

# **An osmotic laxative renders mice susceptible to prolonged *Clostridioides difficile* colonization and hinders clearance**

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**1 Abstract**

2 (Modify depending on target journal, currently abstract submitted to World Microbe Forum)

3 Antibiotics are a major risk factor for *Clostridioides difficile* infections (CDIs) because of their impact

4 on the intestinal microbiome. However, non-antibiotic medications such as the ubiquitous osmotic

5 laxative polyethylene glycol (PEG) 3350, also alter the microbiota, but whether PEG impacts CDI

6 susceptibility and clearance is unclear. To examine how PEG impacts susceptibility, we treated

7 C57Bl/6 mice with 5-day and 1-day doses of 15% PEG in the drinking water and then challenged

8 the mice with *C. difficile* 630 spores. We used clindamycin-treated mice as a control because they

9 consistently clear *C. difficile* within 10 days post-infection (dpi). To examine how PEG treatment

10 impacts clearance, we administered PEG for 1 day to clindamycin-treated, *C. difficile*-challenged

11 mice either immediately following challenge or 3 dpi. We collected longitudinal stool samples

12 to examine *C. difficile* levels in the stool via anaerobic culture and profiled the microbiota by

13 16S rRNA sequencing. PEG treatment alone was sufficient to render mice susceptible to CDI

14 and 5-day PEG-treated mice remain colonized for up to 30 dpi. Additionally, 5-day PEG treated

15 mice remained susceptible to CDI 10-days post treatment. In contrast, 1-day PEG treated mice

16 were transiently colonized, clearing *C. difficile* within 7 dpi. Although 5-day PEG-treated mice

17 exhibited prolonged *C. difficile* colonization, we saw no difference in histological inflammation

18 between PEG- and clindamycin-treated mice. Additionally, administering PEG to mice after *C.*

19 *difficile* challenge prolonged colonization up to 30 dpi in mice that received PEG immediately after

20 challenge and 15 dpi in mice that received PEG 3 dpi. When we examined microbiota composition

21 across our different treatment groups, we found decreased richness in the PEG-treated mice that

22 exhibited prolonged *C. difficile* colonization. Importantly, there were increased Bacteroides and

23 Enterobacteriaceae and decreased Lachnospiraceae and Oscillibacter in most of the PEG-treated

24 mice with prolonged *C. difficile* colonization. Our findings suggest the osmotic laxative PEG 3350

25 alters the mouse microbiota and disrupts colonization resistance to *C. difficile*, as well as clearance

26 in mice with a CDI. Considering that most hospitals recommend not performing *C. difficile* testing

27 on patients taking laxatives and laxatives are used when administering fecal microbiota transplants

28 via colonoscopy to patients with recurrent CDIs, further studies are needed to evaluate if laxatives

29 impact human microbiota colonization resistance.

30 **Introduction**

31 Antibiotics are a major risk factor for *Clostridioides difficile* infections (CDIs) because of their impact  
32 on the microbiota. However, antibiotics are not the only types of medications that disrupt the  
33 microbiota. Although, other medications such as proton pump inhibitors have been implicated as  
34 risk factors for CDIs through epidemiological studies, whether the association is due to their impact  
35 on the microbiome is still unclear (1). Many of the non-antibiotic medications associated with CDIs  
36 are known to modulate intestinal motility, which in turn also strongly impacts microbiota composition  
37 and function.

38 Interestingly our group has previously demonstrated that there are similar microbiota features in  
39 stool samples from CDI patients and patients with diarrhea that tested negative for *C. difficile* with  
40 both groups having lower alpha diversity than non-diarrheal control samples (2). These results  
41 led to our hypothesis that bacterial communities from patients experiencing diarrheal controls  
42 are susceptible, but have not been exposed to *C. difficile* spores. To examine if diarrhea alone  
43 is sufficient to disrupt microbiota colonization resistance, we turned to osmotic laxatives, which  
44 increase intestinal motility and can lead to diarrhea. The ubiquitous osmotic laxative, polyethylene  
45 glycol (PEG) 3350 is found in Miralax, Nulytely, and Golytely and is also commonly used as bowel  
46 preparation for colonoscopies. Interestingly, previous studies has shown that treating mice with  
47 PEG alone rendered the mice susceptible to *C. difficile* infection, altered microbiota composition,  
48 reduced acetate and butyrate and altered the mucus barrier (3–6). The mucus barrier is thought to  
49 mediate protection from *C. difficile* infections by protecting intestinal epithelial cells from the toxins  
50 produced by *C. difficile* (Ref). PEG is also relevant in the context of treating recurrent CDIs via  
51 fecal microbiota transplant (FMT). For FMTs that are delivered via colonoscopy, patients typically  
52 undergo bowel preparation by taking an osmotic laxative prior to the procedure. Many of the FMT  
53 studies to date rationalize the use of laxatives (Ref) based on a case study with 2 pediatric patients  
54 where the authors suggested the laxative may help flush *C. difficile* spores and toxins from the  
55 intestine (7).

- 56 • 2 main questions:
- 57 – Does PEG 3350 prolong colonization and result in more severe *C. difficile* infections?
- 58 – How does PEG 3350 influence *C. difficile* clearance and fecal microbiota transplant

59 (FMT) in mice?

60 **Results**

61 **Laxative treatment alone leads to prolonged *C. difficile* colonization in mice.** We compared  
62 PEG-treated mice to our standard 10 mg/kg clindamycin treatment, which temporarily renders the  
63 mice susceptible to *C. difficile*, with mice typically clearing *C. difficile* within 10 days post-infection  
64 (1, 8). All PEG-treated mice were administered a 15% PEG solution in the drinking water for 5-days,  
65 one group was also treated with clindamycin, and one group was allowed to recover for 10 days  
66 prior to challenge (Fig. 1A). After PEG and/or antibiotic treatment all mice were challenged with  
67  $10^3$  *C. difficile* 630 spores.

- 68 • Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently colonized  
69 with *C. difficile*.
- 70 • Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time  
71 compared to clindamycin-treated mice.
- 72 • Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics  
73 post-infection.
- 74 • Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal  
75 microbiota is altered.
- 76 • Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.
- 77 • Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless of  
78 whether an FMT is also administered.
- 79 • Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in  
80 PEG treated mice.
- 81 • Figure S2. Specific OTUs associated with clearance that are mostly absent in mice with  
82 prolonged *C. difficile* colonization.

83 – Ex. *Muribaculum intestinale*.

84 • Figure 7. Schematic summarizing findings.

85 **Discussion**

86 • Summary of major findings

87 • Discussion of prolonged persistence. C. difficile sequences detected in tissue samples.  
88 Association with mucin-degrading bacteria suggested by recent papers.

89 • Discuss why we might not have observed more severe histology in PEG mice relative to  
90 clindamycin-treated mice

91 – Antibiotics may also impact mucus layer

92 – Strain of bacteria used

93 • Protective bacteria missing in PEG-treated mice

94 • Discuss what these findings might mean for human patients

95 – What's known regarding laxatives and susceptibility to CDIs

96 – Relevance to human FMTs? Unclear what the best administration route is because there  
97 have been no studies designed to evaluate the best administration route for FMTs.

98 **Conclusions**

99 **Acknowledgements**

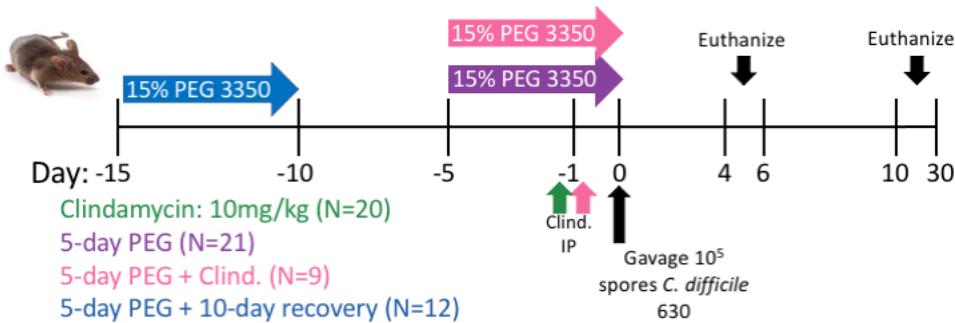
100 We thank members of the Schloss lab for feedback on planning the experiments and data  
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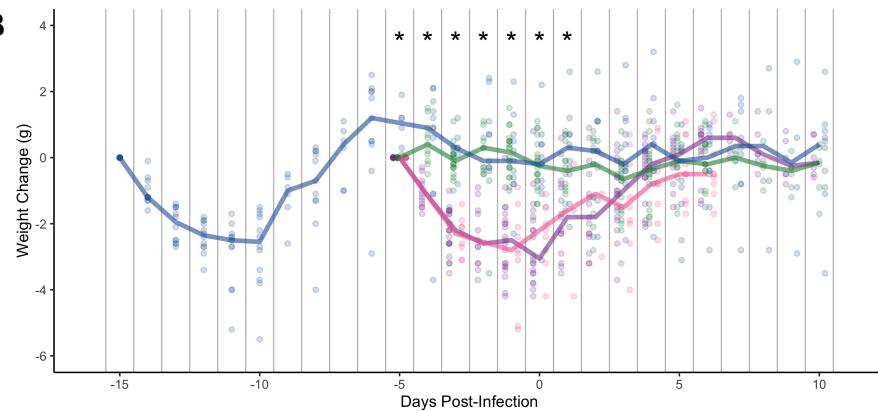
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<sup>107</sup> Postdoctoral Translation Scholars Program (UL1TR002240 from the National Center for Advancing  
<sup>108</sup> Translational Sciences).

<sup>109</sup> **Materials and Methods**

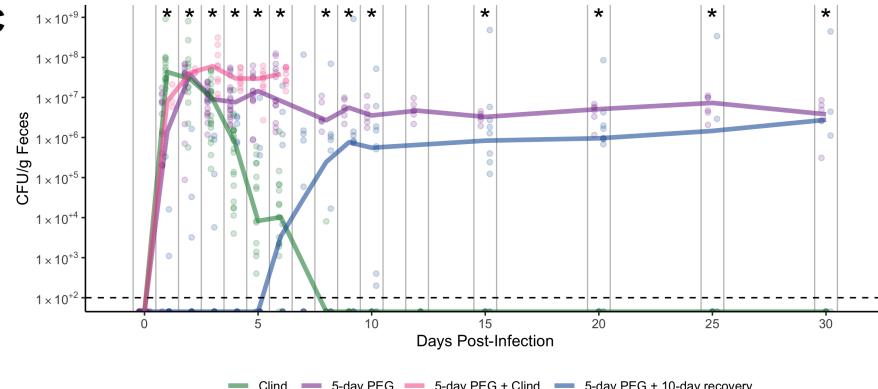
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B



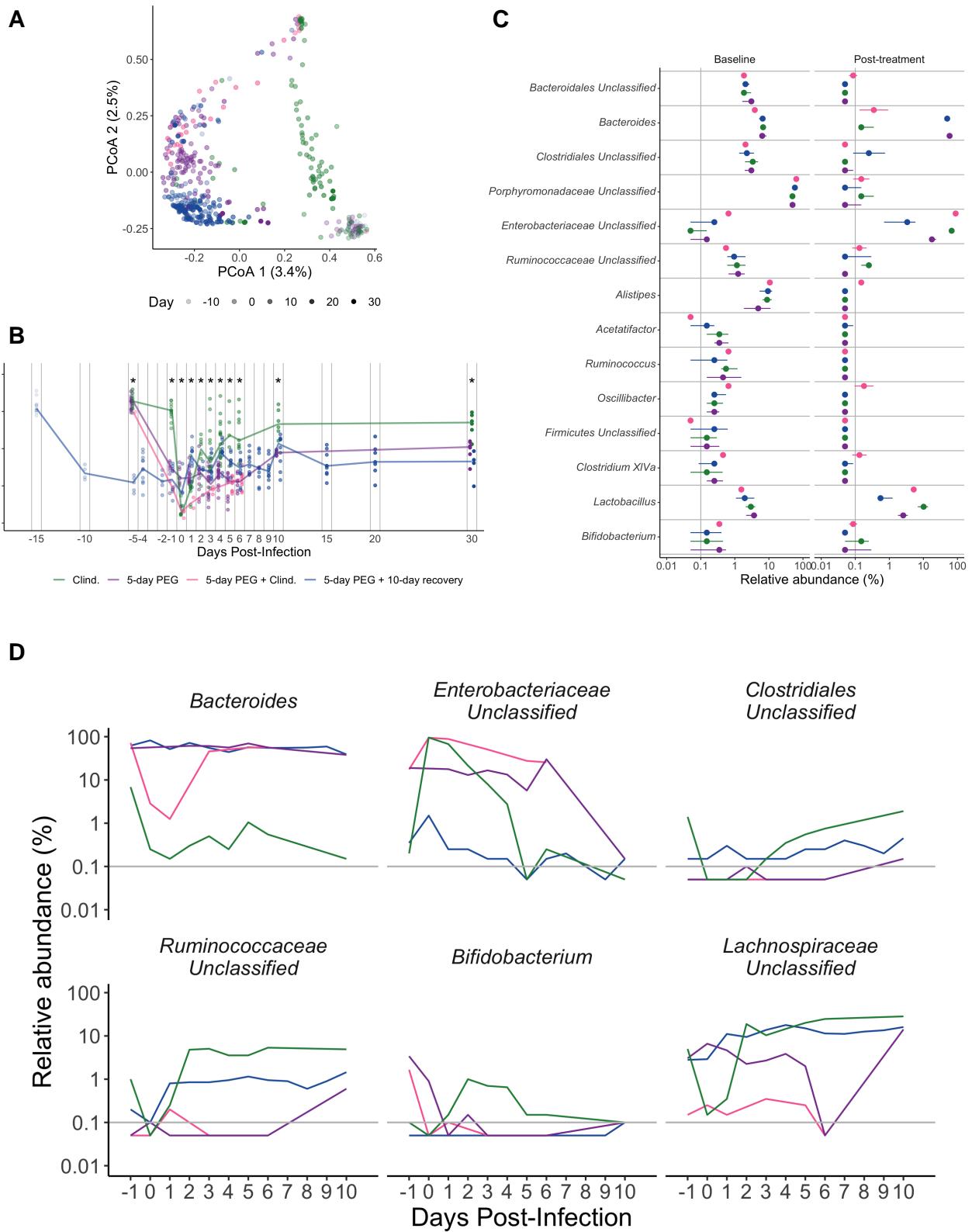
C



110

111 **Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently**  
112 **colonized with *C. difficile*.** A. Setup of the experimental timeline for subset of experiments with  
113 5-day PEG treated mice. B. Weight change from baseline weight in groups after treatment with  
114 PEG and/or clindamycin, followed by *C. difficile* challenge. C. *C. difficile* CFU/gram stool measured  
115 over time (N = 4-(insert variable name) mice per timepoint) via serial dilutions. The black line  
116 represents the limit of detection for the first serial dilution. CFU quantification data was not available  
117 for each mouse due to stool sampling difficulties (particularly the day the mice came off of the

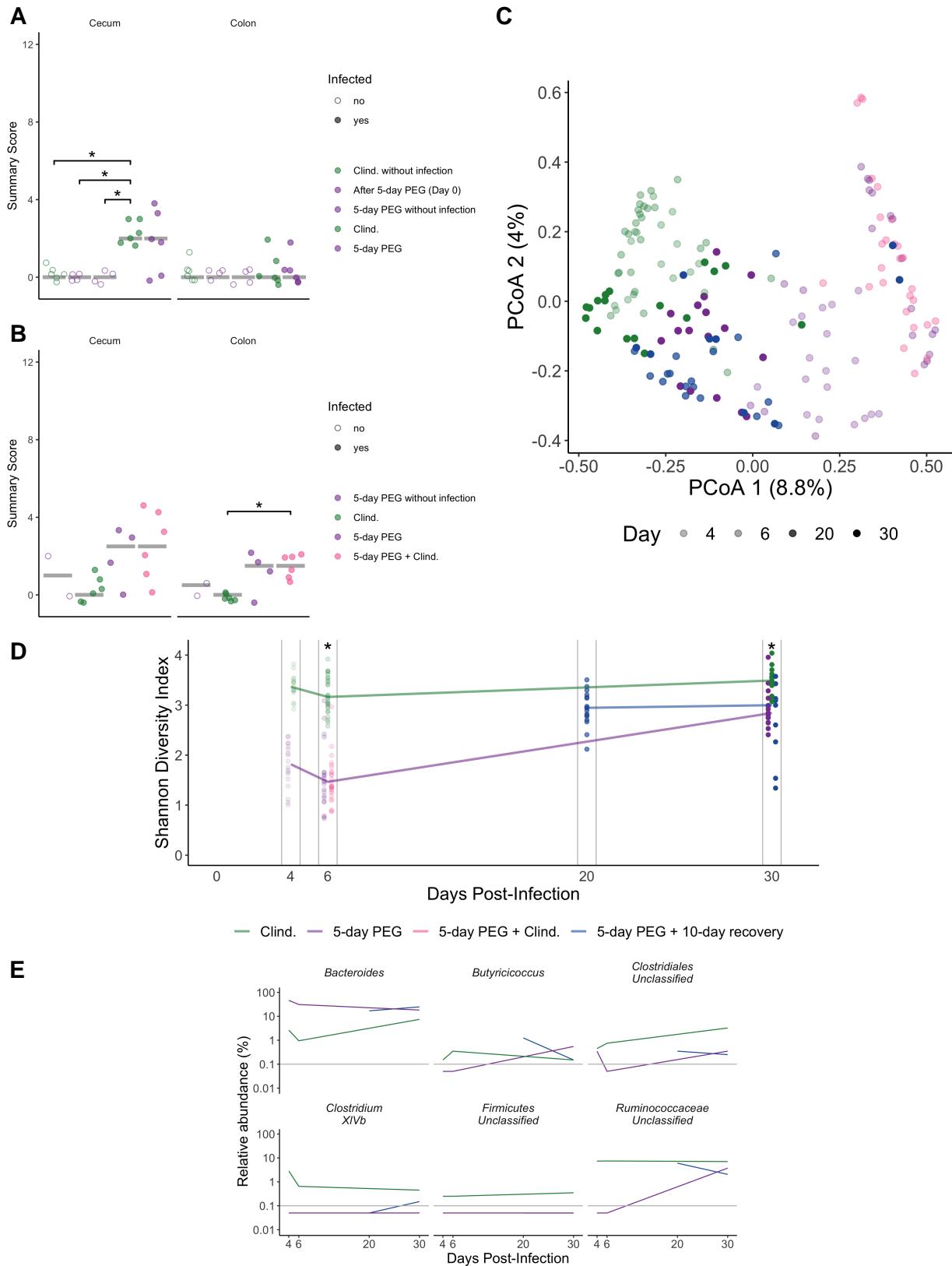
<sup>118</sup> PEG treatment) or early deaths. Lines represent the median for each source and circles represent  
<sup>119</sup> individual mouse samples. Asterisks indicate timepoints where the weight change or CFU/g was  
<sup>120</sup> significantly different between groups by the Kruskal-Wallis test with Benjamini-Hochberg correction  
<sup>121</sup> for testing multiple timepoints.



122

123 **Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time**

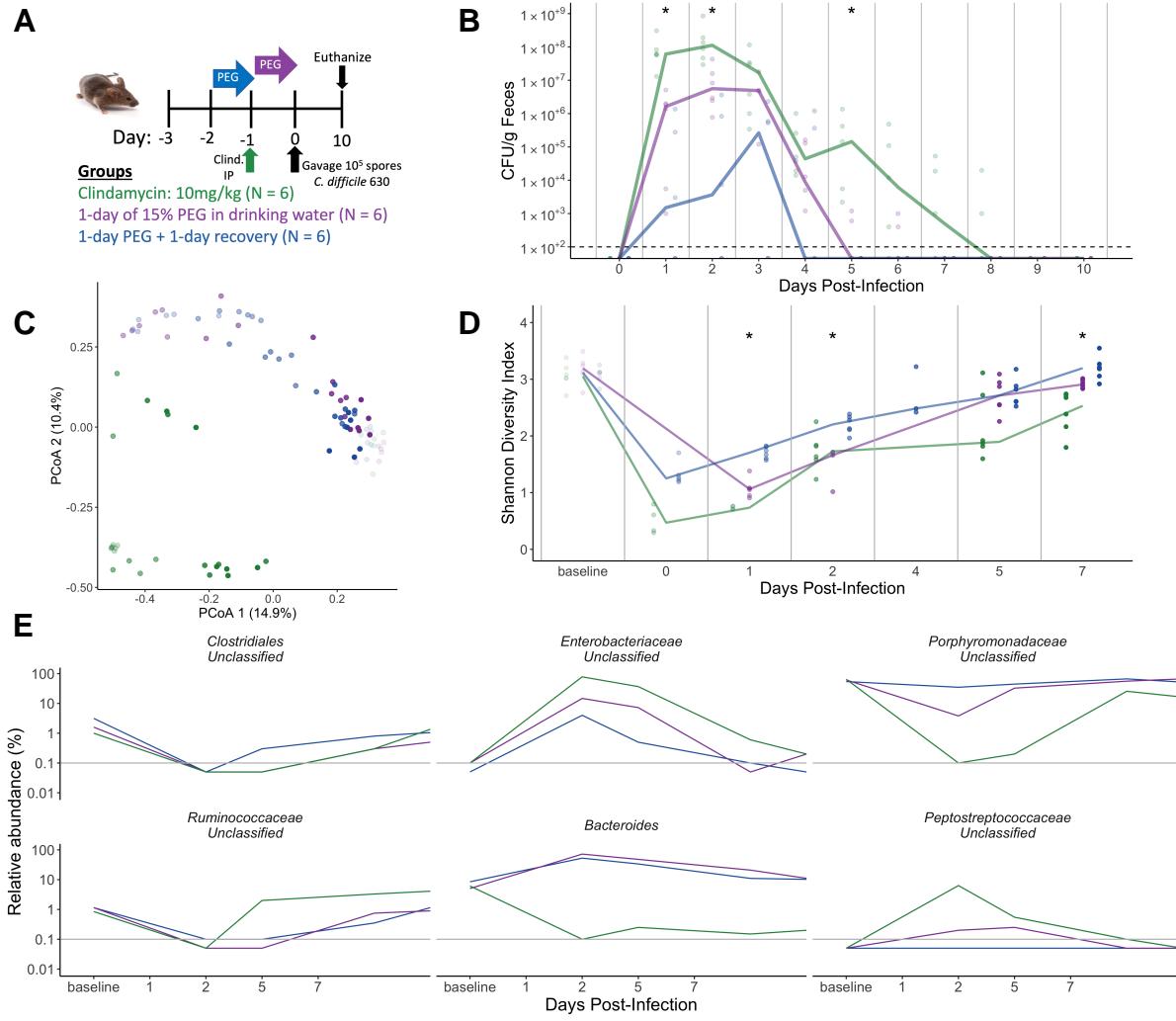
<sup>124</sup> compared to clindamycin-treated mice. A.



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Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal

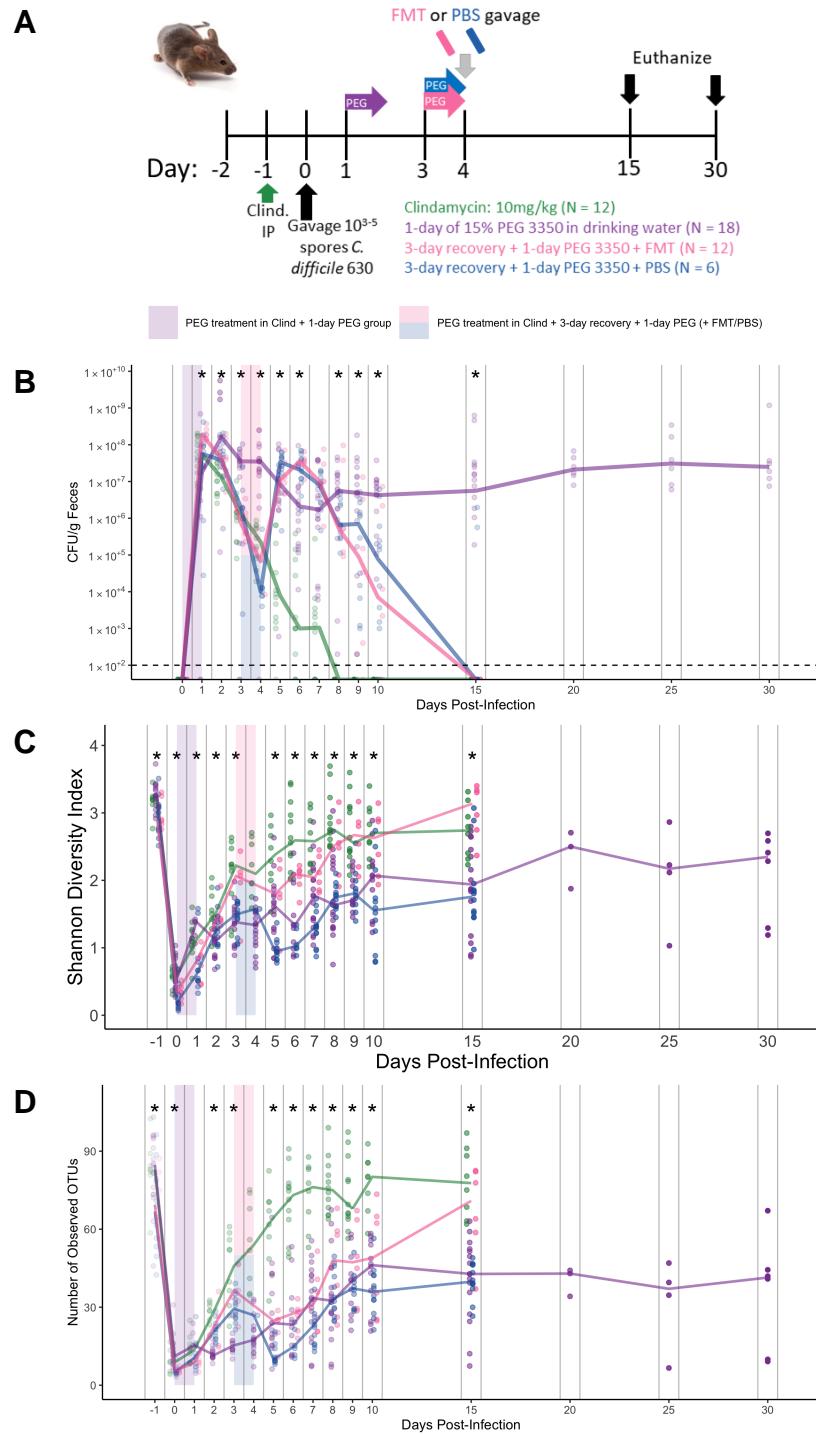
<sup>127</sup> **microbiota is altered.** A.



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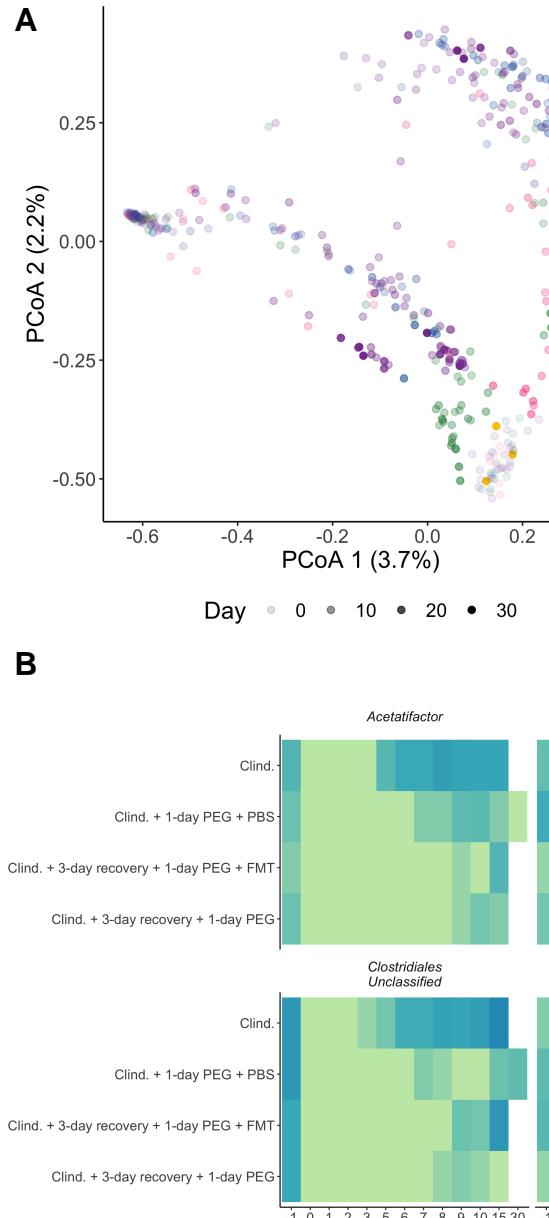
129 **Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile***  
 130 **colonization.** A. Setup of the experimental timeline for the 1-day PEG treated subset of  
 131 mice. B. CFU/gram stool measured over time (N = 6 mice per timepoint) via several dilutions.  
 132 The black dotted line represents the limit of detection for the first serial dilution. Asterisks  
 133 indicate timepoints where the CFU/gram was significantly different between groups using the  
 134 Kruskall-Wallis test with a Benjamini-Hochberg correction for multiple timepoints. C. Principle  
 135 Coordinate Analysis plot of the groups over time with the alpha representing the same time scale  
 136 as in panel D (day:  $R^2 = 0.43$ ; group:  $R^2 = 0.19$ ). D. Shannon Diveristy Index of the groups over  
 137 time. Only days with samples from all groups are shown. Samples for some mice were difficult to  
 138 obtain due to the laxative treatment. The alpha scale follows accordingly with the timeline. E. Line  
 139 plots of relative percent abundance of selected genera over time. Only days with samples from all

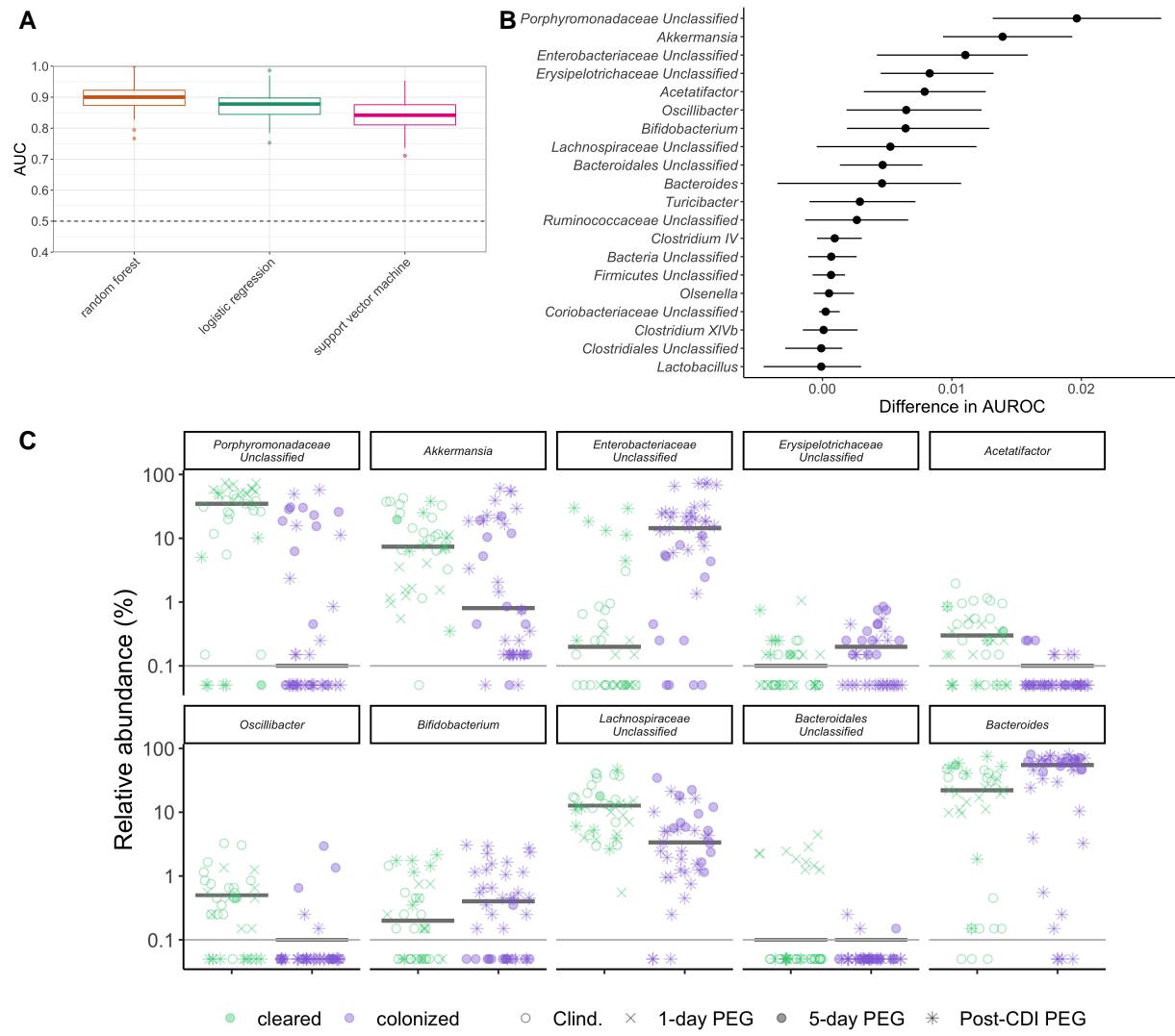
140 groups shown. The gray line represents the limit of detection.



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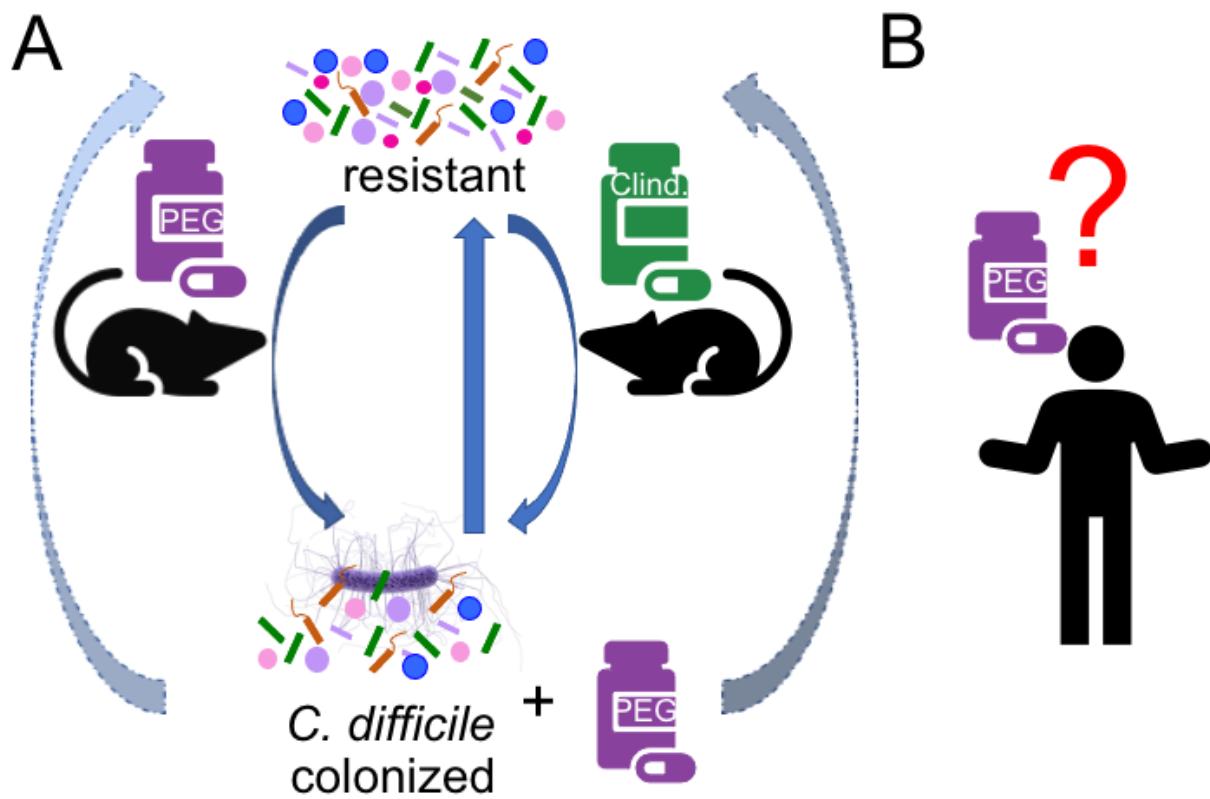
142 **Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless**  
143 **of whether an FMT is also administered. A.**





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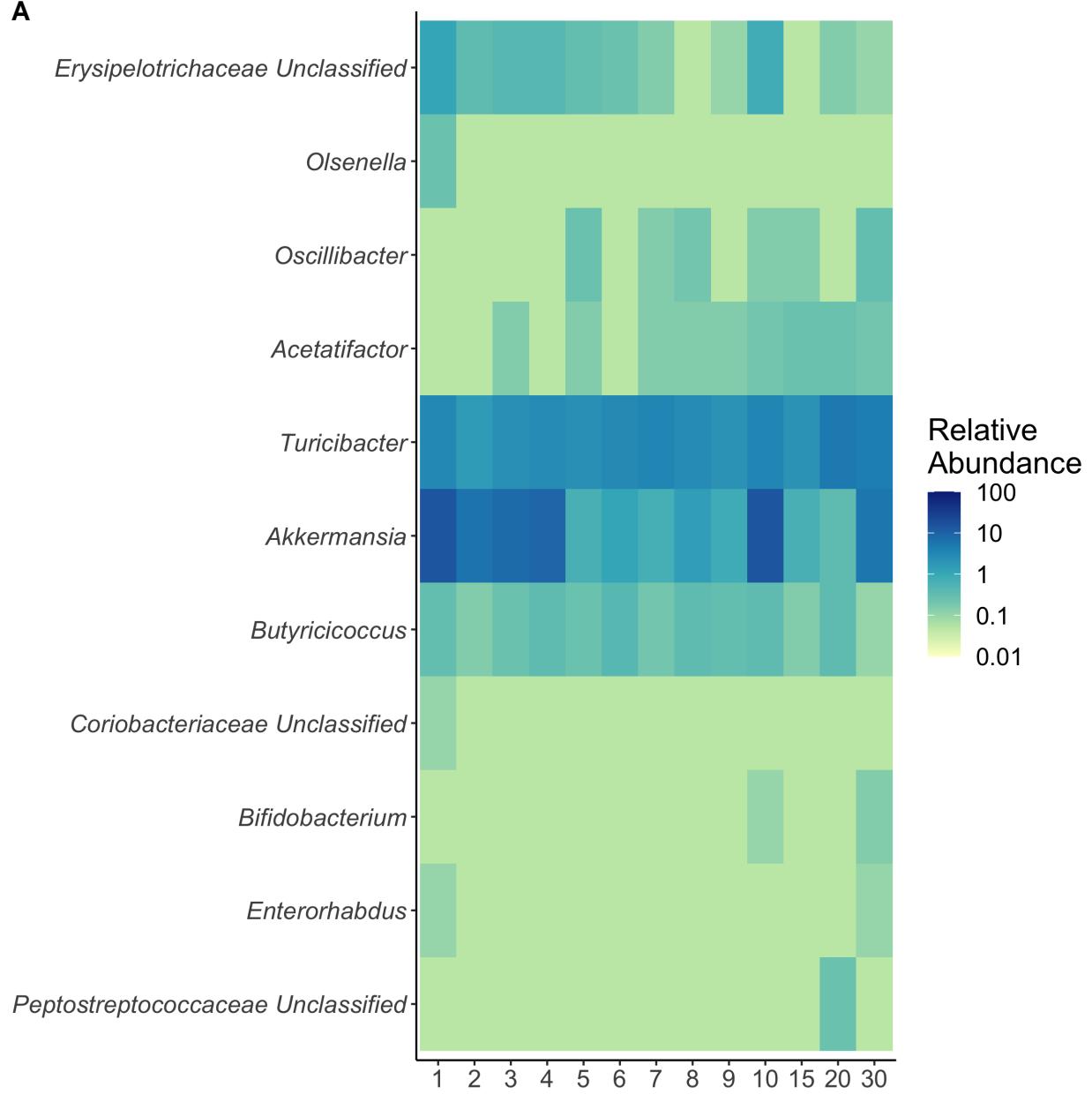
145 **Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization  
146 in PEG treated mice. A.**

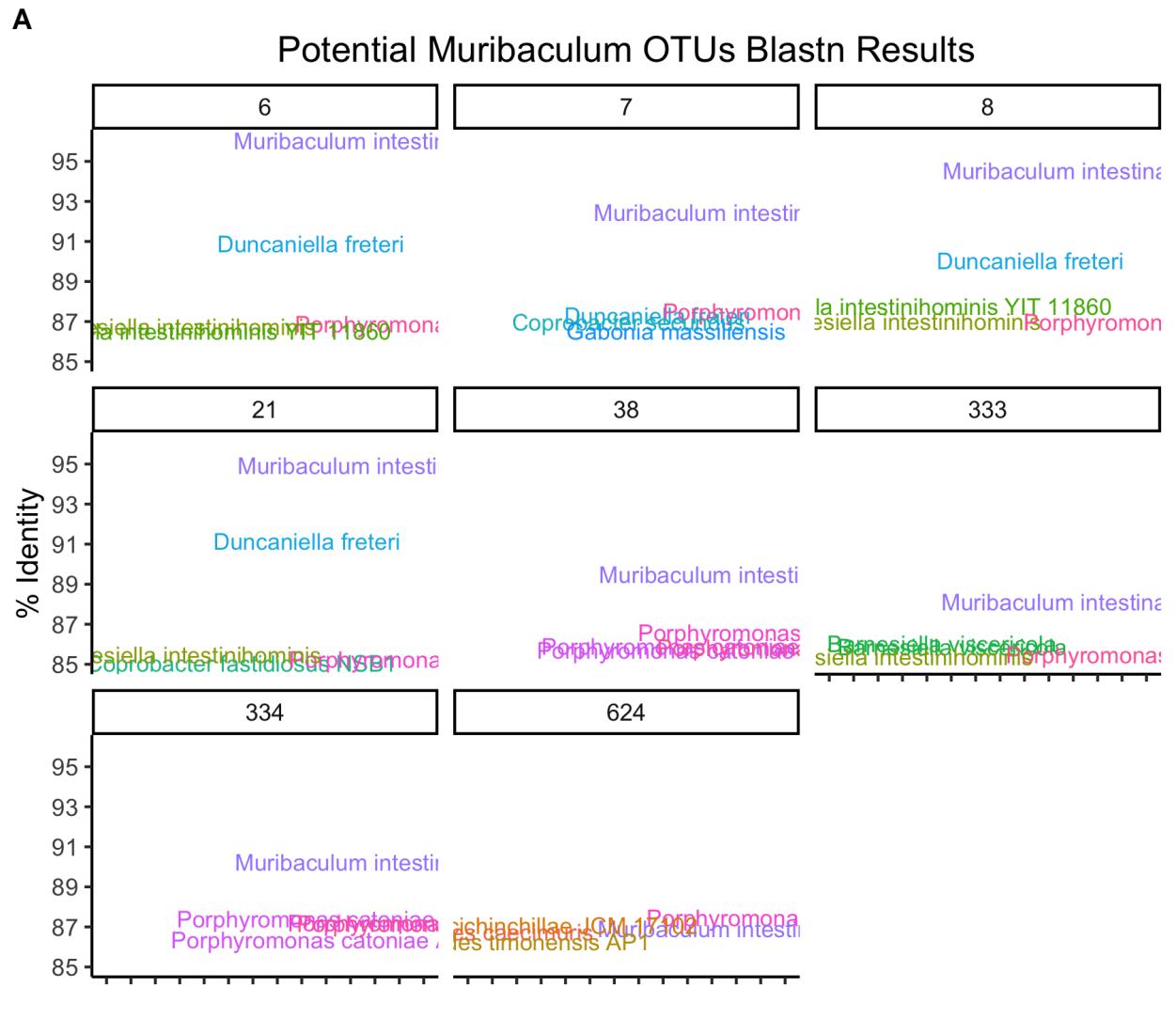


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148 **Figure 7. Schematic summarizing findings. A.**

A





**Figure S2. Specific OTUs associated with clearance that are mostly absent in mice with prolonged *C. difficile* colonization. Ex. *Muribaculum intestinale*. A.**

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