Supplementary Methods

We obtained raw sequence data in FASTQ files from the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>) using the accession numbers provided in each study. We reanalyzed the 16S rRNA gene sequencing data using the QIIME2 platform (v2023.5) (Bolyen et al., 2019). Primers were removed using Cutadapt (Martin, 2011) (via q2-cutadapt), and paired reads were merged with the “merge-pairs” function of VSEARCH (Rognes, Flouri, Nichols, Quince, & Mahe, 2016) (via q2-vsearch). The merged sequences were quality-filtered using the quality-filter (Bokulich et al., 2013) (via q2-quality-filter) followed by denoising with Deblur (Amir et al., 2017) (via q2-deblur). For each study, reads were truncated based on a quality control score of 25. The sequence data were clustered into operational taxonomic units (OTUs) at a 97% sequence identity threshold through closed-reference clustering against the Silva 138 reference database (Quast et al., 2012), implemented using the VSEARCH plugin in QIIME2 with default parameters via the "cluster-features-closed-reference" workflow. Taxonomic assignment of OTUs was conducted by employing the q2-feature-classifier plugin (Bokulich et al., 2018) in QIIME2, implementing the machine learning-driven "classify-sklearn" algorithm to annotate representative sequences against the Silva 138 reference database. The OTU tables from each study were converted to BIOM format, merged using QIIME2, and then imported into R software (version 4.4.1). Singleton OTUs (those consisting of a single read) and OTUs present in only one sample were excluded. OTUs categorized as "Mitochondria," "Chloroplast," "Archaea," and "Eukaryota" were removed from the bacterial community analysis. Finally, we removed samples with fewer than 6,000 reads and rarefied the sequencing depth of the remaining samples to 6,000 reads, after which we used Shannon’s diversity index and ACE to compare the alpha diversity between different treatments and controls.

References

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