The gut microbiota potentiates *Clostridiodes difficile* infection severity.

Running title: Microbiota potentiates *Clostridioides difficile* infection severity

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Abstract

Clostridioides difficile infection has become more common and severe in the last few decades. Patient age, white blood cell count, creatinine levels as well as *C. difficile* ribotype and the presence of toxin genes have been associated with disease severity. However, models based on those data to identify patients with CDI that will develop severe disease have not been robust enough to be broadly applied. These models are input from our understanding of *C. difficile* interactions with the gut environment. The gut bacteria are a key determinant in *C. difficile* infection. CDI is dependent on perturbations in the bacterial community in order to colonize the gut. The gut microbiota also impairs *C. difficile* colonization through bile acid metabolism, nutrient consumption and bacteriocin production. However, it is unclear if the gut bacteria affect the disease severity resulting from that colonization. Here we have shown gut bacterial communities contribute to disease severity variation. We created diverse gut communities by colonizing germ-free mice with human fecal communities. They were then infected with *C. difficile* which resulted in differed disease severities. The severity grouped by the human fecal community they received. Generally, facultative anaerobes with pathogenic potential, such as Escherichia, Helicobacter and Klebsiella, were associated with more severe disease. Bacterial groups associated with dietary fiber degradation, such as Coprobacillus, were associated with reduced disease severity. Lastly, we showed communities that resulted in either low or high histopathologic scores or severe disease had the same outcome when infected with a different *C. difficile* isolate.

Importance

Clostridioides difficile infection (CDI) causes a range of disease from asymptomatic to mild diarrhea to severe outcomes of recurrent infections and even death. Models that

predict severity and treatment decisions are based on clinical factors and *C. difficile* capabilities. But currently the gut microbiome, the primary protector against CDI, has not been accounted for its effect on CDI disease severity. We demonstrated variation in the microbiome of mice colonized with human feces was sufficient to result in a range of disease severities. Our results show groups of bacteria contribute to the development of disease in *C. difficile* infections. Gut bacterial community data from patients with CDI could improve our ability to identify patients more at risk for severe disease and improve interventions which treat the gut bacteria to reduce host damage.

Introduction

Clostridioides difficile infections (CDI) have been increasing in incidence and severity since *C. difficile* was first identified as a pathogen. It was first identified in 1931 in a healthy fecal community [CITATION]. But then in the late 1970s, following the introduction of broad-spectrum antibiotics, *C. difficile* was identified as the cause of antibiotic-associated psuedomembranous colitis [CITATION]. Beginning in the early 2000s, CDI became more problematic with increasing incidences and severity [CITATION]. Disease severities can range from asymptomatic to mild diarrhea to toxic megacolon and death. This severity has been associated with characteristics of *C. difficile* ribotypes and host factors. Over time more pathogenic ribotypes have emerged, such as ribotype 027 in the early 2000s. The presence of the genes for Toxin A/B or fluoroquinolon resistence has been associated with more severe disease [CITATION]. Binary toxin, while alone insufficient to cause damage on it own, when in the presence of Toxin A/B has been associated with increased virulence [CITATION]. Mutations in tcd C, the negative regulator of of toxin transcription, also increased the virulence of *C. difficile* [CITATION]. In addition to the pathogenic potential of the infecting strain, the response by the host also affects the disease severity. Delayed or reduced production of antibodies IgG and IgA can result in more severe disease [CITATION]. Increased neutrophil inflitration can lead to worse outcomes [CITATION]. In addition to the immune response, increased density of the toxin receptor on the host cells can increase the severity of the infection [CITATION]. While there is a thorough understanding of many of the mechanisms driving in CDI incidence and disease severity, there still is not effective tools to reduce the risk of severe disease.

Models have been developed to identify patients at risk for severe CDI but have not been robust for broad application (1). These models utilize patient related data, such as patient age, white blood cell count, serum albumin levels, and creatinine levels, to predict disease severity. In their initial publication, these models perform well enough for publication.

However when Perry et al applied many of the most current models to a multi-center external validation, their performance suffered and often had more false positives than true positives (3). Thus, there seems to be a missing factor contributing to disease severity.

Missing from these models are the gut bacteria which also interact with *C. difficile* during the course of the infection. The indigenous bacteria of a healthy intestinal community provide a protective barrier preventing *C. difficile* from infecting the gut. Only after this community is disrupted from a range of pertubations, such as many classes of antibiotics, medications, or even dietary changes, can *C. difficile* infect the intestine. Once established, the gut bacteria can either promote or inhibit *C. difficile* through producing molecules or modifying the environment [Abbas2020]. Bile acids metabolized by the gut bacteria can inhibit *C. difficile* growth and affect toxin production (5). Bacteria in the gut also can compete more directly with *C. difficile* through antibiotic production or nutrient consumption (7). To eliminate CDI, the primary treatment is a course of antibiotics. Patients who become reinfected and recalitrant to antibiotics, a fecal microbiota transplantion often is sufficient to restore the gut community to eliminate *C. difficile*. While there has been many studies to investigate the interaction between the gut bacteria and *C. difficile* during the infection, it has not been demostrated the bacterial community can modulate the disease severity of CDI. Recently, Tomkovich et al showed the variation in the bacterial communtiies between mice from different colonies and vendors was sufficient to cause differences in the temporal dynamics of the *C. difficile* infection (9). Since the gut bacteria interact with *C. difficile* throughout the infection and community difference resulted in different colonization dynamics, we hypothesized that the gut bacteria contribute to the variation in CDI disease severity. Here, we colonized germ-free C57BL/6 mice with human fecal communities to test if the variablity in the gut bacteria can produce and explain variation in the disease severity caused by a single *C. difficile* isolate.

Results

Germ-free mice colonized with human fecal commmunities have diverse and unique community structures. Based on our previous observation mice with variation in their microbiota showed differences in colonization of *C. difficile* (9), we sought to explore the effect communties with greater variation had on *C. difficile* challenge. To produce communities with greater variation than mouse colonies with their indigenous communties, we used human fecal communities to establish a community in the intestine of germ-free mice. We inoculated a cage of germ-free C57BL/6 mice (2-4 mice per cage) with homogenized feces from one of 15 different human donors via oral gavage. The fecal community was allowed to colonize and stabilize for two weeks post inoculation [CITATION?]. We then assessed the gut bacterial communities by sequencing the V4 region of the 16S rRNA gene extracted from fecal pellets (Figure 1A). The communities established in the mice group most closely to other mice recieving the same human fecal donor community than to either their respective donor or any mice who received stool from a different donor (Figure 1B). Most of the communities were primairily composed of populations of *Clostridia*, *Bacteroidia*, *Erysipelotrichia*, *Bacilli*, and *Gammaproteobacteria*. However, each group of mice harbored unique combination of class abunadances in their gut bacterial communities. *Any alpha? compare to murine variation? Number of unique otus?*

***C. difficile* is able to colonize mice without perturbation.** Our goal was to test the effect of the variation in the microbiota on *C. difficile* infection. A typical mouse model of CDI requires pretreatment with antibiotics such as clindamycin to become susceptible to *C. difficile* colonization [CITATION]. However, we wanted to avoid modifying the communties with an antibiotic to maintain their community structures and differences. Since these communties came from human donors which may have become susceptible through their own exposures, we decided to test if *C. difficile* was able to colonize these communities

without any perturbation. After two weeks post incoluation with human fecal communities, the mice were then challenged with 10^**4?** *C. difficile* ribotype 027 isolate 431 spores (**Add schematic?**) (10). The mice were followed for 10 days post challenge and their stool was collected and plated for *C. difficile* CFU to detect colonization level. We hypothesized that *C. difficile* would most likely be able to colonize the mice with communities that came from donors which had a history of antibiotic use, one of the primary risk factors for developing CDI (TABLE, CITATION). Suprisingly, communities from all donors were colonized (Figure 2). The only two mice that were able to resist *C. difficile* colonization both recieved their community from the same donor. Therefore, the transplanted human fecal communties were susceptible to *C. difficile* colonization without the need for any perturbation.

Infection seveity varies by initial community structure. After challenging the mice with *C. difficile*, we looked at the effect of the community variation on the outcome from the *C. difficile* infection. We followed the mice for signs of disease as well as *C. difficile* colonization and toxin production for 10 days post challenge. Some mice were not initally colonized on the day after *C. difficile* challenge but became colonized with a few days post challenge (**SUPP FIG?**). A subset of mice, all that recieved their comunity from one of 6 donors, suffered from severe disease, which became highly colonized one day post challenge and moribund within 3 days post challenge. The remaining mice, except for one cage, maintained *C. difficile* colonization through the end of the experiment (Figure 2). After the mice were humanely euthanised at the endpoint, day 10 post challenge or earlier if they became moribund, their cecal tissue was collected and scored for histological signs of disease and fecal samples were assayed for toxin (Figure 3). Overall, there was greater toxin activity detected in the stool of the moribund mice (P = 0.003). However, when looking at each group of mice, we saw there was a range in toxin activity for both the mice with non-severe disease and severe disease (Figure 3A). Some donor groups that maintained colonization without severe disease had similarlly high activity levels as was detected in the mice with severe disease. Additionally, some mice with severe disease

did not have toxin activity detected in their stool. These oberserved variations in toxin activity and relation to disease severity have been previously characterized (11). Next we examined the cecal tissue for histopathological damage. For mice with severe disease, we observed high levels of epithelial damage, tissue edema and inflammation (Figure SXXXX), similar to previously reported histopathologic findings for *C. difficile* ribotype 027 (13). As observed with toxin activity, the mice with severe disease had higher histopathologic scores than the mice with non-severe disease (P = 3.0e-9). However, unlike the toxin activity, all moribund mice had consistently high histopathologic scores. The mice which maintained persistent population size, we saw a range in tissue damage from no signs of disease to nearly the same level as the mice with severe disease, which grouped by community donor. Together, the toxin activity, histopathologic score, and moribundity showed variation across the donors but were largely consistent within each group of mice recieving the same human donor fecal community.

Populations of the microbial communities explain variation in CDI disease severity. Since the disease charateristics grouped by human donor fecal community, we next investigated the community composition for its ability to explain the variation in disease severity. First, we tested for associations between taxonomic groups and the outcomes of the *C. difficile* infection. We used the linear discriminant analysis (LDA) effect size (LEfSe) method to identify differences in the genera of the initial bacterial communities between the severity level and toxin production. We split the mice into groups by severity level based on their if they became moribund (severe), or if their clinical score was above 5 (moderate) or below 5 (mild). This analysis revealed 20 genera that were significantly different by the disease severity (Figure 4A). The presence of populations of *Turicibacter*, *Streptococcus*, *Staphylococcus*, *Pseudomonas*, *Phocaeicola*, *Parabacteroides*, *Bacteroides*, and *Escherichia/Shigella* were detected at higher levels in the mice that developed severe disease. Populations of *Anaerotignum*, *Coprobacillus*, *Enterocloster*, and *Murimonas* were increased in the mice that would experience mild disease. There were many

genera that did not have a correlation of relative abundance and disease severity, such as the populations of *Monoglobus* and *Hungatella*, which were decreased or increased, respectively, in only the group of mice that would develop moderate disease. To understand these cases better we used LEfSe to identify the genera differentiating the communities that we detected toxin in from those that we did not (Figure 4B). There were many genera the were associated with toxin production as we would expect from their association with severe disease, such as populations of *Escherichia/Shigella* and *Bacteroides*. Likewise, there were genera such as *Anaerotignum*, *Enterocloster*, and *Murimonas* that were higher in communities that had mild disease and no toxin was produced. However, communities without a linear trend between relative abundance and disease severity can be better understood by their association with toxin production. Populations of *Hungatella* were increased in the group with moderate disease but not with the production of toxin. Lastly, we looked for associations between the histopathologic score and the populations of genera at that same time (Figure 4C). The populatons of *Bacteroides* at the end matched the trend observed in the initial community, increased population correlated with increased histopathologic score. Populations of *Klebsiella* and *Prevotellaceae* positively correlated with clinic score and were increased in the group that produced toxin. These tests have identified which individual populations of bacteria that associated with each disease severity.

We next wanted to understand how the populations in the context of each other lead to the different disease severities. We trained random forest models with the intital bacterial community relative abundance data at each taxonomic rank to predict toxin production, severe disease or final histopathologic score. Overall for predicting toxin production, microbial populations aggregated by their phylum level classificiation performed as well as models using lower taxonomic ranks (AUROC = 0.83, Figure S2). In the initial community populations of *Verrucomicrobia*, *Campilobacterota* and *Proteobacteria* contributed the most to the correct prediction of toxin production (Figure 5A). *C. difficile* was more likely

to produce toxin when the initial community had smaller populations of *Verrucomicrobia* and *Campilobacterota* and had larger popluations of *Proteobacteria*. Next, we assessed the ability of the community to predict severe disease. Class rank classification was sufficient to predict if the mouse would succumb to the infection before the end of the experiment (AUROC = 0.91, Figure S2). Groups of bacteria belonging to *Bacilli*, *Firmicutes*, and *Erysipelotrichia* contributed the most to the correct prediction. Larger populations of *Bacilli* and *Firmicutes* and reduced populations of *Erysipelotrichia* were more likely to result in moribundity (Figure 5B). Aside from *Erysipelotrichia*, the only other population decreased in moribund mice was *Clostridia*, all other features had increased populations in the moribund mice. Lastly, the initial community was able to predict if the mice would have a histopathologic score above or below the median score (median histopathologic score = 5) with the genera relative abundances (AUROC = 0.99, Figure S2). There were no genera with a significantly greater effect on the model performance than any others, indicating the model is reliant on many genera for the correct prediction. The model used some of the genera identified in the LEfSe analysis, such as populations of *Coprobacillus*, *Anaerostipes*, and *Hungatella*. Communities with greater populations of *Hungatella*, *Eggerthella*, *Bifidobacterium*, *Duncaniella* and *Neisseria* were more likely to have greater histopathologic scores.

Disease severity consistent by donor community across strains/isolates. We used a single *C. difficile* isolate, ribotype 027 isolate 431 to characterize the range of disease severity and the features of the bacterial community associated with severity (10). Since we had used the same mice and *C. difficile* isolate in our first set of experiemnts, we next wanted to test if the effect of the community would apply to other *C. difficile* isolates. We selected three communities based on the result from our analysis thus far to select one community we would expect to result in a low clinical score (< 5), a high clinical score (> 5), and one with a high clinical score which becomes moribund. Using genus level data to select the communities, we selected communities based on the relative

abundance patterns of *Sellimonas* and *Anaerotignum* (higher abundance in mice with a low clinical score), *Lachnospiracea* (lower abundance in moribund mice), *Hungatella* and *Eisenbergiella* (higher abundance in moderate mice and lower in moribund mice), Clostridium sensu stricto (higher in moribund mice by both LEfSe analysis and RF model) and *Enterocloster* (lower in moribund mice and a negative linear trend with disease severity by LEfSe analysis) (Figure S3). With this set of human fecal communities selected, we inoculated mice via oral gavage of the human fecal slurry and allowed two weeks for the a community to establish. We then challenged the mice with a different *C. difficile* (10). The disease severity for this set of experiments closely matched that in our first set of experiments with *C. difficile* isolate 431 (Figure 6).

Discussion

Challenging mice colonized with different human fecal communities with a single *C. difficile* isolate allowed us to identify the effect of microbiome variation on *C. difficile* infection disease severity. Our LEfSe analyses and random forest models revealed the relationships between the bacterial community members and the degree of disease severity. We used the relative abundance patterns of community members with strong associations with disease severity to select communities to test if the observed disease severity is conserved when other *C. difficile* isolates are used. The disease outcome from the *C. difficile* chalenge with the new isolate matched the expected disease severities. Overall, these results have demonstrated that the bacterial community is capable of modulating the disease severity of *C. difficile* infection.

CDI severity can range from no symptoms, mild symptoms such as diarrhea, or severe symptoms such as toxic megacolon or death. Physicians use prediction tools to identify patients most at risk of developing a severe infection using white blood cell counts, serum

albumin level, or serum creatine level (14). Those levels are being driven by the activities in the intestine (17). Research into the drivers of this variation have revealed factors that make *C. difficile* more virulent. Strains are categorized for their virulence by the presence and production toxin A and toxin B and the prevelance in outbreaks, such as ribotype 027 and 078 (18). However, other studies have shown that virulence is not necessarily linked with toxin production **???** or the strain **???**. Furthermore, there is variation in the genome, growth rate, sporulation, germination, and toxin production in different isolates of a strain (10). This variation may help explain why severe CDI prediction tools often miss identifying many patients with CDI that will develop severe disease (1). Therefore, it is necessary to gain a full understanding of all factors contributing to disease variation to improve our ability to predict severity.

The state of the intestinal bacterial community determines the ability of *C. difficile* to colonize, persist and even cause recurrent infections. *C. difficile* is unable to colonize an unperturbed healthy gut community and is only able to entry after a perturbation (24). Once colonized, the different communities lead to different metabolic responses and dynamics of the *C. difficile* population (25). The gut bacteria metabolize primary bile acids into secondary bile acids (27). The concentration of these bile acids affect the germination, growth, toxin production and biofilm formation (5). Members of the bacterial community also affect other metabolites *C. difficile* utilizes. *Bacteroides thetaiotaomicron* produce sialidases which release sialic acid from the mucosa for *C. difficile* to utilize (31). The nutrient environemt affects toxin production (33). Thus, many of the actions of the gut bacteria modulate *C. difficile* in ways that would affect the disease severity driven by CDI.

The gut community has not been demostrated to modulate CDI disease severity. Myriad studies have explored the relationship between the microbiome and CDI but none have ventured into the variation of disease severity due to the microbiome. Most CDI studies employ either an *in vitro* or *in vivo* model using a homogenous bacterial community. Collins

et al used multiple human communities to colonize mice, however the communities were pooled prior to gavaging into germ-free mice (34), resulting in a single community. Studies examining difference in disease often use different *C. difficile* strains or ribotypes in mice with similar microbiota as a proxy for variation in disease, such as strain 630 for non-severe and ribotype 027 for severe (18). There has been studies demonstrating variation in severity, but through tapering antibiotic dosage (26) or by reducing the amount of cells or spores used for the challenge (22). Our group has recently been uncovering how variation in the microbiome affects CDI but have been limited to *C. difficile* colonization (26).

With our recent observations that the initial community affects the ability of *C. difficile* to persist in an environment and the existing research describing the myriad ways the microbiome interacts with *C. difficile*, we hypothesized that the microbiome directly and indirectly modulates CDI disease severity. Our data demostrate gut bacterial relative abundances associate with variation in toxin production, histopathologic scoring of the cecum and severe disease. This anaylsis has revealed populations of *Akkermansia*, *Anaerostipes*, *Coprobacillus*, *Enterocloster*, *Lactonifactor*, and *Monoglobus* were more abundant in the microbiome of mice which little to no disease was detected. These genera providing a protective role are supported by previous studies. *Coprobacillus*, *Lactonifactor*, and *Monoglobus* have been shown to be involved in dietary fiber fermentation and associated with healthy communities (37). *Anaerostipes* and *Coprobacillus* produce short chain fatty acids which also have been associated with healthy communities (41). Furthermore, *Coprobacillus*, which is abundant in mice with low histopathologic scores but rare in all other mice, has been shwon to contain putative type I lantibiotic gene cluster and inhibit *C. difficile* colonization (44). *Akkermansia* and *Enterocloster* were also idenitified as more abundant in mice which had a low clinical disease but have contridictory supporting evidence in the current literature. In our data, *Akkermansia* is most abundant in the mice with least disease but there were some mice with severe disease which had increased populations of *Akkermansia*. This could be attributed to either a more protective

mucus layer had developed in the mice with little disease and *Akkermansia* is inhibiting colonization (46) or *Akkermansia* could be crossfeeding *C. difficile* or exposing a niche via its mucus consumption (48). Similarly with *Enterocloster*, in our data this genus was more abundant and associated with low histopathologic scores. It has been associated with healthy populations and has been used to mono-colonize germ-free mice to reduce the ability of *C. difficile* to colonize (51). However, *Enterocloster* has mostly been indentified in infections that result in disease (53). These data demostrate a population of bacteria has the potential to be either protective or harmful. The disease outcome is not likely based on the abundance of a single population of bacteria rather the result of the interactions of many populations.

The groups of bacteria that were associated with either a higher histopathologic score or severe disease are members of the indigenous gut community that also have been associated with disease. These are often referred to as opportunitic pathogens, however based on the the results reported in this study it is more appropriate to apply the damage-response framework for microbial pathogenesis (55). The disease is not driven by a single entity, rather it is an emergent property of the responses of the host immune system, infecting microbe, and the microbes of the indigenous community. Many of the populations with pathgenic potential that associated with worse outcomes are also facultative anaerobes. *Enterococcus*, *Klebsiella*, *Shigella/Escherichia*, *Staphylococcus*, and *Streptococcus* have been shown to expand their population with antibiotic use (57) and are commonly detected in CDI cases (60). In addition to these populations, another set of bacteria, populations of *Eggerthella*, *Prevotellaceae* and *Helicobacter*, associated with worse outcomes have also been associated with intestinal inflammation (64). Recently, *Helicobacter hepaticus* has been demostrated to be sufficient to cause susceptibility to CDI in IL-10ˆ(-/-) B57BL/6 mice (67). In our experiments, when *Helicobacter* was present, the mouse resulted in either severe disease or a high histopathologic score. While we did not use IL-10ˆ(-/-) mice, it is possible the bacterial communtity or host response

are similarly modified by *Helicobacter* allowing *C. difficile* infection and resulting in host damage. However, the populations identified other than *Helicobacter* are not as clear with their relationship between their relative abundance and the resulting disease severity.

The damage-response framework for pathogenesis helps to understand how bacterial populations identified in this study potentiate CDI disease severity through the varied interactions with the host and *C. difficile*. In this set of experiments, we use the same host, C57BL/6 mice, the same infecting microbe, *C. difficile* ribotype 027 isolate 431, with different gut bacterial communities. The members of those communities were often present in multiple levels of disease severity. Thus, it is not merely, the presence of the bacteria but their activity in response to the other microbes and host which determine their effect on host damage. Additionally, while we are using the same host and infecting microbe, they too are reacting to the specific members of the bacterial community. Disease severity is driven by the cummulative effect of the host immune response and the activity of the gut bacteria and *C. difficile*. *C. difficile* can be driving host damage through the production of toxin. The gut microbiota could be contributing to the host damage through the balance of metabolic and competitive activities, such as bacteriocin production or mucin degradation. Low levels of mucin degradation can provide nutrients to other community members producing a diverse non-damaging community []. However, if mucin degradation becomes too great it reduces the protective function of the mucin layer and exposes the epithelial cells []. This over-harvesting contributes to the host damage due to other members producing toxin. Thus disease is a balance of many activities of the community. Host damage is the emergent property of numerous damage-response curves, one for host immune response, one for *C. difficile* activity and another for microbiome community activity, each of which are a composite curve of the individual activities from each group, such as antibody production, neutrophil infiltration, toxin production, sporulation, fiber and mucin degradation. Therefore, while we have identified populations of interest, it is likely targeting one specifically will reduce disease severity.

Many studies have investigated individual activities contributing to CDI. Here we have shown myraid bacterial groups and their relative abundances associate with variation in CDI disease severity. We must continue to explore how the host, microbiota, and *C. difficile* interactions result in severe disease. We can reduce the risk of severity with a better understanding of which interactions, whether be driven by specific community functions or groups of bacteria, reduce the host damage. Approaching this problem from a damage-response framework allows us to experiment with modifying subsets of activities to reduce overall host damage. Our current clinical treatment targets an individual group in this tripartite system. Most commonly, CDI is treated with an antibiotic to eliminate *C. difficile*. Alternatively, another treatment uses antibodies to neutralize *C. difficile* toxins. These treatments are only addressing a single entity of the system, leaving the potential for the others to maintain responses that maintain host damage. However, we can continue this research to elucidate treatments that drive all three entities towards reduced host damage. When a patient is diagnosed with CDI, the gut community composition, in addition to the traditionally obtained information, may improve our severity prediction and appropriate treatment. Treating the microbiome may contribute to reduced severity. From our results, promoting fiber metabolizing bacteria and reducing facultative anerobes could bolster the efficacy of current CDI treatments, reducing disease severities.

Materials and Methods

Animal care. 5. 64 mice, 33F and 31M, ages 6-13 weeks old, 3-4 mice/group (except 2 for C/DA00581 and MOUT/DA10093) each run once except for DA00581 which was run 4 times because we observed colonization resistance in one of the experiments 15 human fecal samples, selected to create a subset list with diverse responses to health questionaire

***C. difficile* challenge.** M.

Sample collection. F.

DNA sequencing. T.

Sequence curation. S.

Statistical analysis and modeling. D.

Code availability. Scripts necessary to reproduce our analysis and this paper are available in an online repository (https://github.com/SchlossLab/Lesniak_XXXX).

Sequence data accession number. All 16S rRNA gene sequence data and associated metadata are available through the Sequence Read Archive via accession XXXX.

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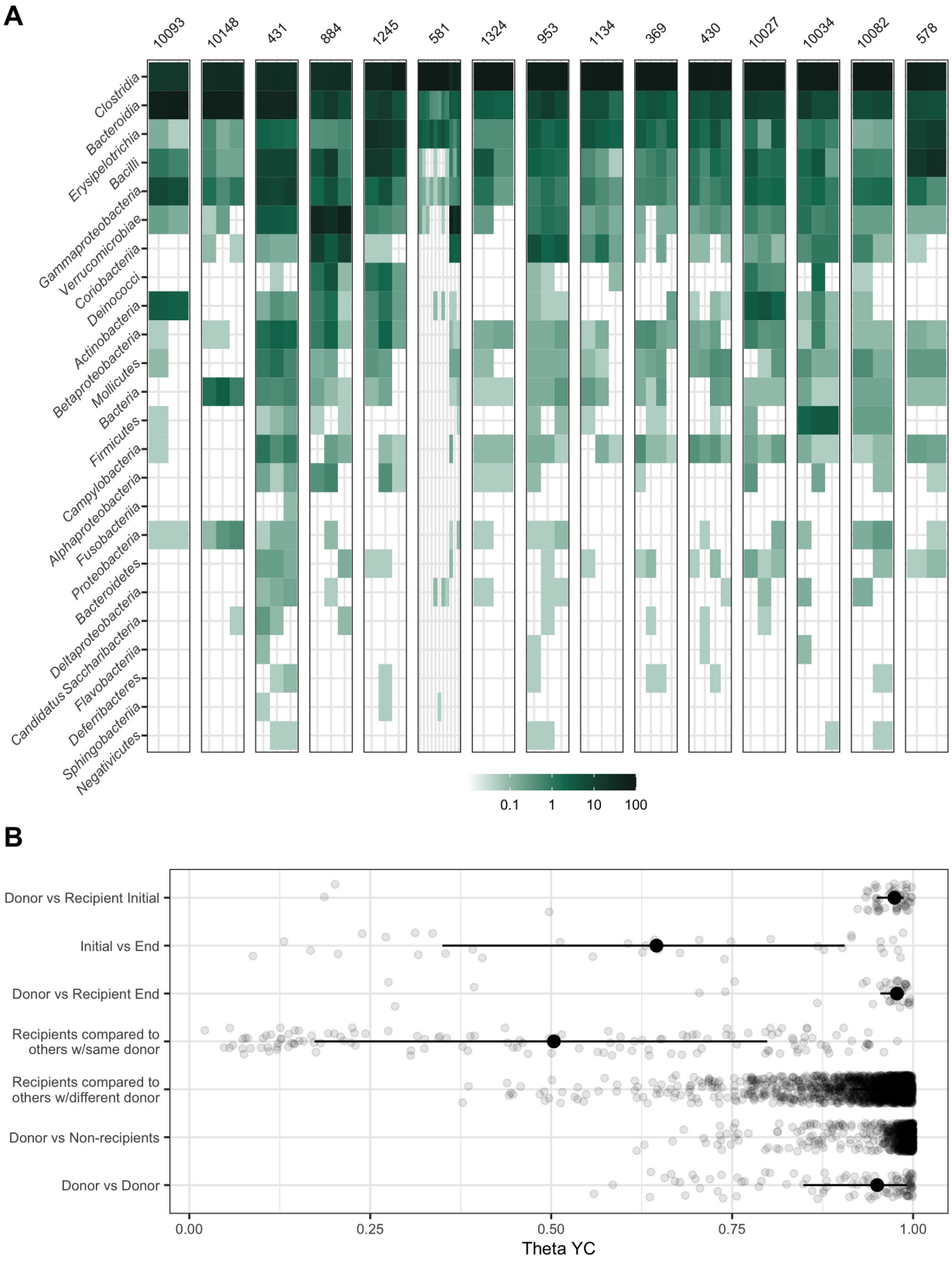
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10034
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Bacteroidia
Erysipelotrichia
Bacilli
Gammaproteobacteria
Verrucomicrobiae
Coriobacteriia
Deinococci
Actinobacteria
Betaproteobacteria
Mollicutes
Bacteria
Firmicutes
Campylobacteria
Alphaproteobacteria
Fusobacteriia
Proteobacteria
Bacteroidetes
Deltaproteobacteria
Candidatus Saccharibacteria
Flavobacteriia
Deferribacteres
Sphingobacteriia
Negativicutes
0.1
1
10
100
B
Donor vs Recipient Initial
Initial vs End
Donor vs Recipient End
Recipients compared to others w/same donor
Recipients compared to others w/different donor
Donor vs Non-recipients
Donor vs Donor
0.00
0.25
0.50
0.75
1.00
Theta YC

639

640 **Figure 1. R.** (A-C).

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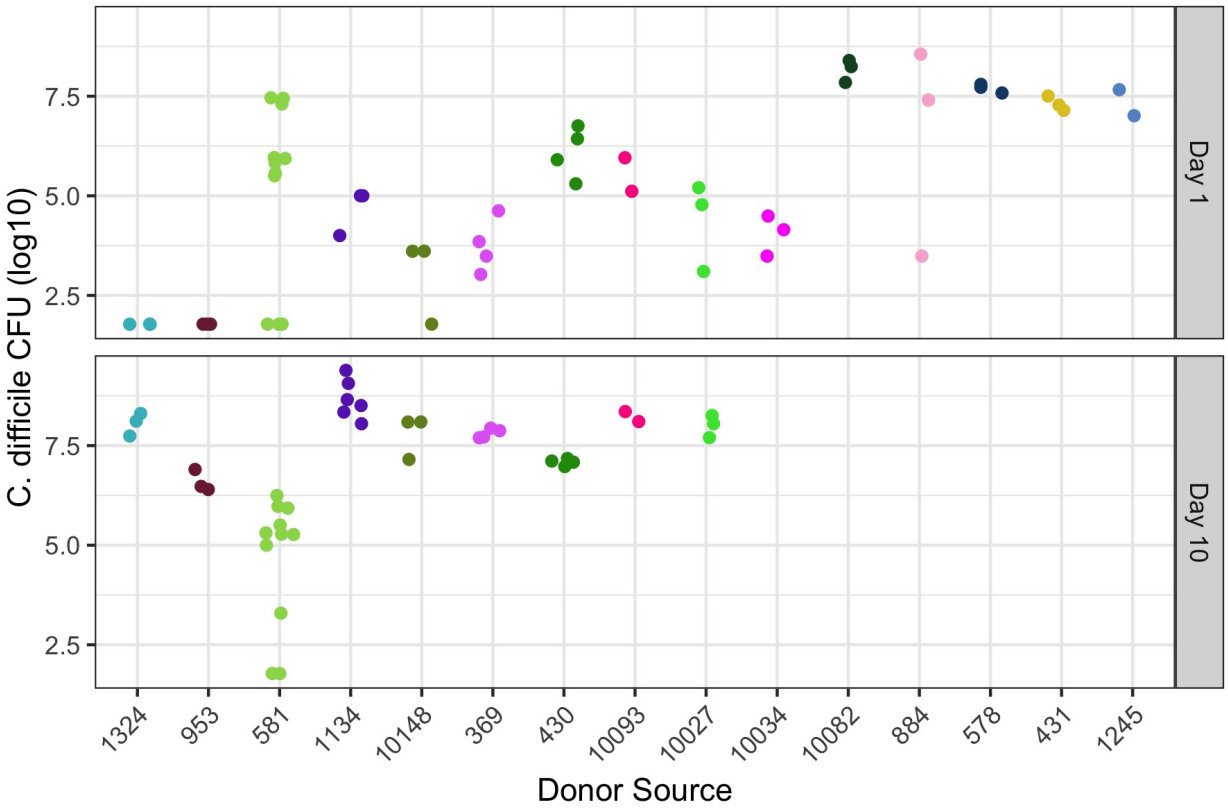

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644 **Figure 2. M.** F.

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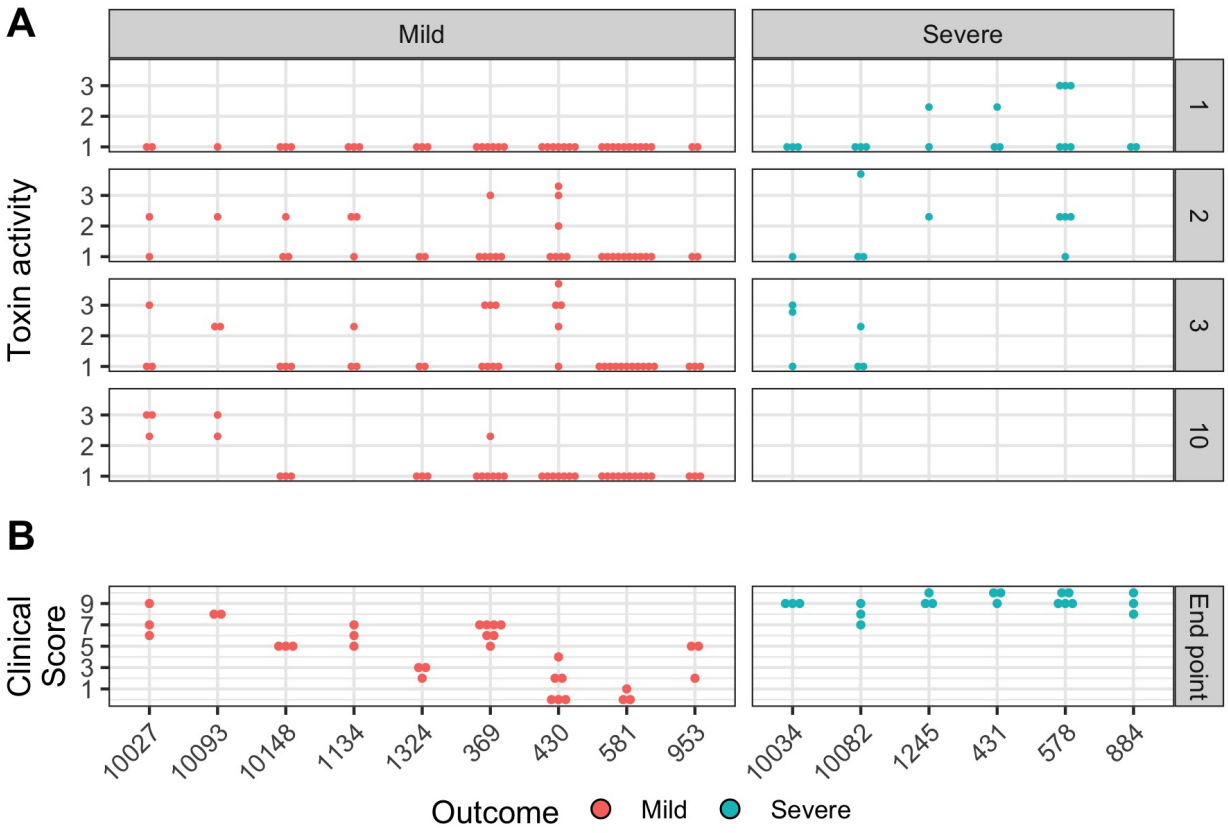

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Figure 3. O. F.

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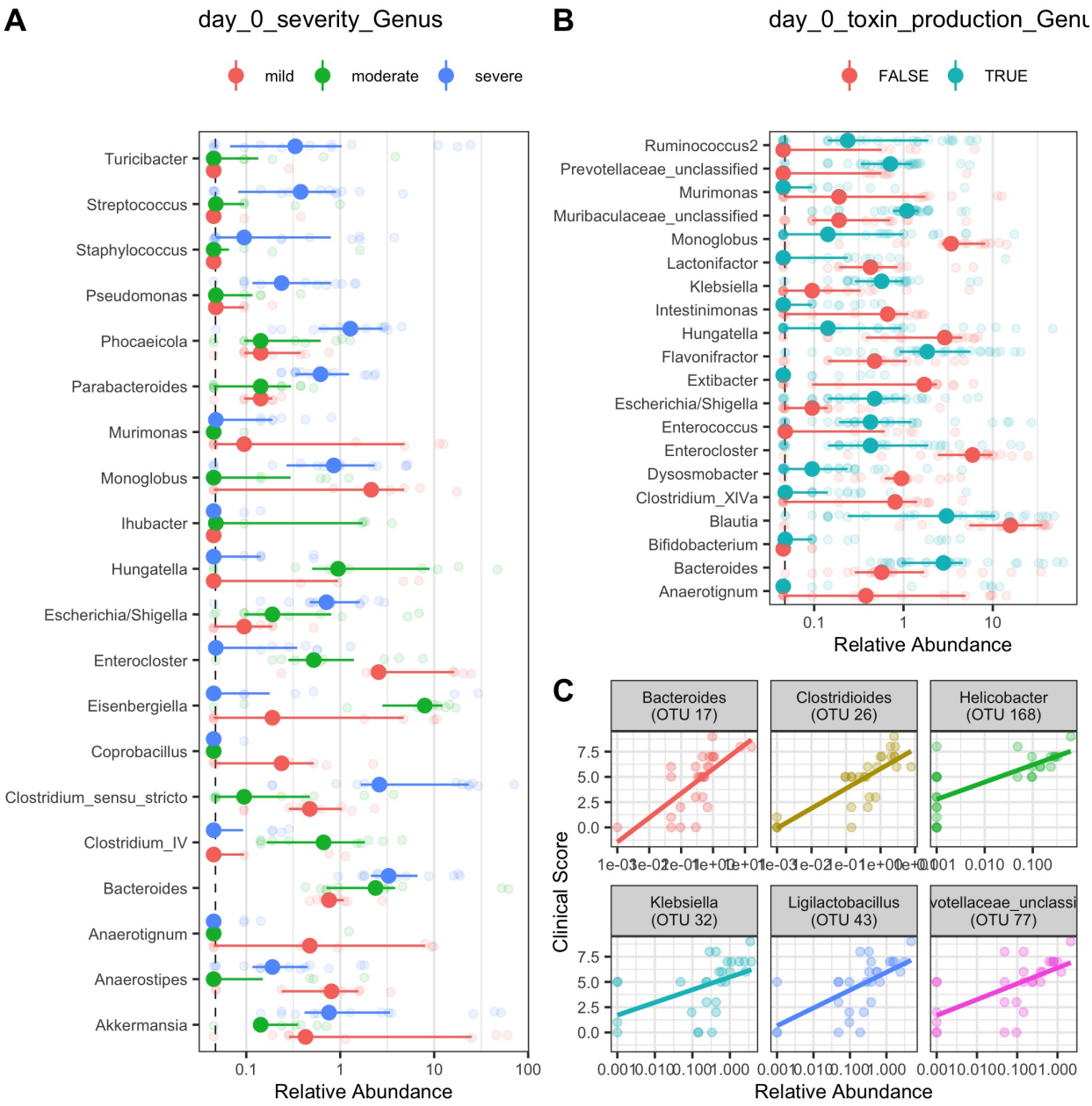
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severe
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Streptococcus
Staphylococcus
Pseudomonas
Phocaeicola
Parabacteroides
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Monoglobus
Ihubacter
Hungatella
Escherichia/Shigella
Enterocloster
Eisenbergiella
Coprobacillus
Clostridium_sensu_stricto
Clostridium_IV
Bacteroides
Anaerotignum
Anaerostipes
Akkermansia
0.1
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FALSE
TRUE
Ruminococcus2
Prevotellaceae_unclassified
Murimonas
Muribaculaceae_unclassified
Monoglobus
Lactonifactor
Klebsiella
Intestinimonas
Hungatella
Flavonifractor
Extibacter
Escherichia/Shigella
Enterococcus
Enterocloster
Dysosmobacter
Clostridium_XlVa
Blautia
Bifidobacterium
Bacteroides
Anaerotignum
0.1
1
10
Relative Abundance
C
Bacteroides (OTU 17)
Clostridioides (OTU 26)
Helicobacter (OTU 168)
Klebsiella (OTU 32)
Ligilactobacillus (OTU 43)
votellaceae_unclassi (OTU 77)
Clinical Score
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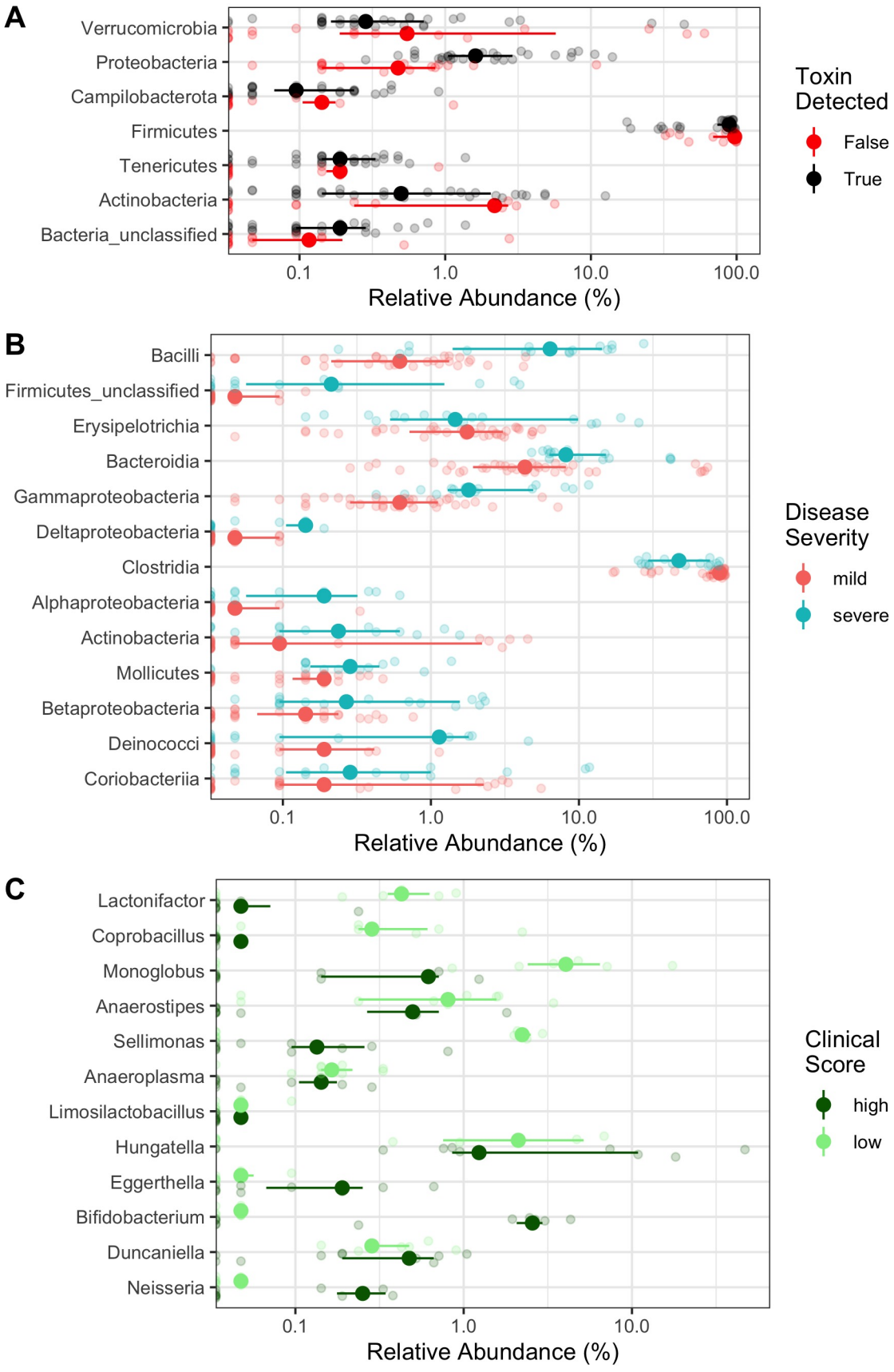
5.0
2.5
0.0
0.001
0.010
0.100
1.000
Relative Abundance

Figure 4. E. A.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Campilobacterota
Firmicutes
Tenericutes
Actinobacteria
Bacteria_unclassified
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1.0
10.0
100.0
Relative Abundance (%)
Toxin Detected
False
True
B
Bacilli
Firmicutes_unclassified
Erysipelotrichia
Bacteroidia
Gammaproteobacteria
Deltaproteobacteria
Clostridia
Alphaproteobacteria
Actinobacteria
Mollicutes
Betaproteobacteria
Deinococci
Coriobacteriia
0.1
1.0
10.0
100.0
Relative Abundance (%)
Disease Severity
mild
severe
C
Lactonifactor
Coprobacillus
Monoglobus
Anaerostipes
Sellimonas
Anaeroplasma
Limosilactobacillus
Hungatella
Eggerthella
Bifidobacterium
Duncaniella
Neisseria
0.1
1.0
10.0
Relative Abundance (%)
Clinical Score
high
low

655

656 **Figure 5. D.** (A) L

657

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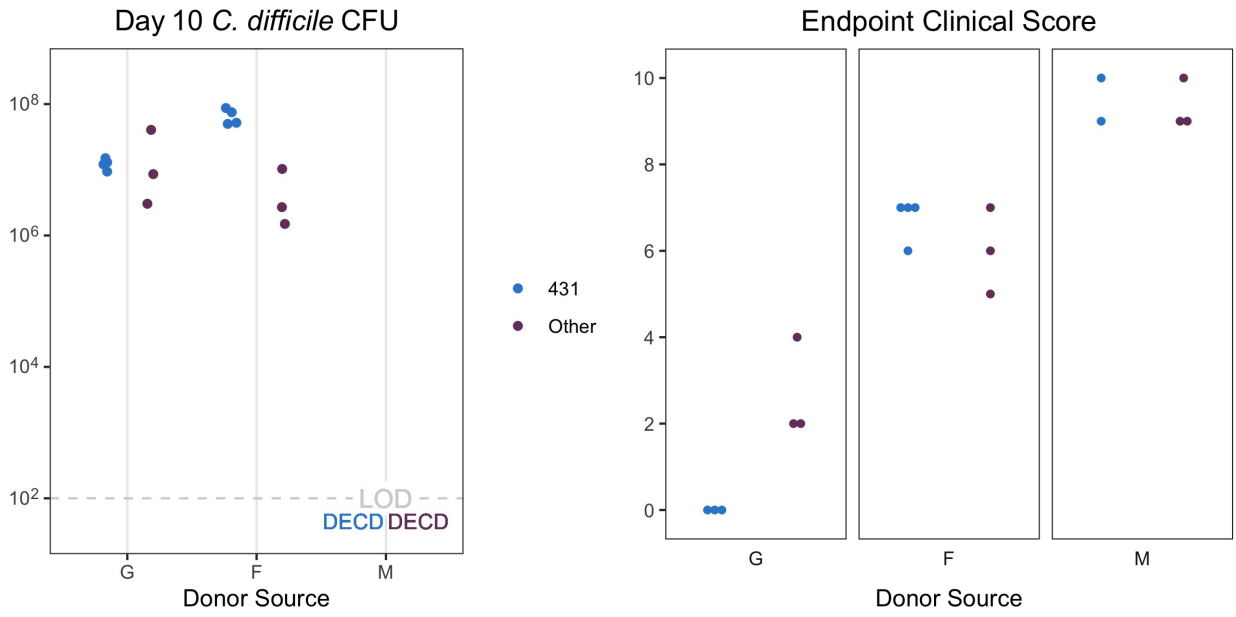

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660 **Figure 6. D.** (A) L

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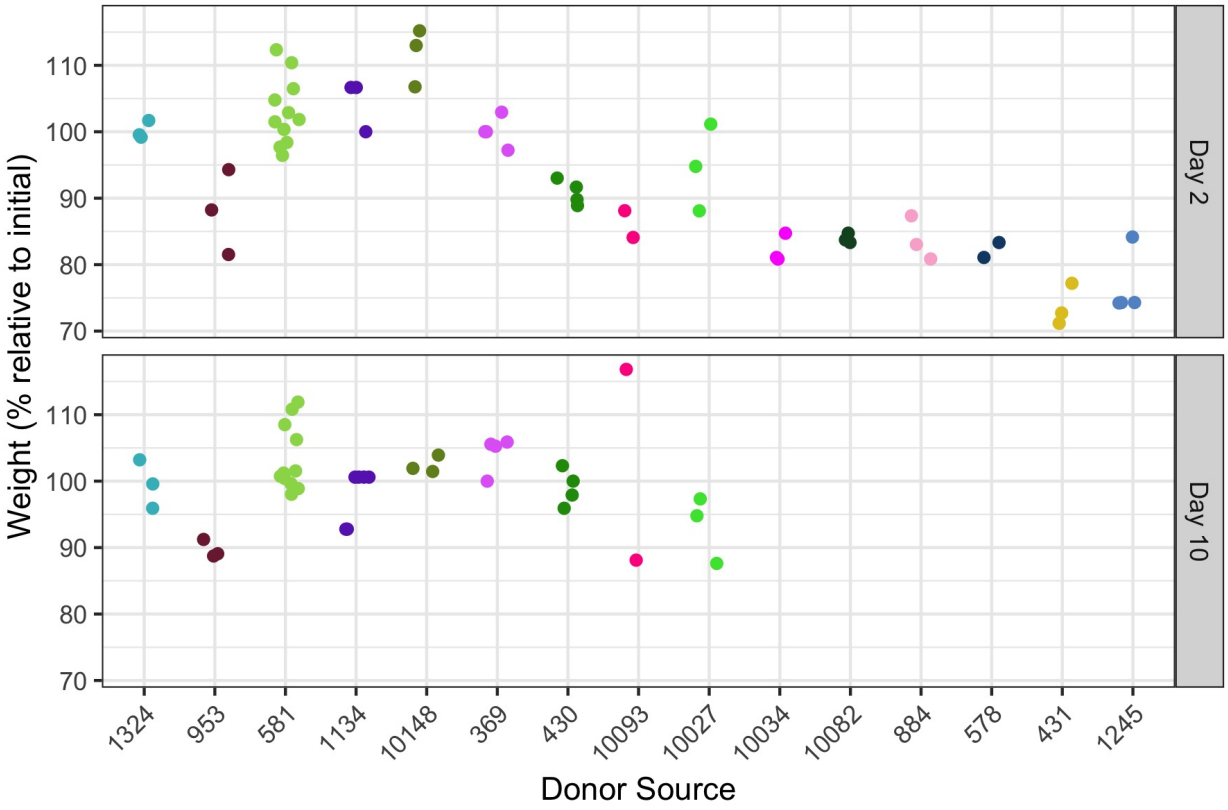

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664 **Figure S1. D.** (A) L

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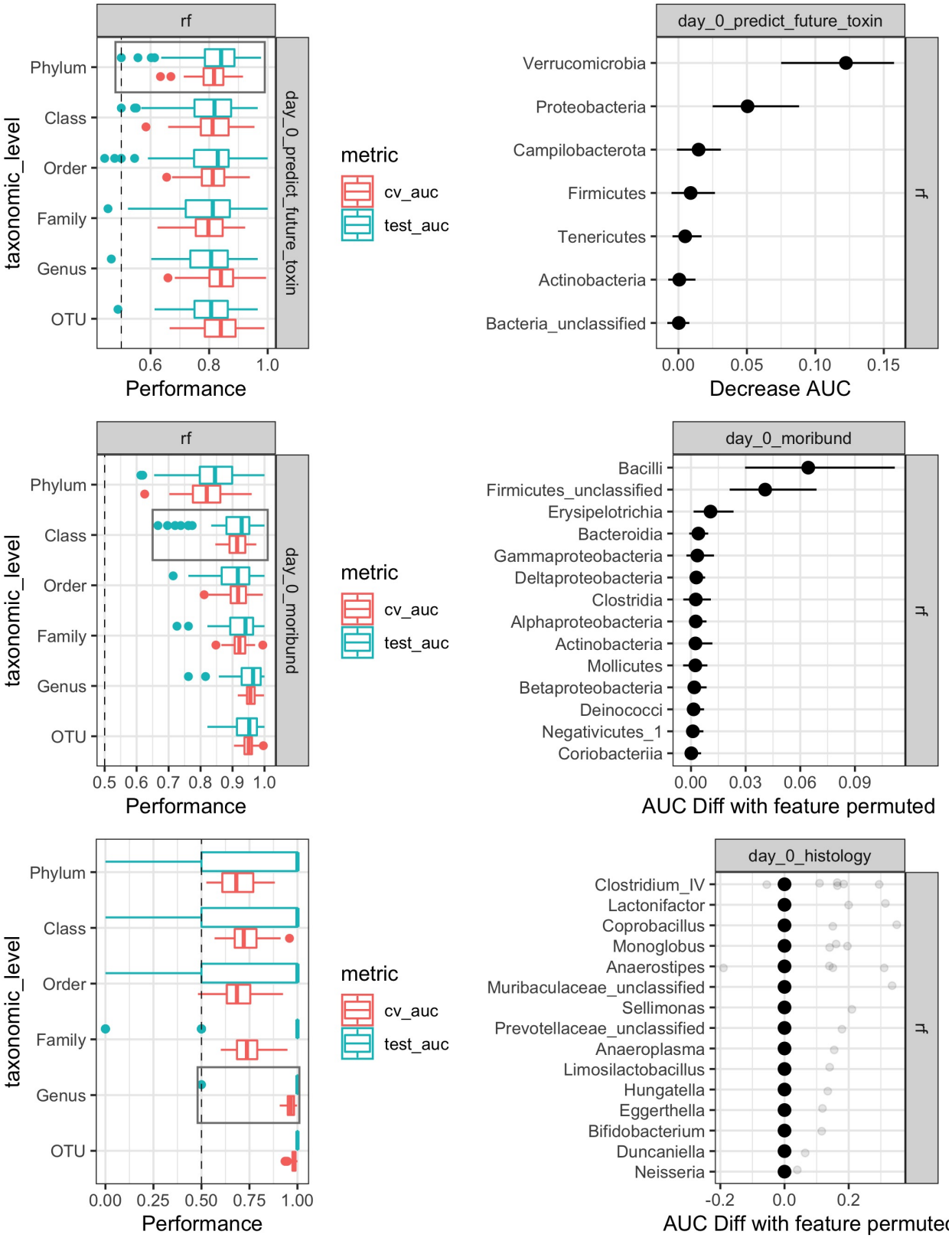
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taxonomic_level
Phylum
Class
Order
Family
Genus
OTU
day_0_predict_future_toxin
0.6
0.8
1.0
Performance
metric
cv_auc
test_auc
day_0_predict_future_toxin
Verrucomicrobia
Proteobacteria
Campilobacterota
Firmicutes
Tenericutes
Actinobacteria
Bacteria_unclassified
rf
0.00
0.05
0.10
0.15
Decrease AUC
rf
day_0_moribund
0.5
0.6
0.7
0.8
0.9
1.0
Performance
metric
cv_auc
test_auc
day_0_moribund
Bacilli
Firmicutes_unclassified
Erysipelotrichia
Bacteroidia
Gammaproteobacteria
Deltaproteobacteria
Clostridia
Alphaproteobacteria
Actinobacteria
Mollicutes
Betaproteobacteria
Deinococci
Negativicutes_1
Coriobacteriia
rf
0.00
0.03
0.06
0.09
AUC Diff with feature permuted
rf
Phylum
Class
Order
Family
Genus
OTU
0.00
0.25
0.50
0.75
1.00
Performance
metric
cv_auc
test_auc
day_0_histology
Clostridium_IV
Lactonifactor
Coprobacillus
Monoglobus
Anaerostipes
Muribaculaceae_unclassified
Sellimonas
Prevotellaceae_unclassified
Anaeroplasma
Limosilactobacillus
Hungatella
Eggerthella
Bifidobacterium
Duncaniella
Neisseria
rf
-0.2
0.0
0.2
AUC Diff with feature permute

667

Figure S2. D. (A) L

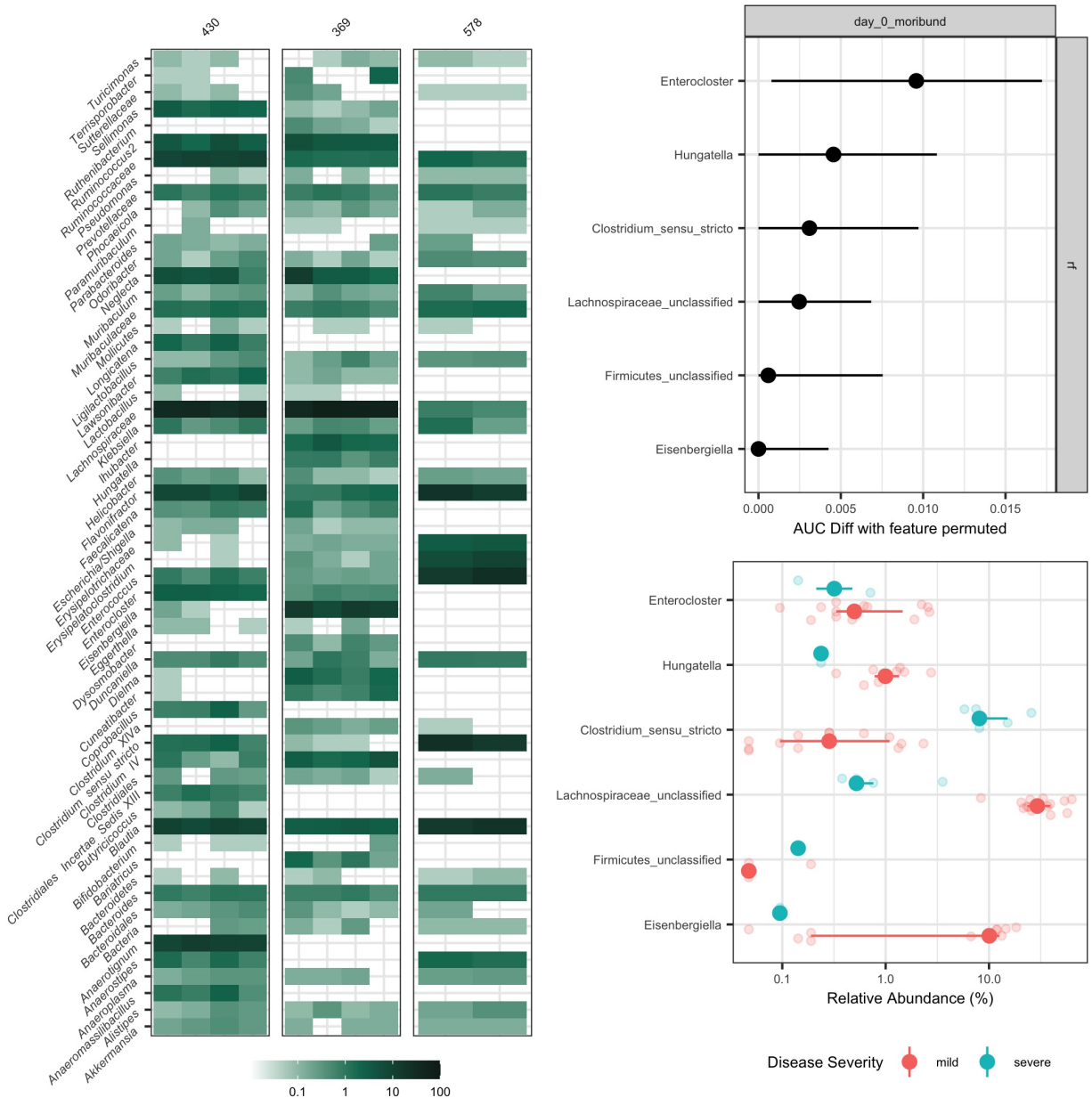

Figure S3. D. (A) L

Table 1. D. (A) L

samples_used <- readxl::read_xlsx('data/raw/humanGF_ids.xlsx') %>% pull(human_source) %>% unique donor_data <- readxl::read_xlsx('data/raw/MIMARKS_cdclinical.xlsx') %>% filter(sample_id %in% samples_used) %>% select(sample_id, biome, age, gender, "antibiotics >3mo", protonpump, antacid, Healthworker, historyCdiff, Surgery6mos, Vegetarian, weight, disease_stat)

Table 2. D. (A) L

read.csv(here('data/raw/Hanna_in_vitro_data_plus_germination.txt')) %>% rownames_to_column('isolate %>% filter(isolate %in% c('DA00299', 'DA00395', 'DA00458', 'DA00431'))