

## Comparison of microbiota analytic techniques

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**Objectives:** Increasingly, there are many bioinformatic and statistical programs available to analyze microbiota data. We aimed to compare different analytic techniques.

**Methods:** We utilized data from a crossover trial in adults (n = 24) who consumed a prebiotic (5 g/d fructooligosaccharides + 5 g/d of galactooligosaccharides; PRE) and a no fiber control (CON) for 4 weeks each. The 16S rRNA V4 region was amplified from extracted fecal DNA with a Fluidigm Access Array prior to high-throughput sequencing on an Illumina HiSeq. The FASTX-Toolkit, DADA2, and QIIME2 were used to process the sequence data. Taxonomy was assigned with the SILVA 138 reference database. Then, microbiota data were analyzed with differential abundances and taxa rankings. Differential abundance analyses were conducted via Wilcoxon signed-rank tests and Linear Discriminant Analysis Effect Size (LEfSe). Compositional taxa rankings were created with DEICODE and Songbird. Qurro (Quantitative Rank/Ratio Observations) was used to visualize taxa rankings and sample log-ratio plots. Wilcoxon rank-sum tests or Welch's t-tests quantified Qurro findings.

**Results:** Using Wilcoxon signed-rank tests, Actinobacteriota increased by 130% in PRE compared to CON ( $P = 0.004$ ,  $q = 0.02$ ). *Bifidobacterium* and *Anaerostipes* increased by 200% ( $P = 0.001$ ,  $q = 0.03$ ) and 100% ( $P = 0.02$ ,  $q = 0.16$ ), respectively, while *Roseburia* and *Ruminococcaceae* CAG352 decreased by 36% ( $P = 0.03$ ,  $q = 0.16$ ) and 21% ( $P = 0.02$ ,  $q = 0.16$ ) in PRE compared to CON. From LEfSe, *Bifidobacterium* increased ( $d = 4.35$ ,  $P = 0.03$ ), while *Dielma* ( $d = 1.95$ ,  $P = 0.03$ ) and the *Eubacterium brachy* group ( $d = 2.08$ ,  $P = 0.04$ ) decreased. With Qurro, the ratio of top to bottom ranked taxa from DEICODE principal component axis 3 increased in PRE compared to CON ( $P = 0.05$ ), with *Bifidobacterium* and *Anaerostipes* among top taxa. From the Songbird treatment differential, ratio of top to bottom ranked taxa increased in PRE compared to CON ( $P = 0.001$ ), with *Bifidobacterium* among top taxa.

**Conclusions:** These findings reveal that *Bifidobacterium* enrichment was consistently detected using various analytic techniques. However, microorganisms that were affected to a lesser degree were not consistent across platforms.

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