

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

Sarah Tomkovich¹, Ana Taylor¹, Jacob King¹, Joanna Colovas¹, Lucas Bishop¹, Kathryn McBride¹, Sonya Royzenblat¹, Nicholas A. Lesniak¹, Ingrid L. Bergin², Patrick D. Schloss^{1†}

† To whom correspondence should be addressed: pschloss@umich.edu

1. Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, USA
2. The Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI, USA

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 R² =
96 0.50, *P* < 0.001).

97 **5-day laxative treatment does not promote more severe CDIs despite altering the mucosal
98 microbiota.** Given previous work has shown that PEG treatment disrupts the mucus layer, we
99 decided to examine the impact of the laxative on the mucosal microbiota and infection severity by
100 evaluating histopathology.

- 101 • Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time
102 compared to clindamycin-treated mice.
- 103 • Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics
104 post-infection.
- 105 • Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal
106 microbiota is altered.
- 107 • Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.
- 108 • Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless of
109 whether an FMT is also administered.
- 110 • Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in
111 PEG treated mice.

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

114 – Ex. *Muribaculum intestinalis*.

- 115 • Figure 7. Schematic summarizing findings.

116 Discussion

- 117 • Summary of major findings

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

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

122 – Antibiotics may also impact mucus layer

123 – Strain of bacteria used

- 124 • Protective bacteria missing in PEG-treated mice

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

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

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

129 Conclusions

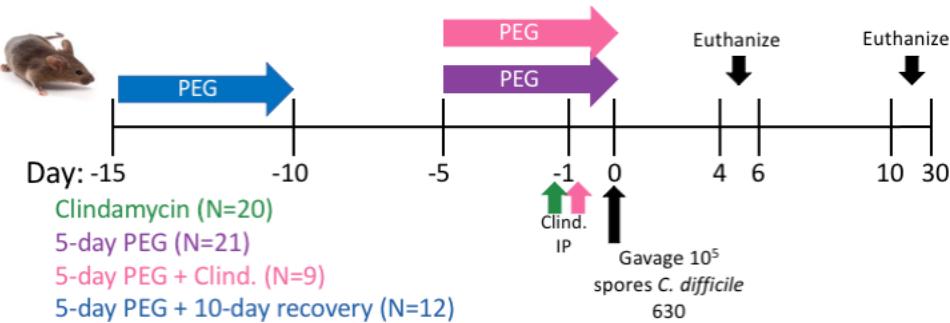
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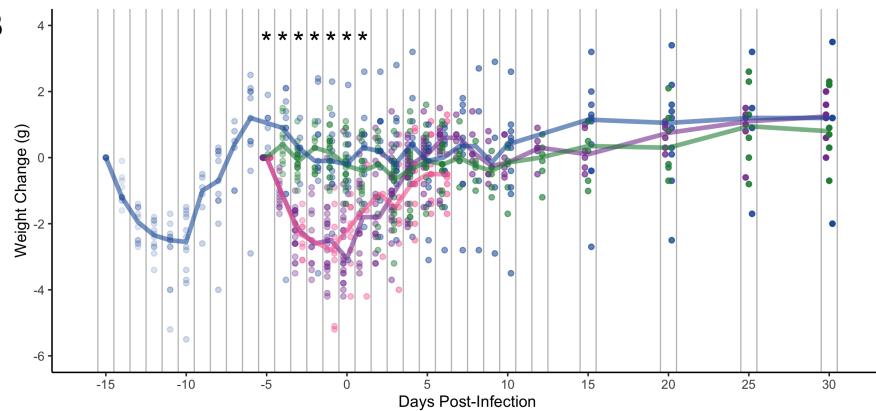
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141 **Materials and Methods**

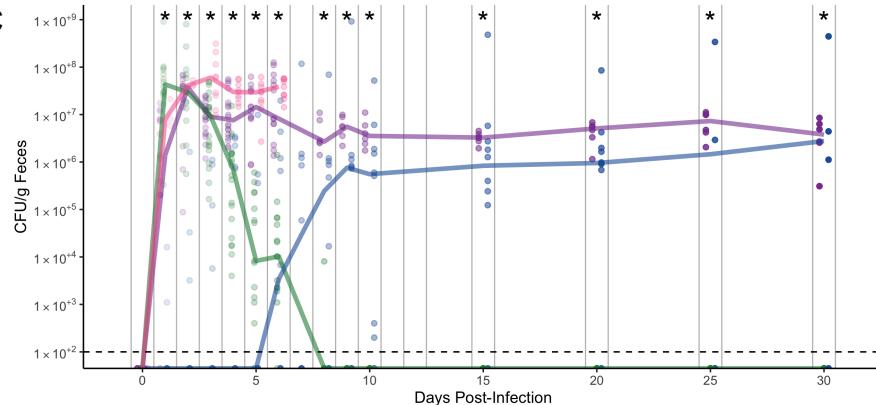
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B



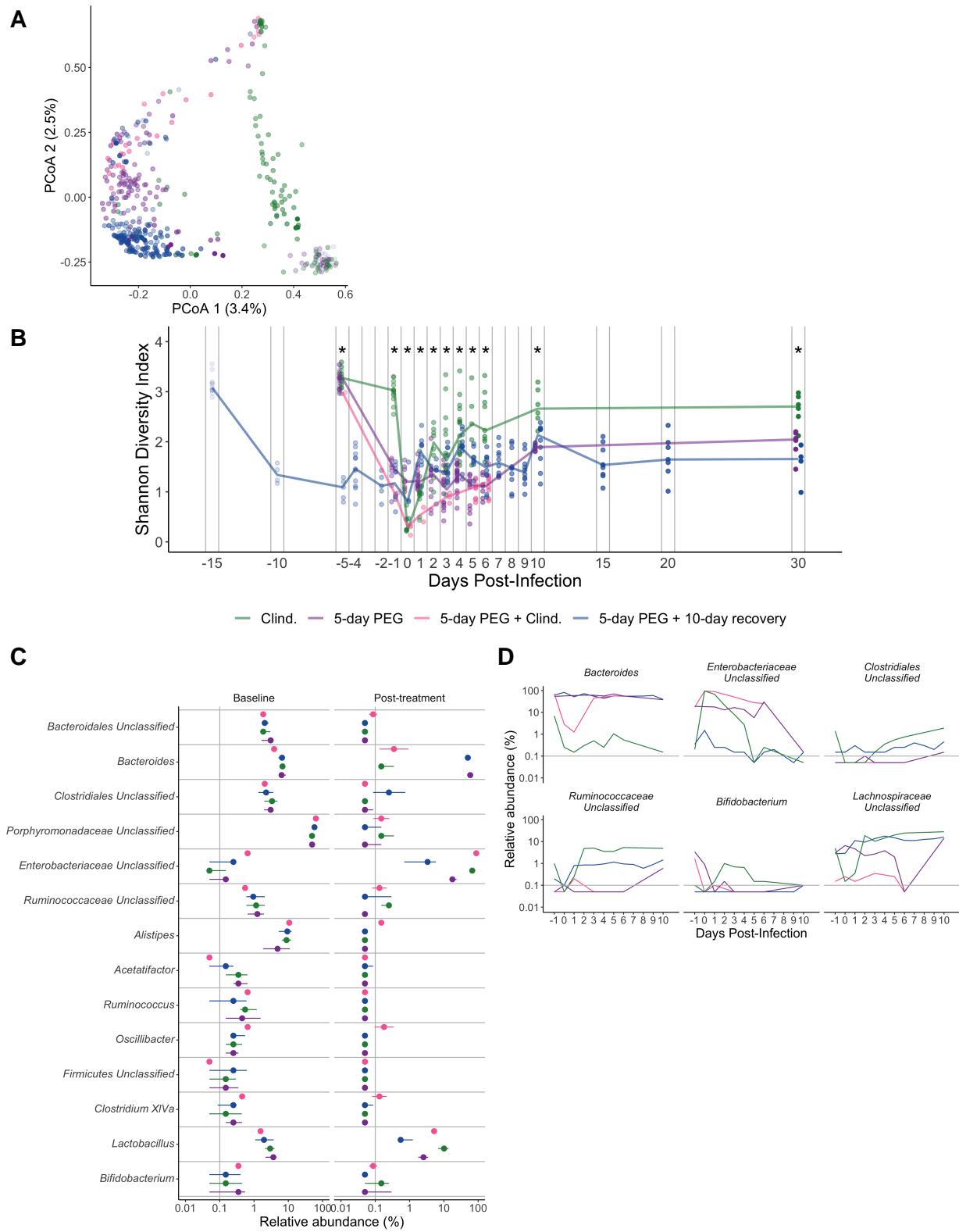
C



142

143 **Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently**
144 **colonized with *C. difficile*.** A. Setup of the experimental timeline for subset of experiments with
145 5-day PEG treated mice. B. Weight change from baseline weight in groups after treatment with
146 PEG and/or clindamycin, followed by *C. difficile* challenge. C. *C. difficile* CFU/gram stool measured
147 over time (N = 4-(insert variable name) mice per timepoint) via serial dilutions. The black line
148 represents the limit of detection for the first serial dilution. CFU quantification data was not available
149 for each mouse due to stool sampling difficulties (particularly the day the mice came off of the

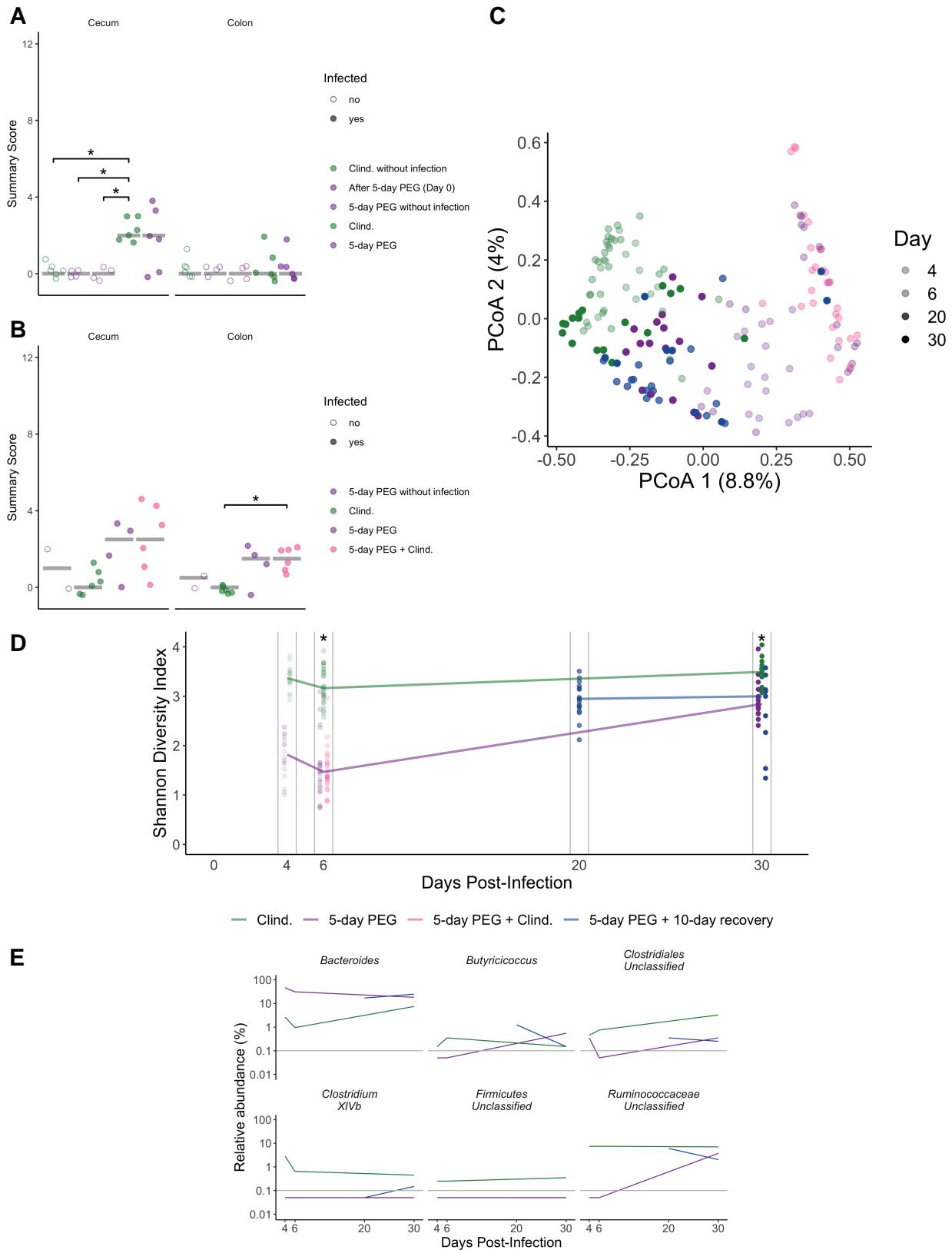
¹⁵⁰ PEG treatment) or early deaths. Lines represent the median for each source and circles represent
¹⁵¹ individual mouse samples. Asterisks indicate timepoints where the weight change or CFU/g was
¹⁵² significantly different between groups by the Kruskal-Wallis test with Benjamini-Hochberg correction
¹⁵³ for testing multiple timepoints.



154

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

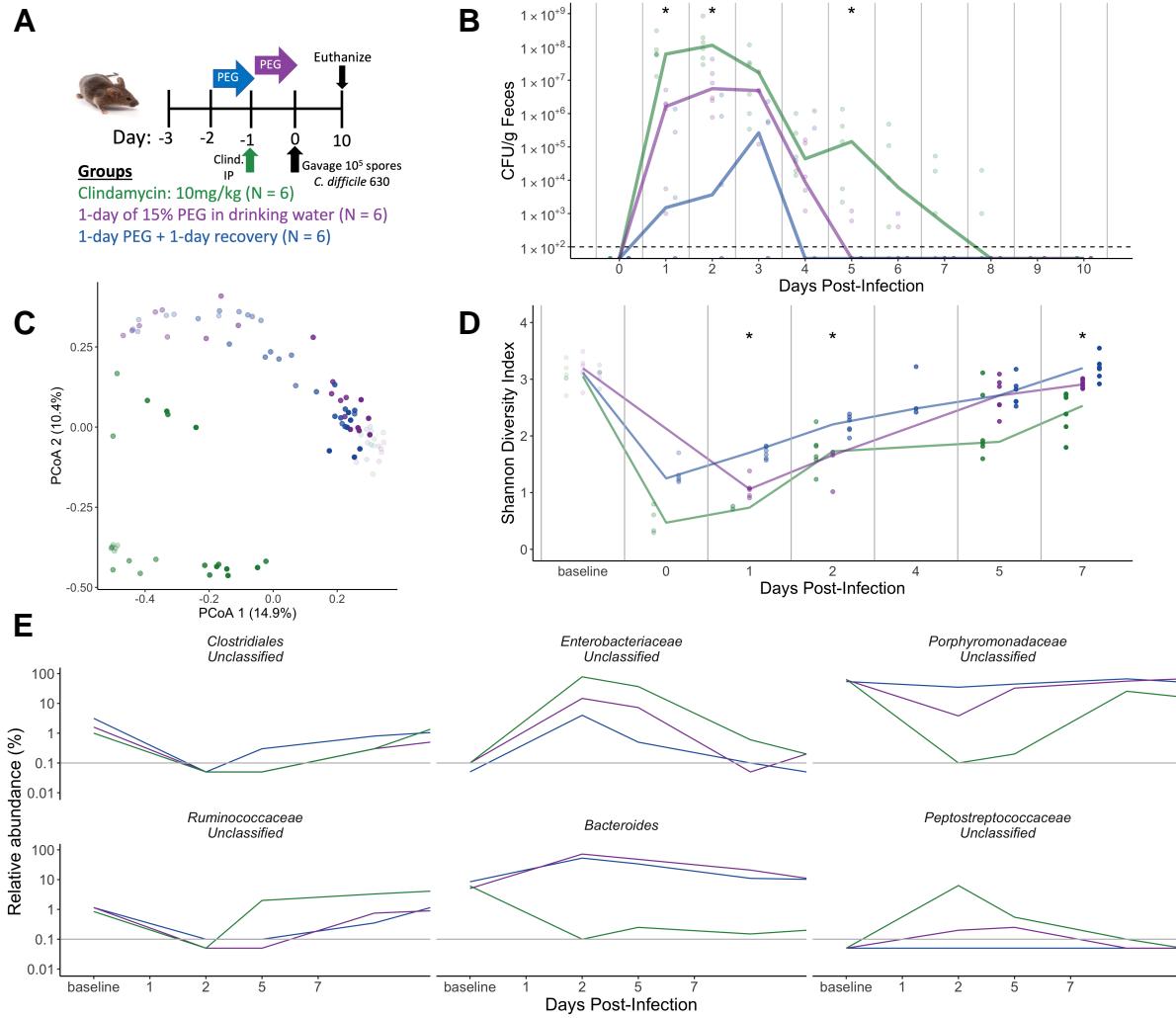
156 compared to clindamycin-treated mice. A.



157

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

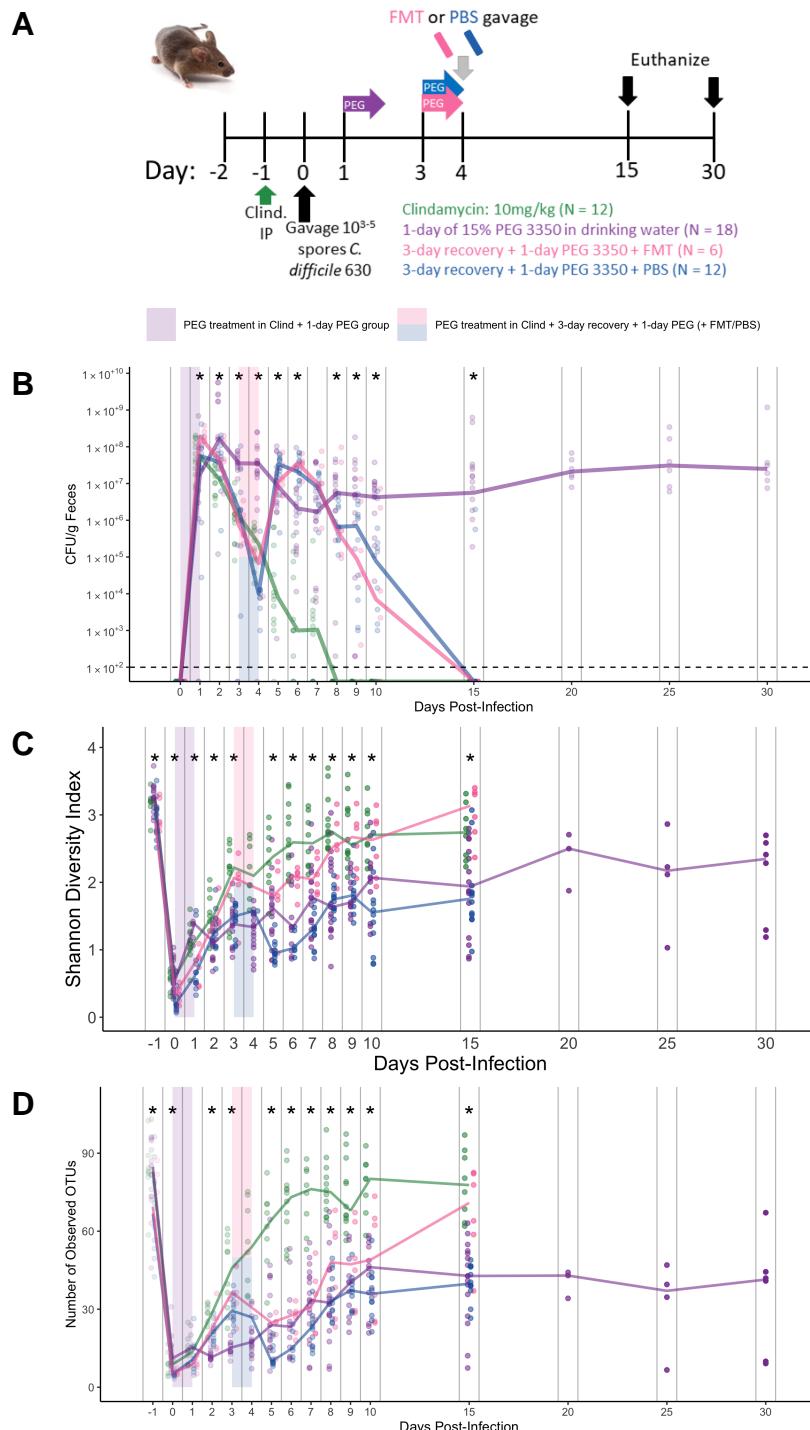
¹⁵⁹ **microbiota is altered.** A.



160

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

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



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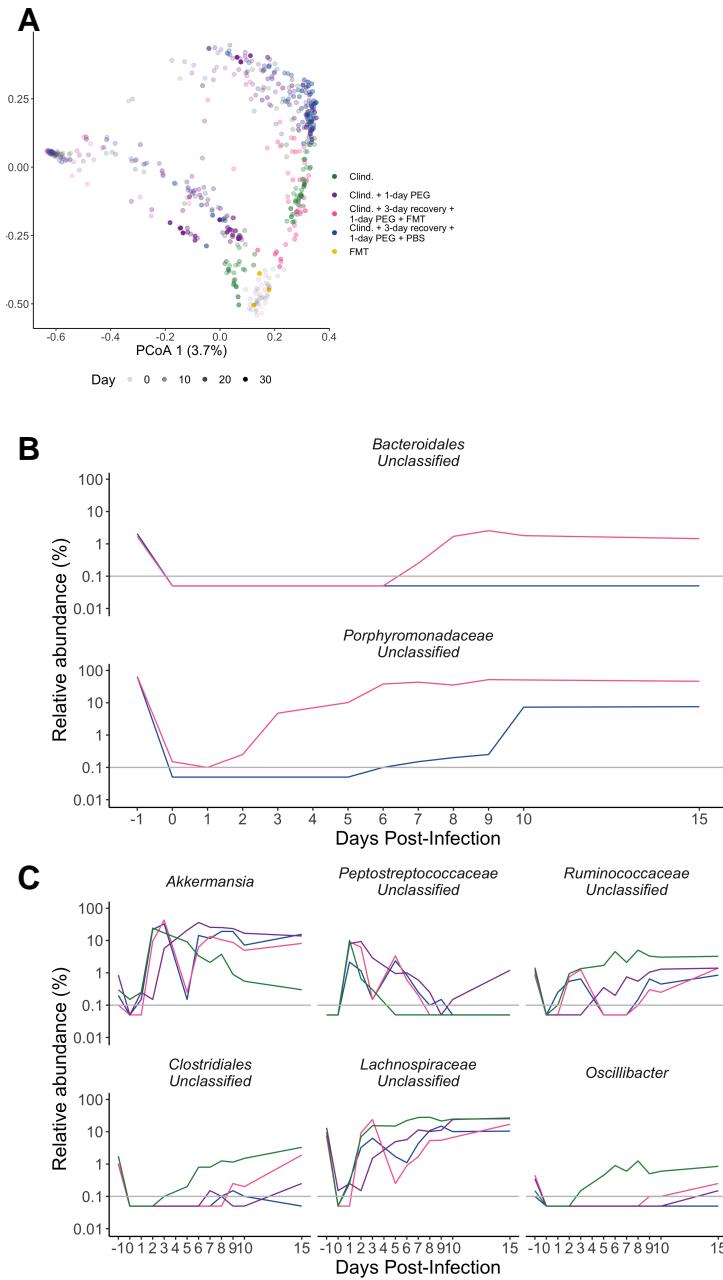
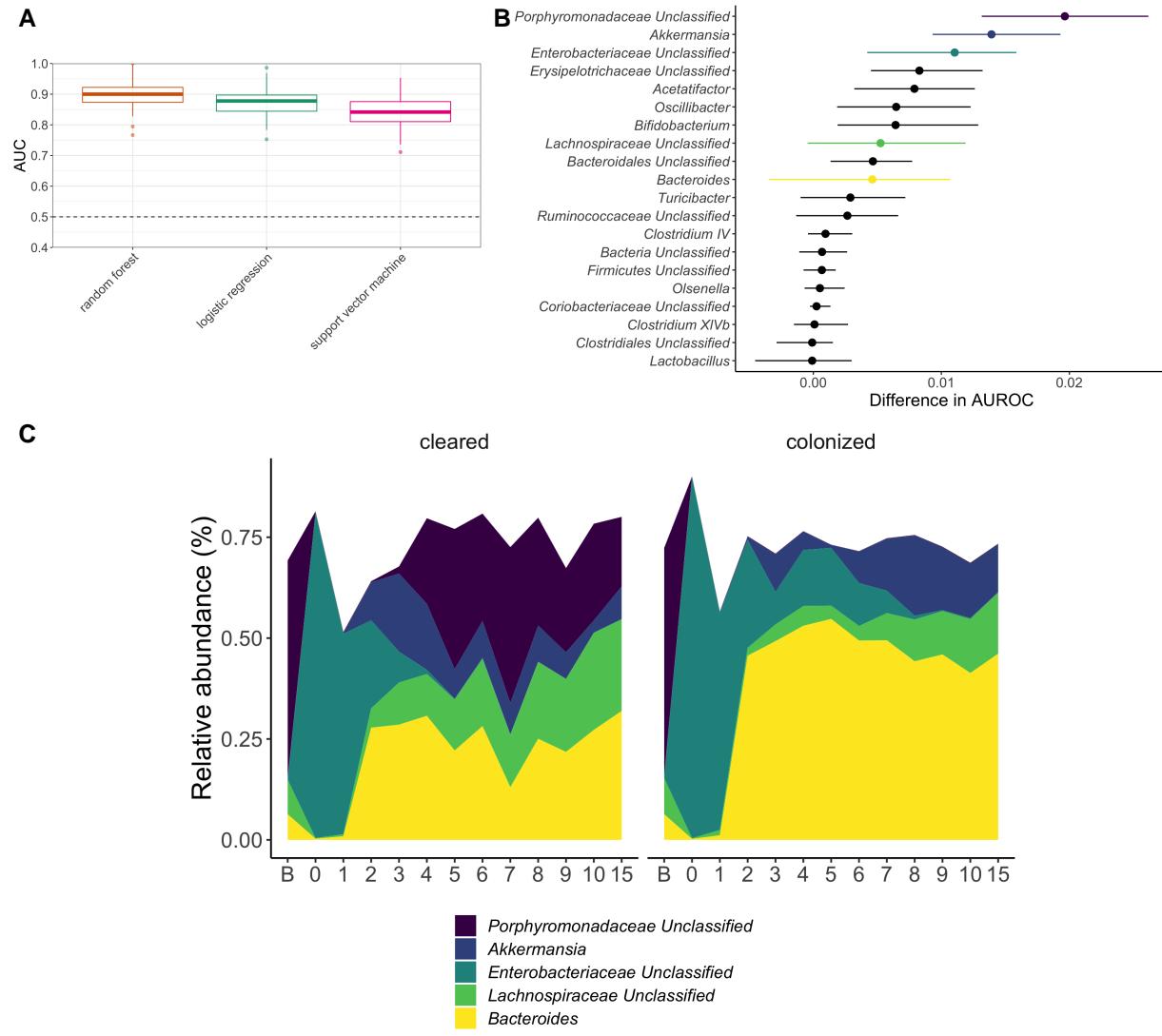


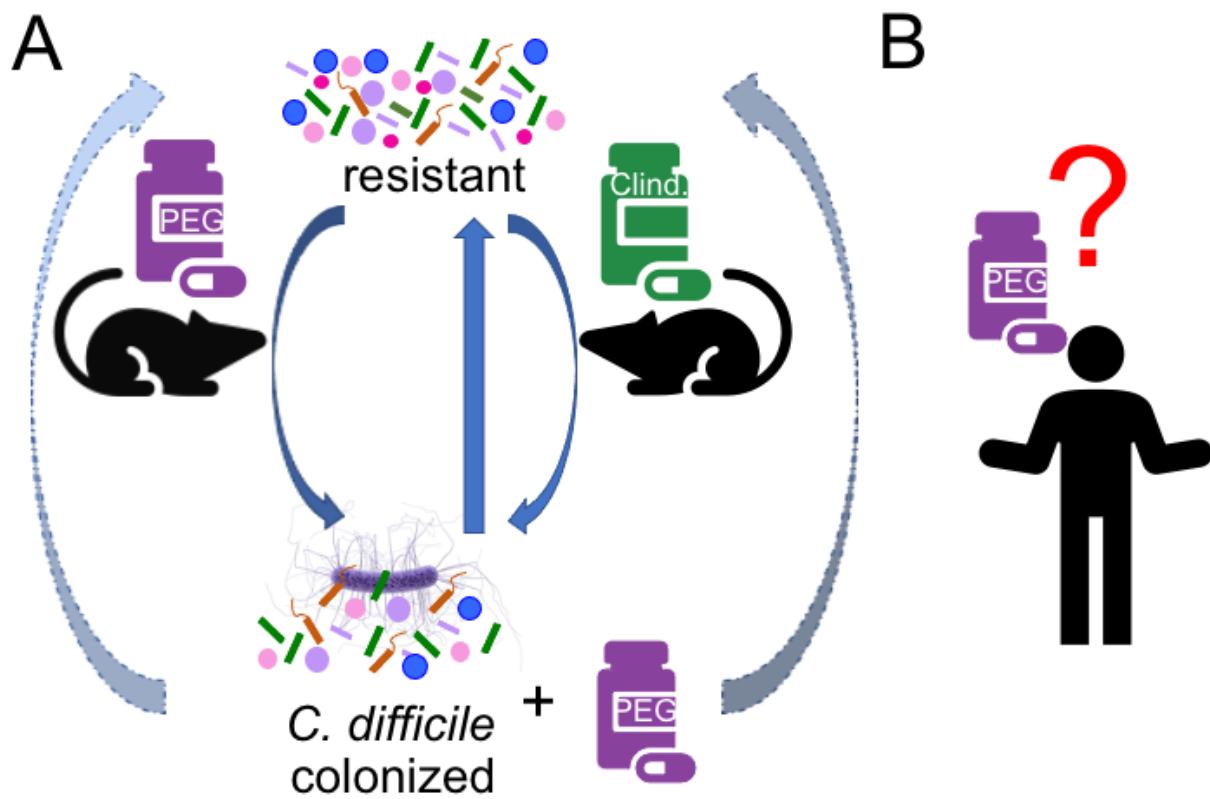
Figure 5. 1-day PEG treatment

174
 175 **post C. difficile challenge prolongs colonization regardless of whether an FMT is also**
 176 **administered. A.**



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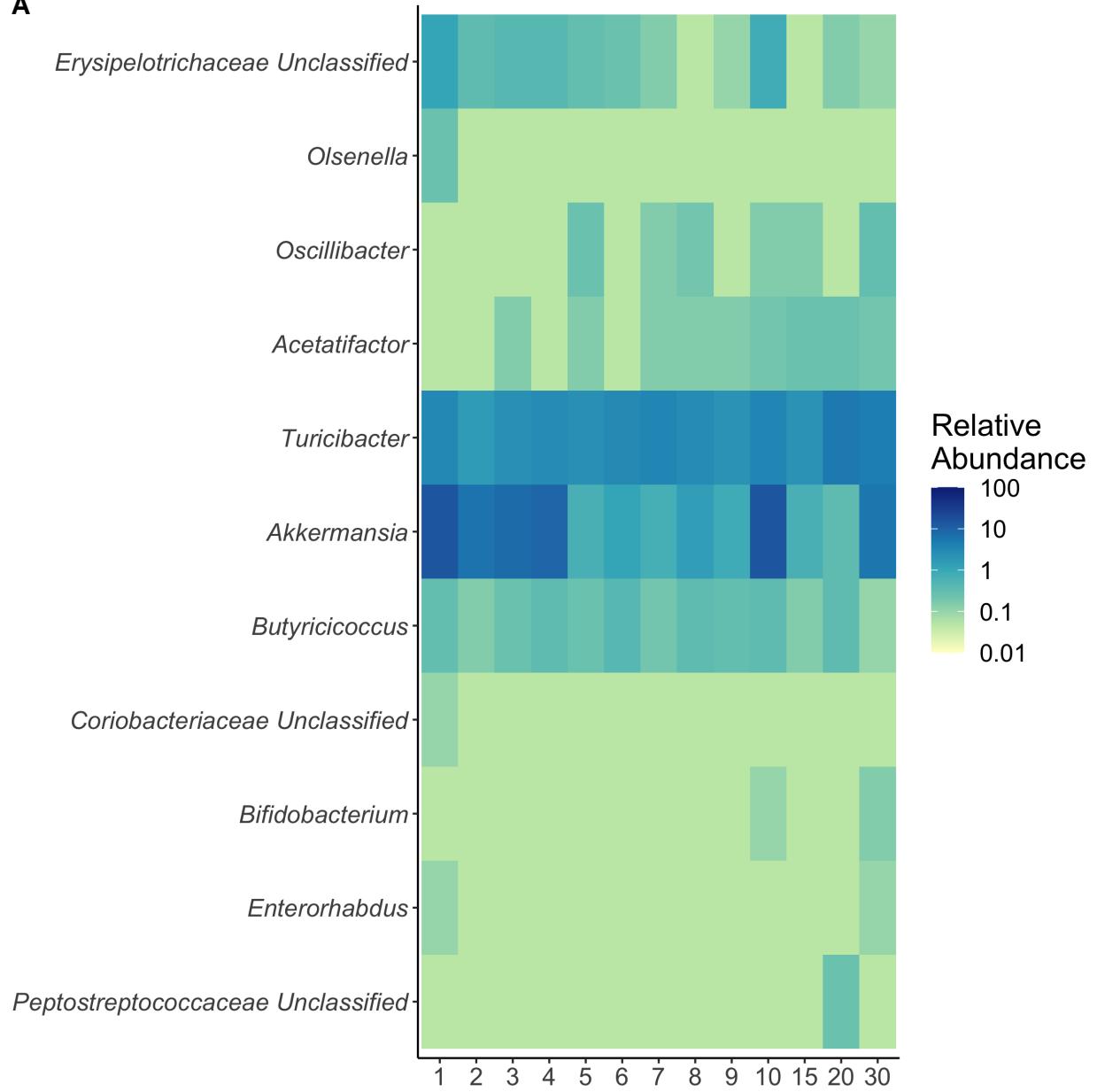
178 **Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization**
179 **in PEG treated mice. A.**



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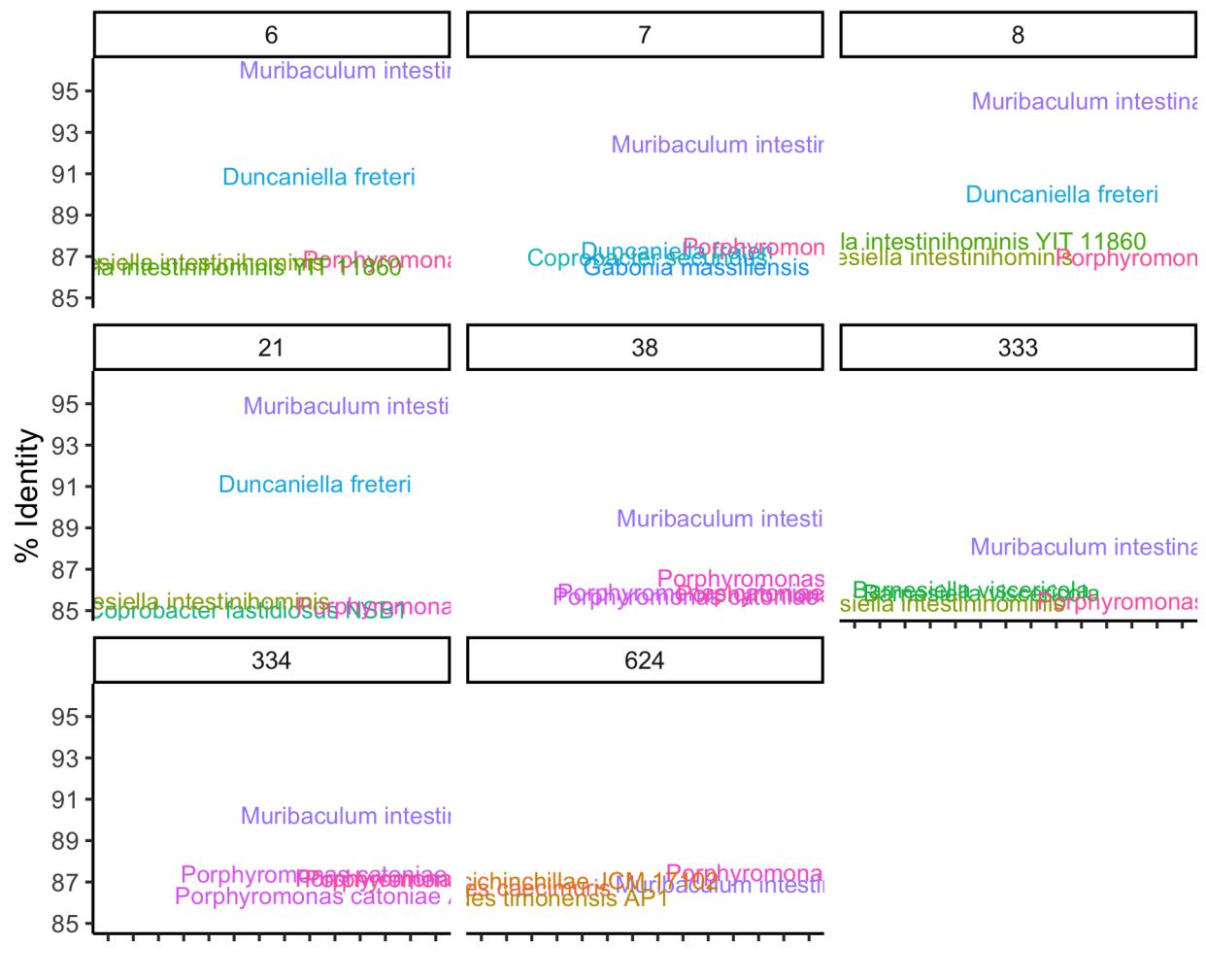
181 **Figure 7. Schematic summarizing findings. A.**

A



A

Potential Muribaculum OTUs Blastn Results



185

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

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