Here Zeng et al. have investigated the gut mycobiota (or gut fungal microbiota) in Crohn's disease (CD), and further how the gut mycobiota differ between different clinical phenotypes of CD. The study includes 45 CD patients and 17 healthy controls. Analyses were conducted both between HC and CD and between different phenotypes within the group of CD patients.

The gut mycobiota is scarcely studied, and this study provides new information on the gut mycobiota in CD phenotypes, giving new insights to this topic. Therefore, I find this study suitable for the PeerJ journal, if the study is revised according to the major and minor comments outlined below.

Major comments:

1. Please include introduction to the clinical phenotypes in Chron's disease since this is now missing from the introduction.
2. Consider performing qPCR and thereby getting absolute abundances instead of relative. Here the relative vs. absolute data is discussed: Jian C, Salonen A, Korpela K. Commentary: How to Count Our Microbes? The Effect of Different Quantitative Microbiome Profiling Approaches. Front Cell Infect Microbiol. 2021 Mar 5;11:627910. doi: 10.3389/fcimb.2021.627910. In addition: in Fig 1 D (and also other places throughout the study): is it mean relative abundance? Relative should be stated in this case. Without absolute abundances and multiple timepoints one cannot state a decrease or increase for example.
3. Please add references to all tools and packages used (no references present in the material and methods part, for example references to FLASH tool, UPARSE, RDP classifier, QIIME, PcoA among others were missing). All packages used in R and tools should be referred to correctly. Similarly, add references to the "statistical analysis" part as well.
4. In the abstract the following is stated: "Stool samples from subjects meeting the inclusion and exclusion criteria were collected for running internal transcribed spacer 2 (ITS2) high-throughput sequencing.", but in the methods this is stated: "The fungal microbiota was identified and analyzed by sequencing the internal transcribed spacer 96 (ITS) fragment, which was the ITS1 amplifieder of ITS1: ITS1F- ITS2R.". What does this mean? Was the ITS1 or ITS2 targeted? Additionally, what primers were used? Please add reference! Both of these sentences are confusing and should overall be revised (what is referred to by "ITS1 amplifieder of ITS 1"?).
5. Please include a strengths and limitations part in the discussion, one strength is for example that the samples have been frozen immediately, minimizing alteration due to being kept at room temperature.
6. Please correct the list of references according to the guidelines of the journal!
7. What background factors were used as confounders in the analysis? Please specify these! All relevant background factors should be checked whether these have an impact on the fungal gut composition and if they do, their impact should be eliminated from the analysis. Background factors/confounders are included to decrease the probability of the results stemming from the background factors, and not from the actual comparison.

Minor comments:

1. References: in the instructions for authors of the PeerJ journal it is stated: "PeerJ uses the 'Name. Year' style with an alphabetized reference list.", please modify accordingly.

2. Line 55: please correct nonself to non-self. Further, non-self-limited intestinal inflammatory disease is somewhat confusing and thus could be clarified.
3. Lines 56-58: “In addition to intestinal bacteria, intestinal fungi are another important microbe to be studied, especially in patients with CD.” why particularly in patients with CD?
4. Line 88: please be more specific on the collection; did you collect one stool sample from each study participant?
5. Line 93: please add reference or description to the CTAB/SDS method used in DNA extraction.
6. Line 103: what further experiments? Would there be a problem choosing samples that only included specific bands? The product from ITS PCR can vary quite heavily in size (200-600 bp) (Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581–3.).
7. Fungal ITS1-2 rDNA Sequence Analysis part of materials and methods: line 111: Pair-end reads… was merged?
8. Line 114: what database was used for annotation – please specify database and version and add this to the list of references.
9. Line 147: disappeared would mean that the two phyla were present in the CD group first but disappeared over time. Please specify to something in style with “was not present”.
10. Line 156: Saccharomyces → *Saccharomyces*
11. Line 167: compared to HC?
12. Line 175: should it be stated in methods?
13. Line 231- The Montreal classifications might be more suitable to be explained in methods than in results
14. Line 245: compared to Saccharomyces, and Dipodascus that were enriched..
15. Line 249: the relative abundances..
16. Line 262: disappeared word used again
17. Line 264: Different how? Different from the results of this study? Or Different from each other? Please specify.
18. Line 265: disappeared: does it mean did not exist or did you have healthy controls who later developed CD and thereby it disappeared?
19. Line 268: did you also characterize the bacteria? Did they have decreased richness and abundance? And did you characterize the absolute abundance? If not, then increased abundance cannot be stated, since that can be characterized only by absolute abundance.
20. Line 271: only last name in an in-sentence citation
21. Line 273: please refer to HC throughout the study
22. Line 297-289: Please revise the sentence: “It is known that the occurrence of CD is associated with increasing bacteria with stronger pathogenic effects and decreasing bacteria with protective effects”, it is difficult to understand.
23. Line 290: Please revise the sentence: “However, there is still a lack of research to support whether fungi have similar characteristics as bacteria as above, as well as mutually cooperative and competitive interactions in fungi, viruses, and bacteria.”, I don’t understand what the message is: what does “as bacteria as above” mean? And is the latter part of the sentence also under the “lack of research”? Please clarify, it is very confusing!
24. Line 294 and 295: results should be in the results part of the article. In the discussion the results are discussed, not stated, nor repeated. Additionally, consider removing the “as” in line 292, it might not be suitable.
25. Line 295: Maybe indicated instead of proved? It is not a proof-of-concept study
26. Line 297: increase in the relative ? abundance of Basidiomycota in what group? compared to what? Or was it a higher relative abundance? An increase is that it has been lower and is now higher.

27. Line 298: again, please include numerical results in the results (referring to the score)
28. Line 305: should Exophiala be in italics? Maybe include what taxonomical level to avoid confusion
29. Line 335: disappeared or was not present?
30. Line 336: They → CD and HC belong to two diverse fungal gut microbiota
31. Line 336: increased or higher? Increased means that it has been lower and is now higher, and to this, absolute abundance and multiple timepoints is required. If higher then the comparison has to be stated: there was a higher relative abundance of fungi belonging to Ascomycota … in WHAT GROUP compared to ?
32. Line 337: decrease or fewer correlations? and 338: reduction?
33. Data availability statement: The raw data have been submitted – Is raw data = sequences? Please specify.
34. Figure 1 C, E, 2 C and 3B: please modify the taxa names: Candida_tropicalis → *Candida tropicalis*
35. Figure 1: Please specify: gut/fecal fungal microbiota composition in patients with Crohn's disease (CD) (n =45) and healthy controls (HC) (n = 17). Might be clearer to open up abbreviations in the text compared to adding it separately in the end.
36. Further: Add gut/fecal microbiota composition. Fungal composition can be from any body site.
37. Figure 2: Add gut/fecal fungal microbiota composition to clarify
38. The quality of the figures is poor, but I am assuming that high-quality figures will be attached in the final submission according to the guidelines of the journal.