

Reviewer Feedback

The study titled, “Microbial biogeography of the wombat gastrointestinal tract,” surveys the microbial communities along the GI tract of two individuals from two wombat species, and compares the communities of the proximal to the distal colon. The study is well-written, provides a clear story, and addresses a clear gap in knowledge. Nonetheless, before its publication, the authors need to be more transparent about their limited sampling and inference. Additionally, there are certain areas of the manuscript where the writing could be clearer or the authors did not include important details, and I have provided suggestions for improving those areas below. I believe the following suggestions will further strengthen the manuscript.

Abstract

The abstract is missing key details, for example the total number of samples, the total number of individuals, and the list of gut regions that were surveyed. I suggest the authors add the following to L27: “Little work has been done to document the composition of GI microbial communities of herbivorous animals at these sites. In this study, we use 16S rRNA gene sequencing to characterize the microbial biogeography along the GI tract in two species of wombats. Specifically, we survey the microbes along XX sites (list gut sites here) in a single bare-nosed wombat (*Vombatus ursinus*) and a single southern hairy-nosed wombat (*Lasiorhinus latifrons*). Our results show that” and then the authors can continue to report the results. It is important that the authors be transparent with the audience about the limited sample sizes upfront.

Amend L34 so that it includes rationale for why authors are comparing the proximal colon to the distal colon, I suggest: “We found several microbial genera that were differentially abundant between the first proximal colon (putative site for plant fermentation) and distal colon regions (which resemble fecal samples)”.

Introduction

Overall, the introduction provided all the relevant details, I have mostly made comments to improve its structure and readability.

L56-58: It is too premature to introduce wombats. Move this sentence further down to when authors start discussing wombat biology.

L58-59, rephrase to something along the lines of, “Additionally, prior research conducted across a range of mammalian species (add citations) found that microbial communities vary along the GI tract.” It is much clearer than what authors currently have written: “The diversity and composition of microbial communities within the gut are known to be site-specific.”

I also suggest the authors merge paragraph 2 into paragraph 1. With the suggestion I made above, the content of lines L64-68 can be deleted, as they do not add new information. The citations from L64-68 could be part of the citations the authors list in the sentence I crafted above.

L82-86: It would be useful to include a figure here depicting the gut morphologies of the two wombat species. The authors can even move the diagram they included in Figure 3 to its own figure and label each gut region. They can reference this figure when they are talking about the different gut regions in the wombat species.

L91-L96: The authors need to include details about their sequencing and sampling, and need to state the study aims before their predictions. I suggest they say, "Here, we used 16S rRNA gene sequencing to investigate the microbial community changes along the GI tract of two wombat individuals, a wild BNW and a SHWN." Then they can state their prediction, "We predicted that the proximal colon would harbor a distinct microbial community than ..." The authors originally had said 'hypothesis' but that is not a hypothesis it is a prediction. Authors should also delete the contents of L94-96 as they are redundant with the Aim sentence.

Methods

The methods are well written and contain the necessary details to be reproducible. Great work!

Results

I have suggestions for improving the organization of this section, so that readers can follow it more easily.

L170-172: Add a sentence or two to remind readers of the study aims and predictions, and list the different gut regions that were surveyed (stomach, small Intestine, proximal colon first, distal colon). Can the authors include a table showing the breakdown of the 31 GI digesta samples in the methods? The authors can then reference that table in the results, along with the figure I suggest they make of the GI tract sites.

L175-179 belong in the Discussion (or Introduction).

Modify L179-181 so that it encompasses all study analyses, I suggest: "To test if these different gut regions influenced microbial communities, we analyzed the microbial composition, richness (the number of ASVs), and beta-diversity of duplicate luminal samples taken throughout the GI tract of each wombat species".

I highly recommend that the authors reorder the paragraphs of this section so that composition appears first, followed by alpha-diversity, and lastly, by beta-diversity. Readers will appreciate reading about the composition (the who is there?) first.

L185 : After the ASV richness sentence, add, “Due to our limited sampling we were unable to run linear models to confirm this pattern statistically, and future studies employing a larger sample size should test the observed pattern.”

Readers (myself included) are expecting results from statistical analyses, and this sentence responds to that.

L187 - 189: If the authors modify the sentences from L179-181 as I suggest (see above), then the sentences from L187-189 will become redundant and can be removed.

L220-221: Authors should remind readers why this analysis was done (e.g. the proximal colon is the primary site of fermentation, the distal colon ...).

Discussion

Minor comments about communicating study limitations regarding sample sizes

L250-253: I suggest the authors rephrase this sentence, as it implies causality (GI region structures microbial communities); their data does not support this direct causality. I suggest they reword to: “Although we had data for only two individual wombats (or “despite our limited sampling”), our results showed that microbial community composition, structure, and diversity varied along the wombat GI tract.” The authors must communicate their sample sizes when they talk about major findings, especially because this study was based on samples from only two individuals.

L276-280 Can the authors replace PC1 with “proximal colon.” It is easier to follow this way. In the methods, they authors make it clear that their analysis focused on proximal colon region 1, so it is fine if they only write “proximal colon” in the discussion.

L301 - Authors should add that their limited sampling did not give them sufficient power to run statistical tests on GI tract alpha or beta diversity. This is a limitation of this study that should be included in this paragraph.

Figures

Figure 1 legend title: rename so that it states, “Microbial diversity (ASV richness) along the wombat GI tract.”

Figure 2 legend title: rename so that it states, “Microbial community structure among sites along the wombat GI tract.”

“Differs” implies differences, which imply statistical differences, which the study does not provide.

Figure 4 legend title is incomplete, “Heat map of the microbial taxa that were found to be differentially abundant.” Among sites? Among wombat individuals? Among the proximal vs distal colon? Please complete the title and add this detail to the actual legend as well.

Figure 5 - please make the text in the Euler diagrams larger; it is currently too small to read.

Supplementary figures: I could not find the legends for the Supplementary Figures.

Data Checks

Thank you to the authors for making their research accessible. I was able to access their data on the NIH SRA, and their code on GitHub.

Minor Comments

L35: present % first and the number of ASVs in parentheses, e.g. 10.6% (99 ASVs)

L52-54 there is a missing citation for this statement

L75-77 and throughout the manuscript: change the acronym of the Southern hairy-nosed wombat so that it is also 3 letters long - so SNW or HNW? The authors can ignore this suggestion if they do not think its valuable.

L100- add (N=2) after “two deceased wild wombats”

L250 - spell out the full species common name for BNW and SHNW

L258-L261 These sentences do not add anything new, please remove.

L262 - spell out PC1 to proximal colon 1 (readers may forget what PC1 is)

L262-263 replace “wombat species” with “wombat individuals.” The authors do not have any statistical power to make any statement about *species differences*.