

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 • Medications have been shown to alter the intestinal microbiota, but how this may influence
32 susceptibility to enteric infections is less clear.
- 33 • The ubiquitous osmotic laxative, polyethylene glycol (PEG) 3350 (Miralax/Nulytely/Golytely)
34 that is also commonly used as bowel preparation for colonoscopies (also one of the possible
35 administration routes used for fecal microbiota transplantation, a therapy for recurrent *C.*
36 *difficile* infection) has been shown to influence the intestinal microbiota and mucosal barrier.
- 37 • Review of previous mouse studies with PEG
- 38 • 2 main questions:
- 39 – Does PEG 3350 prolong colonization and result in more severe *C. difficile* infections?
- 40 – How does PEG 3350 influence *C. difficile* clearance and fecal microbiota transplant
41 (FMT) in mice?

42 **Results**

- 43 • Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently colonized
44 with *C. difficile*.
- 45 • Figure 2. 5-day PEG treatment disrupts the stool microbiota for a longer amount of time
46 compared to clindamycin-treated mice.
- 47 • Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics
48 post-infection.
- 49 • Figure 3. 5-day PEG treatment does not result in more severe CDIs, although mucosal
50 microbiota is altered.
- 51 • Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.
- 52 • Figure 5. 1-day PEG treatment post *C. difficile* challenge prolongs colonization regardless of

53 whether an FMT is also administered.

54 • Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in
55 PEG treated mice.

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

58 – Ex. *Muribaculum intestinale*.

59 • Figure 7. Schematic summarizing findings.

60 ##Discussion

61 • Summary of major findings

62 • Discussion of prolonged persistence. *C. difficile* sequences detected in tissue samples.
63 Association with mucin-degrading bacteria suggested by recent [a[ers]]

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

66 – Antibiotics may also impact mucus layer

67 – Strain of bacteria used

68 • Protective bacteria missing in PEG-treated mice

69 • Discuss what these findings might mean for human patients

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

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

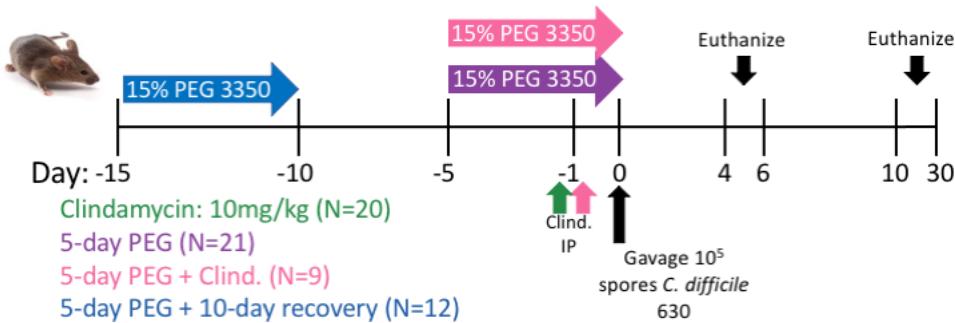
73 **Conclusions**

74 **Acknowledgements**

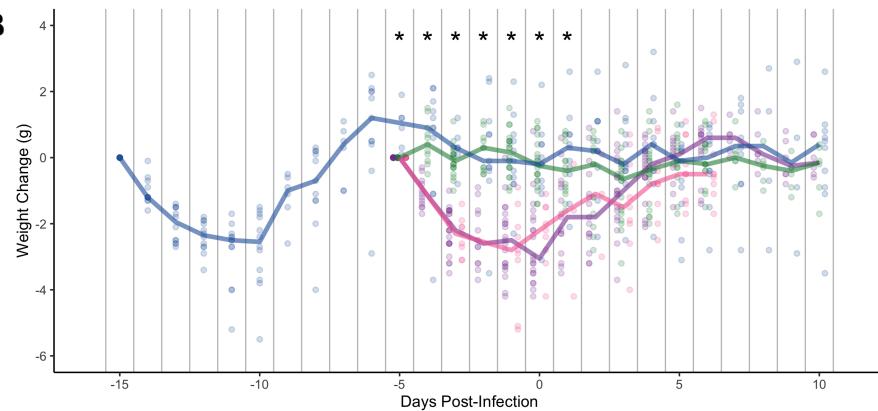
75 We thank members of the Schloss lab for feedback on planning the experiments and data
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77 We also thank the Unit for Laboratory Animal Medicine at the University of Michigan for maintaining
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83 Translational Sciences).

84 **Materials and Methods**

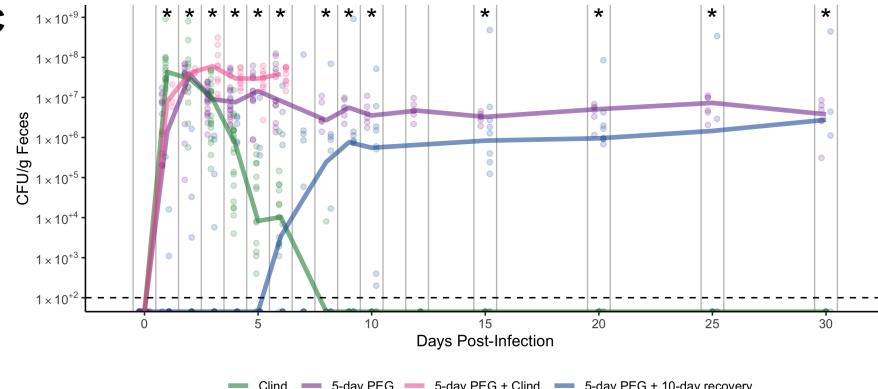
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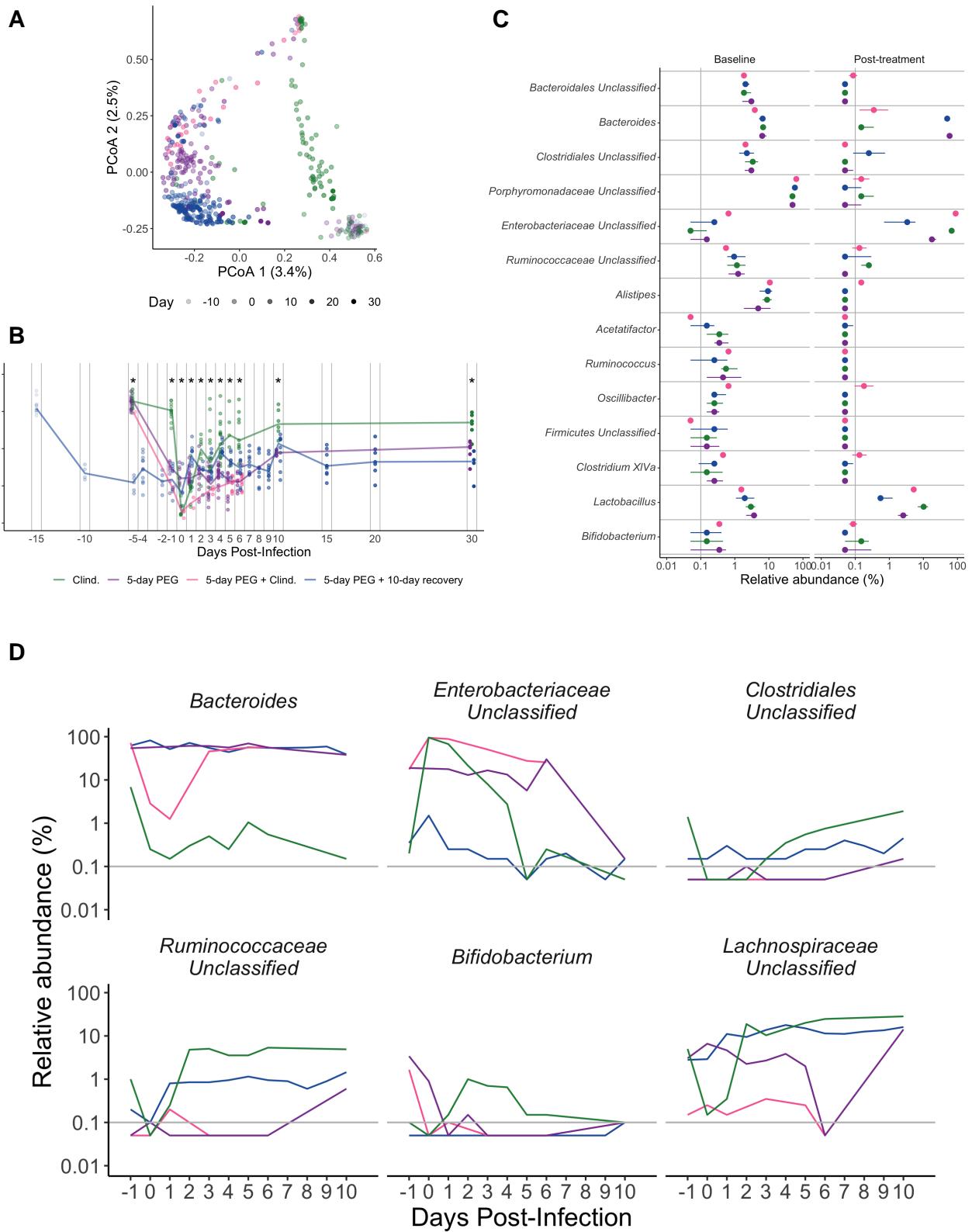
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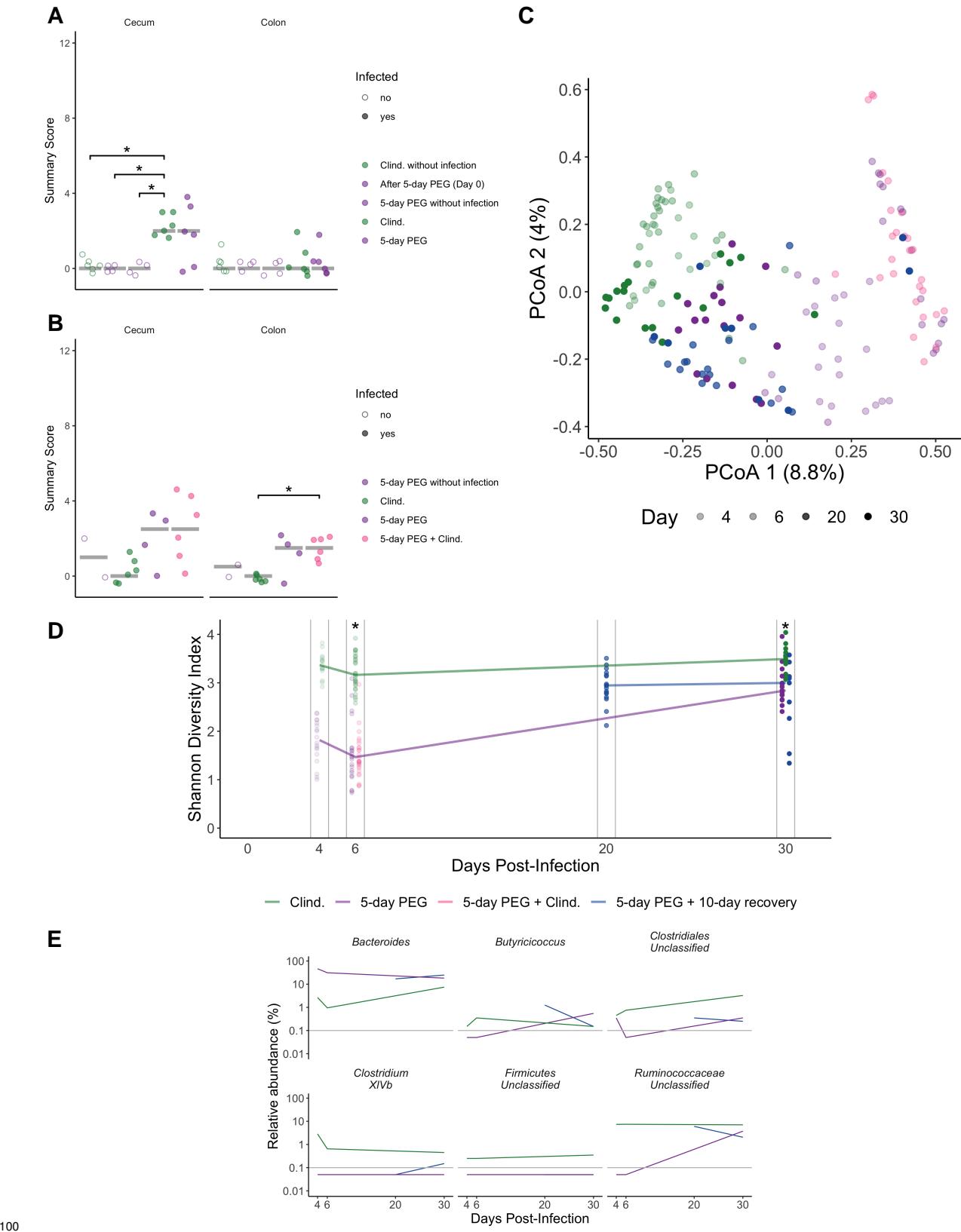
86 **Figure 1. 5-day PEG treatment prolongs susceptibility and mice become persistently**
87 **colonized with *C. difficile*.** A. Setup of the experimental timeline for subset of experiments
88 with 5-day PEG treated mice. B. Weight change from baseline weight in groups after treatment with
89 PEG and/or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

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



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

⁹⁹ compared to clindamycin-treated mice. A.



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

¹⁰² **microbiota is altered.** A.

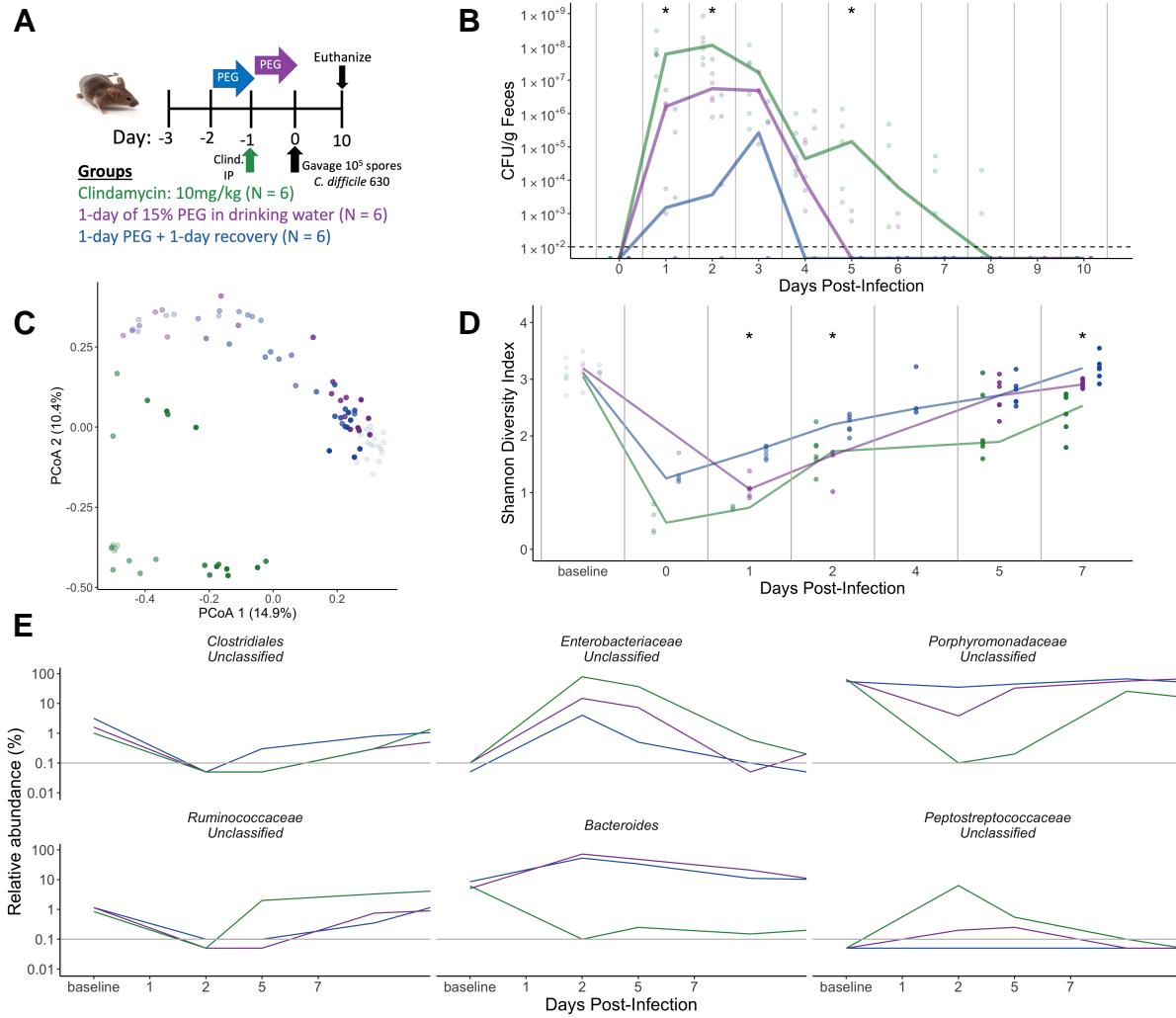
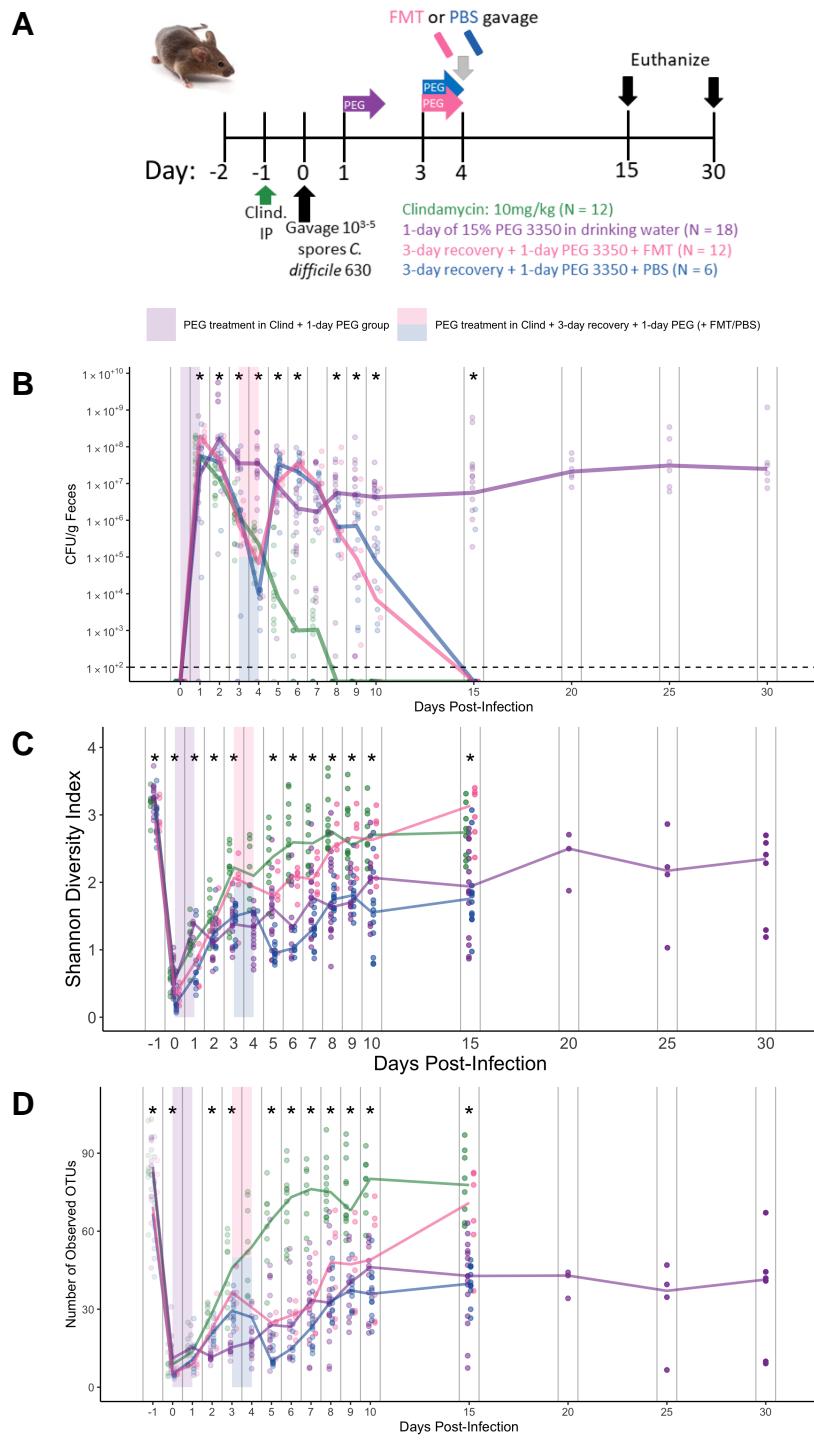


Figure 4. 1-day PEG treatment renders mice susceptible to transient *C. difficile* colonization.

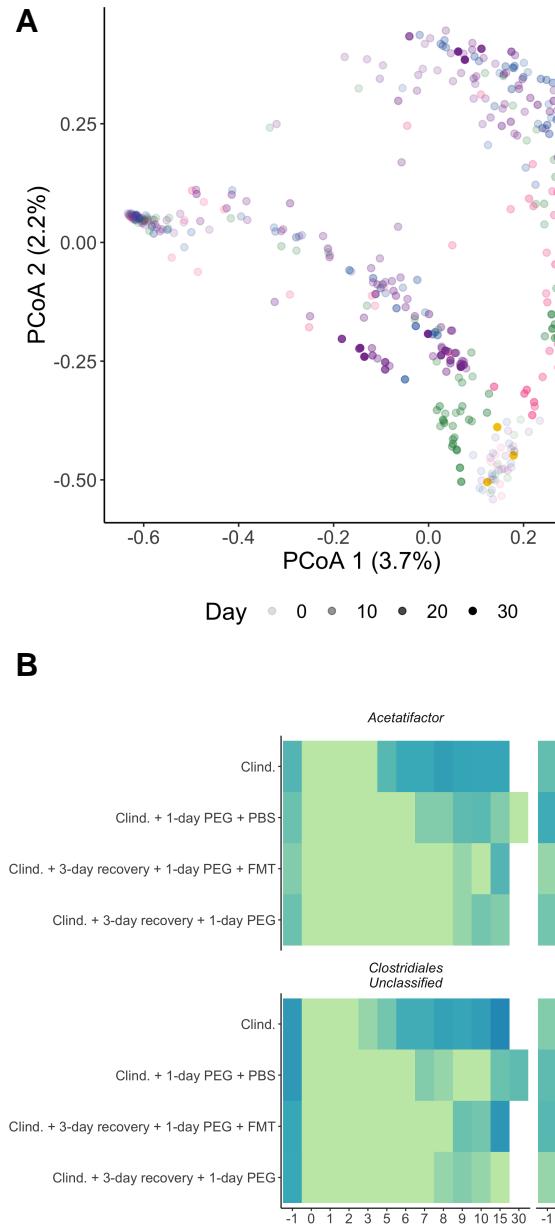
A. Setup of the experimental timeline for the 1-day PEG treated subset of mice. B. CFU/gram stool measured over time ($N = 6$ mice per timepoint) via several dilutions. The black dotted line represents the limit of detection for the first serial dilution. Asterisks indicate timepoints where the CFU/gram was significantly different between groups using the Kruskall-Wallis test with a Benjamini-Hochberg correction for multiple timepoints. C. Principle Coordinate Analysis plot of the groups over time with the alpha representing the same time scale as in panel D ($P = 1$ $R^2 = 1$). D. Shannon Diversisty Index of the groups over time. Only days with samples from all groups are shown. Samples for some mice were difficult to obtain due to the laxative treatment. The alpha scale follows accordingly with the timeline. E. Line plots of relative percent abundance of selected genera over time. Only days with samples from all groups shown. The gray line represents the limit

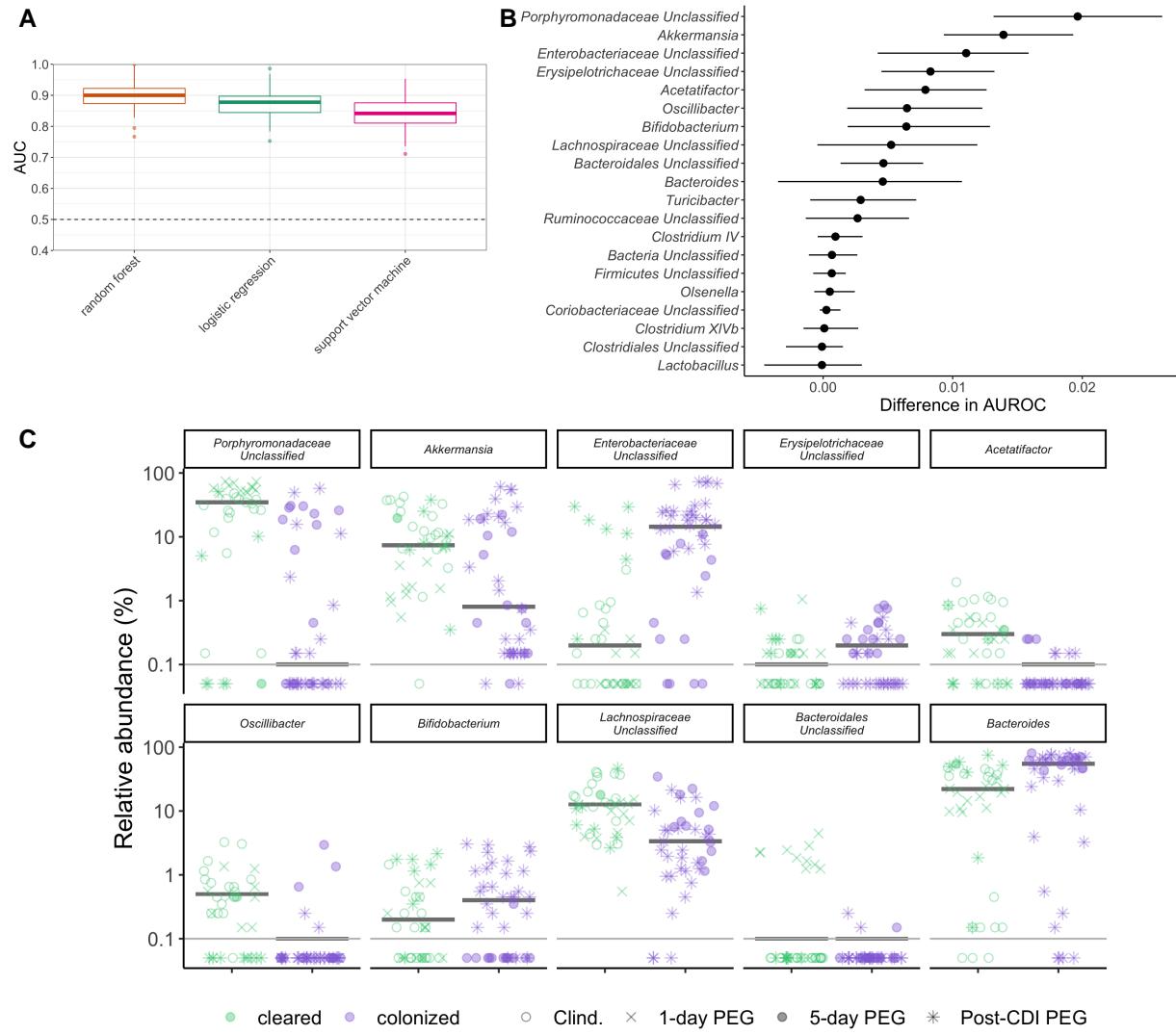
115 of detection.



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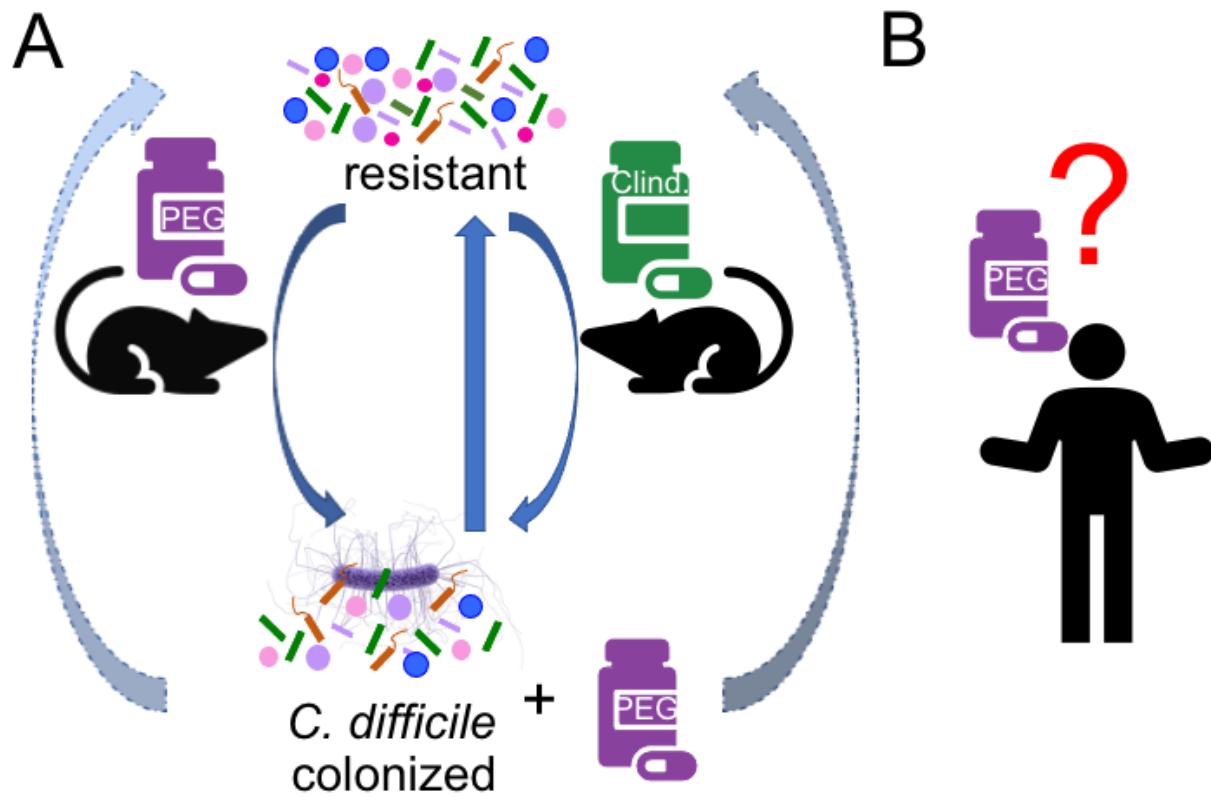
117 **Figure 5. 1-day PEG treatment post C. difficile challenge prolongs colonization regardless**
118 **of whether an FMT is also administered. A.**





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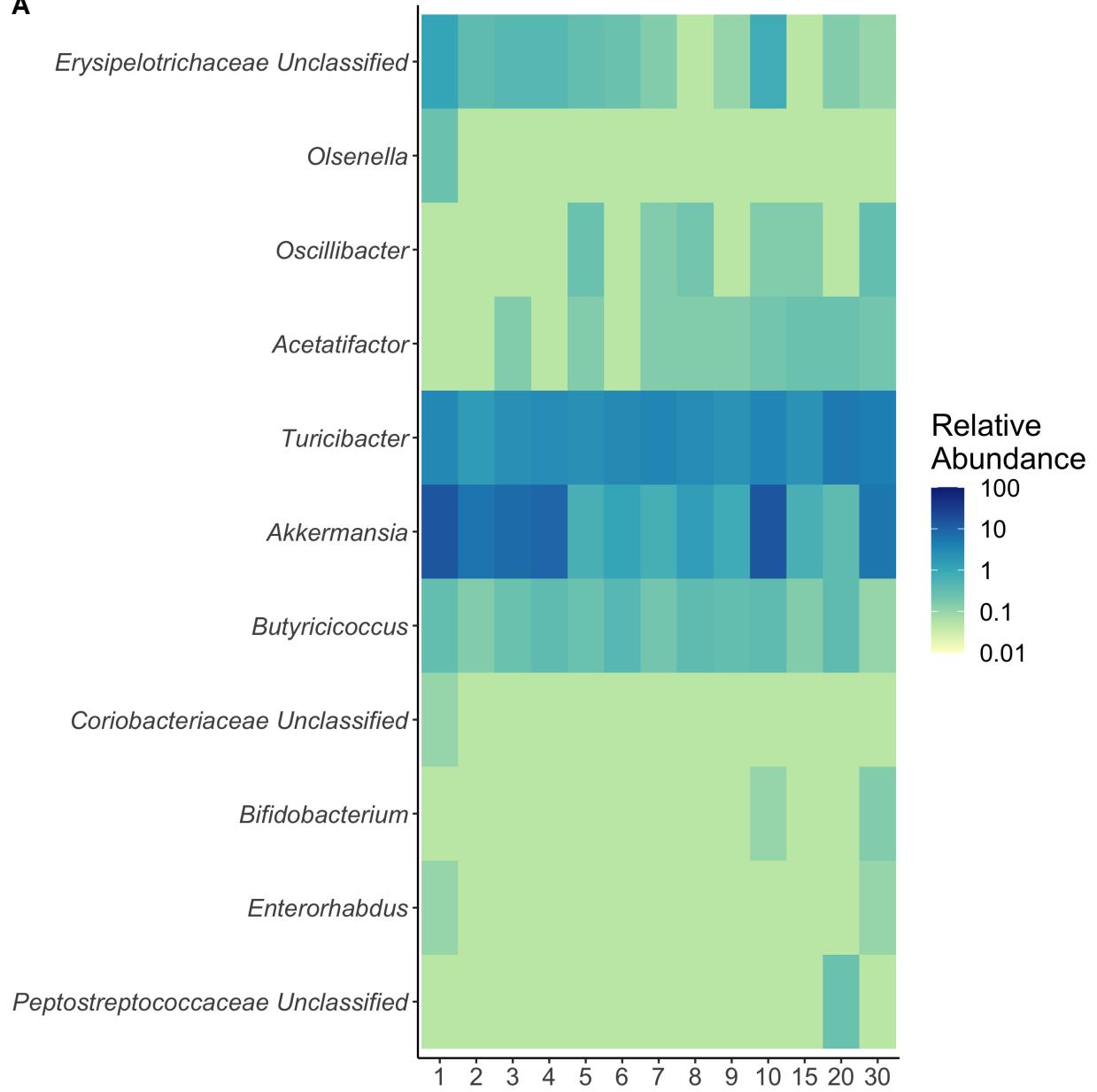
120 **Figure 6. Specific microbiota features associated with prolonged *C. difficile* colonization in
121 PEG treated mice. A.**



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

A

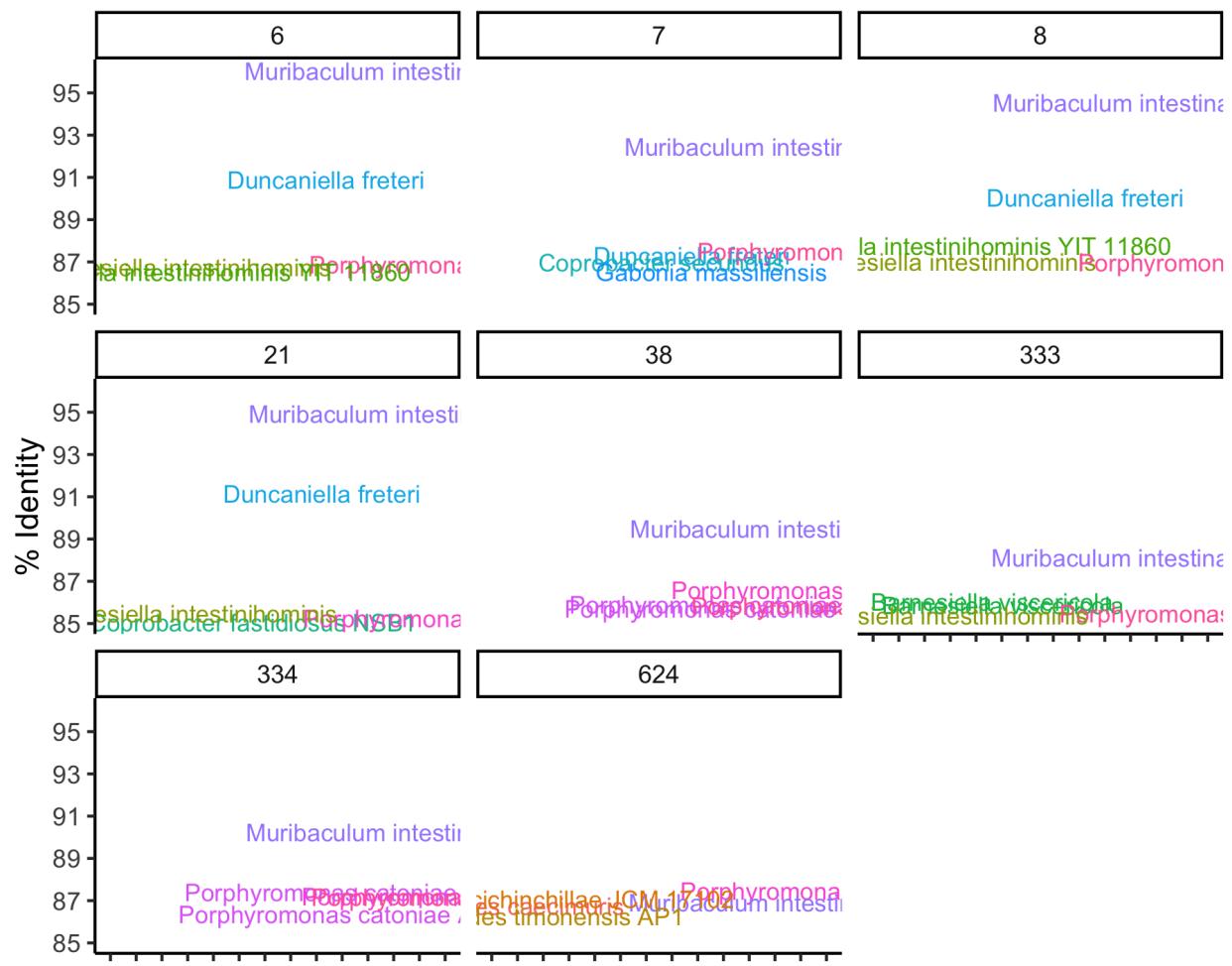


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125 **Figure S1. 5-day PEG treatment plus 10-day recovery mice microbiota dynamics**
126 **post-infection. A.**

A

Potential Muribaculum OTUs Blastn Results



127

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

130 **References**