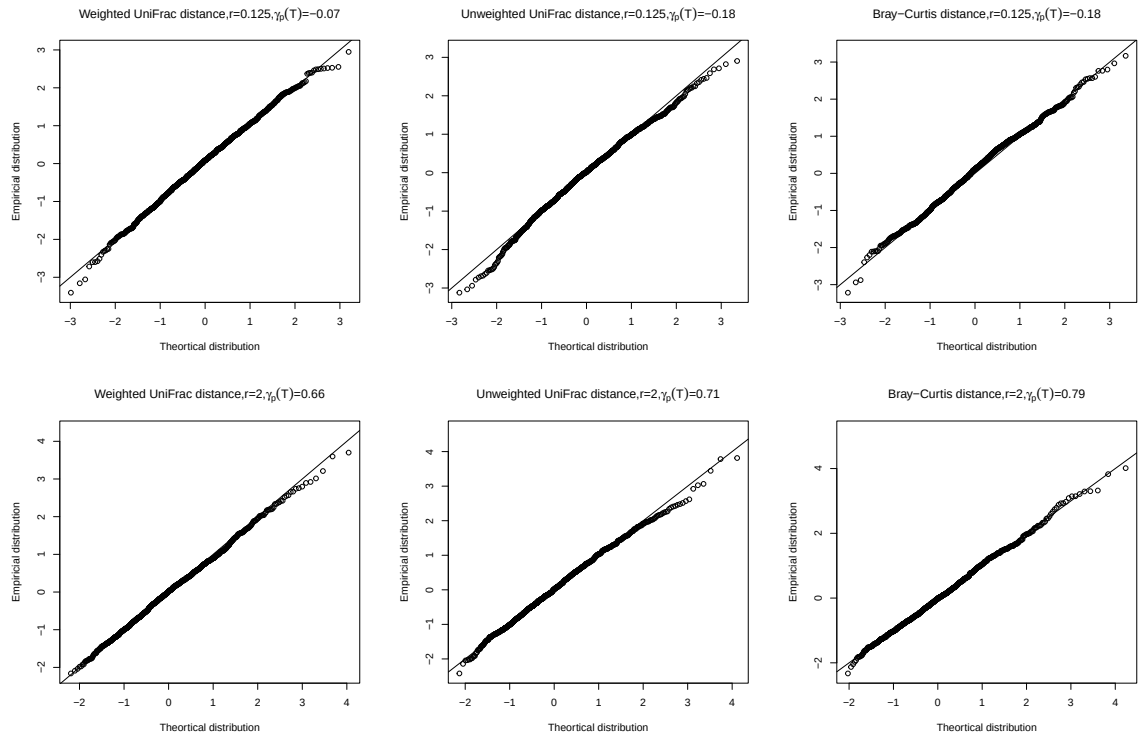


Figure 1: The empirical distribution of $T_{d,r}$ and theoretical Pearson type III distribution with parameter estimated based on the method in Section 1. Different scenarios were considered with binary outcome variable, $r = 0.125, 2$ and $d = 1, 2, 3$ represent two different distances (weight UniFrac distance, Unweighted UniFrac distance and Bray-Curtis distance). All quantile-quantile plots are drawn based on 1000 random samples.

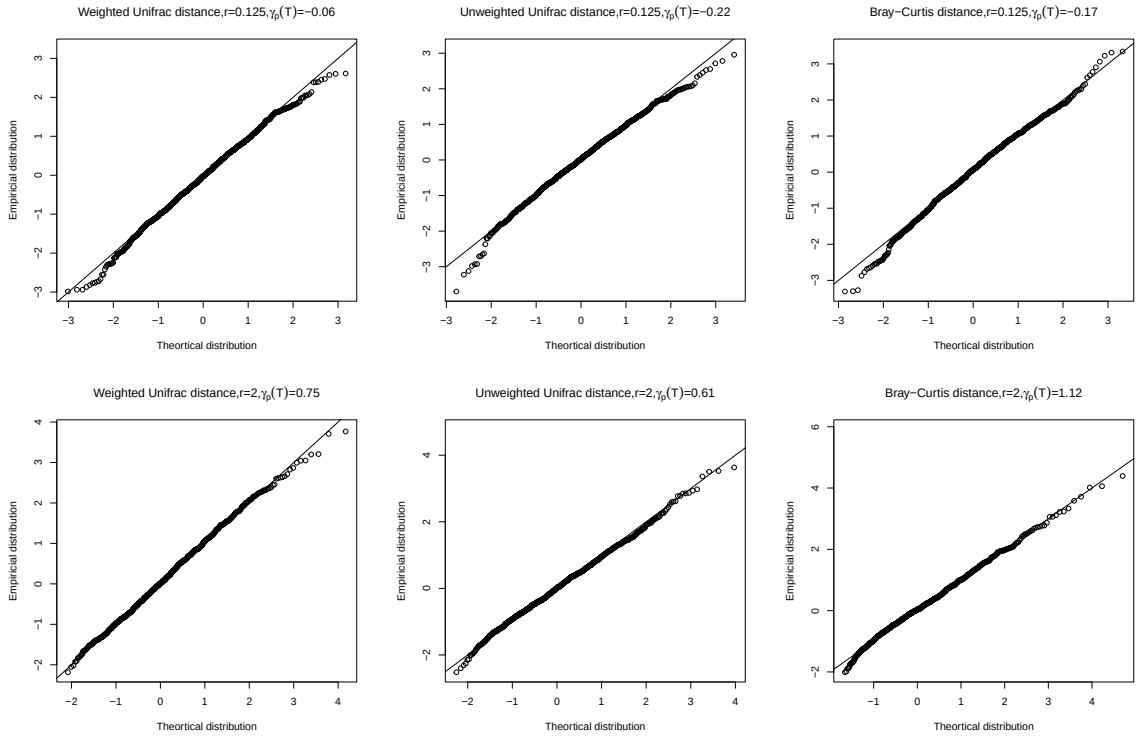


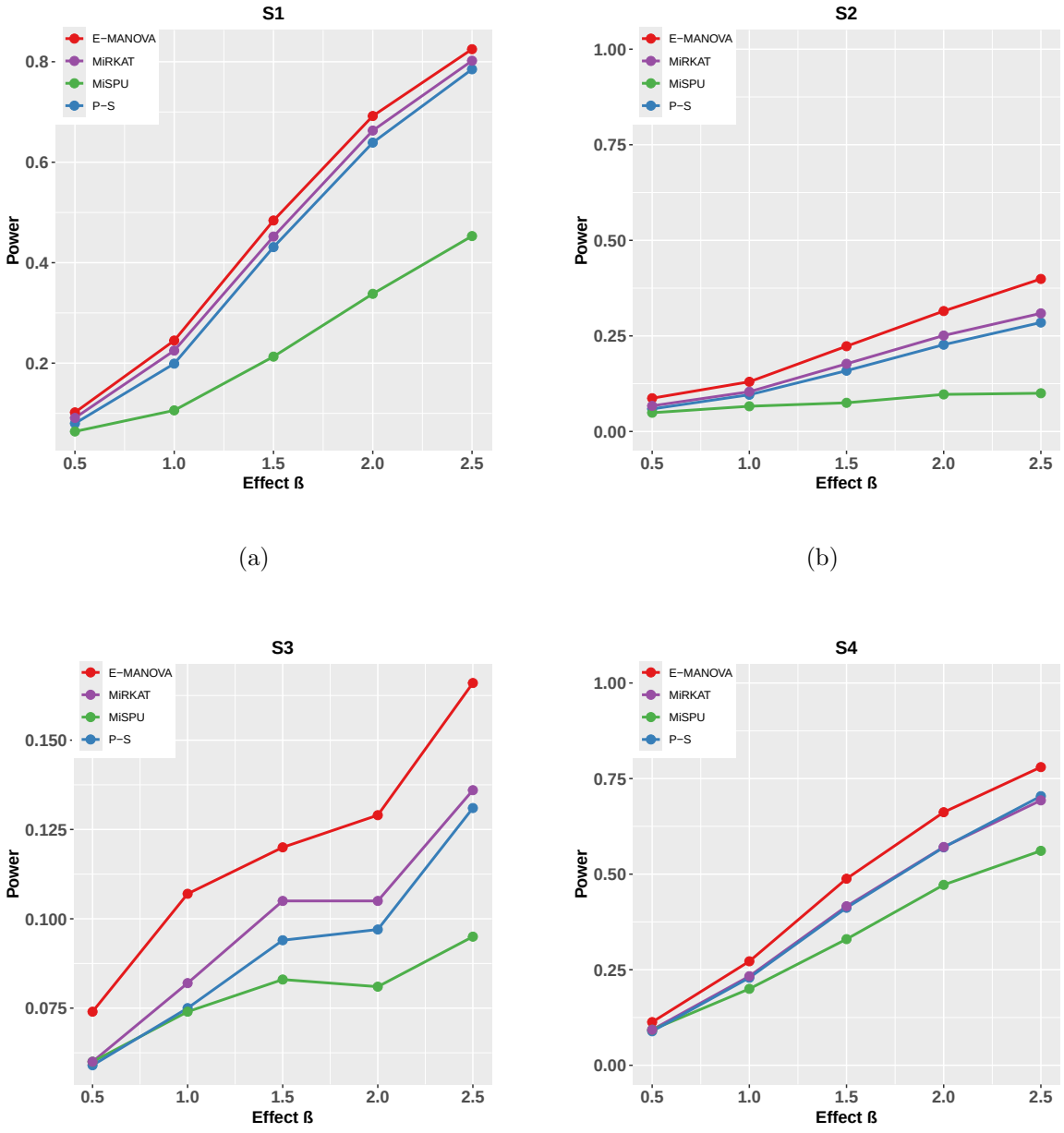
Figure 2: The empirical distribution of $T_{d,r}$ and theoretical Pearson type III distribution with parameter estimated based on the method in Section 1. Different scenarios were considered with continuous outcome variable, $r = 0.125, 2$ and $d = 1, 2, 3$ represent two different distances (weight UniFrac distance, Unweighted UniFrac distance and Bray-Curtis distance). All quantile-quantile plots are drawn based on 1000 random samples.

4 Additional simulation results

This section provides additional simulation results focusing on a binary phenotype. The simulation strategy is outlined in the main text section methods. Table 1 presents the empirical type I error rates, while empirical power results are depicted in Figures 3. The findings from both the table and figures lead to the conclusion that similar results are observed with binary and continuous phenotypes.

Table 1: Type I error rates of binary phenotypes with significance level $\alpha = 0.05$ and $\alpha = 0.01$

		MIRKAT	MISPU	P-S	E-MANOVA
S1, Independent covariates	$\alpha = 0.05$	0.049	0.046	0.042	0.059
S1, correlated covariates	$\alpha = 0.05$	0.056	0.047	0.044	0.062
S2, Independent covariates	$\alpha = 0.05$	0.051	0.048	0.041	0.062
S2, correlated covariates	$\alpha = 0.05$	0.049	0.055	0.042	0.059
S3, Independent covariates	$\alpha = 0.05$	0.045	0.045	0.042	0.052
S3, correlated covariates	$\alpha = 0.05$	0.045	0.047	0.040	0.057
S4, Independent covariates	$\alpha = 0.05$	0.048	0.051	0.044	0.056
S4, correlated covariates	$\alpha = 0.05$	0.055	0.055	0.055	0.059



(a) (b)

(c) (d)

Figure 3: Empirical powers of E-MANOVA(red), MiRKAT(purple), MiSPU(green) and P-S(blue) with bianry phenotypes and independent covariates under scenairo $S1$ to $S4$ with significance level 0.05.

5 Reference

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