



EMORY

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HEALTH

June 8, 2020

Dear Editor,

I am pleased to submit a manuscript entitled “Constraining PERMANOVA and LDM to within-set comparisons by projection improves the efficiency of analyses of matched sets of microbiome data” to *Microbiome* for consideration of publication. All authors have approved this submission.

Matched-set data arise frequently in microbiome studies. For example, we may collect pre- and post-treatment samples from a set of individuals, or use important confounding variables to match data from case participants to one or more control participants. So far, only two methods exist for analyzing matched-set microbiome data and both are limited to the simplest scenario that has *paired* data with a binary trait and no within-pair covariates. Here we presented a new strategy that can be used with both PERMANOVA and the LDM for analyzing a wide range of matched-set data, allowing discrete or continuous traits or interactions to be tested, within-set confounders to be adjusted, and unbalanced data (e.g., having unequal ratio of case-to-control samples per set) to be fully exploited. Our simulations indicated that our proposed strategy outperformed alternative strategies in all of these scenarios. Using simulation, we also explored optimal designs for matched-set studies. Thus, our method can accelerate the search for associations between the microbiome and traits of interest.

We understand that *Microbiome* has very high standards. We believe our work represents a major advance in analytical methods for microbiome association studies and will have a strong impact on current and future microbiome research. Because it presents a very general and timely analytical method that is widely applicable to microbiome association studies in many disciplines (e.g., environmental, agricultural, and biomedical), and adds an important function to existing software programs PERMANOVA and the LDM, it should be of substantial interest to the broad readership of *Microbiome*.

In light of the content, we suggest Dr. **Alexander Alekseyenko** (New York University School of Medicine, email: [alekseye@musc.edu](mailto:alekseye@musc.edu)) to be our Association Editor and the following reviewers: Drs. Huilin Li (New York University School of Medicine, email: [huilin.li@nyumc.org](mailto:huilin.li@nyumc.org)), Michael C. Wu (Fred Hutchinson Cancer Research Center, email: [mcwu@fhcrc.org](mailto:mcwu@fhcrc.org)), or Shyamal Pedadda (University of Pittsburg, email: [spd47@pitt.edu](mailto:spd47@pitt.edu)).

Thank you very much for considering our manuscript.

Sincerely yours,

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