

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

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**1 Abstract**

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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 they disrupt  
32 microbiota colonization resistance (1). However, antibiotics are not the only types of medications  
33 that disrupt the microbiota (2–4). Although, other medications (proton pump inhibitors, osmotic  
34 laxatives, antimotility agents, and opioids) have been implicated as risk or protective factors for CDIs  
35 through epidemiological studies, whether the association is due to their impact on the microbiome  
36 is still unclear (5–9).

37 Many of the non-antibiotic medications associated with CDIs are known to modulate gastrointestinal  
38 motility leading to either increased or decreased colonic transit time, which in turn also strongly  
39 impacts microbiota composition and function (10, 11). Stool consistency often serves as an  
40 approximation of intestinal motility. Our group has shown that when *C. difficile* negative controls are  
41 separated into two groups based on stool consistency, there are shared microbiota features such  
42 as lower alpha diversity in samples from CDI patients and control patients with diarrhea compared  
43 to control samples that were *C. difficile* negative with non-diarrheal consistency (12). These results  
44 led to a hypothesis that bacterial communities from patients experiencing diarrhea are susceptible  
45 to developing CDIs.

46 Osmotic laxatives can lead to diarrhea depending on the administered dose and temporarily disrupt  
47 the human intestinal microbiota (13). The ubiquitous osmotic laxative, polyethylene glycol (PEG)  
48 3350 is found in Miralax, Nulytely, and Golytely and is also commonly used as bowel preparation  
49 for colonoscopies. Interestingly, previous studies have shown that treating mice with PEG alone  
50 altered microbiota composition, reduced acetate and butyrate production, altered the mucus barrier,  
51 and rendered the mice susceptible to *C. difficile* infection (14–17). The mucus barrier is thought to  
52 mediate protection from *C. difficile* infections by protecting intestinal epithelial cells from the toxins  
53 produced by *C. difficile* (Ref). However, whether laxative results in more severe CDIs in mice and  
54 how long mice remain colonized with *C. difficile* after challenge is unclear.

55 Beyond susceptibility, PEG is also relevant in the context of treating recurrent CDIs via fecal  
56 microbiota transplant (FMT) where a healthy microbiota is administered to the patient to restore  
57 colonization. For FMTs that are delivered via colonoscopy, patients typically undergo bowel

58 preparation by taking an osmotic laxative prior to the procedure. Many of the FMT studies to date  
59 rationalize the use of laxatives (Ref) based on a 1996 case study with 2 pediatric patients where the  
60 authors suggested in the discussion that the laxative may help flush *C. difficile* spores and toxins  
61 from the intestine (18).

62 In the past, our group has used C57BL6 mice to characterize how antibiotics including clindamycin  
63 disrupt the microbiota and influence *C. difficile* susceptibility and clearance [ref]. Although, two  
64 groups have now shown PEG treatment alone renders mice susceptible to *C. difficile*, these studies  
65 have raised additional questions regarding the dynamics and severity of infection as well as the  
66 role of laxative treatment in *C. difficile* clearance that should be addressed to better inform how  
67 we think about laxatives in the context of CDIs. Here, we used our C57BL/6 clindamycin model  
68 as a control group to characterize how long PEG-treated mice remain susceptible, whether PEG  
69 treatment results in more severe CDI and sustained *C. difficile* colonization, and if PEG treatment  
70 post-CDI can promote *C. difficile* clearance.

## 71 **Results**

72 **5-day laxative treatment leads to prolonged *C. difficile* colonization in mice.** We compared  
73 PEG-treated mice to our standard 10 mg/kg clindamycin treatment, which temporarily renders the  
74 mice susceptible to *C. difficile*, with mice typically clearing *C. difficile* within 10 days post-infection (9,  
75 19). All PEG-treated mice were administered a 15% PEG solution in the drinking water for 5-days,  
76 one group was also treated with clindamycin, and one group was allowed to recover for 10 days prior  
77 to challenge (Fig. 1A). PEG treatment resulted in weight loss in all 3 groups of PEG-treated mice,  
78 with the greatest change in weight observed on the fifth day of PEG treatment (Fig. 1B). After either  
79 PEG, clindamycin, or PEG and clindamycin treatment all mice were challenged with  $10^3$  *C. difficile*  
80 630 spores. All treatments rendered mice susceptible to *C. difficile* colonization (Fig. 1C), however  
81 PEG-treated mice remained colonized at a high level through 30 days post-infection. In contrast,  
82 the clindamycin-treated mice that cleared *C. difficile* within 10 days post-infection. Surprisingly,  
83 mice were still susceptible to *C. difficile* infection after 10-days of recovery from PEG treatment  
84 although *C. difficile* was not detectable in most of the group in the initial 5 days post-infection (Fig.  
85 1C). From 9 days post-infection onward, the median *C. difficile* stabilized for the 5-day PEG plus

86 10-day recovery group of mice and remained high through 30 days post-infection (Fig. 1C). Thus,  
87 osmotic laxative treatment alone was sufficient to render mice susceptible to prolonged *C. difficile*  
88 colonization and PEG-treated mice remained susceptible for up to 10 days post PEG treatment.

89 **5-day laxative treatment differentially disrupts the fecal microbiota compared to  
90 clindamycin treatment.** Since laxatives and clindamycin have previously been shown to  
91 disrupt the murine microbiota (ref), we hypothesized the different *C. difficile* colonization dynamics  
92 between mice treated with laxatives versus clindamycin were due to their differential effects on the  
93 microbiota. We profiled the stool microbiota over time by sequencing the V4 region of the 16S  
94 rRNA gene to compare changes across treatment groups. We found time and treatment group  
95 explained half of the observed variation between fecal communities (PERMANOVA combined  $R^2 =$   
96 0.50,  $P < 0.001$ ) with the remaining variation explained by interactions between treatment group  
97 and other experimental variables including time, cage effects, and sequencing preparation (Data  
98 Set S1, Sheet X). The impact of cage effects has been well documented due to coprophagy and is  
99 expected.

100 **5-day laxative treatment does not promote more severe CDIs despite altering the mucosal  
101 microbiota.** Given previous work has shown that PEG treatment disrupts the mucus layer, we  
102 decided to examine the impact of the laxative on the mucosal microbiota and infection severity by  
103 evaluating histopathology.

- 104 • Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time  
105 compared to clindamycin-treated mice.
- 106 • Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics  
107 post-infection.
- 108 • Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal  
109 microbiota is altered.
- 110 • Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.
- 111 • Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless of

112 whether an FMT is also administered.

- 113 • Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in  
114 PEG treated mice.

- 115 • Figure S2. Specific OTUs associated with clearance that are mostly absent in mice with  
116 prolonged *C. difficile* colonization.

117 – Ex. *Muribaculum intestinale*.

- 118 • Figure 7. Schematic summarizing findings.

## 119 Discussion

- 120 • Summary of major findings

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

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

125 – Antibiotics may also impact mucus layer

126 – Strain of bacteria used

- 127 • Protective bacteria missing in PEG-treated mice

- 128 • Discuss what these findings might mean for human patients

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

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

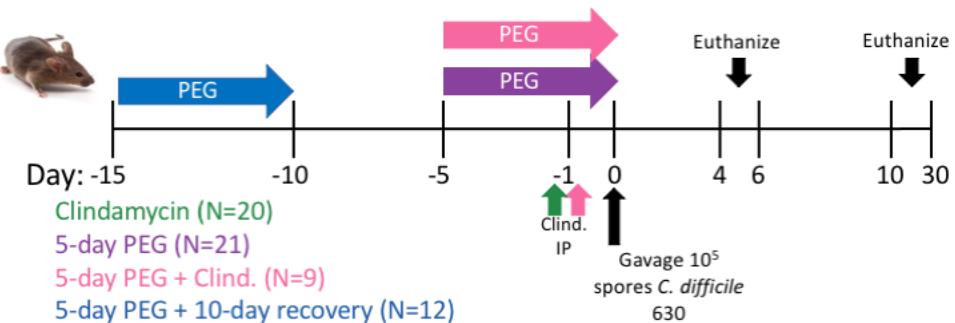
132 **Conclusions**

133 **Acknowledgements**

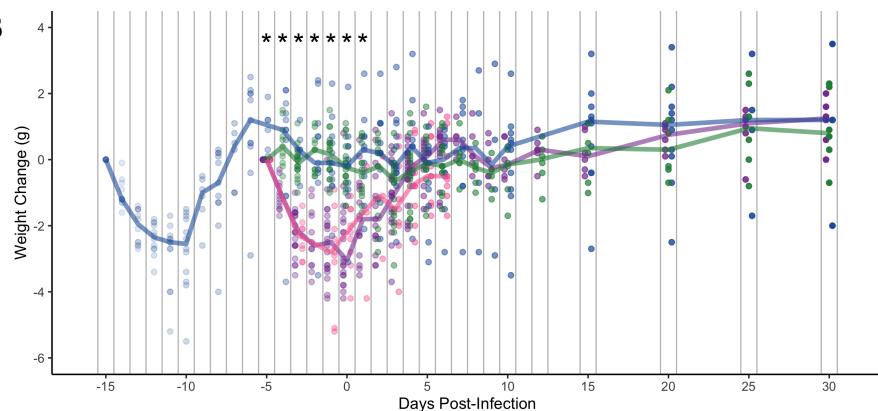
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144 **Materials and Methods**

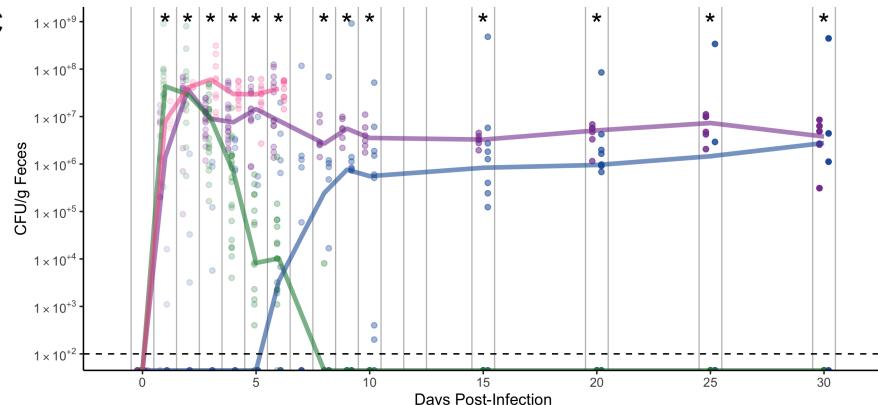
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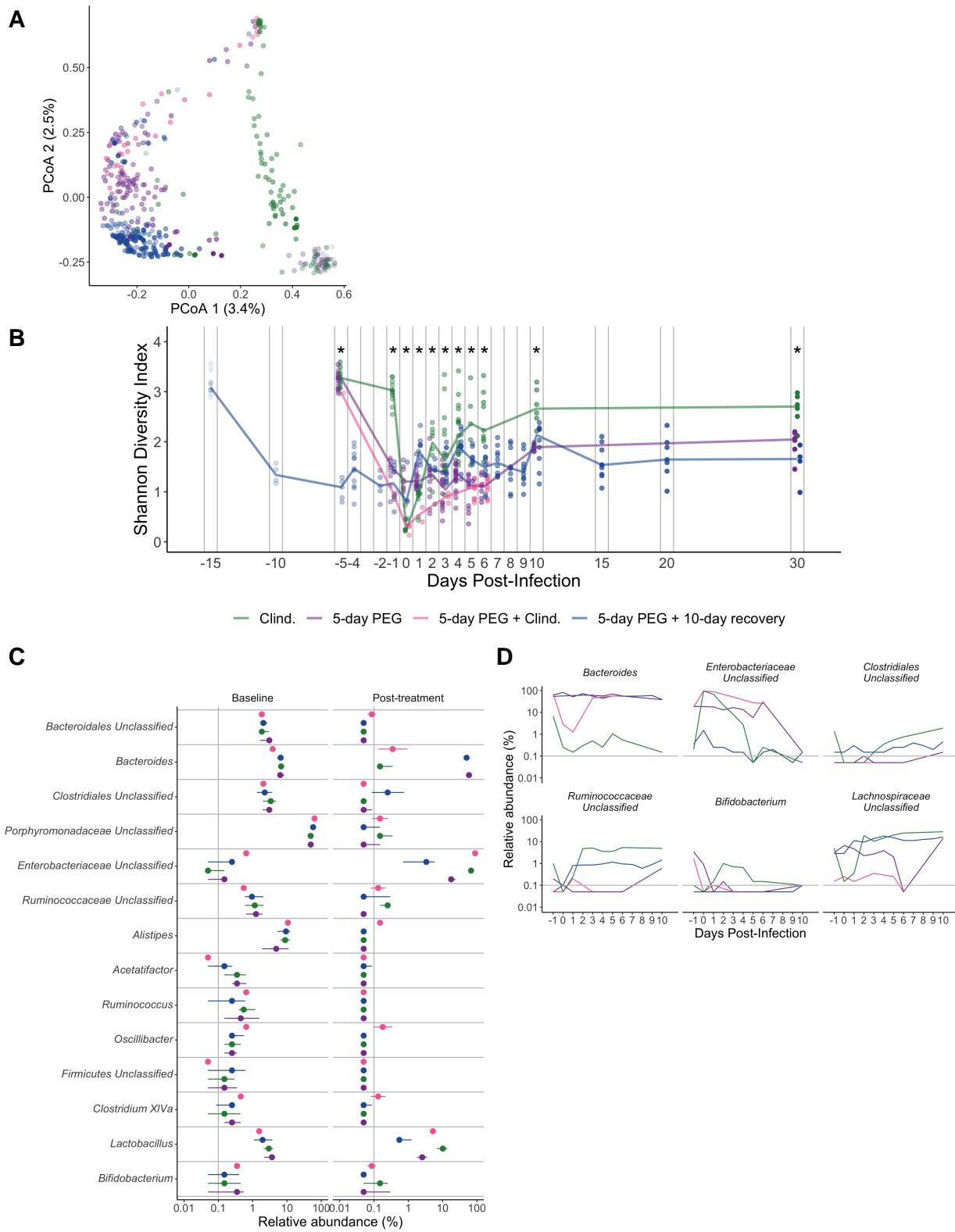
C



145

146 **Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently**  
147 **colonized with *C. difficile*.** A. Setup of the experimental timeline for experiments with 5-day PEG  
148 treated mice. Clindamycin was administered at 10 mg/kg by intraperitoneal injection. 15% PEG  
149 3350 was administered in the drinking water for five days. B. Weight change from baseline weight  
150 in groups after treatment with PEG and/or clindamycin, followed by *C. difficile* challenge. C. *C.*  
151 *difficile* CFU/gram stool measured over time (N = 16-59 mice per timepoint) via serial dilutions. The  
152 black line represents the limit of detection for the first serial dilution. CFU quantification data was

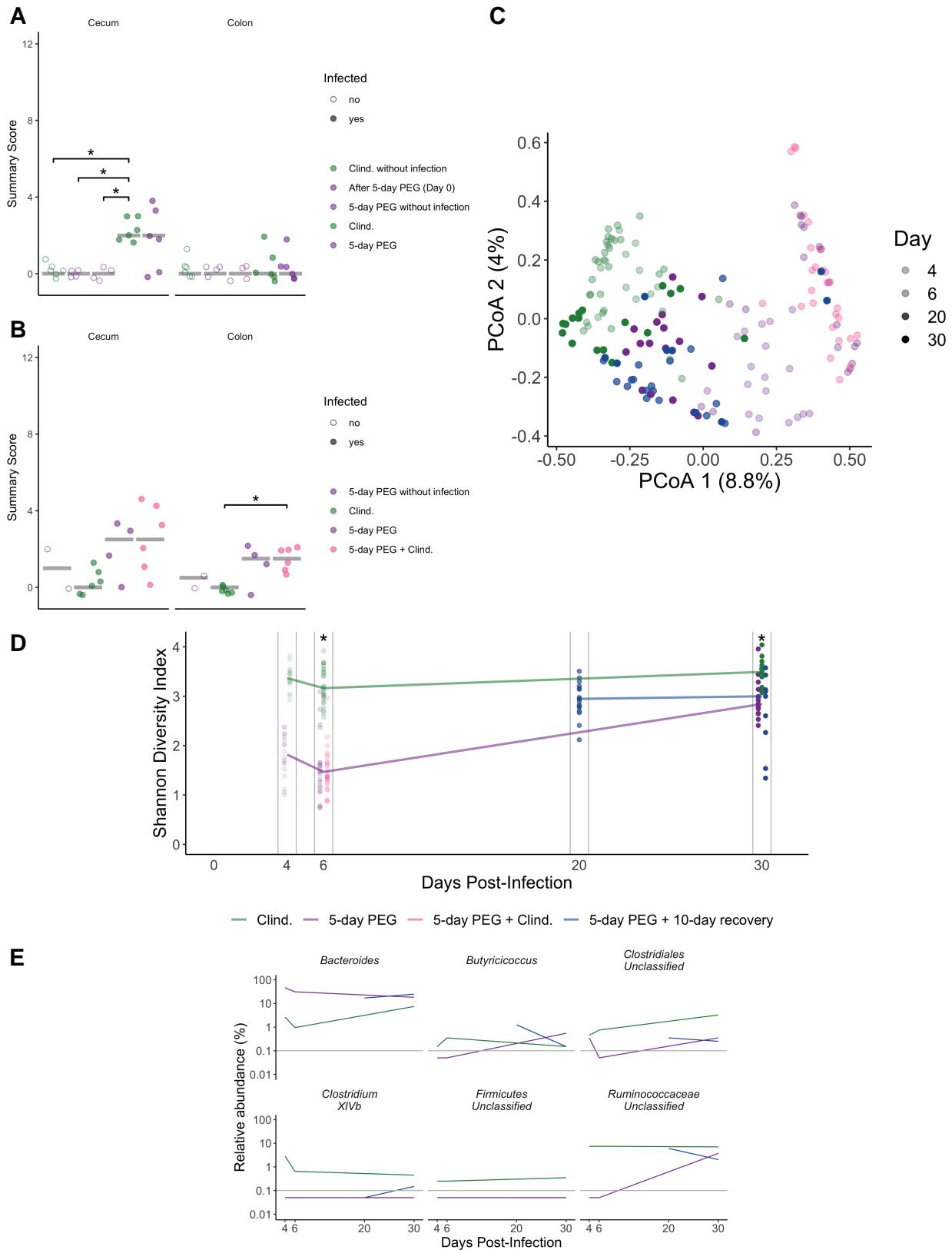
153 not available for each mouse due to stool sampling difficulties (particularly the day the mice came  
154 off of the PEG treatment) or early deaths. For B-C, lines represent the median for each treatment  
155 group and circles represent samples from individual mice. Asterisks indicate timepoints where the  
156 weight change or CFU/g was significantly different between groups by the Kruskal-Wallis test with  
157 Benjamini-Hochberg correction for testing multiple timepoints.



158

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

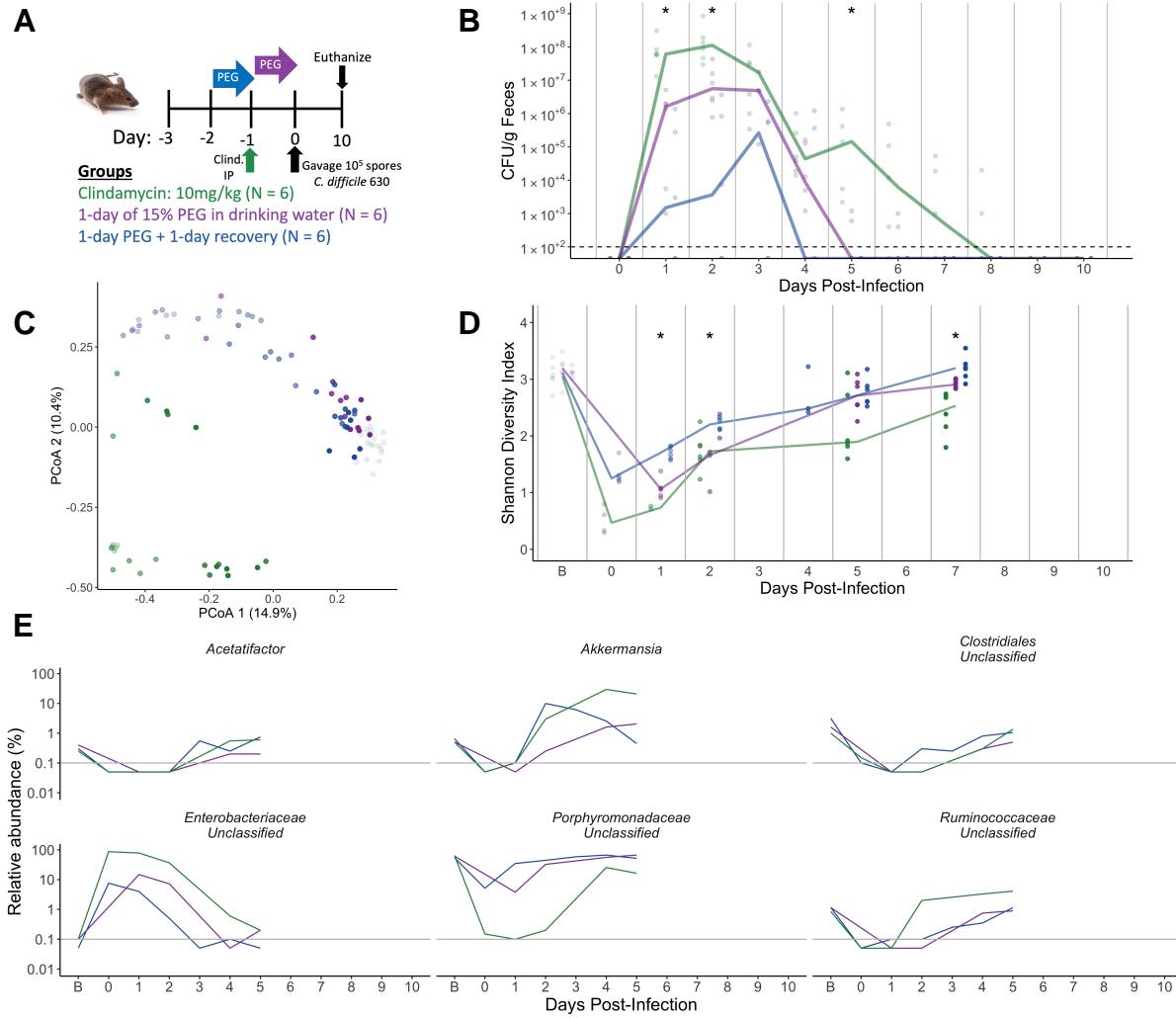
<sup>160</sup> compared to clindamycin-treated mice. A.



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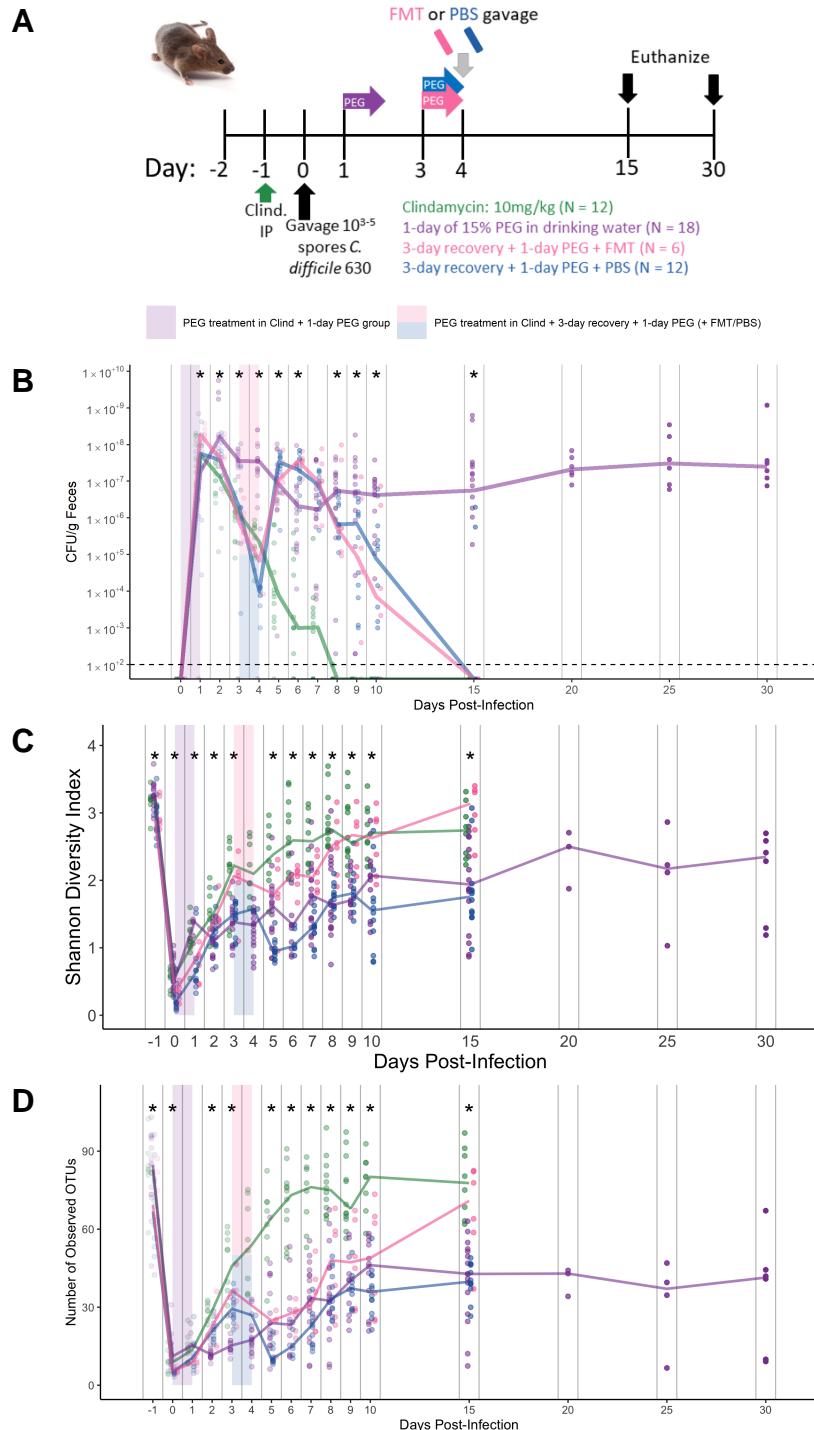
162 **Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal**

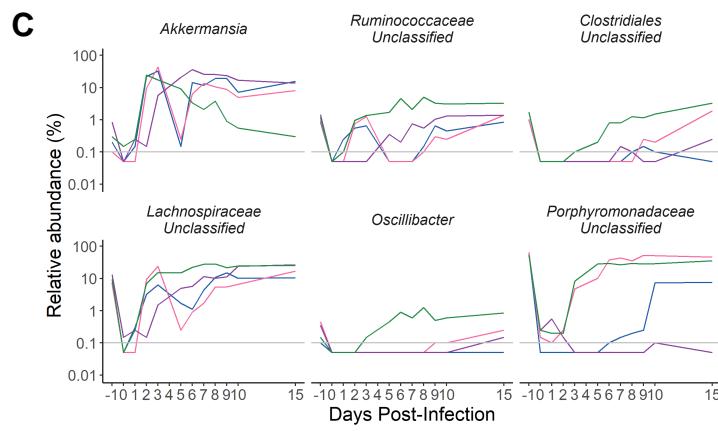
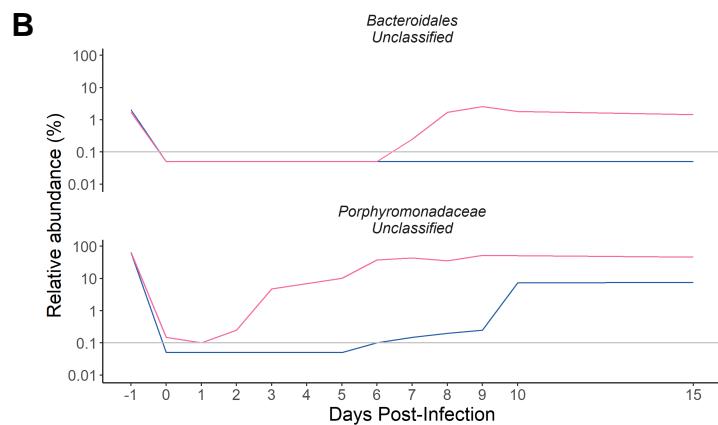
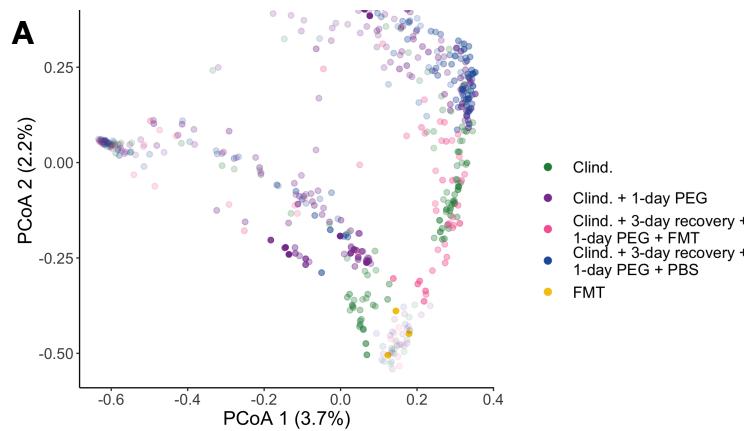
<sup>163</sup> **microbiota is altered.** A.



164

165 **Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile***  
 166 **colonization.** A. Setup of the experimental timeline for the 1-day PEG treated subset of  
 167 mice. B. CFU/gram stool measured over time ( $N = 12\text{-}18$  mice per timepoint) via several dilutions.  
 168 The black dotted line represents the limit of detection for the first serial dilution. Asterisks indicate  
 169 timepoints where the CFU/gram was significantly different between groups using the Kruskall-Wallis  
 170 test with a Benjamini-Hochberg correction for multiple timepoints. C. Principle Coordinate Analysis  
 171 plot of the groups over time with the alpha representing the same time scale as in panel D (day:  
 172  $R^2 = 0.43$ ; group:  $R^2 = 0.19$ ). D. Shannon diversity Index of the groups over time. Only days with  
 173 samples from all groups are shown. E. Line plots of relative percent abundance of selected genera  
 174 over time. Only days with samples from all groups shown. The gray line represents the limit of  
 175 detection.

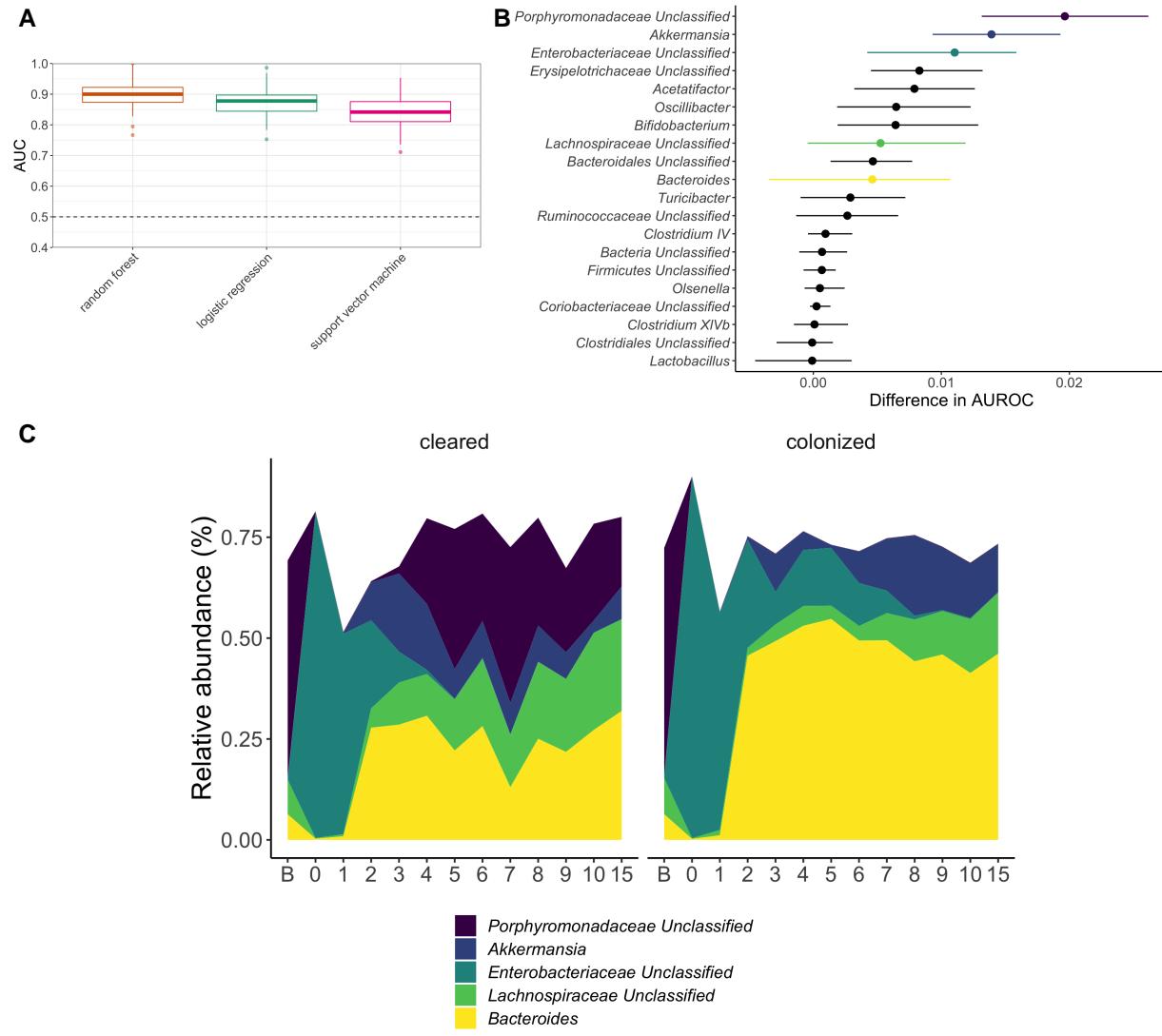




**Figure 5. 1-day PEG treatment**

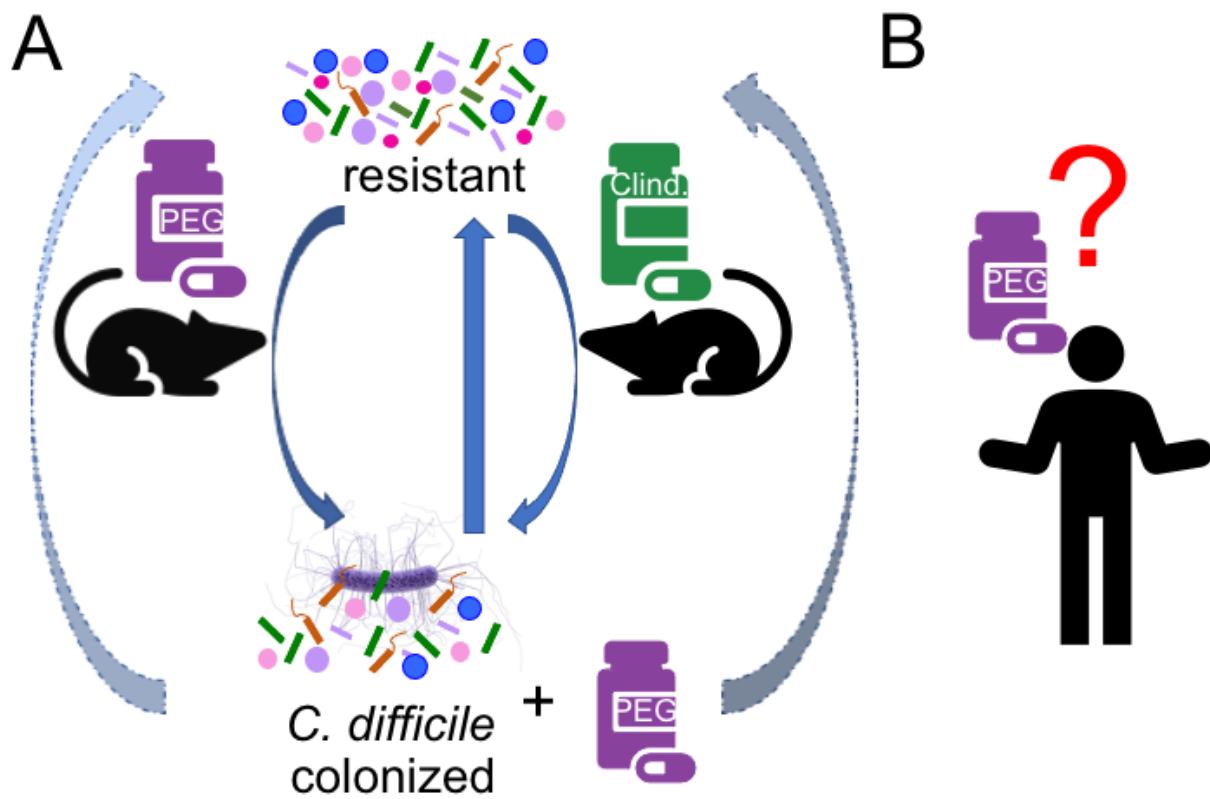
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178 **post C. difficile challenge prolongs colonization regardless of whether an FMT is also  
179 administered. A.**



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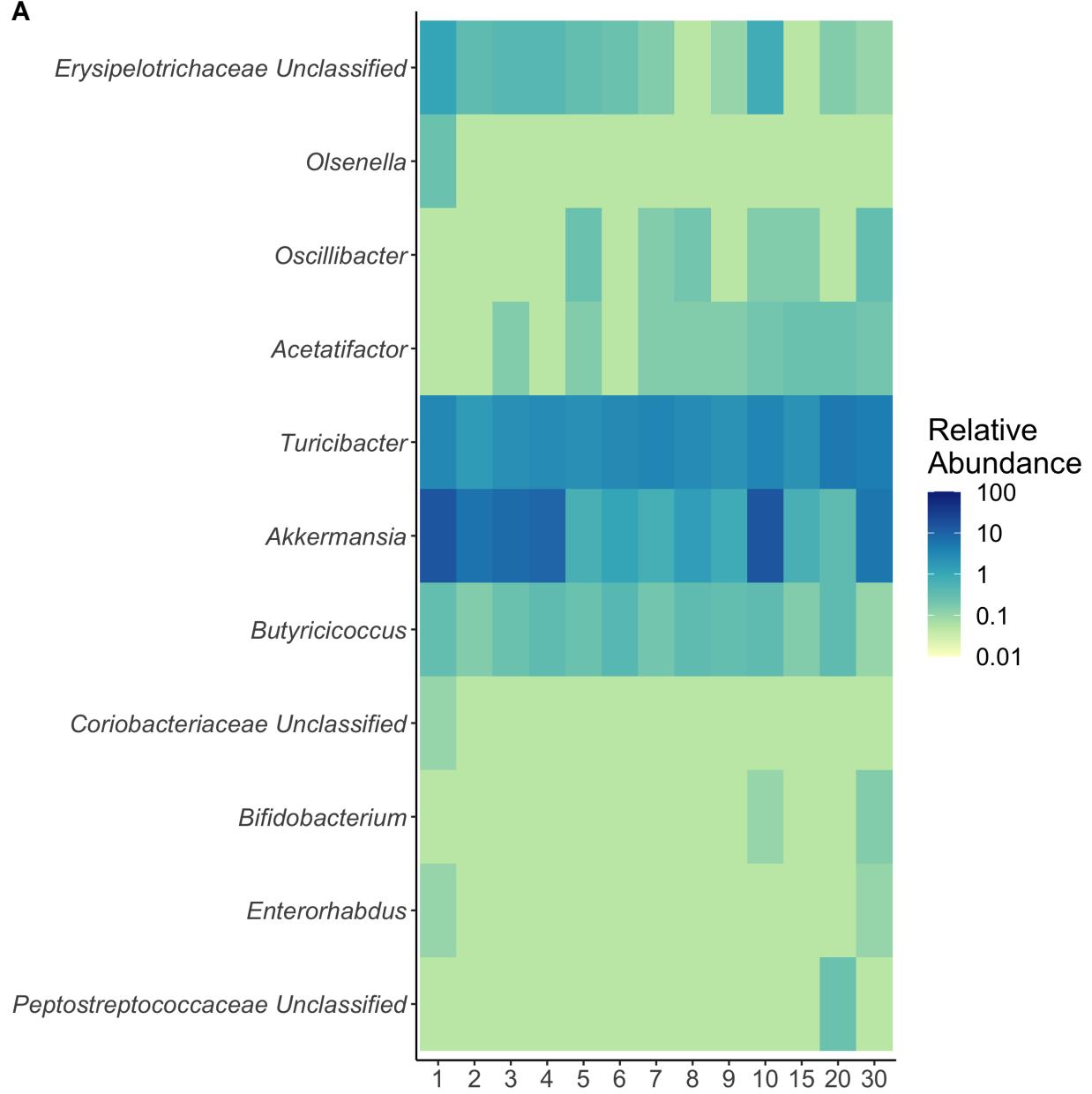
181 **Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization**  
182 **in PEG treated mice. A.**



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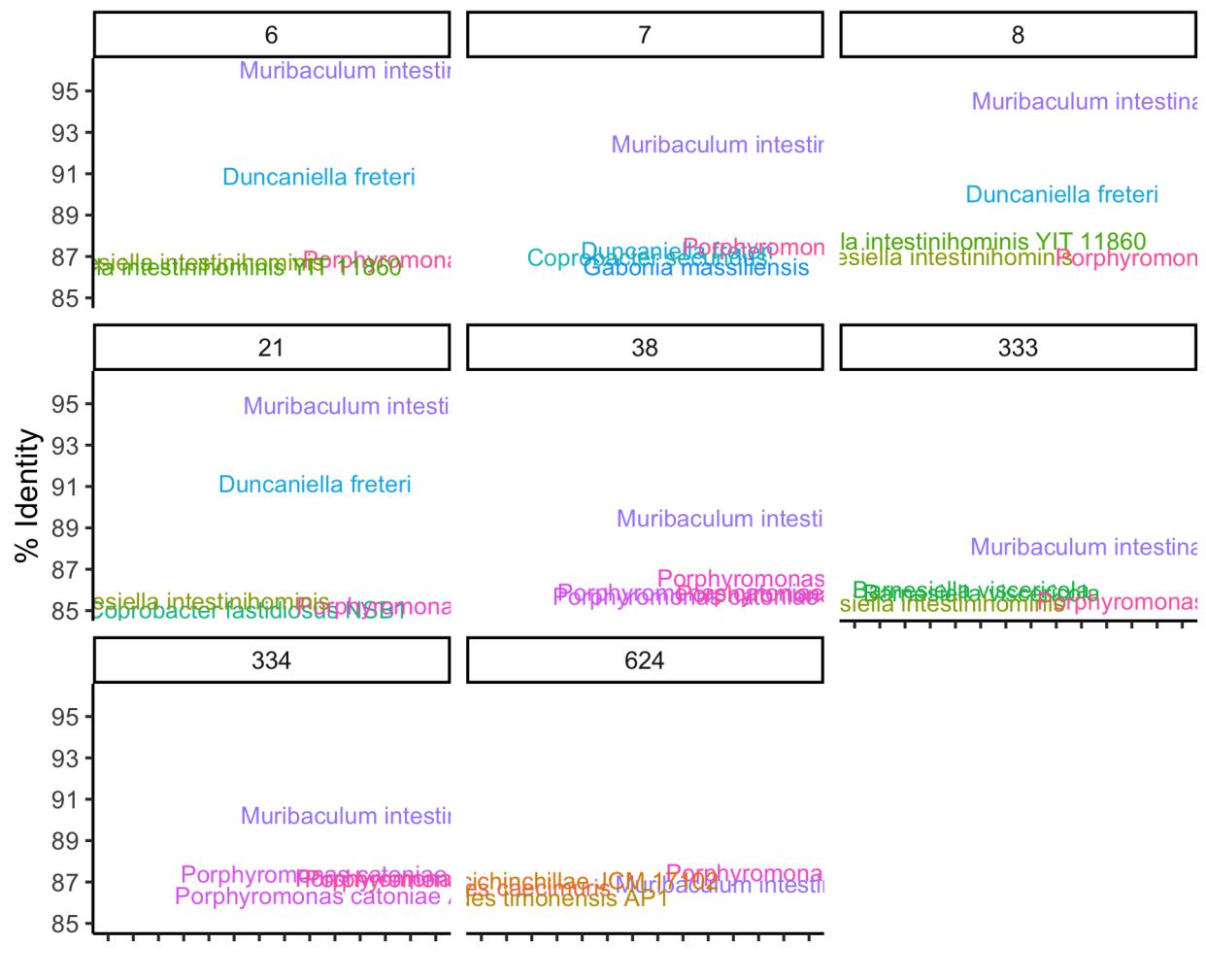
184 **Figure 7. Schematic summarizing findings. A.**

A



A

### Potential Muribaculum OTUs Blastn Results



188

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

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