

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 mice treated with the osmotic laxative PEG 3350 to our standard 10 mg/kg clindamycin treatment,
74 which temporarily renders the mice susceptible to *C. difficile*, with mice typically clearing *C. difficile*
75 within 10 days post-infection (9, 19). All PEG-treated mice were administered a 15% PEG solution
76 in the drinking water for 5-days, one group was also treated with clindamycin, and one group was
77 allowed to recover for 10 days prior to challenge (Fig. 1A). PEG treatment resulted in weight loss in
78 all 3 groups of PEG-treated mice, with the greatest change in weight observed on the fifth day of
79 PEG treatment and the mice regained most of the weight five days after treatment (Fig. 1B). After
80 either PEG, clindamycin, or PEG and clindamycin treatment all mice were challenged with 10^3 *C.*
81 *difficile* 630 spores (Fig. 1A). All treatments rendered mice susceptible to *C. difficile* colonization.
82 However, PEG-treated mice remained colonized with *C. difficile* at high levels through thirty days
83 post-infection (Fig. 1C). In contrast, the clindamycin-treated mice cleared *C. difficile* within ten days
84 post-infection. Surprisingly, PEG-treated mice were still susceptible to *C. difficile* infection after
85 10-days of recovery from treatment although *C. difficile* was not detectable in most of the group

86 in the initial five days post-infection (Fig. 1C). From 9 days post-infection onward, the median *C.*
87 *difficile* stabilized for the 10-day recovery group of PEG-treated mice and remained high through 30
88 days post-infection (Fig. 1C). Thus, osmotic laxative treatment alone was sufficient to render mice
89 susceptible to prolonged *C. difficile* colonization and PEG-treated mice remained susceptible for up
90 to ten days post-treatment.

91 **5-day laxative treatment differentially disrupts the fecal microbiota compared to**
92 **clindamycin treatment.** Since osmotic laxatives and clindamycin have previously been
93 shown to disrupt the murine microbiota (ref), we hypothesized the different *C. difficile* colonization
94 dynamics between mice treated with the osmotic laxative or clindamycin were due to the two
95 drugs having differential effects on the microbiota. We profiled the stool microbiota over time
96 by sequencing the V4 region of the 16S rRNA gene to compare changes across treatment
97 groups. We found time and treatment group explained half of the observed variation between fecal
98 communities with most of the remaining variation explained by interactions between treatment
99 group and other experimental variables including time, cage effects, and sequencing preparation
100 (PERMANOVA combined $R^2 = 0.95$, $P < 0.001$, Fig. 2A, Data Set S1, Sheet X). Cage effects
101 refer to the well-documented phenomenon that mice housed in the same cages have similar
102 microbial communities due to coprophagy (ref), we tried to minimize the impact of cage effects
103 on our experiment by breaking up cagemates when assigning mice to treatment groups and
104 primarily housing only two mice per cage. Importantly, although we conducted a total of 5 separate
105 experiments, the experiment number and its interaction with treatment group was not one of
106 the variables that significantly explained the observed variation in fecal communities (Data Set
107 S1, Sheet X). Interestingly, none of the treatment groups recovered to their baseline community
108 structure either 10 or 30 days post-infection suggesting other community features besides recovery
109 to baseline were responsible for the prolonged *C. difficile* colonization in PEG-treated mice (Fig.
110 2B).

111 Next, we examined alpha diversity by looking at Shannon diversity index over time. Although both
112 clindamycin and PEG treatments decreased diversity, Shannon diversity was lower in the groups of
113 mice that received PEG treatment compared to those that received clindamycin through thirty days
114 post-infection (Fig. 2C). We next examined the bacterial genera that shifted after PEG treatment by

115 comparing the baseline samples of mice treated with only PEG to samples from the same mice one
116 day post-treatment. We found 18 genera that were altered by PEG treatment (Data Set S1, Sheet
117 X). The majority of the bacterial relative abundances decreased after PEG, but *Enterobacteriaceae*
118 and *Bacteroides* increased and the increase in *Bacteroides* was unique to PEG treatment as
119 *Bacteroides* actually decreased in clindamycin treated mice (Fig. 2D). Finally, we examined the
120 bacteria that differ across treatment groups over multiple timepoints. We found 24 genera were
121 different over multiple timepoints out of the 33 that were different between treatment groups (Fig.
122 2E, Data Set S1, Sheet X). Thus, PEG has a significant impact on the fecal microbiota that was
123 maintained over time and distinct from clindamycin treatment.

124 Surprisingly, *C. difficile* was not immediately detectable in the stools of the PEG-treated mice
125 that were allowed to recover for 10 days prior to challenge. We decided to examine the
126 bacteria that changed during the post-infection period when the group median *C. difficile* shifted
127 from undetectable at 1 day post-infection to detectable in the stool samples with the median
128 stabilizing around 8 days post-infection (Fig. S1A). Interestingly, we found *Erysipelotrichaceae*,
129 *Enterobacteriaceae*, and *Akkermansia* were changing during the time period when *C. difficile* was
130 becoming detectable in the stools (Fig. S1B), although none of the bacteria were significant after
131 multiple hypothesis correction (Data Set S1, Sheet X). Although we did not identify a clear signal to
132 explain the delayed appearance of *C. difficile* in the 5-day PEG mice that were allowed to recover
133 for 10 days prior to challenge, the delay is striking and could reflect changes in microbial activity or
134 metabolites that were not examined in this study.

135 **5-day laxative treatment does not promote more severe CDIs despite altering the mucosal**
136 **microbiota.** Given the findings from a previous study that demonstrated that PEG treatment
137 disrupts the mucus layer and alters the immune response in mice (16), we decided to examine the
138 impact of PEG treatment on the mucosal microbiota and CDI severity. To evaluate the mucosal
139 microbiota, we sequenced snips of tissue collected from the cecum, proximal colon, and distal
140 colon. Similar to what was observed with the stool samples, alpha diversity was lower in the
141 PEG-treated mice compared to clindamycin treatment (Fig. 3A). Although alpha diversity continued
142 to increase over time based on the communities from PEG-treated mice collected at 20 and 30 days
143 post-infection (Fig. 3A, Data Set S1, Sheet X). Group, day of the experiment, and their interactions

144 with other variables (cage, experiment number, and sample type) explained the majority of the
145 variation observed in mucosal communities (PERMANOVA combined $R^2 = 0.83$, $P < 0.05$, Fig.
146 3B, Data Set S1, Sheet X). We saw the greatest difference in the mucosal microbiota between
147 treatment groups at 6 days post-infection with 16 (insert code instead) genera that were significantly
148 different in all three of the tissue types we collected (cecum, proximal colon, and distal colon; Fig.
149 S3A, Data Set S1, Sheet X). However, at 30 days post-infection only *Bacteroides*, *Clostridiales*,
150 *Firmicutes*, and *Ruminococcaceae* were different between treatment groups and just in the cecum
151 tissues (Fig. 3C, Fig. 2E, Data Set S1, Sheet X). Interestingly, one of the genera that was different
152 between treatment groups at 6 days post-infection was *Peptostreptococcaceae* (the genera with
153 sequences that match *C. difficile*). *Peptostreptococcaceae* was primarily only present in the 5-day
154 PEG treatment group of mice and decreased in the proximal and distal colon tissues over time (Fig.
155 S2B). Thus, PEG treatment had a significant impact on the mucosal microbiota and we detected *C.*
156 *difficile* sequences in the cecum, proximal colon, and distal colon tissue communities.

157 Next, we examined CDI severity by evaluating cecum and colon histopathology (20) and found there
158 was no difference in cecum and colon scores between clindamycin and PEG-treated mice that were
159 challenged with *C. difficile* at 4 days post-infection (Fig. 3D), the timepoint typically examined in
160 *C. difficile* 630 challenged mice (ref). We also looked at 6 days post-infection because that was
161 when we started to see a large difference in *C. difficile* colonization levels between PEG- and
162 clindamycin-treated mice (Fig. 1C). Although, there was a slight difference in the colon between
163 PEG and clindamycin-treated mice, there was no difference in the cecum and the overall score is
164 still relatively low given that the max possible summary score is 12 (Fig. 3E). Thus, although PEG
165 treatment had a profound impact on the mucosal microbiota, the impact of *C. difficile* 630 infection
166 on the cecum and colon was similar between PEG and clindamycin treated mice.

167 ***C. difficile* challenge does not have a synergistic disruptive effect on the microbiota of**
168 **PEG-treated mice**

Because *C. difficile* itself can have an impact on the microbiota (21), we also
sequenced the tissue and stools of mock-challenged clindamycin and 5-day PEG treated mice.
Examining the stools of the mock-challenged mice revealed similar bacterial disruptions as the *C.*
difficile challenged mice (Fig. S2A-C). Similarly, there was no difference between the tissues of
mock and *C. difficile* challenged mice (Fig. S2D-F). Thus, most of the microbiota alterations we

173 observed in the PEG-treated mice were a result of the laxative and not an interaction between the
174 laxative and *C. difficile*.

175 **1-day laxative treatment results in transient *C. difficile* colonization and minor microbiota**
176 **disruption** Next, we decided to examine how a shorter osmotic laxative perturbation would impact
177 the microbiome and susceptibility to *C. difficile*. We administered mice either a 1-day PEG treatment,
178 a 1-day PEG treatment with a 1-day recovery period, or a clindamycin treatment before challenging
179 them with *C. difficile* 630 (Fig. 3A). In contrast to the 5-day PEG treated mice, the 1-day PEG
180 treated mice were only transiently colonized and cleared *C. difficile* by 7 days post-infection (Fig.
181 3B). The stool communities of PEG-treated mice were also only transiently disrupted, with Shannon
182 diversity close to baseline by 7 days post-infection (Fig. 3C-D). We found 14 bacteria were
183 impacted by treatment but recovered close to baseline levels within 7 days post-infection including
184 *Enterobacteriaceae*, *Clostridiales*, *Porphyromonadaceae*, and *Ruminococcaceae* (Fig. 3E, Data
185 Set S1, Sheet X). These findings suggest the duration of treatment matters when considering the
186 impact of the laxatives, with shorter treatments resulting in a transient loss of *C. difficile* colonization
187 resistance.

188 **Post-CDI laxative treatment disrupts clearance in clindamycin-treated mice regardless of**
189 **whether an FMT is also administered** Since 1-day PEG treatment resulted in a more mild
190 microbiota perturbation, we decided to use the 1-day treatment to examine the hypothesis that
191 PEG helps to flush *C. difficile* spores from the intestine. We were also interested in exploring
192 whether PEG might help with engraftment in the context of FMTs. To examine the impact of PEG
193 treatment on *C. difficile* clearance and FMT treatment, we treated 4 groups of mice with clindamycin
194 and then challenged all mice with *C. difficile* before administering the following treatments: no
195 additional treatment, 1-day PEG immediately after challenge, and 1-day PEG treatment 3 days
196 after challenge followed by either administration of an FMT or PBS solution via gavage (Fig. 5A). In
197 contrast to our hypothesis, all groups of mice that received PEG actually exhibited prolonged *C.*
198 *difficile* colonization (Fig. 5B).

- 199 • Draft of results
200 – Prolonged *C. difficile* colonization in post-CDI PEG treated mice (Fig. 5B)

- FMT appears to partially restore alpha diversity (Shannon, but not richness Fig. 5C-D)
- PCoA (necessary?) Could comment on the clustering of Clindamycin and PEG-treated mice that received FMT (Fig. 6A)
- Only 2 genera significantly impacted by FMT treatment, likely not as important for clearance (Fig. 6B)
- Bacteria that consistently differ between groups over time, associated with prolonged colonization (Fig. 6C)

Five-day post-infection community data can predict mice that will have prolonged *C.*

difficile colonization

After identifying bacteria associated with the 5-day, 1-day and post-CDI 1-day PEG treatments, we decided to examine the taxa that were influencing prolonged *C. difficile* colonization. We trained 3 types of machine learning models (random forest, logistic regression, and support vector machine) with input bacterial community data from 5 days post-infection to predict whether the mice were colonized with *C. difficile* 10 days post-infection. We chose 5 days post-infection because that was the earliest timepoint where we would see a treatment effect in the mice that were given 1-day PEG treatment three days after *C. difficile* challenge and then administered an FMT or PBS gavage. The random forest model had the highest performance (AUROC = 0.90, Data Set S1, Sheet X), so we next performed permutation importance to examine the bacteria that were driving performance. We selected the top 10 bacteria contributing to our models performance (Fig. 7A) and examined their relative abundance at 5 days post-infection, the timepoint used to predict *C. difficile* colonization status on day 10 (Fig. 7B). Next, we focused on the 5 genera that were > 1 % relative abundance in either the cleared or colonized mice and examined the relative abundance dynamics of these bacteria over time. We found , , and _ tended to have a higher relative abundance and , had a lower relative abundance in the mice with prolonged colonization compared to the mice that cleared *C. difficile* (Fig. 7C). Previous work examining the impact of PEG on the murine microbiota found that PEG treatment resulted in the permanent loss of S24-7, also known as *Muribaculum intestinalis* (16). We decided to check our *Porphyromonadaceae* OTUs because *Muribaculum intestinalis* is known to be classified as *Porphyromonadaceae* by the Ribosomal Database Project (RDP) database (22), *Porphyromonadaceae* was a top feature in the random forest model predicting prolonged *C. difficile* colonization, and had a high relative

230 abundance in the communities of mice that cleared *C. difficile* within 10 days. We identified 4
231 OTUs that had at least (insert minimum percent identity) to *Muribaculum intestinalis* and examined
232 their abundance in mice that either cleared or were still colonized with *C. difficile* at 10 days
233 post-infection (Fig. S4).

234 **Discussion**

- 235 • Summary of major findings (Fig. 8A)
- 236 • Discussion of prolonged persistence. *C. difficile* sequences detected in tissue samples.
237 Association with mucin-degrading bacteria suggested by recent papers.
- 238 • Discuss why we might not have observed more severe histology in PEG mice relative to
239 clindamycin-treated mice
 - 240 – Antibiotics may also impact mucus layer
 - 241 – Strain of bacteria used
- 242 • Protective bacteria missing in PEG-treated mice
- 243 • Discuss what these findings might mean for human patients (Fig. 8B)
 - 244 – What's known regarding laxatives and susceptibility to CDIs
 - 245 – Relevance to human FMTs? Unclear what the best administration route is because there
246 have been no studies designed to evaluate the best administration route for FMTs.

247 **Conclusions**

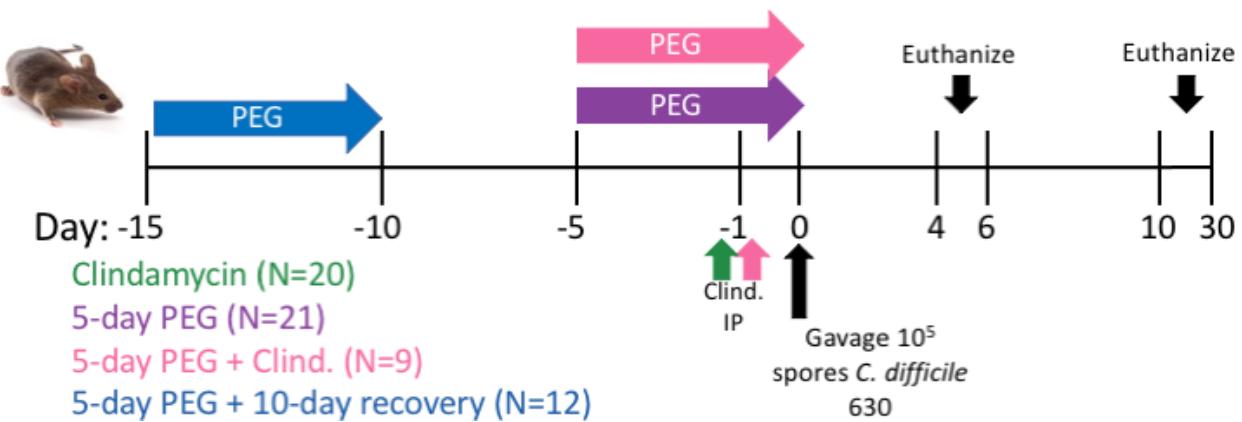
248 **Acknowledgements**

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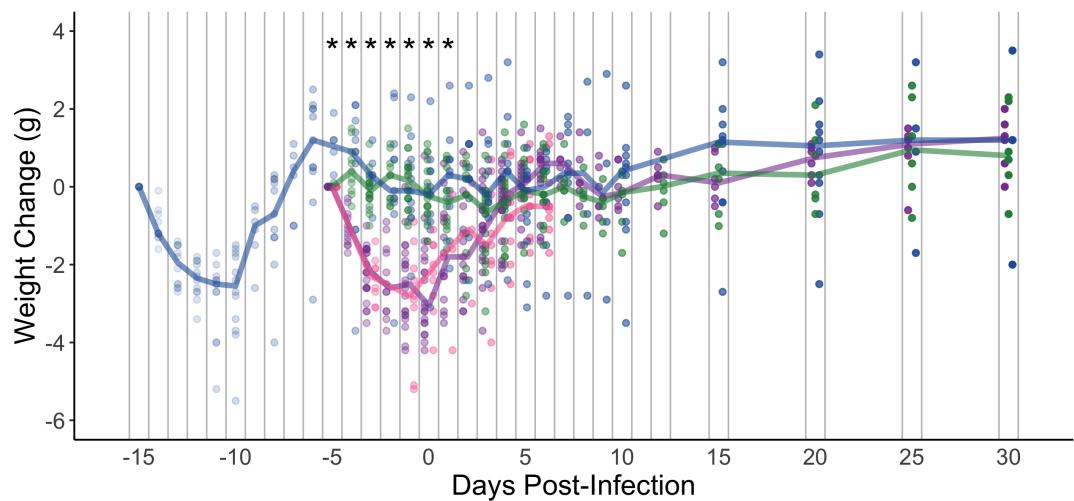
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259 **Materials and Methods**

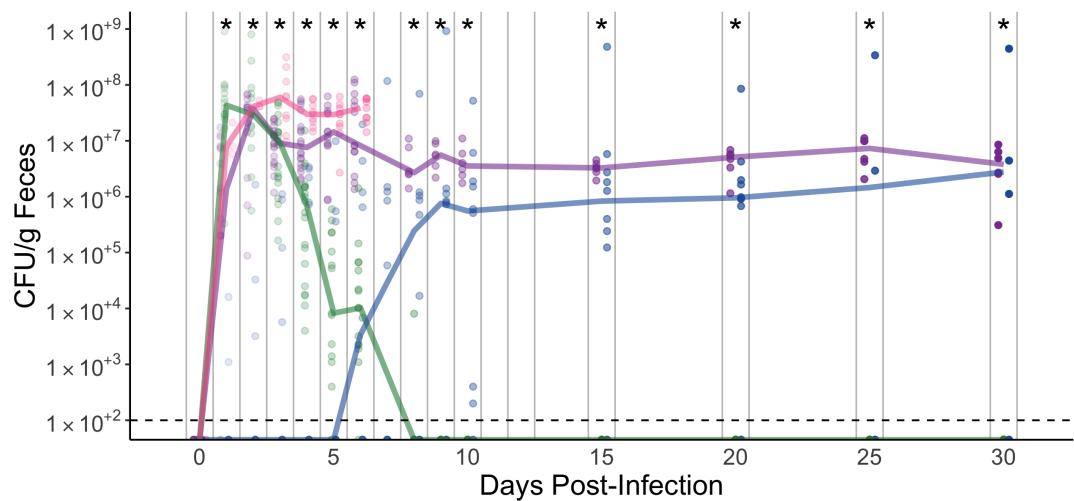
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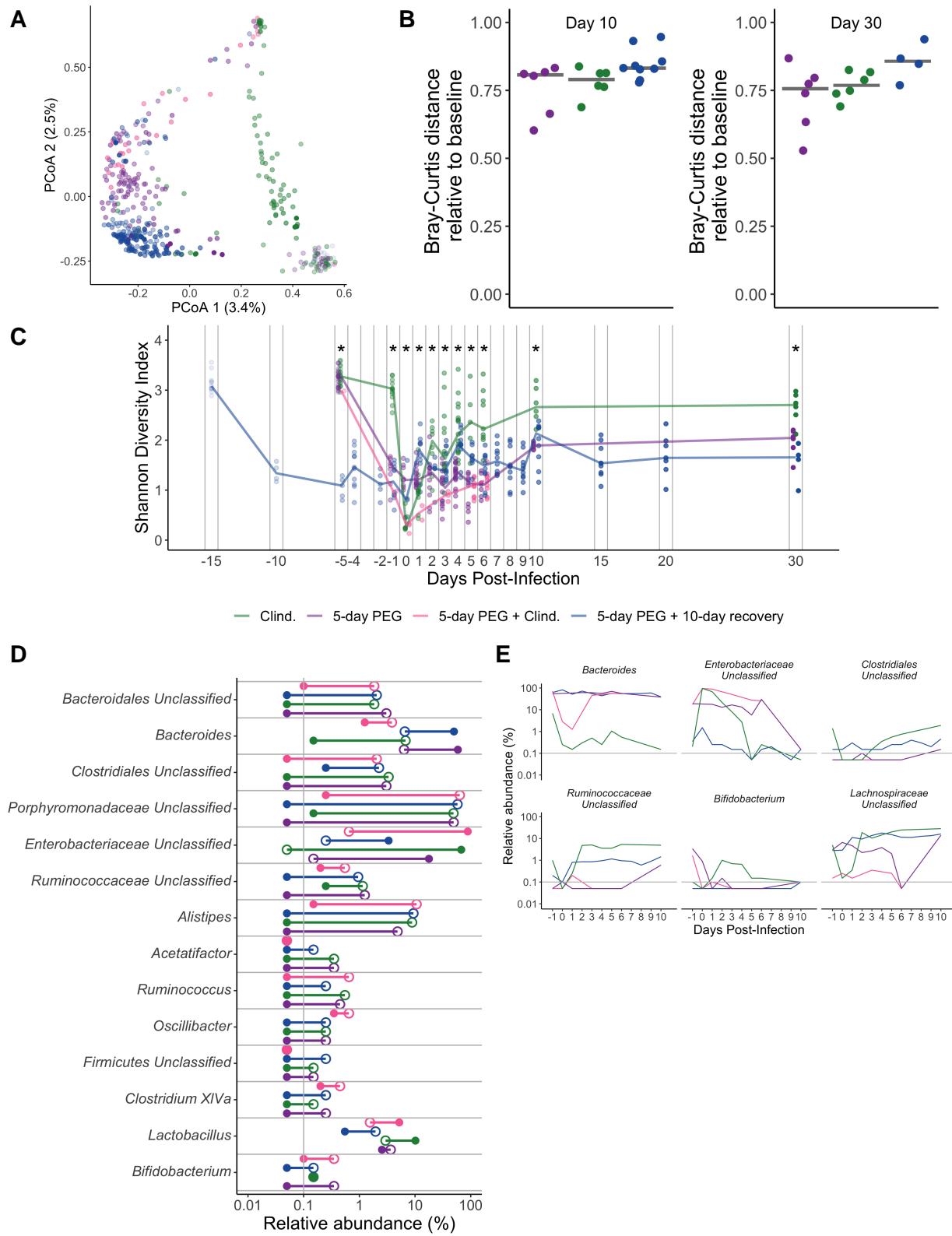
C



260

261 **Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently**
262 **colonized with *C. difficile*.** A. Setup of the experimental timeline for experiments with 5-day PEG
263 treated mice. Clindamycin was administered at 10 mg/kg by intraperitoneal injection. 15% PEG

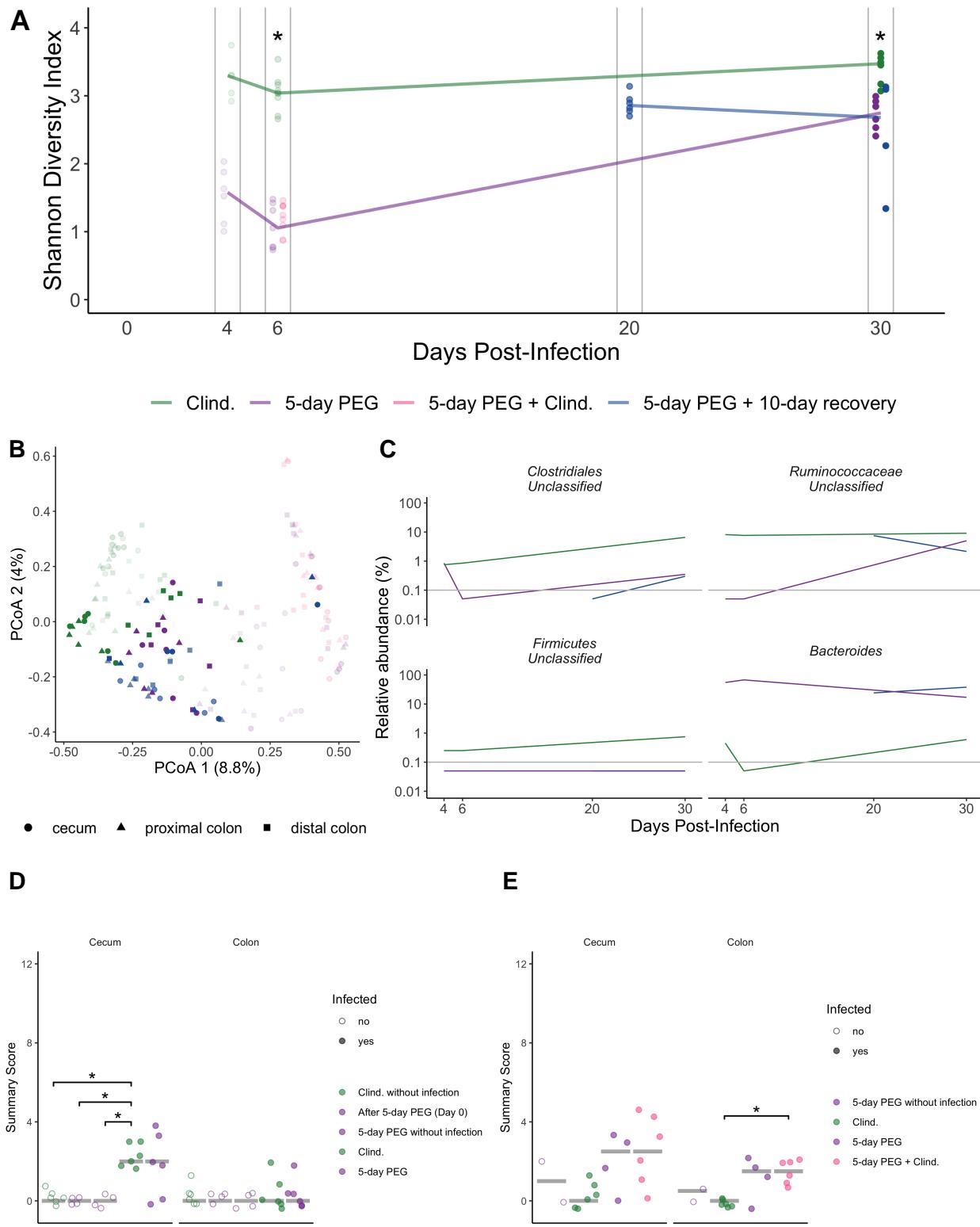
264 3350 was administered in the drinking water for five days. B. Weight change from baseline weight
265 in groups after treatment with PEG and/or clindamycin, followed by *C. difficile* challenge. C. *C.*
266 *difficile* CFU/gram stool measured over time (N = 16-59 mice per timepoint) via serial dilutions. The
267 black line represents the limit of detection for the first serial dilution. CFU quantification data was
268 not available for each mouse due to stool sampling difficulties (particularly the day the mice came
269 off of the PEG treatment) or early deaths. For B-C, lines represent the median for each treatment
270 group and circles represent samples from individual mice. Asterisks indicate timepoints where the
271 weight change or CFU/g was significantly different between groups by the Kruskal-Wallis test with
272 Benjamini-Hochberg correction for testing multiple timepoints. The data presented are from a total
273 of 5 separate experiments.



274

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

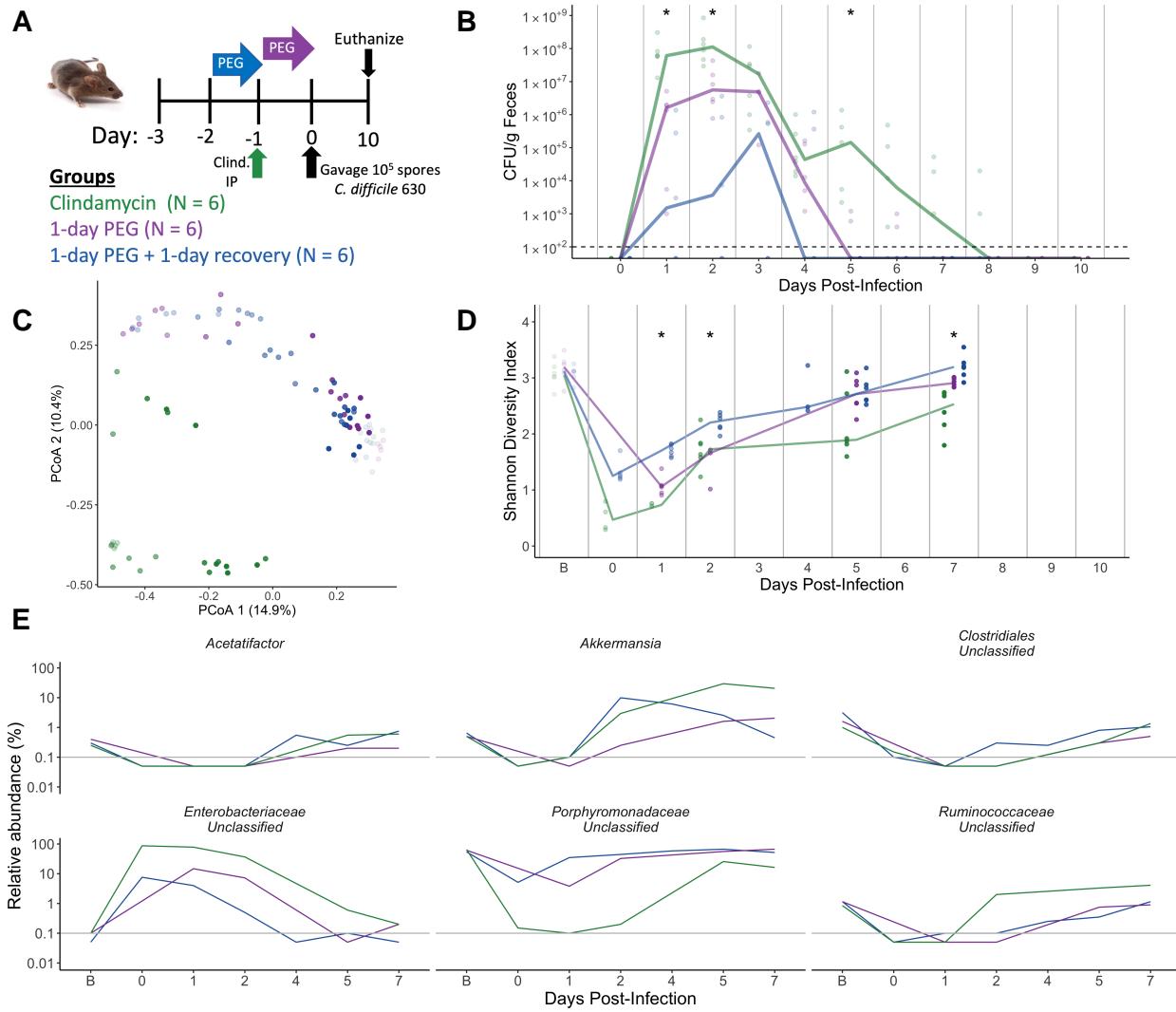
²⁷⁶ **compared to clindamycin-treated mice.** A. PCoA of Bray-Curtis distances from stool samples
²⁷⁷ collected throughout the experiment.



278

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

280 **microbiota is altered.** A.



281

282 **Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile***
283 **colonization.** A. Setup of the experimental timeline for the 1-day PEG treated subset of
284 mice. B. CFU/gram stool measured over time (N = 12-18 mice per timepoint) via several dilutions.
285 The black dotted line represents the limit of detection for the first serial dilution. Asterisks indicate
286 timepoints where the CFU/gram was significantly different between groups using the Kruskall-Wallis
287 test with a Benjamini-Hochberg correction for multiple timepoints. C. Principle Coordinate Analysis
288 plot of the groups over time with the alpha representing the same time scale as in panel D (day:
289 $R^2 = 0.43$; group: $R^2 = 0.19$). D. Shannon diversity Index of the groups over time. Only days with
290 samples from all groups are shown. E. Line plots of relative percent abundance of selected genera
291 over time. Only days with samples from all groups shown. The gray line represents the limit of

²⁹² detection.

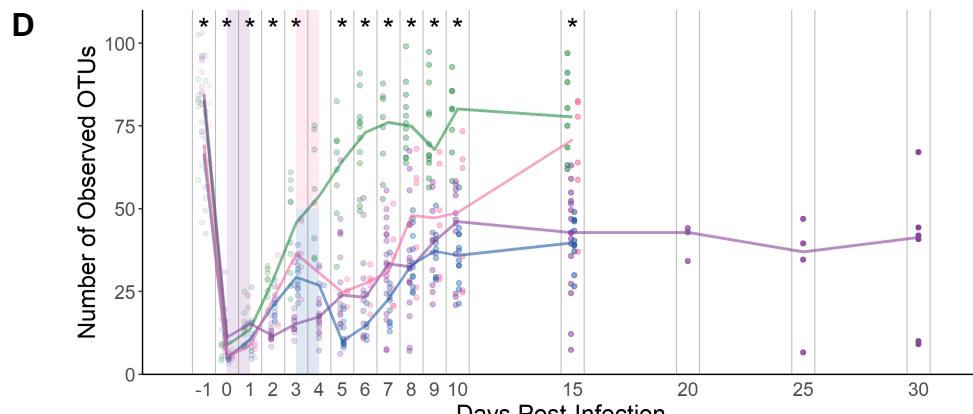
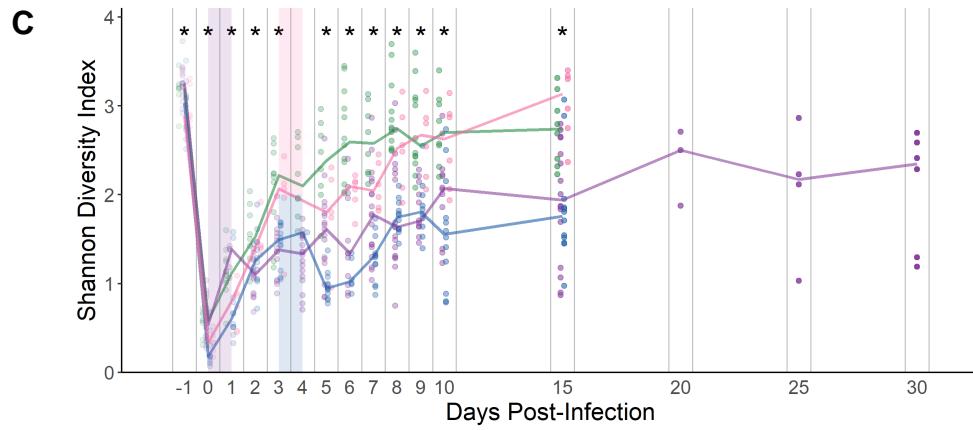
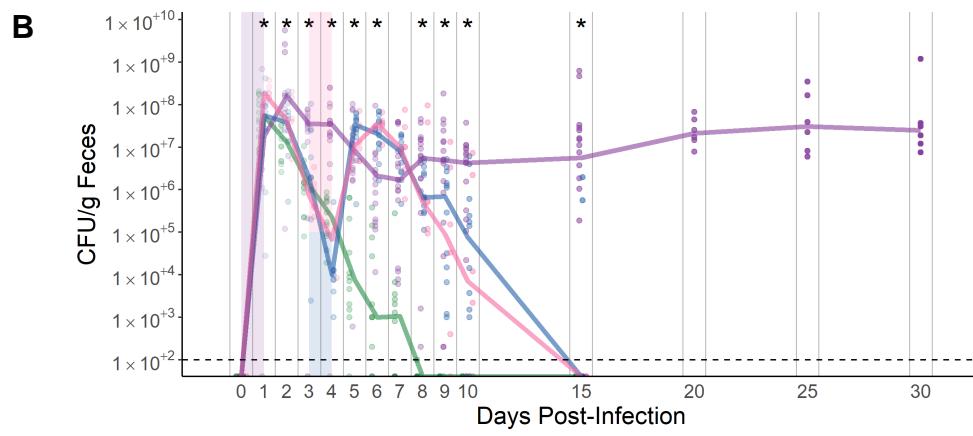
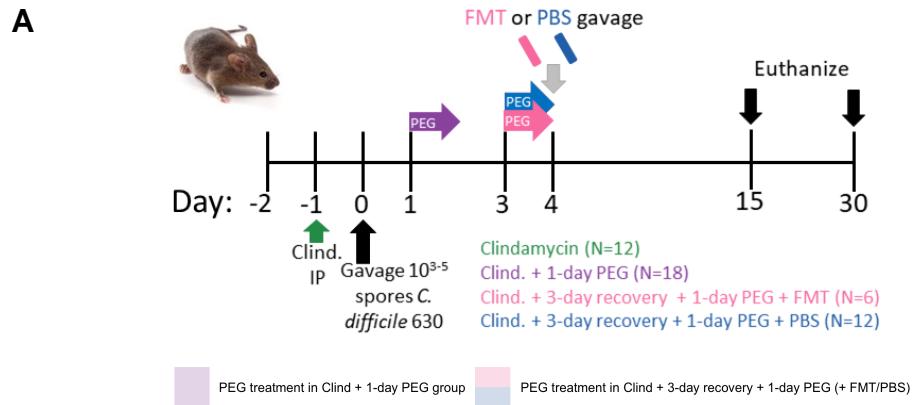
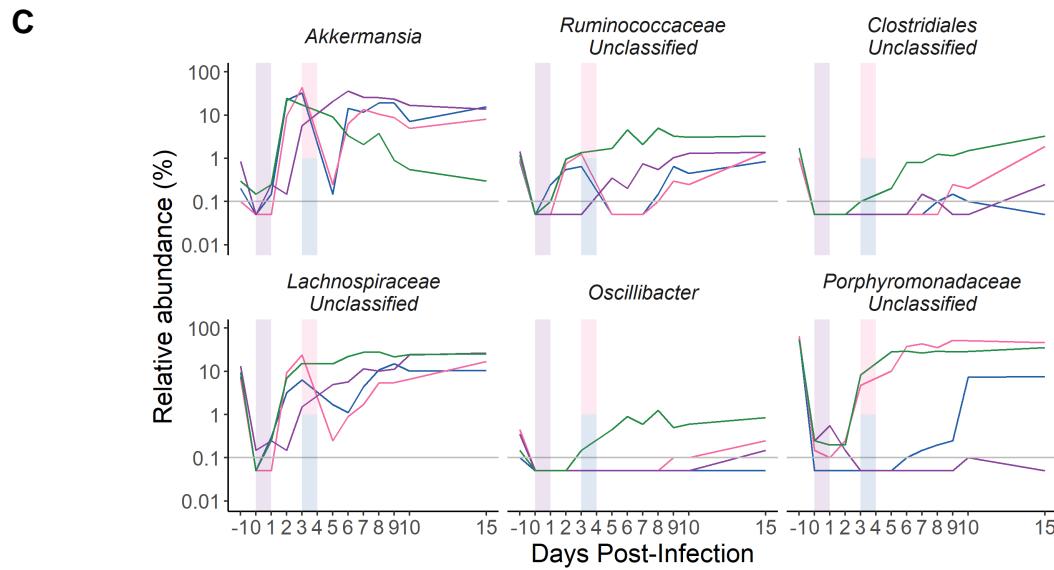
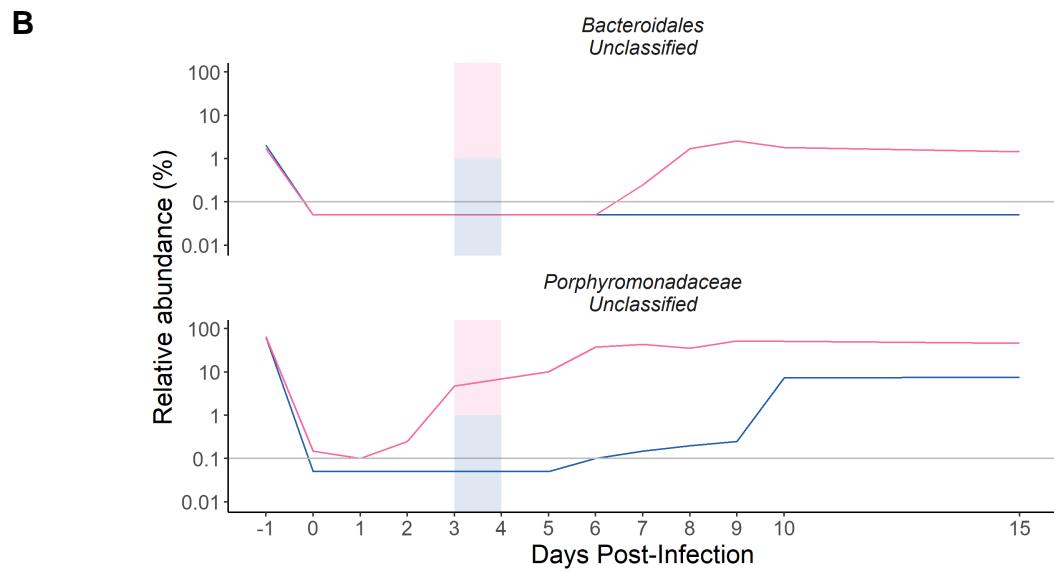
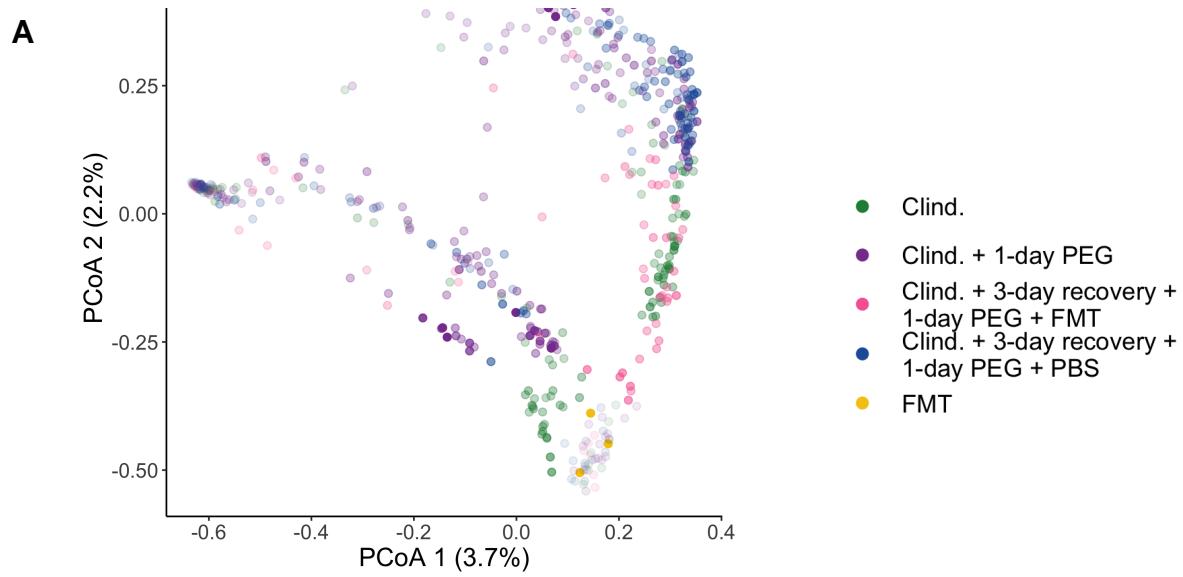


Figure 5.

293 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless of
294 20

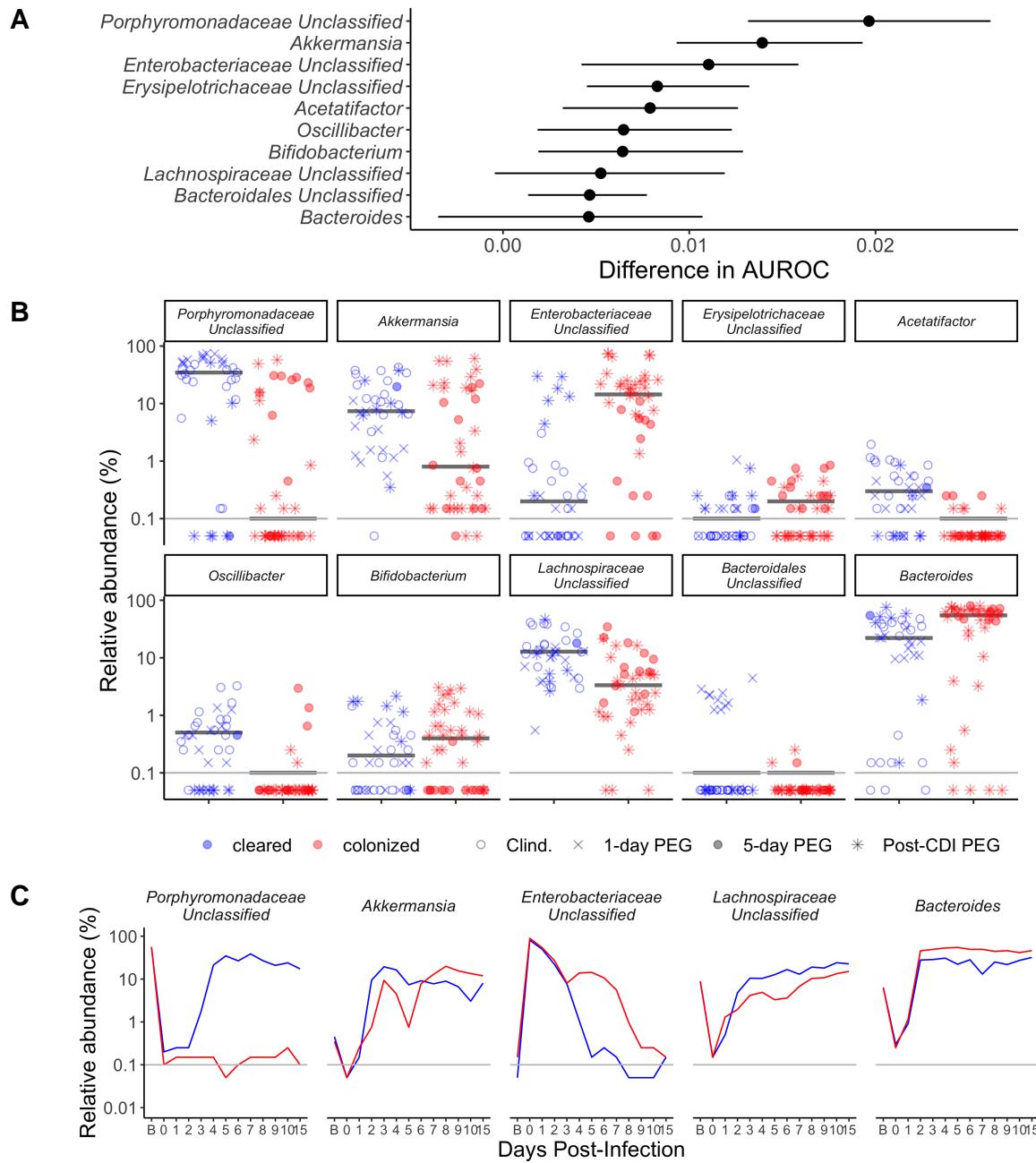
295 **whether an FMT is also administered.** All mice were administered 10 mg/kg clindamycin
296 intraperitoneally (IP) 1 day before challenge with *C. difficile* 630 spores on day 0. 15% PEG 3350
297 was administered in the drinking water for 1 day. Mice feces were collected daily through the end of
298 the experiment (30 days post-infection for Clind. + 1-day PEG group, 15 days post-infection for all
299 others). CFU quantification data was not available for each mouse due to stool sampling difficulties
300 (particularly the day the mice came off of the PEG treatment) or early deaths. B. CFU/g of *C.*
301 *difficile* stool measured over time via serial dilutions. C. Median and individual Shannon Diversity
302 measured over time. D. Median and individual richness measured over time. B-D. Asterisks
303 indicate time points with significant differences between groups using Kruskall-Wallis tests with a
304 Benjamini-Hochberg correction for testing multiple times points. Black line represents the limit
305 of detection for the first serial dilution. Background shading indicates PEG treatment period for
306 applicable groups. Opacity of points reflects time point. The data presented are from a total of ***
307 separate experiments.



308

309 **Figure 6. For 1-day PEG treatment post *C. difficile* challenge mice that also receive an FMT**
22

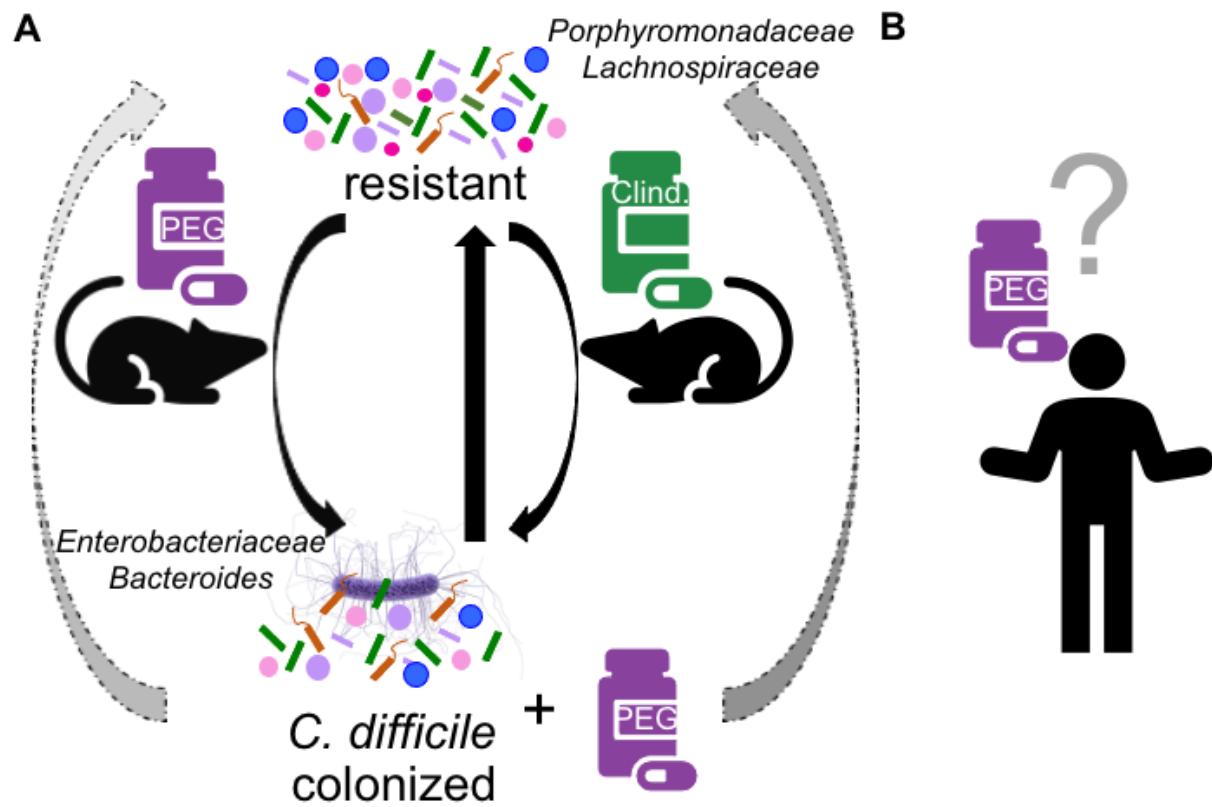
310 **only some bacterial genera were restored.** A. PCoA of all groups + FMT plotted over time.
311 Opacity of points reflects time point. B. Relative abundance of genera significantly different over
312 multiple time points post FMT or PBS treatment, plotted over time. Limit of detection is .1%. C.
313 Relative abundances of top 6 significant genera ranked by number of days significant, plotted over
314 time. Limit of detection is .1%.



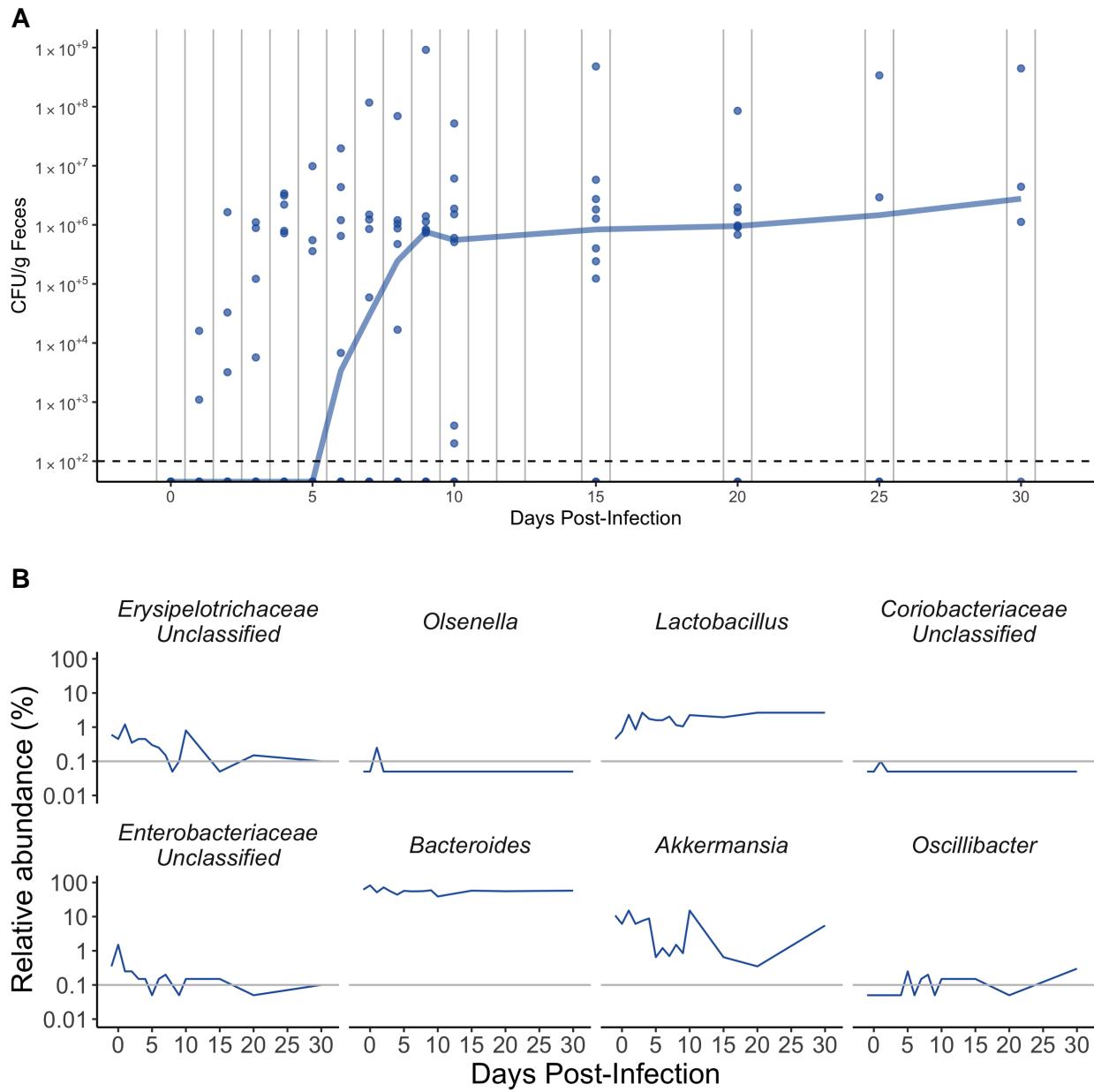
315

316 **Figure 7. Specific microbiota features associated with prolonged *C. difficile* colonization
317 in PEG treated mice. A.**

318

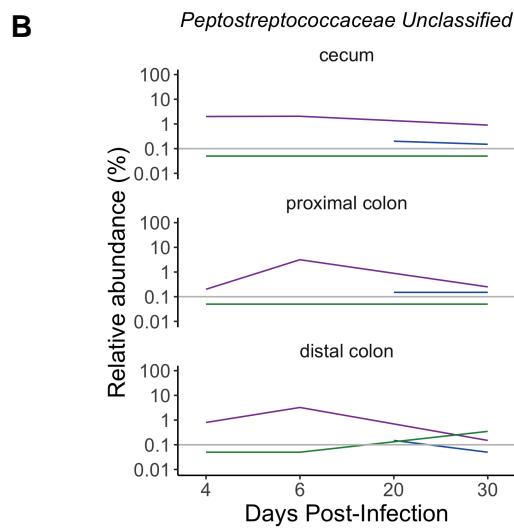
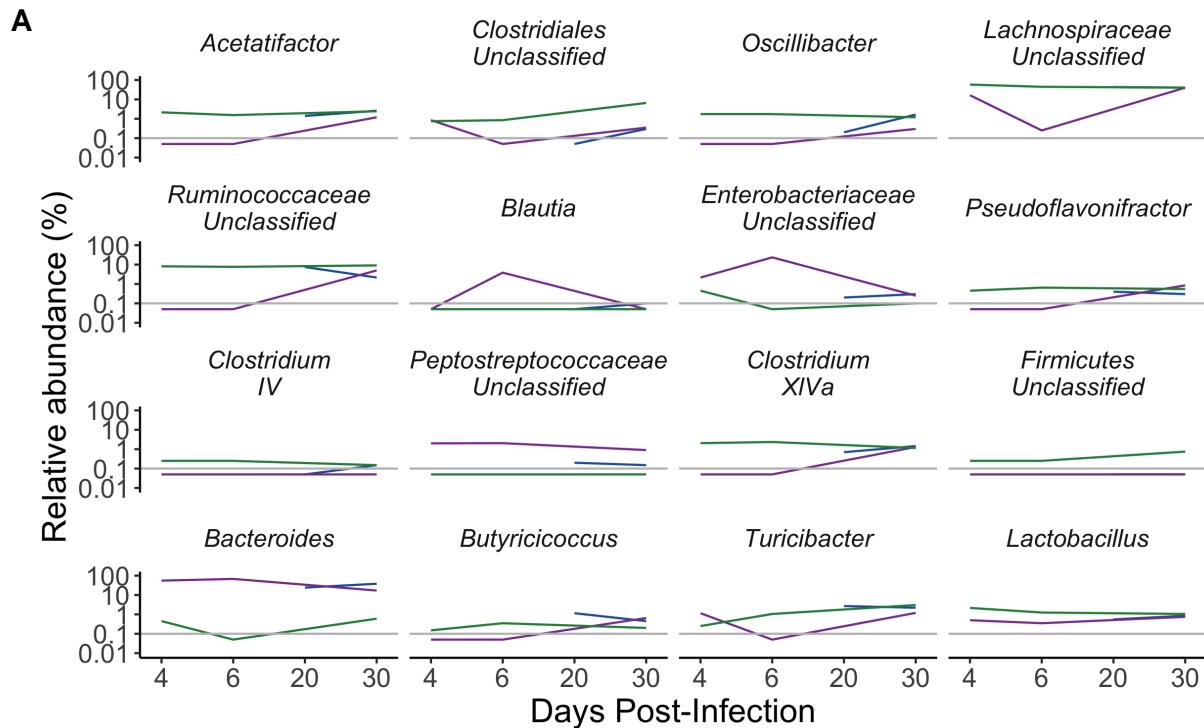


319 **Figure 8. Schematic summarizing findings. A.**



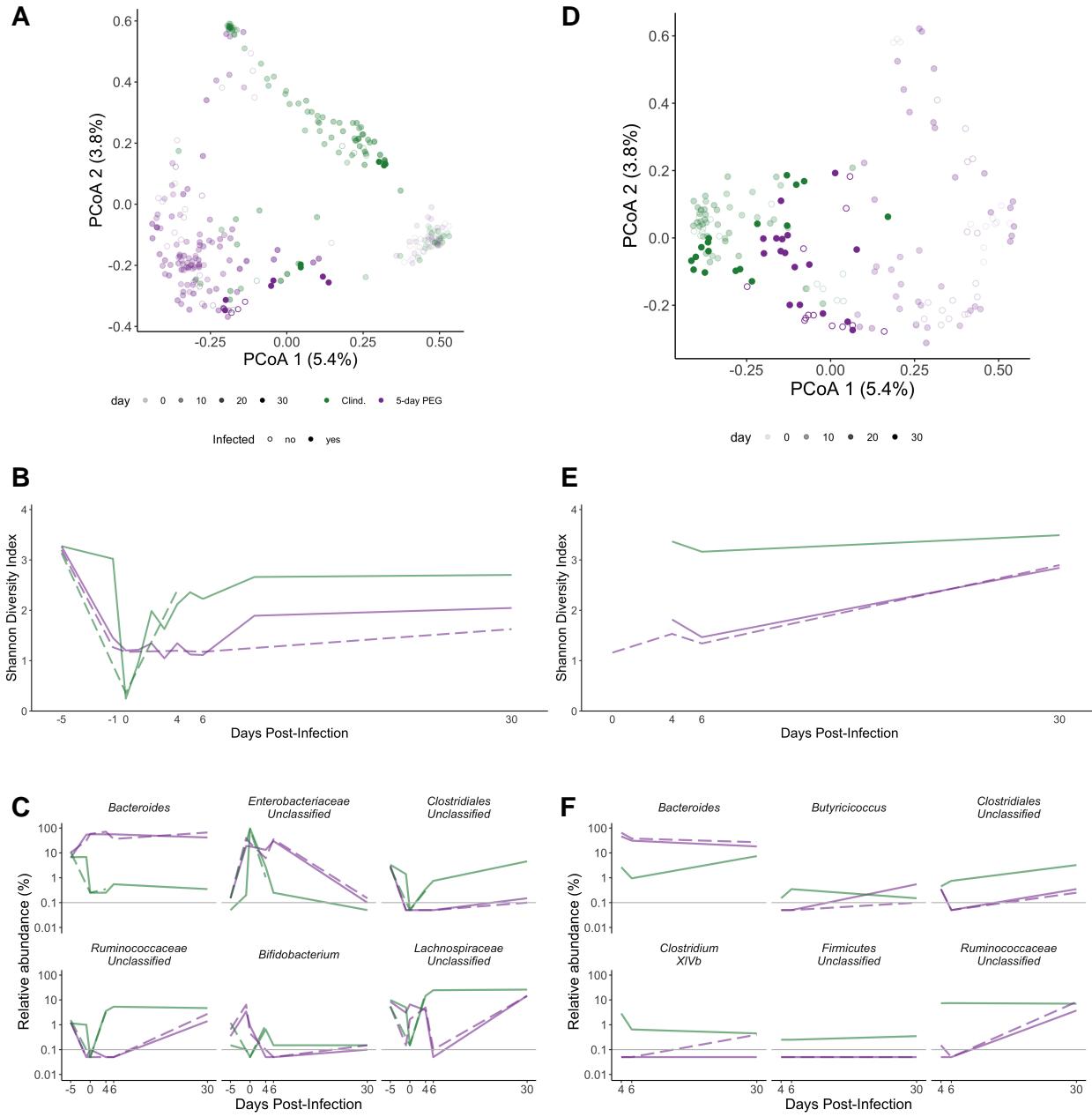
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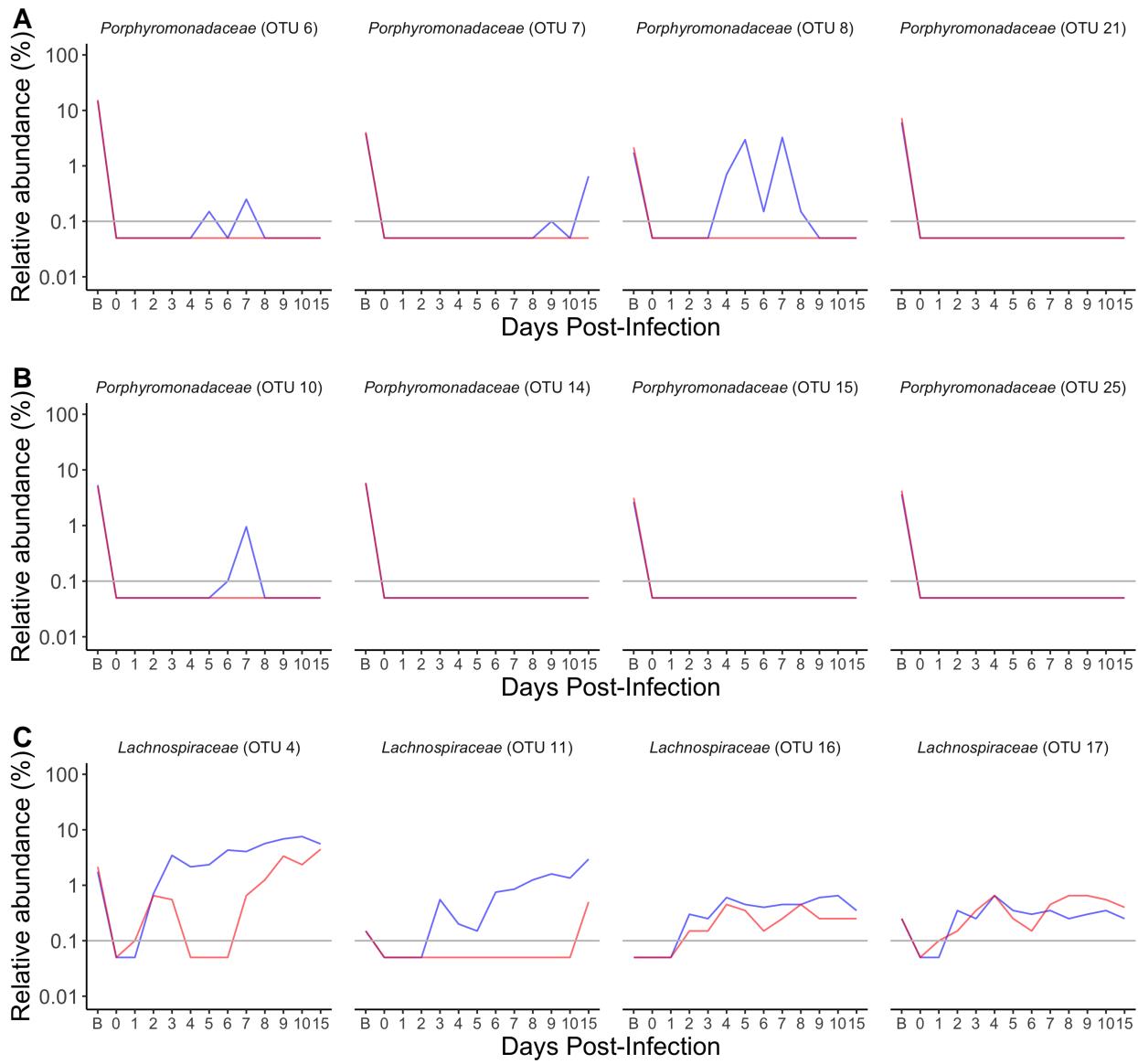
321 **Figure S1.** 5-day PEG treatment plus 10-day recovery mice microbiota dynamics
 322 post-infection. A.



324 **Figure S2. Impact of PEG treatment on the mucosal microbiota is greatest 6 days
325 post-infection**

326





330

331 **Figure S4. Specific OTUs associated with clearance that are mostly absent in mice with**
 332 **prolonged *C. difficile* colonization. Ex. *Muribaculum intestinalis*. A.**

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