Detailed response to **REVIEWER 3:**

This study obtains a metagenomic signature after machine learning-based analysis with predictive capacity according to the presence of inflammatory bowel disease (IBD) in faecal samples and validates the signature in two external cohorts: Ulcerative colitis (UC) and Crohn's disease (CD). Key findings include a strong relationship between the intestinal microbiome and IBD, and high performance in predictions. Moreover, two subtypes of IBD such as UC and CD present common profiles in the microbiome. However, some significant issues need to be addressed.

1. The authors indicated that "On one hand, glmnet has low performance for both cohorts (0.5478 of AUC in Gevers; 0.5532 of AUC in Morgan) whereas RF model achieve better results in both (0.7588 of AUC in Gevers; 0.7391 of AUC in Morgan).". Please also provide the data for glmnet model in Figure 2.

We would like the reviewer´s appreciation, for this reason we added a new panel in Figure 2, corresponding to ROC obtained in glmnet model.

2. The authors mentioned that "Other genera showing a clearly stratified distribution in these two subsets of patients are: Lachnobacterium, Butyricicoccus and Acidovarax.". The main problem is that the descriptive conclusion is not supported by the evidence presented in Figure 3A.

We agree with this suggestion. In particular, the genus Lachnobacterium does not show a clear stratification, as shown in Figure 3A. In contrast, we consider that in the other two genera, Butyricicoccus and Acidovaras, a clear pattern of disease status can be distinguished.

Following the reviewer's indications, we reformulate this sentence to provide more clarity to the readers:

"Other two genera, such as Butyricicoccus and Acidovarax also exhibit a stratified distribution in the two subsets of patients."

3. The most important result is that there is a clear pattern of the presence of Akkermansia in undiagnosed patients of the CD cohort. Although the authors summarized that "Ulcerative colitis and Crohn´s disease shared common patterns at genus level", no similar result was observed in the UC cohort.

In relation to this consideration, we believe it is appropriate to specify and clarify this point. When we refer in the manuscript to the fact that both subtypes of the disease present common patterns, we mean that the same signature can be used for the identification, in this case, of these two IBD subtypes.

As reflected in Figure 3A, the expression patterns associated with each of the genera within the signature are not replicated across the two IBD subtypes. We believe this is for two main reasons:

1. Firstly, the heterogeneity that exists within the cohorts adds a degree of complexity to observing common patterns. Aspects such as the different sequencing platforms, the depth of sequencing, and other more general aspects, strongly influence in this respect.

2. Secondly, and most importantly, the selection of features was not done with the intention of obtaining genera that play a major role in the occurrence of the disease. Even if it is true that, probabilistically, some genus present in the signature is directly involved in the development of the disease, this has not been the main objective of the work. In our case, and as can be seen in the predictions of the model, it is the total set of genera included in the signature that provides the necessary information for the classification of patients. Therefore, when looking at the heatmaps in Figure 3, it is expected that the presence of different expression patterns will be observed in the two cohorts.

Just as our signature is associated with clinical aspects such as antibiotic use, appendix removal or alcohol consumption, we expect it to be correlated with the genera (see supplementary materials, added in the new version of the manuscript) biologically associated with the disease.

In line with the reviewer's comment, we considered reformulating some of the paragraphs of the results in order to clarify this issue in the manuscript (in red in the text).

4. Based on Figure 3B, the evidence is not strong enough for demonstrating that the presence of the genera Parabacteroides, Coprococcus and Ruminococococcus are associated with disease occurrence. It also lacks evidence that Parvimonas and Butyricicoccus have a protective action.

This question is closely related to the previous section. However, we agree with the reviewer's assessment, and consider that it would be better to reformulate this paragraph to avoid confusion among readers.

"As for the UC cohort, as shown in Figure 3B, the presence of the genera Parabacteroides, Coprococcus and Ruminococococcus seems to be more present in patients with the disease, while Parvimonas and Butyricicoccus seems to be more abundant in disease-free patients"

5. For "Commons drugs and/or probiotics treatments can be works in both subtypes" on page 10 line 346, recommend for discussing more details on the probiotics used in UC and/or CD patients. Is there any association with the identified metagenomic signature in this study?

This is a complex issue that we would like to clarify. The results obtained in this study show that the signature identified in IBD can be generalised to the two major subtypes (CD and UC). This signature was identified only for predicting the presence of disease, not for the response to certain drug treatments or probiotics.

We believe the reviewer's comment is fair, and therefore consider that the statement he makes should not be included in the conclusions section, but rather in the discussion of the manuscript.

In addition, in order to clarify this issue, we have carried out different analyses to observe the correlations between the signature and clinical variables such as antibiotic or probiotic intake. The analysis was performed with the data present in the AGP cohort, as unfortunately we did not find other public cohorts with these data available.

First, the correlation of signature with antibiotic and probiotic intake was examined. For this we used model predictions from both the train and test subsets. Both comparisons were included in supplementary material in this new manuscript version. In summary, in train set,

there is a significant relationship between model predictions and probiotics and antibiotics intake. In contrast, in test set, due to sample size, we cannot observe these correlations.

Some minor concerns:

1. Please check the abbreviation of ROC on Page 6 line 181. The first time you use an abbreviation in the text, please present both the spelled-out version and the short form.

Corrected

2. Please correct “Figure ??D” on Page 7 line 223.

Corrected