Meta-analysis identifies microbial signatures of disease in murine models of inflammatory bowel disease

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Running Head: Meta-analysis of murine IBD models

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ABSTRACT

The gut microbiota plays a central role in modulating intestinal inflammation, but the identification of specific inflammation-associated microbes has remained elusive. Here, we perform a meta-analysis on metagenomic data from 12 different studies of murine colitis triggered by a variety of genetic and environmental factors with the goal of finding bacterial taxonomic groups that can act as signatures of health or disease across studies, and that can be used to discriminate between healthy and diseased mice. We leveraged recent developments in 16S analysis tools to identify amplicon sequence variants (ASVs) instead of the traditional Operational Taxonomic Units, and used the EZTaxon reference database that distinguishes between currently unnamed and uncharacterized 16S phylotypes. Random Forest model and differential abundance analysis were used to detect microbial signatures that could consistently differentiate healthy from diseased mice, and a 'dysbiosis index' was constructed from these. This dysbiosis index was able to correctly distinguish samples derived from inflamed and non-inflamed mice in the majority of studies and significantly outperformed other frequently used metrics of dysbiosis including alpha-diversity, proteobacterial abundance, and the ratio of Bacteroidetes to Firmicutes. 10 of 12 bacteria we identify as associated with the diseased state are members of the order Bacteroidales, including several species from the abundant but poorly understood S24-7 family. The implications of these findings are discussed.

INTRODUCTION

The human gut contains vast numbers of bacteria, viruses and fungi that collectively make up the gut microbiota, which plays a pivotal role in the host's health (1). While the microbiome is important for fundamental host processes like digestion (2), metabolism (3) and

immune system development (4), changes in the microbiome, often termed dysbioses (5), have been linked to several diseases, including inflammatory bowel disease (IBD) (6). IBD is a group of relapsing and remitting inflammatory disorders that mainly manifest as Crohn's disease or ulcerative colitis (7, 8). Human studies have mostly been retrospective cohort ones, which limits their utility in elucidating causal links between changes in the microbiome and disease onset. Regardless, understanding these changes remain important, especially if there is a particular "dysbiotic" microbiome, or microbiome members, associated with disease. Although 16S sequencing of the bacterial microbiota have allowed comprehensive investigations of such changes (9), inter-individual variability within studies, and a lack of standardized techniques across studies (differing extraction and sequencing protocols, 16S variable regions, analysis pipelines and taxonomic reference databases) hinders comparisons of large sample sets to find consistent microbial signatures of disease (10). In order to compare and synthesize results from different human studies, meta-analyses, starting from the original sequencing data, have been conducted, resulting in the discovery of consistent microbial signatures in IBD patients (11, 12). Such meta-analyses have significant potential for designing non-invasive sequencing based diagnostic tools for IBD onset (12). They also reveal interesting insights into the relationship between the microbiota and disease; for example, meta-analyses of microbiome studies investigating obesity and IBD suggest that although metrics like the Bacteroidetes to Firmicutes ratio and alpha diversity may be significant in some studies, they do not seem to be consistent across studies (11, 13). In spite of the contributions of human meta-analyses to clinical diagnosis and broad inferences, causal and mechanistic inference remains challenging.

To shed mechanistic insight on the link between IBD and the microbiota, murine models have been relied upon heavily (6, 14, 15). Yet, despite the professed advantages of

reproducibility and well-defined conditions, murine samples also seem to have significant inter-individual variability, lack of standardization across studies, and sample sizes are often small (14, 16). Furthermore, there are multiple widely-used mouse models for IBD, including Dextran Sodium Sulfate (DSS) induced colitis, 2,4,6-trinitrobenzenesulfonic acid (TNBS) induced colitis, T-cell adoptive transfer, and Interleukin-10 deficiency (17, 18). Studies investigating the role of various immunomodulatory genes in IBD also often report changes in the microbiota (19, 20).

The existing literature mostly consists of descriptions of microbiota changes associated with colitis in various mice models (19, 21, 22), with only some assessing colitogenic potential of particular microbiotas, or honing in on particular microbes (23, 24). The lack of standardization prevents meaningful comparison of the changes reported, and the question remains as to whether there are any consistent markers of inflammation in mice models. Reviews published have been descriptive (25, 26). Since there is little overlap between the human and murine microbiota, host-specific analysis is paramount (27). In the context of a poorly catalogued murine microbiota with limited cultured isolates (28), identification of a microbial signature can help focus isolation efforts and mechanistic studies on the best microbial candidates for further research in mouse models.

Here we report a meta-analysis of 12 studies/datasets that utilize 16S sequencing to describe a link between development of colitis and changes in the microbiota in murine models. We aimed to find bacterial taxonomic groups that are consistent signatures of health and disease across studies, and that can be used to discriminate between healthy and diseased mice. We only included studies whose raw 16S sequencing read files were available and thus were able to standardize the analysis and directly compare the studies. We leveraged recent developments in 16S analysis tools to identify amplicon sequence variants (ASVs) instead of the traditional

Operational Taxonomic Units (OTUs) (29), and used the EZTaxon reference database that distinguishes between currently unnamed and uncharacterized 16S phylotypes (30). We used a Random Forest model and differential abundance analysis to detect any consistent microbial signatures that differentiate healthy from diseased mice, and constructed a "dysbiosis index" from these. Finally, since alpha diversity, Bacteroidetes-to-Firmicutes (BF) ratio and Proteobacteria levels are often used as markers of microbiome health, we investigated the utility of these in discriminating colitic from healthy samples (11, 13, 31).

RESULTS

Study Search, Inclusion and Data Aggregation

To identify studies that investigated the murine intestinal microbiota in the context of intestinal inflammation, we conducted a systematic search of NCBI PubMed for articles that contained terms relating to microbiota, intestinal inflammation and murine models in the title and abstract, published between 2012 and 2016, and was not a review. We followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines to limit inclusion bias (32). We screened the title and abstracts of 816 articles yielded by the search for eligibility, and obtained 2 additional studies from knowledge of published literature and our own currently unpublished dataset (Martin A. et al. unpublished) (819 total); 79 full-text articles were then assessed; 44 were retained to be checked for data availability; 10 had publicly available data and 2 provided access by the time of publication (19–22, 33–39) **(Table 1, Fig 1)**.

Search terms and screening and inclusion strategy are outlined in Methods. Briefly, we looked for studies that did a 16S sequence-based analysis of non-synthetic murine microbiota in various IBD models before and after onset of colitis. We excluded infection-based inflammation

and samples administered antibiotics as these are likely to have changes that go well beyond colitis associated ones. Studies that only assessed microbiota before inflammation onset were excluded because we were interested in a microbial signature associated with the *onset* of colitis, rather than a microbiota that is associated with increased *susceptibility* to colitis.

Selection of relevant samples within the 12 studies yielded 601 samples, of which 434 were healthy and 167 had colitis. We used a standardized custom data-processing pipeline to detect Amplicon Sequence Variants (ASVs) using the DADA2 algorithm that leverages quality information from sequence reads for sequence inference. Taxonomy was assigned using a custom script and the EZTaxon database to be able to distinguish and name currently uncultured, but sequenced, phylotypes. Only classified taxons were kept and after filtering for rare taxa and merging datasets, we obtained 1558 unique taxonomic groups (detailed methods in Materials and Methods).

Beta Diversity

Beta diversity, the between-sample diversity, can provide insight as to whether mice with colitis have a different microbial community structure compared to healthy mice (40). We calculated the Bray-Curtis distances (41) between samples and used Principal Coordinates Analysis (PCoA) to visualize the microbial communities. Plotting the samples on the first three coordinates suggested that samples tended to cluster by study more than by disease status (**Fig 2a, b)**. However, a Permutational Analysis of Variance (PERMANOVA) (42) suggested that microbiome composition differed by both disease status and study ($p < 0.001$). Furthermore, within each study, PERMANOVA revealed significant differences due to disease status in all except one study (**Fig S1**). Visually, within each study, disease status often provided a stark

differentiation in PCoA plots, which was not replicated in the pooled data. However, the significant PERMANOVA result suggested the possibility of differences between communities based on disease status, across studies.

Alpha Diversity

Alpha diversity, the diversity of the microbiome within each sample, is regularly investigated as a marker of "health" in both human and mouse studies. While individual studies have found associations between reduced alpha diversity and obesity and IBD in humans, meta-analyses have found the evidence for such relationships to be weak (13, 43). Another oft-used, yet inconsistent, within-sample marker is the ratio of Bacteroidetes to Firmicutes ratio. Lastly, a bloom of Proteobacteria has also been associated with dysbiosis. We investigated the utility of using these markers in discriminating between healthy and diseased mice.

In our meta-analysis, we do not find a consistent relationship between alpha diversity and colitis in mice. Seven of the 11 studies had significant differences in the Shannon index (*H*) between healthy and diseased mice, with 5 having higher values of *H* (lower diversity) in healthy, and 2 having higher diversity in healthy **(Fig 3a).** Similarly, there was no consistent relationship between colitis and the Bacteroidetes to Firmicutes ratio; 2 studies had a significantly lower ratio in healthy, while 2 had a significantly higher ratio in healthy **(Fig 3b).** Finally, there was also no consistent relationships between disease status and the relative abundance of Proteobacteria **(Fig 3c)**. For all three measures, the pooled data did not show a significant difference between the healthy and diseased mice when tested using random effects models ($p > 0.05$).

Random Forest Models

Given the significant PERMANOVA results, inconsistent alpha diversity metrics, and the success of statistical learning techniques in previous studies, we hypothesized that a random forest model would be able to discriminate between diseased and healthy mice (12, 43, 44). A random forest model works by building hundreds of decision trees, with a cut-off value (abundance) for a particular feature (taxon) being selected at each split to maximize the correct classification of the outcome (disease status) among the samples being used for training. For a new sample, the trees are used to classify it as diseased or healthy (45). Model performance in discrimination can be summarized using the area under the receiver operating curve (AUROC), with 0.5 being as good as random, and 1 being perfect prediction. We built a cross-validated random forest model on a randomly selected 70% of the samples, which yielded an AUROC of 0.975. When this model was used to predict the remaining 30% of the samples, the AUROC was 0.972, suggesting a lack of overfitting and the presence of a detectable microbial signature of disease across studies included in this analysis **(Fig 4a).**

To test the generalizability of this approach and whether the individual studies contributed complementary or unique information, we conducted a leave-one-out analysis. One by one, each study was left out, and the samples left out were predicted using a random forest classifier trained on the remaining set of samples ("n-1"). Furthermore, we assessed the cross-validated performance of the "n-1" classifiers, as well as that of models trained on the study left out and tested on the "n-1" set. The performance of the models varied greatly depending on which study was left out, as measured by the AUROC **(Fig 4b)**. Samples from Lamas et al. (35), Laubitz et al. (19), and Yeom et al. (22) were perfectly predicted by models trained on all other samples, suggesting that they contained "overlapping" information. All other scenarios had

performance worse than the full model, with the Whitfield-Cargile et al. (36) samples having AUROC less than 0.5 (0.268). The worse-than-random prediction of the Whitefield-Cargile et al. samples suggests that it contributes information that runs counter to those in the other studies. Potential reasons for this are considered in the discussion. Assessing from the opposite direction, we also found great variability in the performance of models trained on one study and used to predict the rest. None of these had very high AUROC values, which is likely due to small sample sizes within each study. Indeed, He et al., Corsi et al. and Yeom et al. had few samples, and poorly predicted other studies. On the other hand, Lamas et al. performed well even with small sample sizes. Despite this variability, we found that removing one study did not change the overall model performance, as assessed by repeated cross-validation, with AUROC values staying consistently around 0.9 **(Fig 5).**

Variable Selection and "Dysbiosis" Index

As the overall random forest model had a robust predictive performance, we wanted to identify the taxons that contributed to this predictive power. To do this, we used the Boruta feature selection algorithm. This algorithm creates random probes (i.e. taxons with shuffled abundance values across samples), and tests their performance relative to the true features (i.e. taxons with observed abundance values). An iterative process retains only the taxons that are significantly better at prediction than their random counterparts (46). The algorithm yielded 184 taxons that were confirmed as being important for disease status prediction, out of the total 1558 tested **(Supplementary Table 1).** Ninety-eight of these had a higher mean abundance in mice with colitis (colitis-associated taxons), while 86 had a higher mean abundance in healthy mice (health-associated taxons). To narrow this list further in order to identify relevant taxons that

were the most prevalent, we only included ones that were present in half or more healthy (if health-associated) or colitis samples (if colitis-associated). This filtering retained 33 taxons, 12 of which were colitis-associated, and 21 of which were health-associated **(Table 2).**

Most of the microbes (10 of 12) associated with the diseased state were members of the order Bacteroidales, including several species from the abundant but poorly understood S24-7 family. However, microbes associated with the healthy state also included members of the order Bacteroidales (8 of 21) along with a number of Firmicutes (12 of 21 including *Lactobacilli* and *Clostridia sp.*) and one Actinobacteria (*Bifidobacteria*). These findings loosely align with the notion that there may be a bias towards a greater abundance of Firmicutes in the non-diseased state, and that Lactobacilli and Bifidobacteria may be markers of gut health (e.g. both are marketed as probiotics). As it relates to how different Bacteroidales species respond to inflammation and dysbiosis, however, there is clearly much that remains to be understood.

We hypothesized that the combined information contained in the relative abundances of this list of taxons was more relevant for disease status than the Shannon index, BF ratio or abundance of any one taxon. Thus, we created a "dysbiosis index", which is the log transformed ratio of the relative abundances of colitis-associated taxa to health-associated taxa. When dysbiosis index values was calculated for each sample, we found significantly higher values for colitis samples in 8 studies (t-test, $p < 0.05$). Two of the remaining had higher, non-significant mean values for mice with colitis, while one had a significantly higher value for healthy samples **(Fig 6)**. A random effects model for the pooled data, with study as the random effect, indicated that mice with colitis had significantly higher dysbiosis index values, suggesting that this was a much better indicator of disease status than the other metrics (Shannon diversity index, B/F ratio, proteobacterial abundance) tested above.

DISCUSSION

Animal models, especially murine models, have been extensively used in research to understand human health and disease. For IBD in particular, a multitude of chemically induced, cell-transfer, and genetic models in mice have been used to understand aspects of intestinal immunology (18). More recently, there has been increasing interest in how the gut microbiota plays an important role in the onset of IBD in humans and colitis in these murine models. The prevalence of IBD in human populations and its murky etiology, combined with breakthroughs in microbiome research techniques, has fostered a plethora of studies investigating the role of the gut microbiota in this disease. Thus, these studies not only represent a potential for understanding the causes of IBD, but also the role of the microbiome more generally (47). Since it is difficult to interrogate causality and mechanisms of disease onset in humans, murine models provide such work, despite differences in some anatomical features, dietary habits and the microbiota.

In studying complex systems like microbiomes, where there are a nearly endless number of dimensions to be explored in terms of both membership and abundance, summary statistics that enable comparison along one dimension are often attractive. Alpha diversity metrics aim to summarize information regarding the how many members are present, as well as how evenly their abundances are distributed. Here we test the Shannon diversity index and find that even though individual studies can have significant results, they are not consistently in the same direction, and in the pooled data, there is no significant evidence of a relationship. Similarly, the BF ratio and Proteobacteria levels were also found to be inconsistent markers of gut health. Thus, while these one-dimensional metrics have the advantage of simplicity, they should be

249 employed with caution. Moreover, universal generalizations linking a diverse ecosystem to
250 health may be unwarranted in this context.

251 Another frequently accepted principle is that the functional features of the microbiota can
252 be usefully predicted from 16S level phylogenetic analysis at the family or genus level. Here,
253 again, our study urges caution. While our analysis flagged an increase in specific Bacteroidales
254 during inflammation as a common feature, other closely related Bacteroidales were reduced. Of
255 particular interest are members of the S24-7 family, which are abundant members of the gut
256 microbiota and yet are understudied and poorly understood and may play an important role in the
257 resilience of the microbiota in the face of different abiotic stresses including osmotic-induced
258 diarrhea (48). Given that different members of the S24-7 family were associated with either the
259 disease or healthy state, the assumption that there is functional similarity between microbes at
260 the phylum, family, or even genus level is an oversimplification that may prevent a more
261 nuanced and complete understanding of the specific forces that shape the membership/abundance
262 of gut microbial communities.

263 When analyzing high-dimensional data, the use of statistical learning techniques can be
264 useful to "pick out" patterns that are not immediately observable – even from highly
265 heterogenous datasets derived from different studies across multiple animal facilities. The
266 results of our leave-one-out analyses indeed highlights the heterogeneity that exists between each
267 of the studies. Given the relatively limited scope of our analysis, we were greatly encouraged by
268 the very high predictive performance of our combined random forest model, which may suggest
269 that there may indeed be some 'universal' microbial signatures that enable us to discriminate
270 between healthy and diseased samples across multiple unrelated studies. It would be interesting
271 to investigate what factors contribute to the observed heterogeneity between some studies,

272 especially after having relatively stringent inclusion criteria. This would only be feasible,
273 however, if a much greater number of studies with available data and large samples sizes became
274 available.

275 We note that one study chosen (Whitfield-Cargile) was a distinct outlier in our analysis
276 from the other eleven studies. Unlike the other genetic and chemical models of dysbiosis
277 included in this meta-analysis, the NSAID-induced inflammation in the Whitfield-Cargile study
278 largely manifests in the distal jejunum and ileum and less the large intestine (i.e. it is not a true
279 "colitis" *per se*). Furthermore, the trigger of dysbiosis used in that study, the NSAID
280 indomethacin, is itself an anti-inflammatory agent that inhibits cyclooxygenase enzymes – which
281 would further change the nature of the resulting inflammation. This indicates that care must be
282 taken when comparing studies that may be superficially similar, but that have important
283 differences in their underlying mechanisms or locations within the host. Accordingly, we
284 believe the fact that the Whitfield-Cargile study performed differently than the other studies,
285 indicates that the dysbiosis index derived from our meta-analysis is robust and able to distinguish
286 colitis from other types of intestinal pathology.

287 The vast differences in the composition of the human and murine microbiota mean that
288 translation between the two cannot be made directly in terms of individual microbes. On one
289 hand, this means that it might require considerable effort in order to find any potential human
290 counterpart of murine microbes causally implicated in colitis. On the other hand, this has meant
291 that efforts to catalog the human gut microbiome has not helped much with cataloging the
292 murine microbiome and robust human meta-analyses to synthesize evidence on the IBD-
293 microbiome interaction cannot be readily used to guide future studies in mouse models.

In this context, the meta-analysis of animal studies can be useful. Generally, such studies are conducted for preclinical animal trials to select robust candidates for clinical trials, and prevent excessive replication (49, 50). However, animal meta-analyses can also play a valuable role in guiding research avenues in murine models of complex systems like microbiomes that are often studied at a macro-level, without a systematic approach to investigating host-microbe or microbe-microbe interactions at a micro level. In our meta-analysis, we find that simple metrics to summarize microbial diversity and composition may not be consistent; we also identify a microbial signature of disease that is relatively robust across studies, and report a list of microbes that may be good candidates for focused isolation and characterization efforts.

Most importantly, through the quantitative synthesis of published literature, we identified a number of organisms that seem to be consistently associated with health or disease in murine models of IBD. The colitis-associated microbes are likely to be good candidates for screening in mono-association or infection studies, whereas the healthy-associated ones are likely to be good candidates for probiotic studies. Many of the identified strains are phylotypes that are yet to be isolated. In a rapidly evolving field where mice microbiomes have been under increasing attention for systematic approaches to cataloguing and strain isolation, this study provides a tool that can be used to prioritize efforts.

Finally, our experience suggests that trying to standardize microbiome studies and make the data publicly accessible is of paramount importance. One of the main time-consuming steps in our analysis was the custom processing of datasets generated by a diversity of sequencing approaches, and the resolution of our taxonomic classification was limited by the diversity of 16S primers used. While we identified 44 studies of interest for the meta-analysis, we were only able to obtain data from 12, suggesting that only a small fraction of published sequencing data is

317 actually deposited in a publicly accessible system. Journal policies on data-sharing can help
318 rectify this.
319
320 **METHODS**
321 **Study Search and Inclusion**
322 To identify studies that investigated the murine intestinal microbiota in the context of
323 intestinal inflammation, we conducted a systematic search of NCBI PubMed for articles that
324 contained terms relating to microbiota, intestinal inflammation and murine models in the title and
325 abstract, published between 2012 and 2016, and was not a review. We followed the preferred
326 reporting items for systematic reviews and meta-analyses (PRISMA) guidelines to limit
327 inclusion bias (51). The detailed search term was: "((microbiota[Title/Abstract] OR
328 microbiome[Title/Abstract]) AND (colitis[Title/Abstract] OR (inflammation[Title/Abstract]
329 AND (mucosa*[Title/Abstract] OR epitheli*[Title/Abstract] OR colon*[Title/Abstract] OR
330 gut[Title/Abstract] OR intestin*[Title/Abstract]))))) AND ("2012/01/01"[PDAT] :
331 "2016/12/30"[PDAT]) AND (mice[Title/Abstract] OR mouse[Title/Abstract] OR
332 murine[Title/Abstract]) NOT review[Publication Type]". We screened the title and abstracts of
333 816 articles yielded by the search for eligibility. In addition, we obtained three studies from
334 knowledge of published literature, and a currently unpublished dataset of an IL-10 knockout
335 model of colitis from a collaborator.
336 Seventy-nine full-text articles were then assessed, and 44 were retained to be checked for
337 data availability. Ten studies had read files accessible in Sequence Read Archive, European
338 Nucleotide Archive, MG-RAST or personal collaboration; 32 studies had no publicly available
339 data or metadata, and only two provided access to data by the time of publication after contact.

Studies were excluded if they: did not do a 16S sequence-based analysis of the microbiome; used a synthetic microbiota (e.g. Altered Schaedler Flora, humanized); only had outcomes of low-grade inflammation or aging associated "inflammaging"; used pathogenic infection for inflammation; used bacterial treatment (e.g. probiotics); used antibiotic treatment; used non-murine models; did not have an outcome of colonic inflammation; did not have non-colitis controls; was not a primary article; or that failed to assess the microbiota before onset of inflammation. We had the final exclusion criteria because many studies aim to answer the question of whether a particular microbiota is associated with increased susceptibility to colitis; however, we were interested in a microbial signature associated with the *onset* of colitis. We retained articles if they contained a subset of samples that were eligible, contingent on non-colitis controls being present. Within the included studies, we excluded any samples that met relevant exclusion criteria outlined above (e.g. low-grade inflammation, antibiotic treatment).

Data Processing

For each study, we used a standardized bioinformatics pipeline to generate counts for taxonomic groups. Quality filtering criteria was determined on a study-by-study basis depending on the sequencing platform used and inspection of read quality (52, 53). Reads were filtered and resolved to amplicon sequence variants (ASVs) using the DADA2 pipeline. The advantage of using DADA2 over traditional clustering methods is that it resolves differences of as little as one nucleotide to determine exact sequences based on an error model for the sequencing run (54). Resolved paired-end sequences were merged where applicable, chimeras removed and a RSV-abundance tables built (equivalent to OTU-tables).

RSVs were assigned microbial taxonomies using a custom R script (55). Sequences were searched against the EZTaxon database (January 2017 Update) (30) using vsearch (56) and assigned the taxonomy (up to species level) of the highest identity match >97%. Tied hits were assigned ambiguous classification (e.g. Shigella/Escherichia). Use of EZTaxon allows classification of uncultured bacteria because it has unique identifiers assigned to manually curated phylotypes. RSVs classified as "Streptophyta" at phylum level were filtered out. RSV counts were standardized by calculating relative abundances. Within each study, RSVs that did not make up more than 0.2% of the community in at least one sample, as well as taxonomic groups that were detectable in only one sample, were removed.

To enable merging of datasets from different studies, that used different 16S regions, RSVs unclassified at the species level were removed. Next, RSV abundance tables were collapsed to the highest taxonomic resolution possible (e.g. x/y from one study was collapsed into x/y/z group if another study could not resolve between x/y/z). Data handling, merging and filtering was done using the phyloseq package (57).

Alpha and Beta Diversity Analysis

For each sample, we estimated the Shannon diversity index using the vegan package (58), as well as the ratio of Bacteroidetes abundance to Firmicutes abundance (BF ratio). Following normalizing transformations, t-tests were used to detect significant differences between healthy and mice with colitis, within each study. For the pooled data, a linear random effects model (implemented using the lme4 package (59)) with random slopes and intercepts was used to determine if there was a statistically significant association between the measures and disease status.

385 Diversity across samples was investigated using Principal Coordinates Analysis (PCoA)
386 and permutational analysis of variance (PERMANOVA) (adonis function; 999 permutations)
387 using the vegan package. This was done separately for each study, as well as the pooled data.
388
389 **Random Forest Models**
390 A random forest (RF) model was built with the pooled data using the caret package (60)
391 (10-fold cross validated, repeated 5 times; 250 trees), and area under the receiver operating
392 characteristic curve (AUROC) was sued to assess model performance in discriminating healthy
393 from diseased mice. To investigate the generalizability of using RF models, we conducted a
394 leave-one-out analysis, repeatedly building models on data from n-1 studies, and testing model
395 performance in predicting the study left out, using AUROC.
396
397 **Variable Selection and "Dysbiosis" Index**
398 To identify which taxonomic groups are most important in discriminating between
399 healthy and diseased mice within a random forest framework, the Boruta feature selection
400 algorithm was used with 500 runs using the Boruta package (46). Next, the selected taxa were
401 labelled as being associated with healthy or diseased status based on whether they had higher
402 mean abundance in healthy or diseased mice, respectively (statistical significance not
403 considered). This labelled list was pruned to contain only taxa present in 50% of diseased or
404 healthy mice. The dysbiosis index was calculated by log transforming the ratio of the colitis-
405 associated taxa to the health-associated taxa.

406 As with the alpha diversity metrics, within each study, t-tests were used to determine the
407 utility of the index in discriminating between healthy and diseased mice, and a similar random
408 effects model was used for the pooled data.

409 All data was visualized using the ggplot2 package (61).

410

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420

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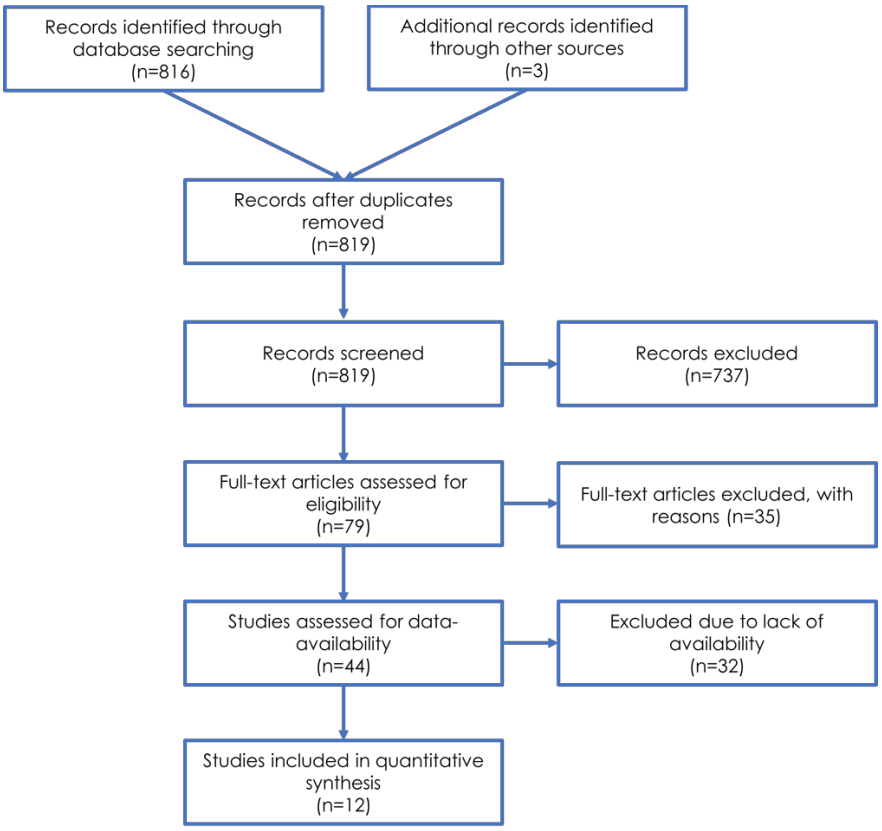

573

574 **FIG 1:** Flow diagram of literature search and review for inclusion in meta-analysis, represented

575 according to PRISMA guidelines.

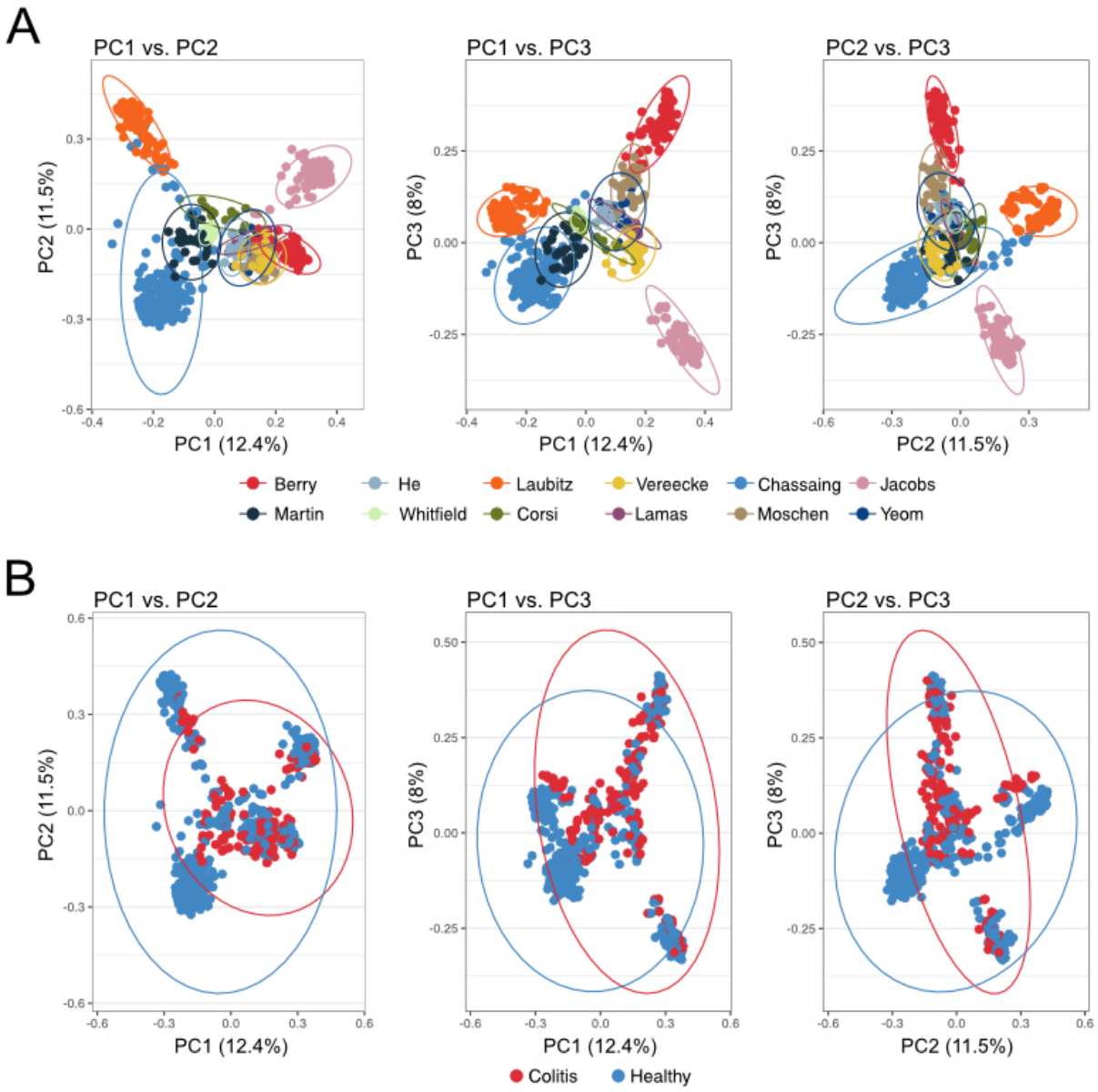

FIG 2: Principle Coordinates Analysis (PCoA) plots depicting the relationships between sample microbial compositions along the first three principal coordinates. Colored points and the associated ellipses distinguish between the different studies/datasets (A) or disease status (B).

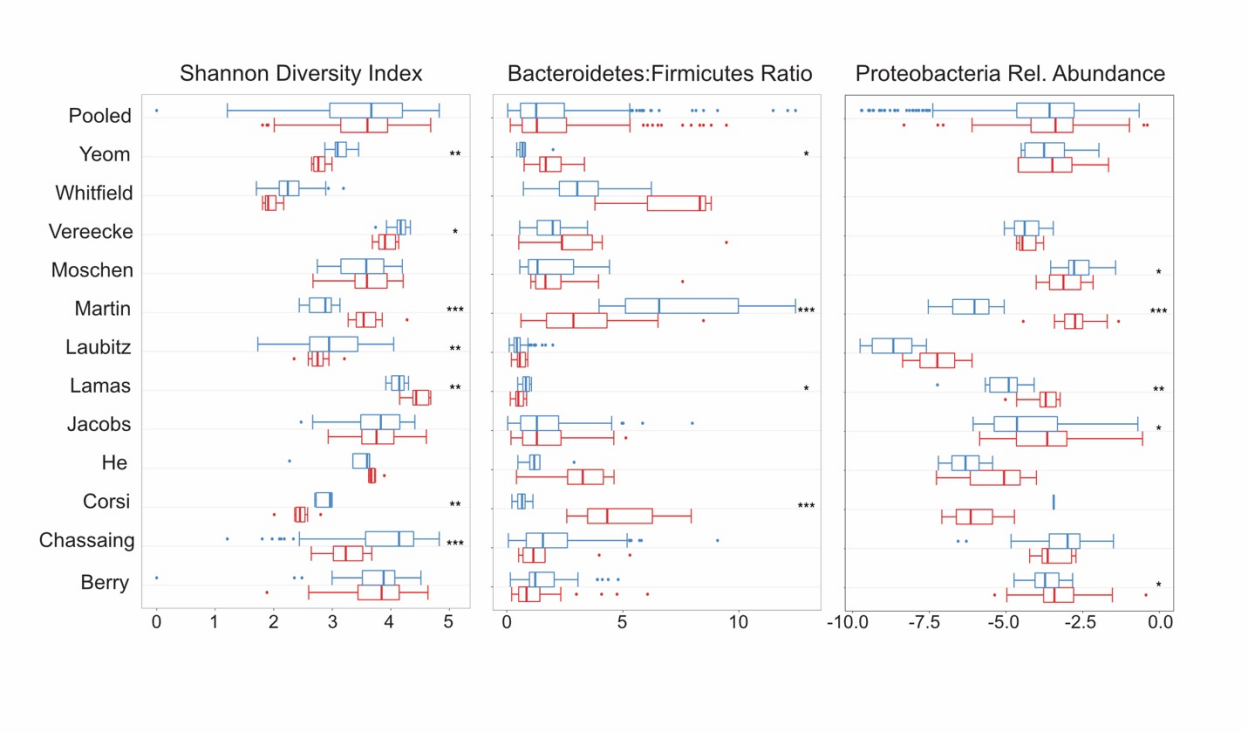

FIG 3: Boxplots of commonly used diversity and composition metrics. From left, each panel shows distribution and statistical significance of Shannon Diversity Index, Bacteroidetes-Firmicutes Ratio and Relative Abundance of Proteobacteria, within each study and for pooled samples. Blue represents healthy samples and red represents colitic samples. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

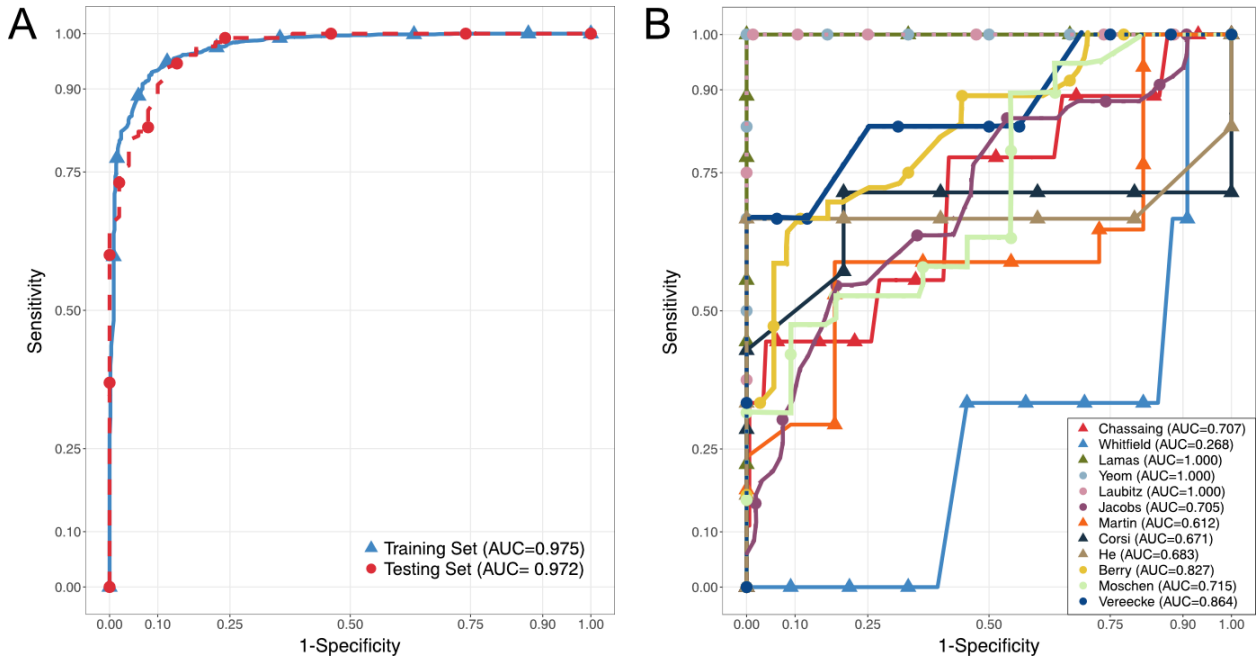

FIG 4: Receiver-Operator Curves demonstrating predictive performance of Random Forest Models, with Area-Under the Curves (AUC) reported. (A) shows cross-validated performance on training set consisting of a random 70% of all samples (blue) and performance on remaining 30% of samples in the testing set (red). (B) shows performance of models trained on (n-1) studies on the study that was left out. Each color-shape and name in the legend refers to the study that was left out of, and predicted by, the model.

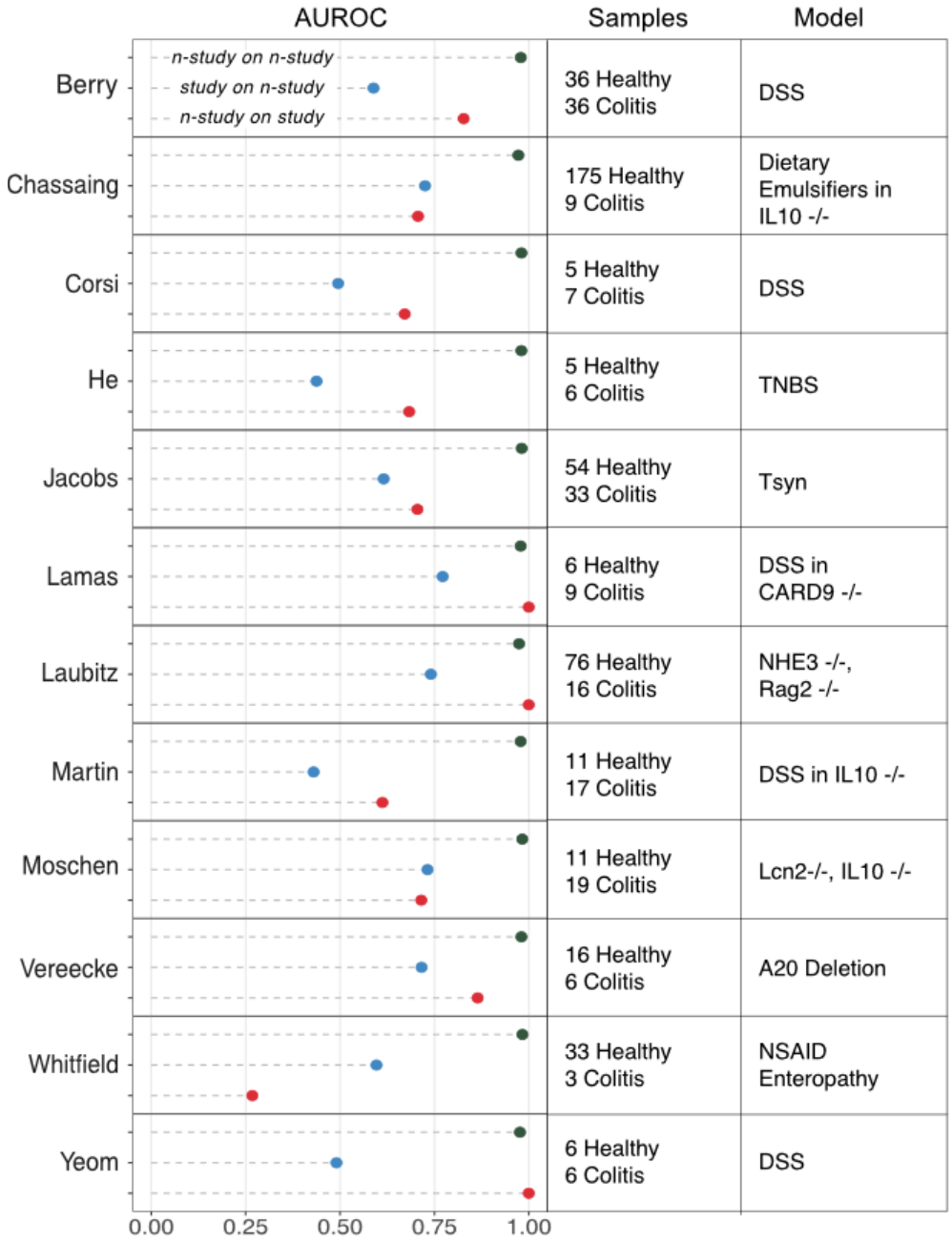

FIG 5: Summary of model performances in terms of Area Under the Receiver-Operator Curve (AUROC) and characteristics for each study. The top line (green) shows cross-validated performance of model when the named study was left out (e.g. for first panel, cross-validated AUROC for model with all studies except Berry et al.). The second line (blue) shows

599 performance of model trained on named study on predicting all other studies (for first panel,
600 AUROC for model trained on Berry et al. and tested on all studies except Berry et al.). The third
601 line (red) shows performance of model trained on all studies except the named one, on predicting
602 the named one (for first panel, AUROC for model trained on all studies except Berry et al. and
603 tested on Berry et al.).

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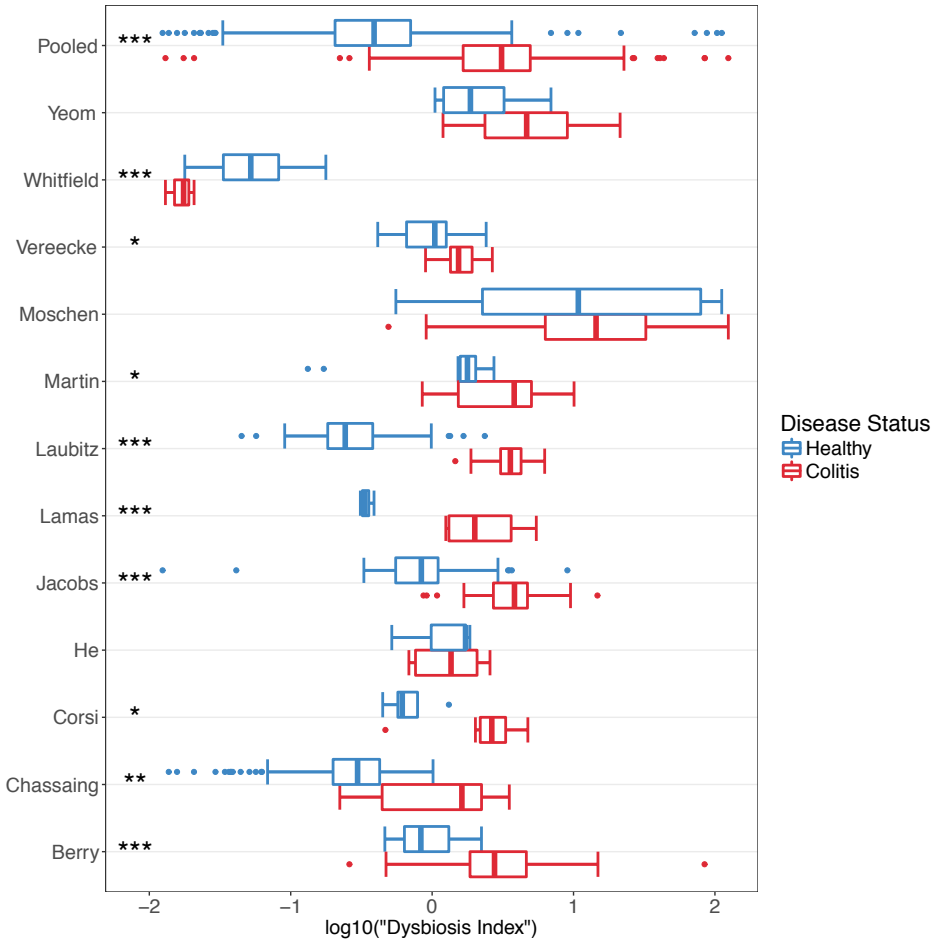

606

607 **FIG 6:** Boxplots showing distribution and statistical significance of "dysbiosis index" within

608 each study and for pooled samples. Blue represents healthy samples and red represents colitic

609 samples. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

610

Study, Year	Title	Colitis Model	Mouse Strains	DNA extraction	Region	Platform	Biological Source	Sample counts	Data Source
Jacobs et al. 2017[19]	Microbial, metabolomic, and immunologic dynamics in a relapsing genetic mouse model of colitis induced by T-synthase deficiency.	Tsyn mice (C1galt1 or T-synthase deleted)	C57BL/6	MO Bio PowerSoil DNA Isolation Kit	V4	Illumina HiSeq	Intestinal lavage and wash	54 Healthy 33 Colitis	Public, PRJNA318692
Chassaing et al. 2015[20]	Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome.	Dietary emulsifiers in IL10-/- mice	C57BL/6	MO Bio PowerSoil DNA Isolation Kit	V4	Illumina MiSeq	Fecal Samples	175 Healthy 9 Colitis	Public, PRJEB8035
Laubitz et al. 2016[21]	Reduced Epithelial Na+/H+ Exchange Drives Gut Microbial Dysbiosis and Promotes Inflammatory Response in T Cell-Mediated Murine Colitis.	NHE3-/-, Rag2-/-	129S6/SvEv	Beads + Proteinase, Phenol-chloroform	V4	Illumina MiSeq	Fecal Samples	76 Healthy 16 Colitis	Public, 10.17605/ OSF.IO/UWFAP
Yeom et al. 2016[22]	Sasa quelpaertensis leaf extract regulates microbial dysbiosis by modulating the composition and	DSS	C57BL/6	Fast DNA SPIN Kits	V1-V3	Roche 454	Fecal samples	6 Healthy 6 Colitis	Public, PRJEB13815

	diversity of the microbiota in dextran sulfate sodium-induced colitis mice.								
Lamas et al. 2016[32]	CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands.	DSS in CARD9-/- mice	C57BL/6J	Beads in FastPrep, isopropanol.	V3-V4	Illumina MiSeq	Fecal samples	6 Healthy 9 Colitis	Public, PRJEB9079
Whitfield-Cargile et al. 2016[33]	The microbiota-derived metabolite indole decreases mucosal inflammation and injury in a murine model of NSAID enteropathy.	NSAID (indomethacin)	C57BL/6J	MO Bio PowerSoil DNA Isolation Kit	V4	Illumina MiSeq	Fecal samples	33 Healthy 3 Colitis	Public, PRJNA290483
Martin, unpublished	Experiments showing IL10-/- deletion caused spontaneous colitis in some mouse cages but not others	DSS in IL10-/- mice	C57BL/6	DNA from soil kit (Macherey-Nagel)	V4	Illumina MiSeq	Fecal samples	11 Healthy 17 Colitis	Request
Sassone-Corsi et al. 2016[34]	Microcins mediate competition among Enterobacteriaceae in the inflamed gut.	DSS	C57BL/6 Slc11a1^{+}	QIAamp DNA Stool Kit	V4	Illumina MiSeq	Fecal samples	5 Healthy 7 Colitis	Public, PRJEB15700

Moschen et al. 2016[35]	Lipocalin 2 protects from inflammation and tumorigenesis associated with gut microbiota alterations.	Lcn2-/-, IL10-/-	C57BL/6J	FastDNA SPIN Kit, Precellys 24 homogenizer	V1-V2	Illumina MiSeq	Cecal content	11 Healthy 19 Colitis	Public, ERP014639
Berry et al. 2015[36]	Intestinal microbiota signatures associated with mice experiencing recurring colitis.	DSS	C57BL/6	Phenol-chloroform, bead	V6-V9	Roche 454	Intestinal flush, fecal samples	36 Healthy 36 Colitis	Request
Vereecke et al. 2014[37]	A20 controls intestinal homeostasis through cell-specific activities.	A20 deletion	C57BL/6	QIAamp DNA Stool Mini Kit	V3-V5	Roche 454	Cecal content	16 Healthy 6 Colitis	Request
He et al. 2016[38]	Dysbiosis of the fecal microbiota in the TNBS-induced Crohn's disease mouse model	TNBS	BALB/c	Phenol-chloroform, bead	V5-V4	Ion Torrent PGM	Fecal samples	5 Healthy 6 Colitis	Public, ERP011541

TABLE 1 Summary of studies included in the meta-analysis. DSS = Dextran Sulfate Sodium, IL10 = Interleukin 10, NSAID = Non-Steroidal Anti-Inflammatory Drug, TNBS = 2,4,6-trinitrobenzene sulfonic acid.

Median Importance	Phylum	Class	Order	Family	Genus	Species	EZTaxon ID	Associated Status	Proportion Present
8.467	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	AB626912_g	EU452880_s	107581	Healthy	0.502
7.015	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	HM124280_g	EF603706_s	103736	Healthy	0.829
6.873	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus_gallinarum/Lactobacillus_hamsteri/Lactobacillus_kitasatonis/Lactobacillus_acidophilus/Lactobacillus_gasseri/Lactobacillus_ultunensis/Lactobacillus_helveticus/Lactobacillus_johnsonii/Lactobacillus_hominis/Lactobacillus_rodentium/FN667084_s/Lactobacillus_amylovorus/Lactobacillus_crispatus/Lactobacillus_kalixensis/Lactobacillus_taiwanensis	117587/127919/85874/85875/85904/95031/95041/95044/95067/95231/95239/95242/95646/95665/95889	Healthy	0.654
6.829	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Eisenbergiella	AB626943_s/EU457259_s/EF603797_s/EU622677_s/EU457126_s	103742/107624/107627/109826/84929	Healthy	0.528
6.557	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	DQ815871_g	EF603701_s/EU474208_s/EF099993_s/HM124175_s/EU456490_s	101731/103735/107617/108613/123282	Healthy	0.611
5.916	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillibacter	FJ880499_s/EF602810_s/EU505076_s/GU302582_s/FJ880976_s/FJ881211_s/EU454374_s/EU771312_s/EU454366_s/EU453793_s/KE159714_s	103700/107590/107598/107599/109224/110626/116255/116284/116308/121628/139962	Healthy	0.613
5.416	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	HM124247_g	EF603835_s/EF097965_s/EU455014_s	101684/103744/107601	Healthy	0.657
4.652	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	EF602759_g/EU622749_g	EF604981_s/HM123985_s/EU450917_s	103776/107567/123229	Healthy	0.778
4.558	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae/Lachnospiraceae	Pseudoflavonifractor/Butyricicoccus/Eisenbergiella/FJ374222_g	Pseudoflavonifractor_capillosus/Eubacterium_desmolans/JQ191036_s/EF404944_s/KI535319_s/FJ881243_s/HM124177_s/EF603680_s/EF604701_s/AB606283_s/EF097039_s/ADDX_s/FJ374222_s/Flavonifractor_plautii/AB606233_s/AY244908_s/FJ879530_s/EF603862_s/EF603786_s/AY858452_s/AB626937_s/EU622686_s/BCAB_s/AB606341_s/EU794285_s/FJ879507_s/AB606380_s/GQ897291_s/EF400624_s/EF071402_s/PAC000182_s/JQ083832_s/EU505160_s/AB606266_s/AB606386_s/FJ880805_s/EU456711_s/DQ015070_s/AY992183_s/JQ084492_s/JQ084301_s/EU773377_s/DQ456429_s/HQ716472_s/EF404855_s/FJ510897_s/DQ057387_s/EU509811_s/JQ084116_s/DQ456157_s/JQ084120_s/EF096610_s/HQ750839_s/Intestinimonas_butyriciproducens/GQ867588_s/FJ880402_s/FJ368283_s/EU454100_s/EU4	100395/100646/101556/101664/101669/101672/102250/102321/102325/103732/103741/103748/103773/103892/104268/104284/106717/107596/107620/107724/108101/109210/109226/109289/109472/109831/110754/110830/111111/112154/113204/113242/114211/116208/116209/116250/116273/116312/120648/120687/123220/123283/127181/127401/127454/127589/127827/130498/134093/134182/135159/135163/135164/135166/135171/135178/135303/	Healthy	0.601

						65687_s/EU460161_s/EU504346_s/EU344341_s/EF644509_s/EU009861_s/EU009822_s/AM278900_s/EF096916_s/DQ815545_s/AB606256_s/JN713225_s/HM123968_s/HQ782969_s/HQ821334_s/JX198570_s/JX047097_s/HQ759796_s/JQ599692_s/EU775346_s/EU888823_s/AB062828_s/AY916184_s/EU542517_s/JQ084175_s/JN680614_s/DQ795333_s	136192/137750/138265/139755/140361/141122/142006/80589/81115/84772/84784/84792/84801/84831/84849/84853/84926/85961/90333/92836/94458/94607/94957/95439/97493/97598/99582/99594		
4.526	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	AY305316_g	EU456172_s	107613	Healthy	0.507
4.4567	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillibacter	AB606367_s/EF602808_s/JQ084467_s	103699/135173/84845	Healthy	0.666
4.316	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus_apodemi/Lactobacillus_animalis/Lactobacillus_faecis/Lactobacillus_murinus	85634/86083/95249/95520	Healthy	0.726
4.180	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	DQ815871_g	DQ815395_s	100637	Healthy	0.751
4.149	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Eisenbergiella	EF603669_s	103730	Healthy	0.507
3.949	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	KE159538_g	KE159538_s	139953	Healthy	0.620
3.924	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	FJ881296_g	EF406456_s	102343	Healthy	0.793
3.354	Actinobacteria	Actinobacteria_c	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium_choerinum/Bifidobacterium_pseudolongum	130483/130566/92228	Healthy	0.551
3.311	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	EF406773_g	EF406773_s	102358	Healthy	0.551
3.197	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillibacter	AB606333_s/AB606363_s/HM124063_s/FJ881219_s/AB606362_s/JQ085218_s/EU457459_s/EU509241_s	107630/109277/116309/123250/135196/84826/84842/84843	Healthy	0.574
3.165	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus_reuteri/Lactobacillus_panis/Lactobacillus_pontis/Lactobacillus_antri/Lactobacillus_vaginalis/Lactobacillus_oris/Lactobacillus_frumenti	143355/85903/86957/89050/91592/95057/95077	Healthy	0.562
2.718	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	EF602759_g	DQ815429_s	100640	Healthy	0.567
12.406	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides_fragilis/Bacteroides_salyersiae/Bacteroides_acidifaciens/Bacteroides_finegoldii/Bacteroides_thetaiotaomicron/Bacteroides_xylanisolvens/AB021165_s/DQ798855_s/Bacteroides_faecichinchillae/Bacteroides_faecis/AY986255_s/FJ371693_s/FJ368968_s/JH815484_s/HQ769253_s/HQ804309_s/Bacteroides_ovatus/HQ789817_s/DQ805799_s	100427/100475/101203/113206/113213/127510/127651/127740/130536/80812/82489/86035/88159/90221/91731/94946/95194/95223/96915	Colitis	0.557
7.897	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	FJ880046_g	EF406536_s	102347	Colitis	0.880
6.354	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	HM124117_g	EF097184_s	101677	Colitis	0.713
5.869	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes	DQ815748_s/FJ510995_s	100663/114212	Colitis	0.647
5.044	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	Parabacteroides_goldsteinii	91730	Colitis	0.503

4.991	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	EF406806_g	EF603121_s	103716	Colitis	0.623
4.688	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	EF406806_g	EF603904_s	103750	Colitis	0.557
4.676	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	HM124247_g	EF406368_s	102341	Colitis	0.844
4.029	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	EF406712_g	EF406712_s	102356	Colitis	0.635
3.462	Firmicutes	Clostridia	Clostridiales	EU234093_f	AB606326_g	AB606326_s/EF604610_s	103758/84822	Colitis	0.533
3.287	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_f	HM124247_g	EF603149_s	103717	Colitis	0.587
3.180	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Anaerotruncus	DQ168656_s/KE159677_s	139961/98138	Colitis	0.551

TABLE 2 Thirty-three taxa selected through Boruta feature selection for inclusion in dysbiosis index. The median importance is the median importance score calculated by the Boruta algorithm. EZTaxon ID refer to the ID number in the EZTaxon database. Proporotion present refers to the proportion of healthy or colitis samples the taxon was present in.