

# **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 (1). However, antibiotics are not the only types of medications that disrupt the  
33 microbiota (2–4). Although, other medications have been implicated as risk factors for CDIs through  
34 epidemiological studies, whether the association is due to their impact on the microbiome is still  
35 unclear (5–9). Many of the non-antibiotic medications associated with CDIs are known to modulate  
36 intestinal motility, which in turn also strongly impacts microbiota composition and function (10, 11).

37 Stool consistency often serves as an approximation of intestinal motility. Our group has shown that  
38 when *C. difficile* negative controls are separated into two groups based on stool consistency, there  
39 are microbiota features such as alpha diversity that overlap between samples from CDI patients and  
40 control patients with diarrhea (12). These results led to our hypothesis that bacterial communities  
41 from patients experiencing diarrhea are susceptible, but may not have been exposed to *C. difficile*  
42 spores.

43 Osmotic laxatives can lead to diarrhea depending on the administered dose and temporarily disrupts  
44 the intestinal microbiota in humans (13). The ubiquitous osmotic laxative, polyethylene glycol (PEG)  
45 3350 is found in Miralax, Nulytely, and Golytely and is also commonly used as bowel preparation  
46 for colonoscopies. Interestingly, previous studies have shown that treating mice with PEG alone  
47 rendered the mice susceptible to *C. difficile* infection, altered microbiota composition, reduced  
48 acetate and butyrate and altered the mucus barrier (14–17). 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). However, whether laxative results in more severe CDIs in mice is  
51 unclear.

52 Beyond susceptibility, PEG is also relevant in the context of treating recurrent CDIs via fecal  
53 microbiota transplant (FMT) where a healthy microbiota is administered to the patient to restore  
54 colonization. For FMTs that are delivered via colonoscopy, patients typically undergo bowel  
55 preparation by taking an osmotic laxative prior to the procedure. Many of the FMT studies to date  
56 rationalize the use of laxatives (Ref) based on a 1996 case study with 2 pediatric patients where the  
57 authors suggested the laxative may help flush *C. difficile* spores and toxins from the intestine (18).

58 In the past, our group has used C57BL6 mice to characterize how antibiotics disrupt the microbiota  
59 and influence *C. difficile* susceptibility and clearance [ref]. Although, two groups have now shown  
60 PEG treatment alone renders mice susceptible to *C. difficile*, these studies have raised additional  
61 questions that should be addressed given their relevance to CDIs. Here, we used our C57BL/6  
62 clindamycin model as a control group to characterize how long PEG-treated mice remain susceptible,  
63 whether PEG treatment results in sustained *C. difficile* colonization, and if PEG treatment post-CDI  
64 can promote *C. difficile* clearance.

## 65 Results

66 **5-day laxative treatment leads to prolonged *C. difficile* colonization in mice.** We compared  
67 PEG-treated mice to our standard 10 mg/kg clindamycin treatment, which temporarily renders the  
68 mice susceptible to *C. difficile*, with mice typically clearing *C. difficile* within 10 days post-infection (9,  
69 19). All PEG-treated mice were administered a 15% PEG solution in the drinking water for 5-days,  
70 one group was also treated with clindamycin, and one group was allowed to recover for 10 days prior  
71 to challenge (Fig. 1A). PEG treatment resulted in weight loss in all 3 groups of PEG-treated mice,  
72 with the greatest change in weight observed on the fifth day of PEG treatment (Fig. 1B). After either  
73 PEG, clindamycin, or PEG and clindamycin treatment all mice were challenged with  $10^3$  *C. difficile*  
74 630 spores. All treatments rendered mice susceptible to *C. difficile* colonization (Fig. 1C), however  
75 PEG-treated mice remained colonized at a high level through 30 days post-infection. In contrast,  
76 the clindamycin-treated mice that cleared *C. difficile* within 10 days post-infection. Surprisingly,  
77 mice were still susceptible to *C. difficile* infection after 10-days of recovery from PEG treatment  
78 although *C. difficile* was not detectable in most of the group in the initial 5 days post-infection  
79 (Fig. 1C). From 9 days post-infection onward, the median *C. difficile* stabilized for the 5-day PEG  
80 plus 10-day recovery group of mice and remained high through 30 days post-infection (Fig. 1C).  
81 Thus, osmotic laxative treatment alone was sufficient to render mice susceptible to prolonged  
82 *C. difficile* colonization and PEG-treated mice remained susceptible for up to 10 days post PEG  
83 treatment. **5-day laxative treatment differentially disrupts the fecal microbiota compared to**  
84 **clindamycin treatment.** Given **5-day laxative treatment does not promote more severe CDIs**  
85 **despite altering the mucosal microbiota.** Given

- 86 • Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently colonized  
87 with *C. difficile*.
- 88 • Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time  
89 compared to clindamycin-treated mice.
- 90 • Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics  
91 post-infection.
- 92 • Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal  
93 microbiota is altered.
- 94 • Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.
- 95 • Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless of  
96 whether an FMT is also administered.
- 97 • Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in  
98 PEG treated mice.
- 99 • Figure S2. Specific OTUs associated with clearance that are mostly absent in mice with  
100 prolonged *C. difficile* colonization.  
101 – Ex. *Muribaculum intestinale*.
- 102 • Figure 7. Schematic summarizing findings.

103 **Discussion**

- 104 • Summary of major findings
- 105 • Discussion of prolonged persistence. *C. difficile* sequences detected in tissue samples.  
106 Association with mucin-degrading bacteria suggested by recent papers.
- 107 • Discuss why we might not have observed more severe histology in PEG mice relative to  
108 clindamycin-treated mice

- 109            – Antibiotics may also impact mucus layer
- 110            – Strain of bacteria used
- 111            • Protective bacteria missing in PEG-treated mice
- 112            • Discuss what these findings might mean for human patients
- 113                – What's known regarding laxatives and susceptibility to CDIs
- 114                – Relevance to human FMTs? Unclear what the best administration route is because there
- 115                  have been no studies designed to evaluate the best administration route for FMTs.

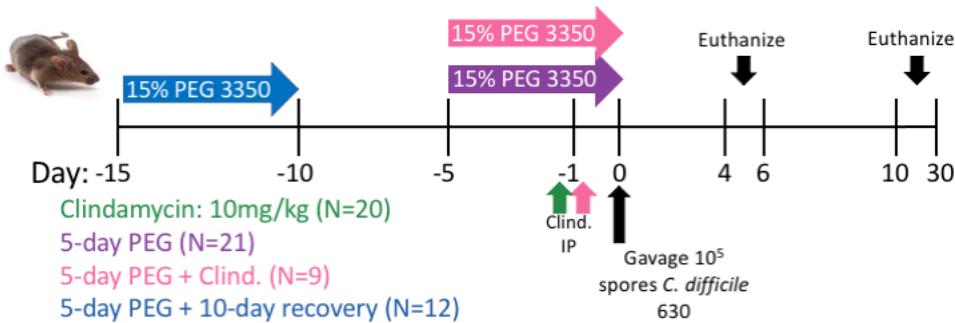
## 116      **Conclusions**

## 117      **Acknowledgements**

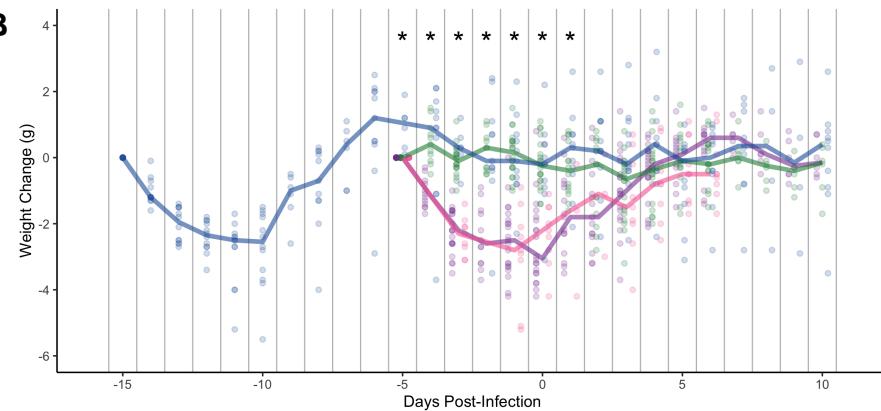
118      We thank members of the Schloss lab for feedback on planning the experiments and data  
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## 127      **Materials and Methods**

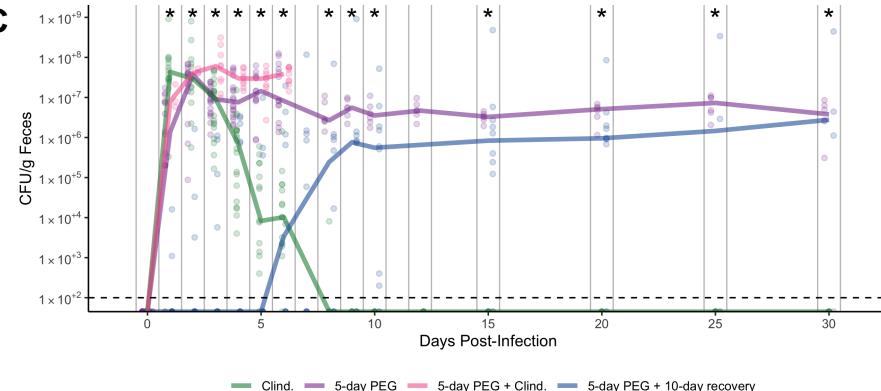
A



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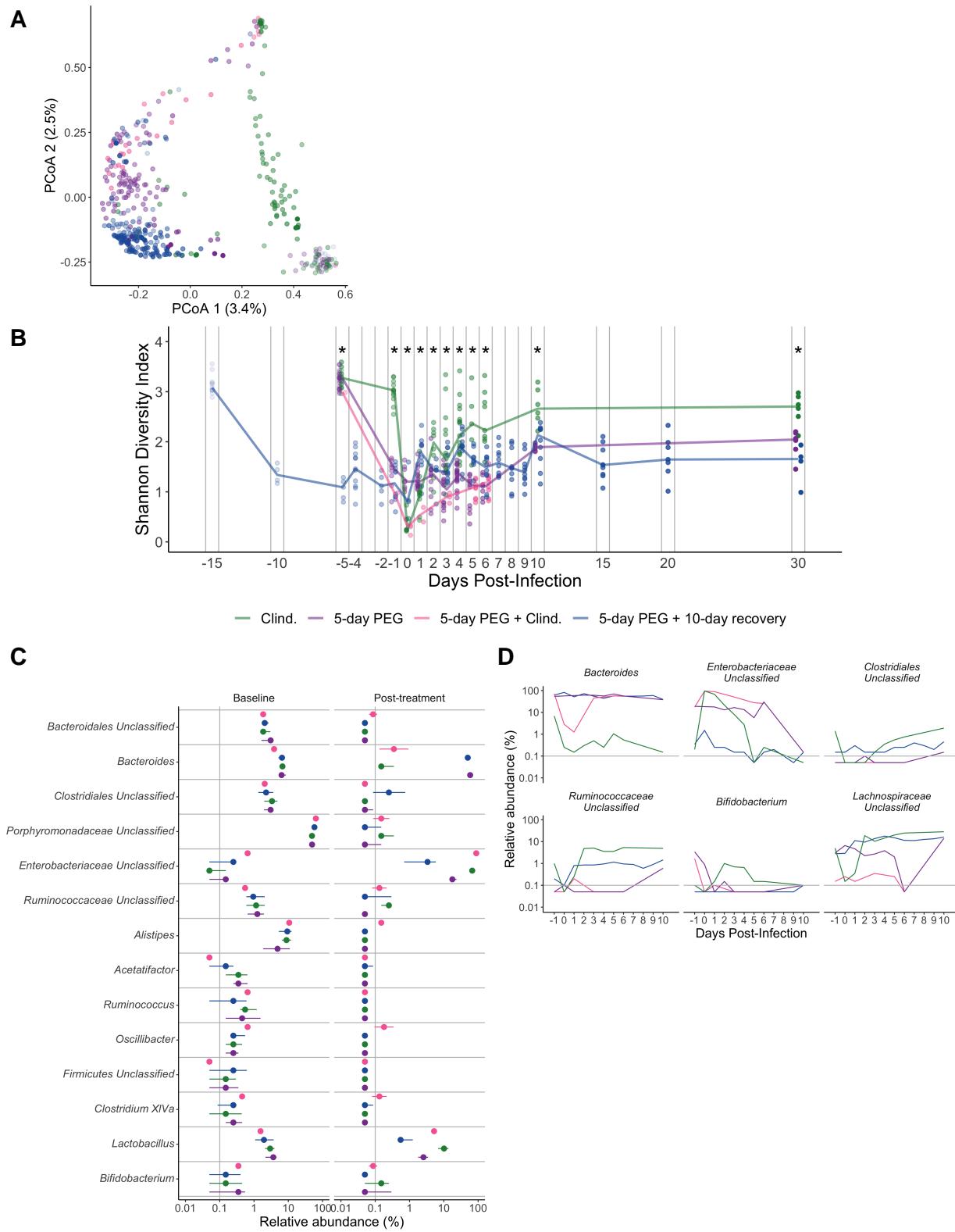
C



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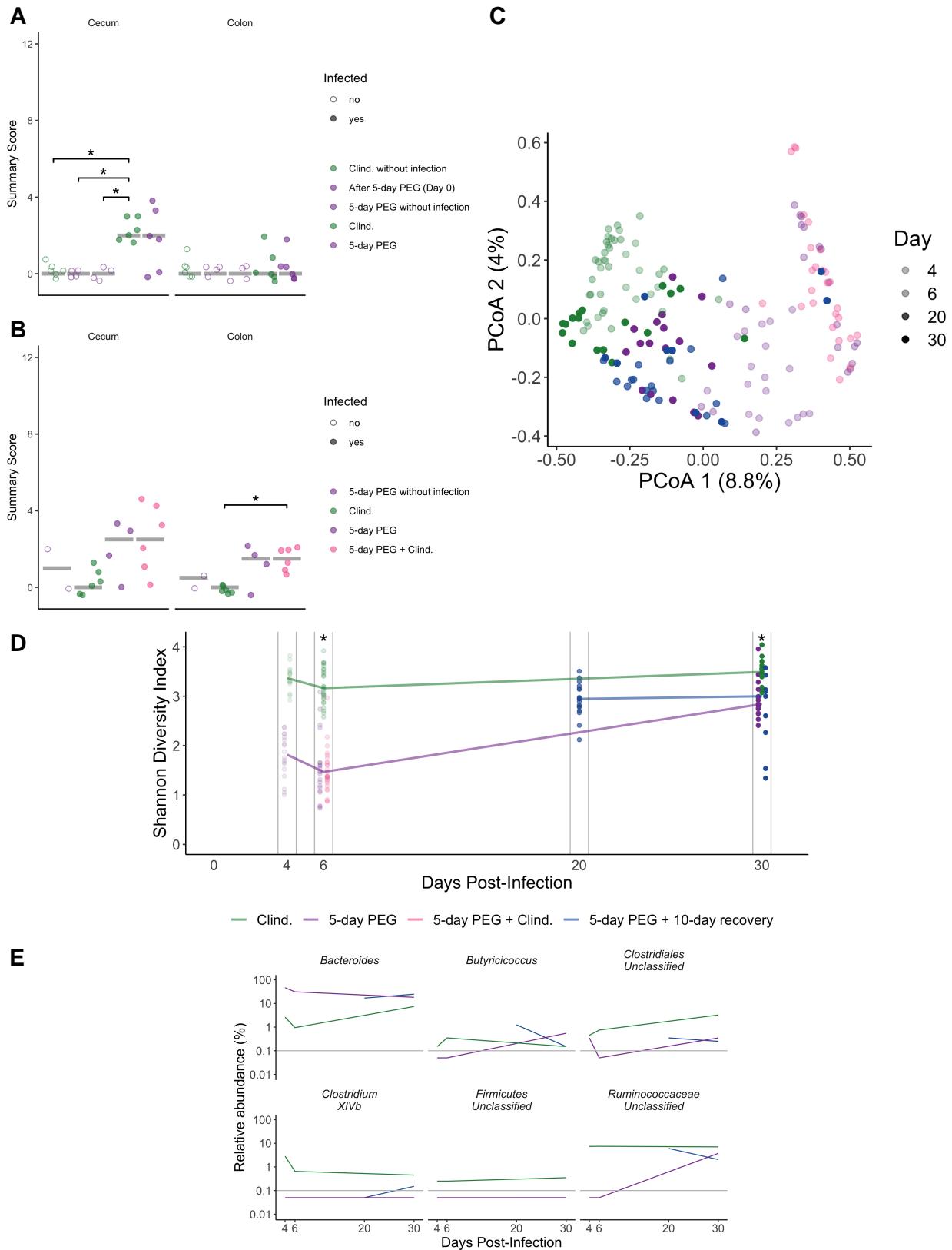
129 **Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently**  
130 **colonized with *C. difficile*.** A. Setup of the experimental timeline for subset of experiments with  
131 5-day PEG treated mice. B. Weight change from baseline weight in groups after treatment with  
132 PEG and/or clindamycin, followed by *C. difficile* challenge. C. *C. difficile* CFU/gram stool measured  
133 over time (N = 4-(insert variable name) mice per timepoint) via serial dilutions. The black line  
134 represents the limit of detection for the first serial dilution. CFU quantification data was not available  
135 for each mouse due to stool sampling difficulties (particularly the day the mice came off of the

<sup>136</sup> PEG treatment) or early deaths. Lines represent the median for each source and circles represent  
<sup>137</sup> individual mouse samples. Asterisks indicate timepoints where the weight change or CFU/g was  
<sup>138</sup> significantly different between groups by the Kruskal-Wallis test with Benjamini-Hochberg correction  
<sup>139</sup> for testing multiple timepoints.



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

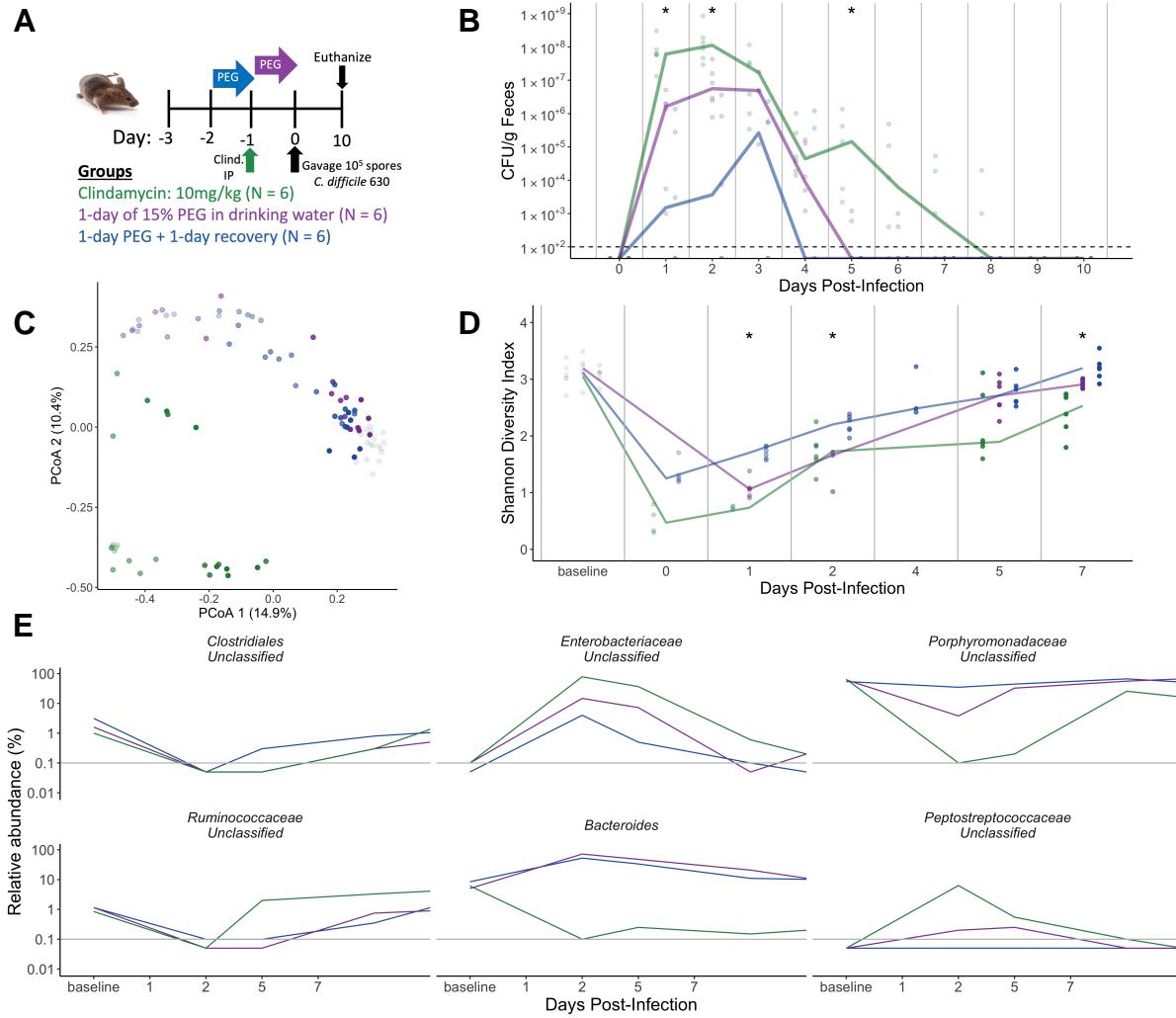
<sup>142</sup> compared to clindamycin-treated mice. A.



143

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

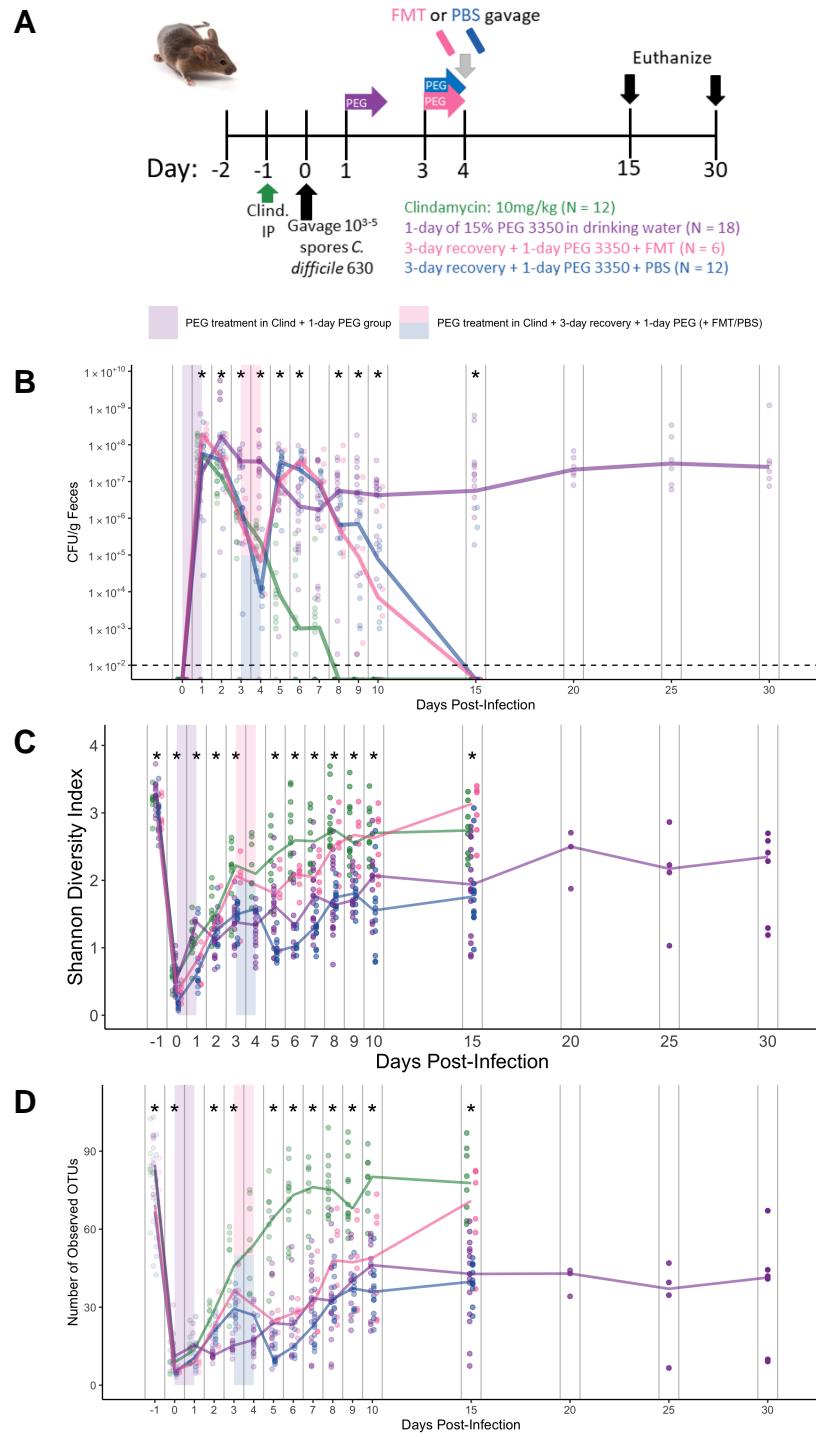
<sup>145</sup> **microbiota is altered.** A.



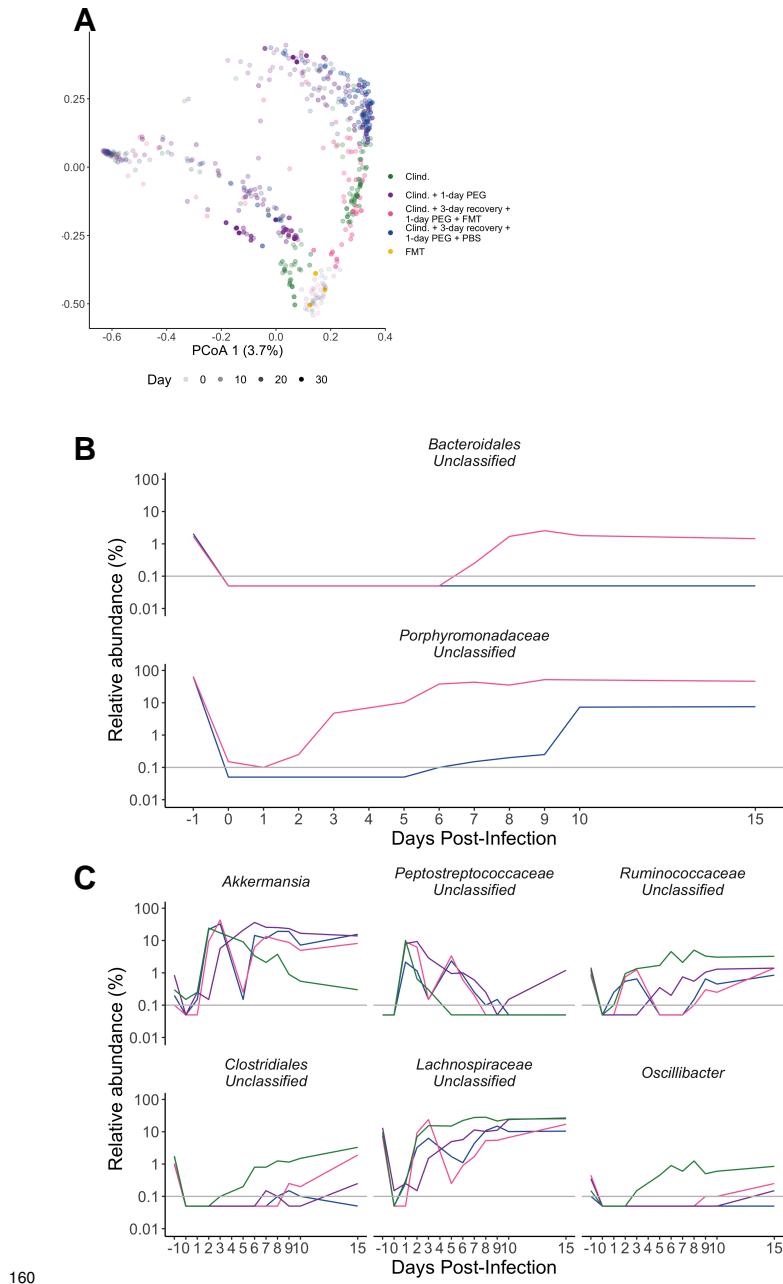
146

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

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

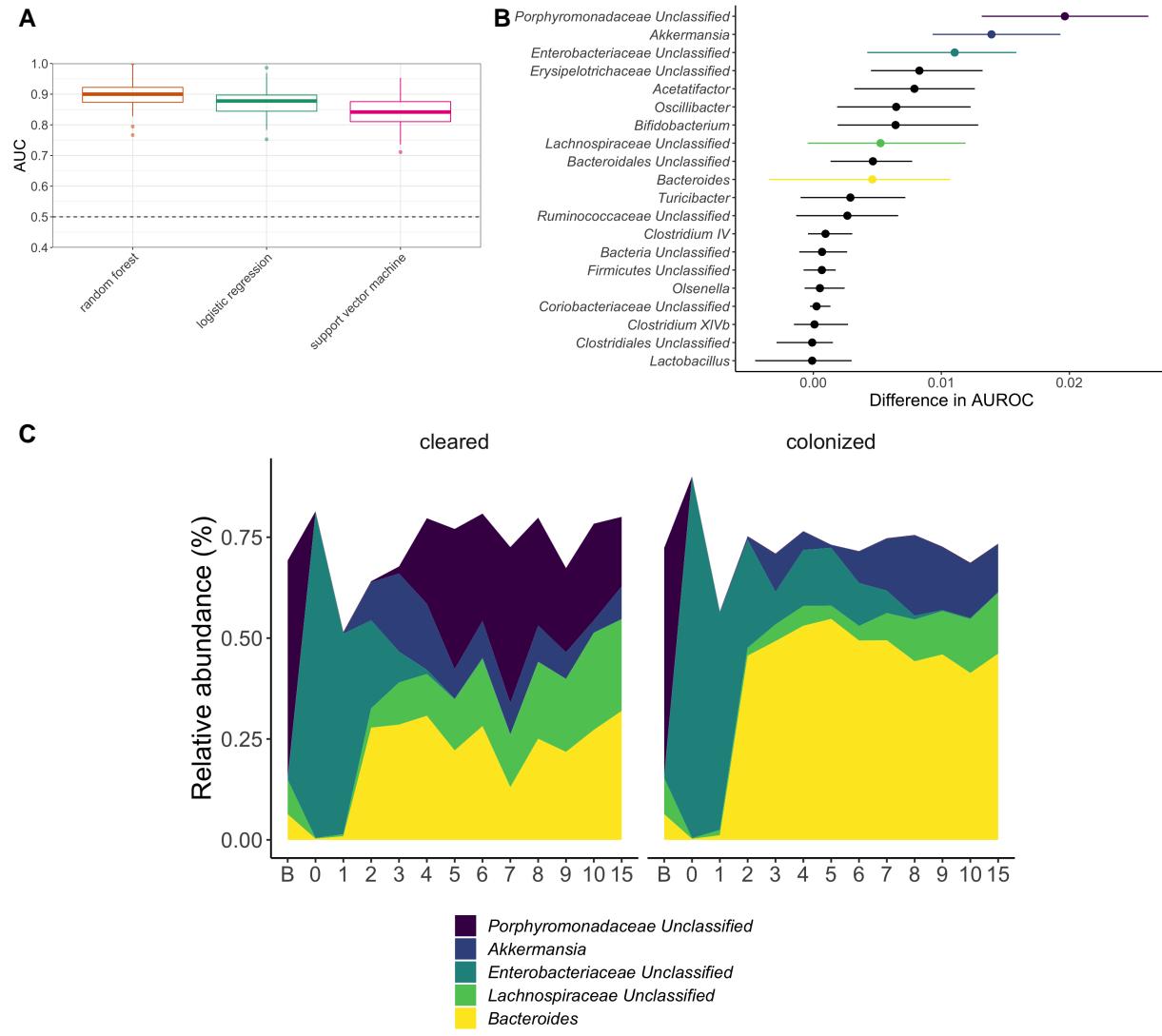


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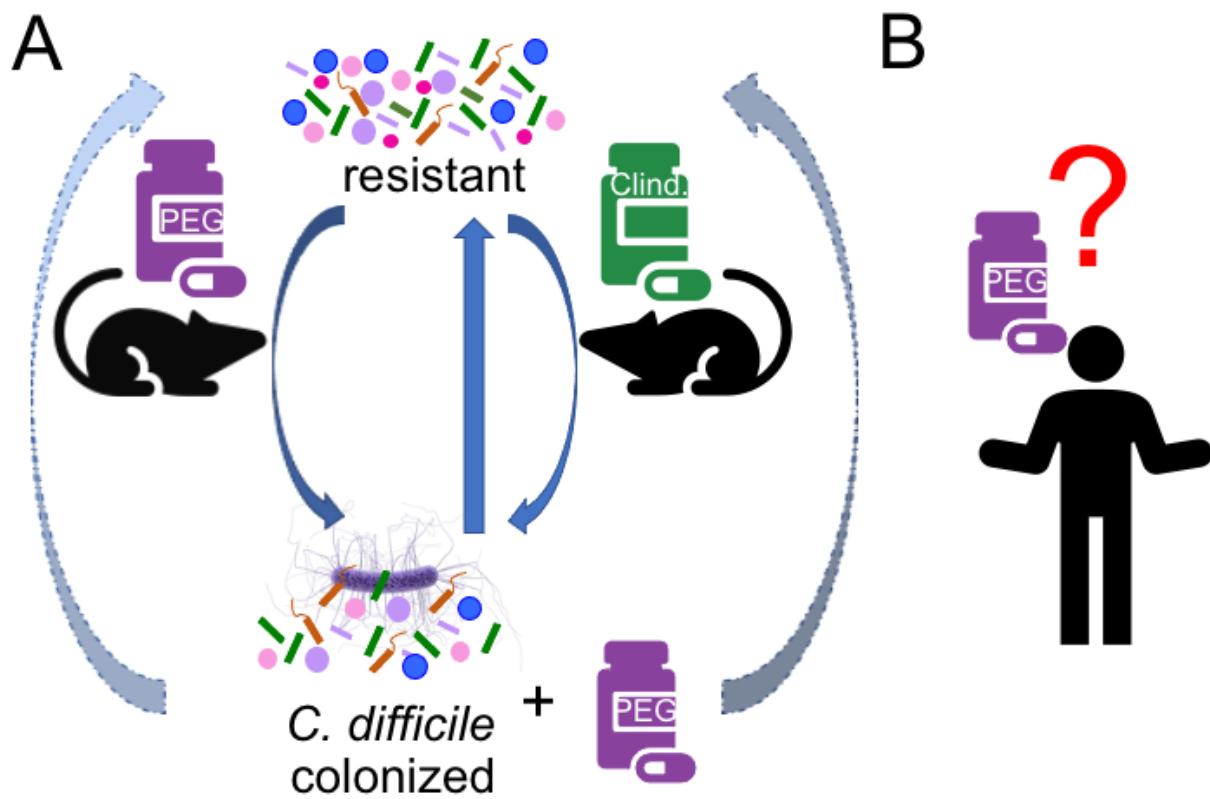
**Figure 5. 1-day PEG treatment**

160  
 161 **post C. difficile challenge prolongs colonization regardless of whether an FMT is also  
 162 administered. A.**



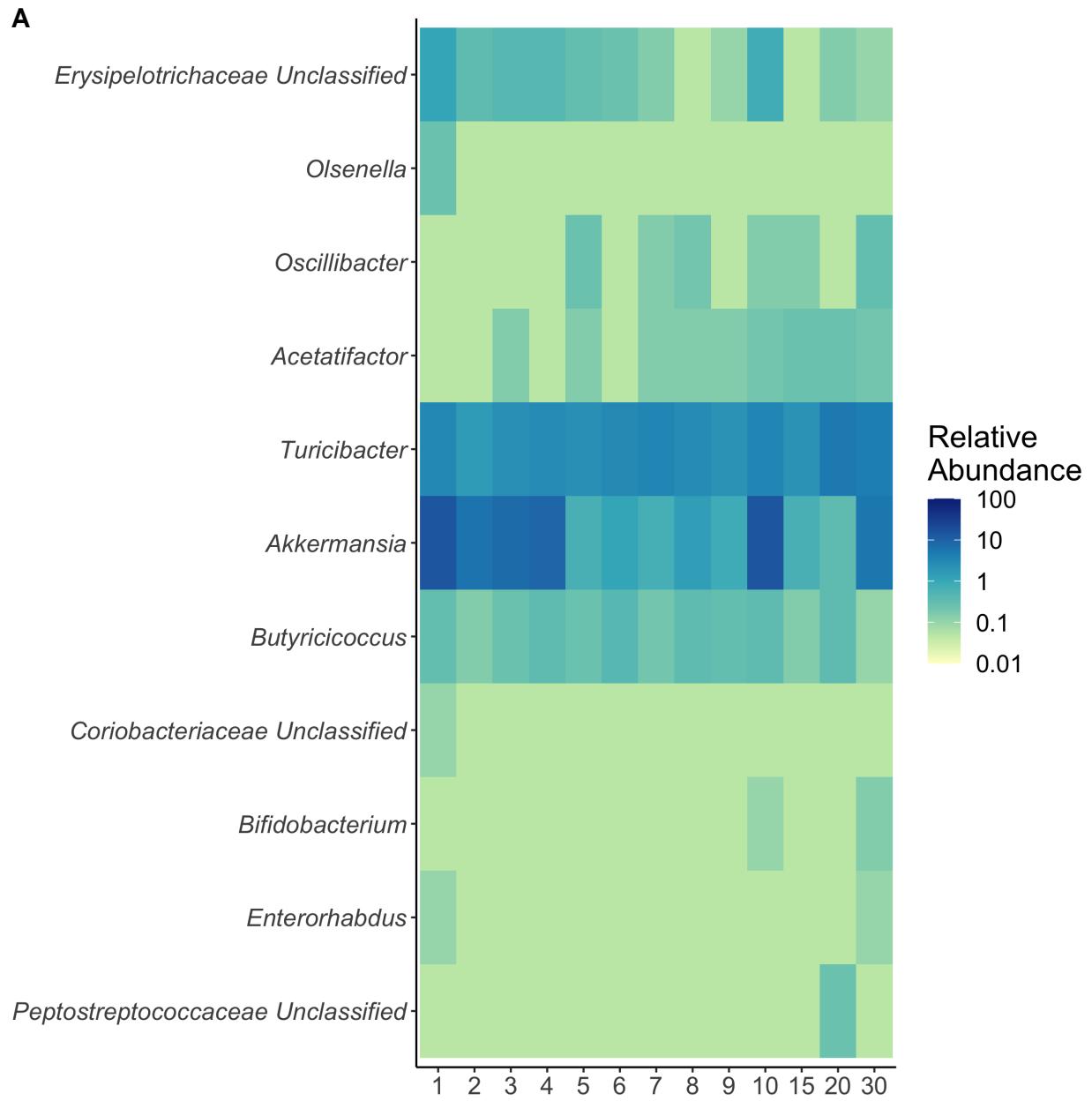
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164 **Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization**  
165 **in PEG treated mice. A.**



166

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

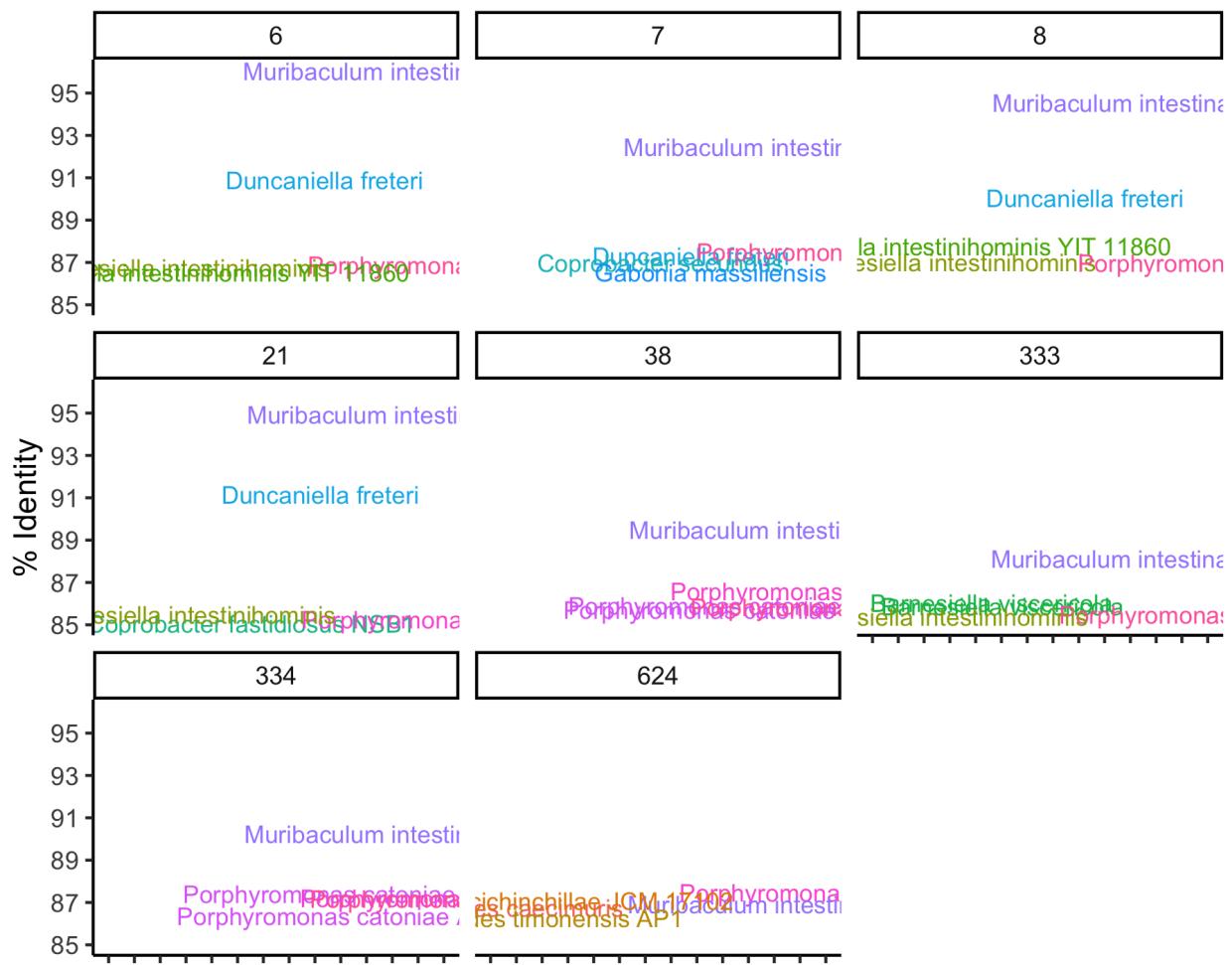


168

169 Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics  
170 post-infection. A.

A

### Potential Muribaculum OTUs Blastn Results



171

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

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