Dynamics of Bacterial and Viral Transmission in Experimental Microbiota Transplantation

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Abstract

Mouse models are vital tools for discerning the relative contributions of host and microbial genetics to disease, often requiring the transplantation of microbiota between different mouse strains. Transfer methods include antibiotic treatment of recipients and colonization using either co-housing with donors or the transplantation of fecal or cecal donor material. However, the efficiency and dynamics of these methods in reconstituting recipients with donor microbes is not well understood. We thus directly compared co-housing, fecal transplantation, and cecal transplantation methods. Donor mice from Taconic Biosciences, possessing distinct microbial communities, served as the microbial source for recipient mice from Jackson Laboratories, which were treated with antibiotics to disrupt their native microbiota. We monitored microbial populations longitudinally over the course of antibiotics treatment and reconstitution using 16S rRNA gene sequencing, quantitative PCR, and shotgun sequencing of viral-like particles. As expected, antibiotic treatment rapidly depleted microbial biomass and diversity, with slow and incomplete natural recovery of the microbiota in non-transplanted control mice. While all transfer methods reconstituted recipient mice with donor microbiota, co-housing achieved this more rapidly for both bacterial and viral communities. This study provides valuable insights into microbial transfer methods, enhancing reproducibility and informing best practices for microbiota transplantation in mouse models.

Introduction

As our appreciation continues to grow for the diverse range of phenotypes the trillions of microbes inhabiting our bodies, termed the microbiota, can mediate, so does our reliance on manipulatable small animal models to experimentally interrogate these effects. The gut microbiota, a complex community of microorganisms residing in the gastrointestinal tract, plays a pivotal role in host health and physiology[1,2]. These microbial communities are involved in critical functions such as nutrient metabolism[3,4], immune system development[5–7], and protection against pathogens[8–10]. Dysbiosis, or the disruption of the normal microbiota balance, has been implicated in a range of diseases including obesity[11,12], malnutrition[13,14], and inflammatory bowel diseases[15,16]. Understanding the intricate relationships between the microbiota and host systems is therefore essential for developing therapeutic strategies targeting microbiome-related diseases.

To unravel these complex host-microbe interactions, researchers heavily leverage animal models, particularly mice. Controlled studies using mouse models enable the manipulation of microbial communities to observe resultant effects on host physiology and disease outcomes. A key aspect of such research is the generation of gnotobiotic mice—animals that are germ-free or colonized with specific, defined microbial communities[17]. These models provide a unique platform to study the microbiota's role under controlled conditions.

Despite the utility of germ-free mice for numerous applications, however, their use is associated with some drawbacks. For investigators studying numerous mouse genotypes, executing experiments not amenable to the use of a gnotobiotic isolator such as those involving particular pathogens or behavioral testing, or performing studies requiring a normal microbiota during development which is then disrupted, germ-free mice may be impractical or inappropriate. Antibiotics treatment represents an accessible alternative for the depletion of the microbiota, and this approach has been broadly used by many investigators to examine many of the same phenotypes explored in germ-free mice, often with strong concordance between the two models[17].

Establishing gnotobiotic mice or reconstituting the microbiota after perturbation necessitates reliable and efficient methods for microbial transfer. Common techniques include fecal microbiota transplantation (FT), cecal content transplantation (CT), and co-housing (CH). Fecal transplantation involves transferring fecal material from a donor to a recipient, while cecal transplantation is similar but utilizes the cecal contents, which may have a different complex microbial composition due to variation along the gastrointestinal tract. Co-housing leverages the natural coprophagic behavior of mice, allowing for the transfer of microbes through direct contact and shared environments. Despite their widespread use, experiments designed utilizing these methods are generally based on historical precedent rather than a comprehensive understanding of their efficacy or the specific dynamics of microbial colonization they promote.

This reliance on convention can lead to assumptions that impact experimental outcomes and reproducibility, a major challenge facing the field[18–21]. For instance, variation in microbial transfer efficiency can affect the establishment of the desired microbiota, influencing the host's physiological responses and potentially skewing results[22,23]. Furthermore, while bacterial community dynamics have been relatively well-studied, the transfer and establishment of viral communities (the virome) remain less understood. Given the growing recognition that bacteriophages and other viruses shape microbial communities and influence host health[24–30], it is crucial to consider both bacterial and viral components when evaluating these methods.

Herein, we systematically compare fecal transplantation, cecal transplantation, and co-housing as methods for transferring microbes to microbially-depleted mice, characterizing this process longitudinally. Recipient mice from Jackson Laboratories were treated with antibiotics for one week to disrupt their native microbiota. Donor mice from Taconic Biosciences, known to have distinct microbial communities from Jackson, served as the source of bacteria and viruses for transplantation[31]. By employing 16S rRNA gene sequencing and quantitative PCR (qPCR), we monitored bacterial load, diversity, and composition. Additionally, we analyzed

viral community composition and diversity through short-read shotgun sequencing of viral-like particles.

Our findings indicate that co-housing facilitates a more rapid transfer of both bacterial and viral communities compared to fecal and cecal transplants, which performed similarly to each other. While all methods resulted in bacterial communities similar to the donor by day 15 post-transplantation, viral communities exhibited greater diversity following co-housing. These results highlight method-specific influences on microbial community dynamics and underscore the importance of method selection in experimental design.

By carefully characterizing these commonly used methods, our study provides valuable insights that can enhance reproducibility and consistency in microbiota research. This work informs best practices for replacing the enteric microbiota of mice and contributes to our understanding of the mechanisms underlying microbial transfer and establishment in experimental models. Such insights are essential for advancing microbiota research and for the development of interventions targeting microbiome-related diseases.

Results

Effect of antibiotic treatment on bacterial load, diversity, and community composition

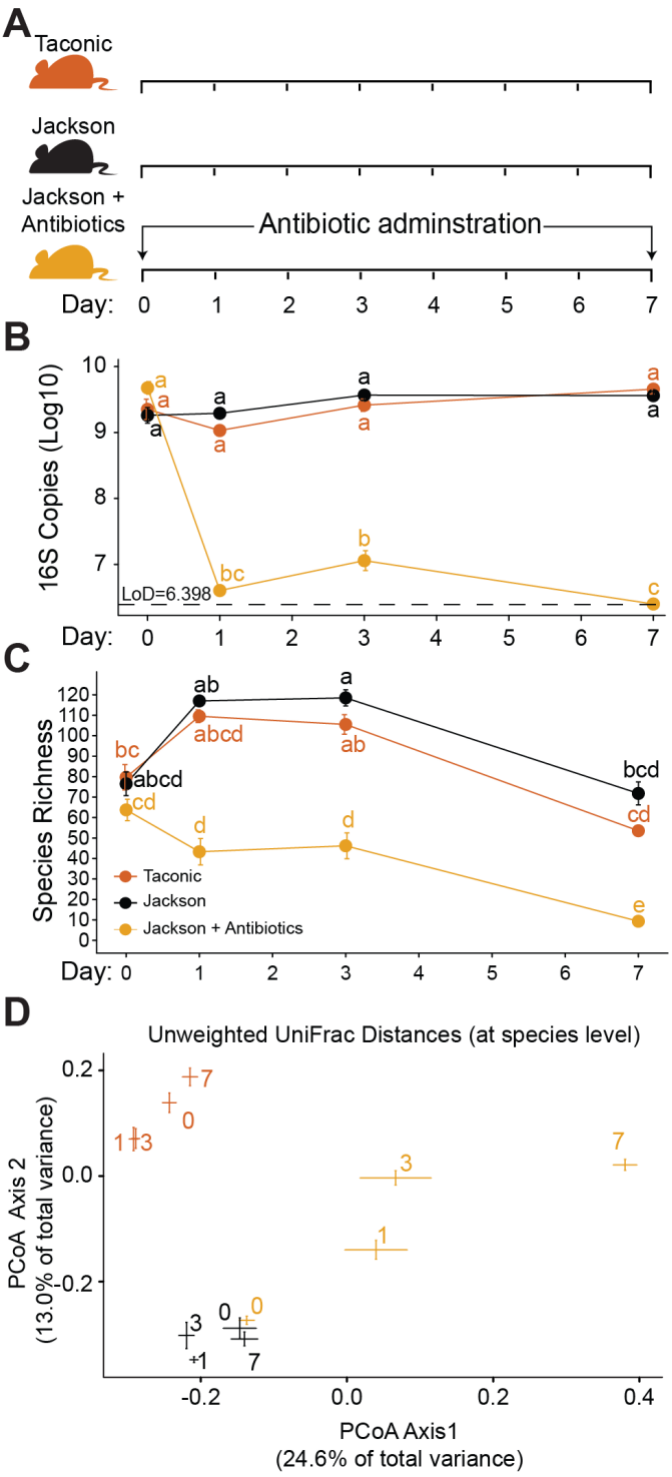
Based upon prior studies leveraging antibiotic treatments of mice, we elected to use a simplified cocktail of vancomycin, neomycin, and ampicillin, which we and others have shown mediates robust depletion of the microbiota within three to seven days of treatment initiation and is also well-tolerated by recipients[10,11]. We used C57BL/6 mice from Jackson Laboratory (JAX) as our recipients and C57BL/6 mice from Taconic (TAC) as our donors for microbiota transfer, as mice from these different vendors are well-established to have distinct enteric microbial communities[12,13]. Antibiotics were administered to JAX recipient mice (n=13, 16, and 16 respectively in three independent experiments, subdivided into four groups corresponding to method of transplantation as described below) for 7 days, while TAC donor mice (n=4 per experiment) and control groups of JAX (n=4 per experiment) and TAC (n=4 per experiment) mice were left untreated (**Figure S1A**).

Fecal samples were collected on days 0, 1, 3, and 7 and subjected to 16S rRNA gene qPCR and sequencing to characterize the microbial community prior to and during antibiotic treatment. The bacterial load as determined by 16S qPCR was not significantly different in JAX and TAC mice prior to antibiotic treatment (**Figure S1B**, Day 0, $p > 0.05$). Antibiotics rapidly and significantly reduced the bacterial load in JAX mice 1 day after treatment initiation (**Figure S1B**, Day 1, $p < 0.05$) and 16S levels were below the limit of detection following 7 days of antibiotic treatment (**Figure S1B**). The number of 16S copies did not change significantly over the first 7 days of the experiment in untreated JAX and TAC mice.

We next evaluated sequencing data for the V4 region of the 16S rRNA gene for these samples. Sequencing reads were processed using the DADA2 pipeline implemented in Nephele to generate amplicon sequence variants (ASVs)[32,33]. Bacterial species were defined by grouping ASVs with greater than 97% sequence identity. ASV richness was similar between JAX and TAC mice at Day 0 (mean ± SE: TAC=~80 ± 6 OTUs, JAX=66 ± 5; **Figure S1C**). Treatment with antibiotics significantly reduced the number of bacterial species present in the stool of JAX mice within 1 day (mean ± SE= 43 ± 6; **Figure S1C**). Bacterial richness

was not significantly different on day 3 of treatment but was reduced to one-third of the original level by day 7. Although the 16S levels were below the limit of detection in antibiotic-treated mice on day 7, sequencing suggested that some of the native bacteria persisted at a low level.

To understand how community composition was affected by antibiotic treatment and the subsequent reduction in bacterial biomass, the abundance of bacterial species was used to calculate the unweighted UniFrac distance between all samples collected over the full course of the experiment, and dimensionality reduction via principal coordinate analysis (PCoA) was performed. The data were then subset to samples collected during the first seven days to visualize the effect of antibiotic treatment on community composition. PCoA axis 1 explained 24.6% of the total variance in bacterial community composition, with variance along this axis driven by antibiotic treatment of JAX mice (**Figure S1D**). The variance along PCoA axis 2 (13.0%) split untreated JAX and TAC mice. Antibiotic treatment contributed more to overall variance in the bacterial community composition than the vendor. One week of treatment with vancomycin, neomycin, and ampicillin was sufficient to substantially reduce bacterial load and diversity and drastically alter bacterial community composition.



Supplementary Figure 1. One-week treatment with antibiotic cocktail significantly alters fecal bacterial biomass and community composition. (**A**) Mice were obtained from Jackson or Taconic labs. Mice from Jackson Labs were provided vancomycin, neomycin, and ampicillin in their drinking water for one week (Jackson + Antibiotics). Untreated Jackson control mice and Taconic mice were maintained on normal drinking water. (**B**) Bacterial load measured by qPCR of 16s rDNA gene throughout the first week of the experiment. The letters next to each point indicate significant groupings, as those with the same letter were not significantly different from one another by two-way ANOVA followed by Tukey's HSD post-hoc test for pairwise comparisons with an alpha of 0.05. (**C**) Bacterial species richness, the number of unique bacteria species observed per mouse, throughout the first week of the experiment

via V4-16S rRNA gene sequencing. The letters next to each point indicate significant groupings, as those with the same letter were not significantly different from one another by two-way ANOVA followed by Tukey's HSD post-hoc test for pairwise comparisons with an alpha of 0.05. (**D**) Principal Coordinate Analysis of unweighted UniFrac distances calculated from species counts. Error bars (± SE) intersect at centroid of all samples collected for each group of mice on the day post-antibiotics treatment, indicated by the number on the plot.

Route of transfer regulates community reconfiguration during transplantation following antibiotic treatment

We next compared the outcomes of different transfer methods for the transplantation of TAC microbiota into antibiotic-treated JAX mice. Cessation of antibiotics occurred on day 7, and antibiotic-treated JAX mice were either (i) maintained with no intentional transfer of microbes (n=12, 8-12 samples per timepoint combined across three experiments), or gavaged with prepared (ii) cecal (n=12, 7-12 samples per timepoint) or (iii) fecal (n=12, 8-12 samples per timepoint) contents from untreated TAC mice, or (iv) co-housed (n=11, 6-11 samples per timepoint) with untreated TAC donors and sampled over the following two weeks (**Figure 1A**). Additionally, TAC (n=3, 0-3 samples per timepoint) and JAX (n=10, 6-10 samples per timepoint) mice that did not receive antibiotics were maintained and sampled over these two weeks to monitor changes over time in the background communities.

Bacterial load was significantly increased two days after cessation of antibiotics (Day 9) in all mice that received microbial transplantation relative to those that did not, regardless of method (**Figure 1B**). Although the bacterial load returned to detectable levels in mice that did not receive a microbial transplant on day 9 it was nearly two orders of magnitude lower than that of untreated mice or of those that received a microbial transplant. By day 11, there was no significant difference in the number of 16S copies detected between the methods of transfer or untreated mice from JAX or TAC. After one week (day 14), the bacterial load was similar between all groups for the duration of the experiment. Although increasing steadily post-transplantation, the recovery of bacterial alpha-diversity (species richness), was slower than that of overall bacterial load, regardless of transplantation approach (**Figure 1C**).

The effect of the method of transplant on community composition was evaluated next. Samples were separated based on the day they were collected and the treatment group to which the mouse belonged. Unweighted UniFrac distances were calculated between all samples to assess global changes in community composition and beta diversity throughout the experiment. PCoA computed on this full set enabled the visualization of microbial community recovery and transfer following antibiotic treatment over time between days 9 and

21 (**Figure 1D**). Confidence ellipses designating the samples from untreated JAX mice and TAC mice represent the original recipient and donor community compositions in feces collected from mice that did not receive antibiotics from days 0 through 7.

The bacterial communities of JAX antibiotic-treated mice administered either fecal or cecal transplant gradually approach the TAC donor community composition between days 9 and 11, converging on the donor community profile by day 15 (**Figure 1D**). Thus, although fecal or cecal transplant restored bacterial loads to levels similar to TAC donors within two days (**Figure 1B**), the composition of the bacterial community was not fully recapitulated by day 9 with either of these transfer methods. In contrast, after only two days (day 9), the bacterial composition of co-housed mice was equivalent to that of TAC donors (**Figure 1D, E**). The bacterial community of JAX mice that received antibiotics but no transfer gradually returned towards that of untreated JAX mice, eventually achieving a similar state to the original JAX microbiota by experimental day 21.

The unweighted UniFrac distances of the treatment arms were statistically compared to untreated Taconic and Jackson control mice throughout the experiment using PERMANOVA. To account for drift in community composition over time in the control communities, as well as low sample sizes from untreated Taconic controls, samples collected on each day of the experiment for the treatment arms were compared to pooled control samples collected within the same week. The fecal bacterial communities of antibiotic-treated animals were significantly different than JAX controls following 1 day of antibiotic treatment, the difference between these groups remained significant and increased through the end of antibiotic treatment on day 7 (**Figure 1E**). Additionally, the bacterial community of all these groups of mice was significantly different than the TAC controls, also increasing until day 7 (**Figures 1D, E**). By day 9, the earliest timepoint sampled following transplantation, the bacterial microbiota of antibiotic-treated JAX mice co-housed with TAC donors were no longer significantly different from TAC controls and were not significantly different for the remainder of the experiment. Mice that received cecal or fecal transfer were significantly different from the Taconic donors until Day 14, suggesting differences in the efficiency of bacterial transfer

across the three methods. Like the trends observed in the 16S qPCR data (**Figure 1B**), the bacterial community of JAX mice that were treated with antibiotics that did not receive transplantation gradually recovered and was not significantly different from the JAX controls by day 21 (**Figures 1D, E**). However, the average distance between these two groups was still elevated (0.69 ± 0.1 [mean ± SD]) and had not returned to baseline (0.47 ± 0.1 [mean ± SD]). Interestingly, although co-housed mice displayed the most rapid transition to a Taconic-like bacterial community, they were not significantly different from JAX controls on days 14 and 21. This may be indicative of incomplete microbial transfer and/or recovery of JAX bacteria in co-housed mice resulting in an 'intermediate' community state.

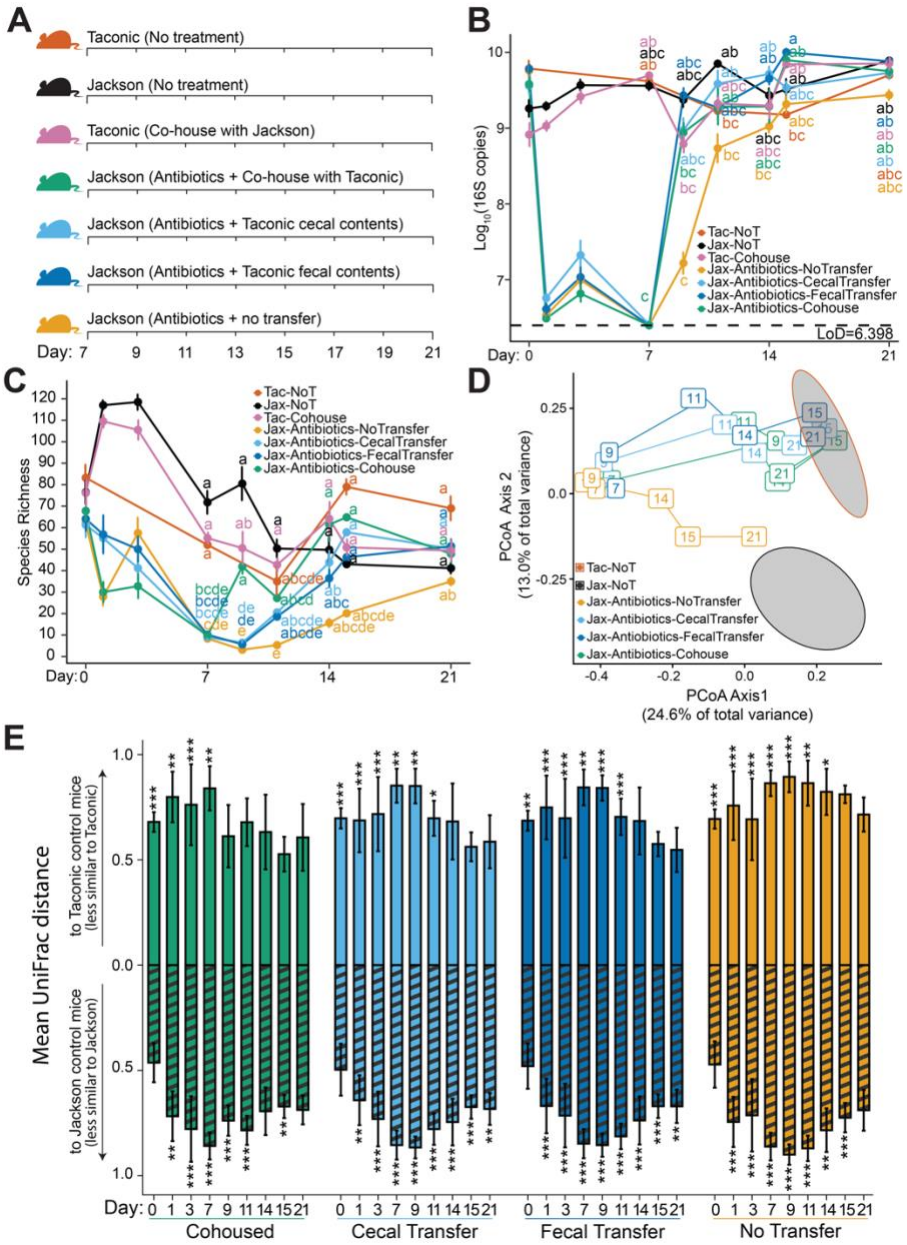


Figure 1. Cohousing of mice reconstitutes the bacterial microbiota more rapidly than fecal or cecal transplant. (**A**) Antibiotic-treated Jackson mice were either allowed to recover naturally (Jax-Antibiotics-NoTransfer), or were colonized with Taconic-associated microbes via gavage of cecal (Jax-Antibiotics-CecalTransfer) or fecal (Jax-Antibiotics-FecalTransfer) material, or co-housing (Jax-Antibiotics-Cohouse). Jackson (Jax-NoT) and Taconic (Tac-NoT) mice that did not receive antibiotics were maintained as controls. (**B**) Bacterial load measured by qPCR of the 16S rRNA gene throughout the final two weeks of the experiment shows the rapid recovery of bacterial load following transplantation. The letters next to each point indicate significant groupings, those with the same letter were not significantly different from one another by two-way ANOVA followed by Tukey's HSD post-hoc test for pairwise comparisons with an alpha of 0.05. (**C**) Bacterial species richness, the number of unique bacterial species observed per mouse, throughout the first week of the experiment via v4-16s sequencing. The letters next to each point indicate significant groupings, those with the same letter were not significantly different from one another by two-way ANOVA followed by Tukey's HSD post-hoc test for pairwise comparisons with an alpha of 0.05 (**D**) Principal Coordinate Analysis of unweighted UniFrac distances calculated from species counts across all three weeks of the experiment illustrate the change in community composition following antibiotic treatment and

microbiota transplantation. Ellipses (95% confidence) represent the baseline fecal bacterial communities of untreated Jackson (Jax-NoT) and Taconic (Tac-NoT) mice during the first week of the experiment. (**E**) Bar heights indicate the mean Unweighted UniFrac distance was calculated between the fecal bacterial community of Jackson mice treated with antibiotics that received microbial transplantation via co-housing, cecal transfer, fecal transfer, or no transfer, and the fecal bacterial community of control Jackson and Taconic mice that never received antibiotics, pooled by week (days pooled: [0,7), [7,14), [14,21]). Striped bars below the x-axis indicate the distance from untreated Jackson controls while solid bars above the x-axis indicate the distance from untreated Taconic controls. Error bars indicate the standard deviation of all pairwise comparisons between the indicated groups. A PERMANOVA test was performed on the unweighted UniFrac distance matrix for the comparisons shown followed by Bonferroni's corrections for multiple comparisons (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Co-housing rapidly transfers Taconic-associated bacterial taxa and their predicted functional capabilities

To better understand the dynamics of bacterial transfer following transplantation, ASVs were classified as being either Taconic- (162 ASVs) or Jackson-associated (124 ASVs), or neither based on their prevalence and abundance in samples collected from untreated mice over the first week of the experiment (**Figure 2A**). These subsets of ASVs were then tracked throughout the course of the experiment (**Figure 2B**). As expected, Taconic ASVs were higher in Taconic mice than in Jackson mice that didn't receive transplantation, significantly so on days 7 and 8. Although there was a transient increase in the richness of Taconic ASVs on days 1 and 3 in all groups measured, by day 7 the Taconic-associated ASV richness in all Jackson mice (mean = 0.7 – 2.6 Taconic ASVs per sample) was significantly lower than in Taconic donors (~36.3 Taconic ASVs per sample) (**Figure 2B**). By day 9, the number of Taconic ASVs had substantially increased in co-housed mice (mean ± SE = 26.9 ± 2.0) and was significantly higher than untreated Jackson controls (mean ± SE = 0.7 ± 0.1) and not significantly different than Taconic co-housed donors (mean ± SE = 30.8 ± 4.5). These were samples from mice originally from Taconic that shared a cage with antibiotic-treated Jackson recipient mice. At this time, mice that received cecal or fecal transplants did not have significantly higher richness of Taconic-associated ASVs (means ± SEs = 4.6 ± 0.8 and 4.3 ± 0.4, respectively). By day 15, the richness of Taconic-associated ASVs in Jackson mice that received antibiotics followed by cecal or fecal transplant was significantly elevated relative to

Jackson mice that received antibiotics and no subsequent transplantation and remained relatively steady until the end of the experiment on day 21. Overall, these data are consistent with our observation that co-housing more rapidly introduces donor-specific bacteria than cecal or fecal transplant.

To complement our taxonomic description of this transplantation process, we next sought to characterize the functional capacity of these bacterial communities. The functional capabilities of microbial communities can be similar even if there are distinct taxonomic differences at the ASV level. PICRUSt2 was used to predict the abundance of MetaCyc pathways in each of our samples[34,35]. Antibiotic-treated Jackson mice and Taconic co-housed donors were compared to Jackson and Taconic controls grouped by week to identify the total number of differentially abundant pathways using DESeq2 (**Figure 2C**)[36]. A larger number of differentially abundant pathways indicates that the predicted functional capabilities of the communities are more disparate. Consistent with our prior taxonomic results, by day 9, the number of differentially abundant pathways between co-housed mice and Taconic control mice dropped off precipitously, from over 200 on day 7 to less than 100 on day 9. Mice receiving a cecal transplant didn’t drop below 100 until day 14, while mice receiving a fecal transplant didn’t drop below 100 until day 21.

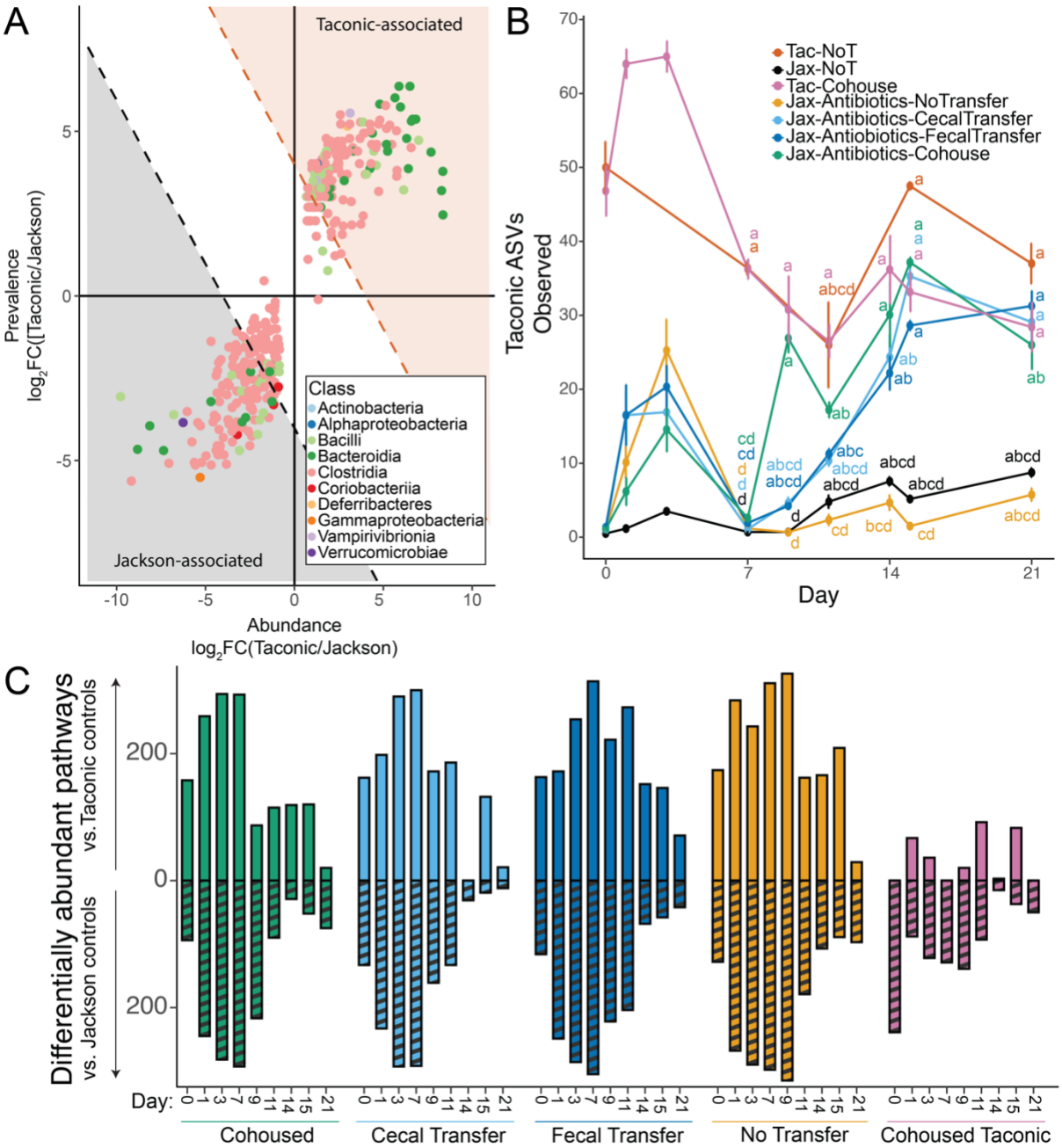


Figure 2. Defining and tracking Taconic-associated ASVs and their predicted functional capacities. (**A**) Bacterial ASVs were defined as being Taconic or Jackson associated based on their prevalence and abundance in samples collected from antibiotics-free mice over the first week of the experiment. ASVs that had an absolute combined $\log_2$(Fold Change) greater than 4 were consider Taconic-associated while those that had an absolute combined $\log_2$(Fold Change) less than -4 were considered Jackson-associated. (**B**) Richness of Taconic ASVs was monitored throughout the course of the experiment, plot show mean richness in each group at a given timepoint, error bars represent the standard error, compact letter display indicates results of pairwise Kruskal-Wallis followed by Bonferroni's multiple comparison correction with an alpha threshold of 0.05. (**C**) Abundance of functional pathways were predicted using PICRUSt2. Bar heights indicate the number of pathways determined to be significantly differentially abundant using DESeq2 (alpha = 0.05) to compare between the fecal bacterial community of Jackson mice treated with antibiotics that received microbial transplantation via co-housing, cecal transfer, fecal transfer, or no transfer, and Taconic co-

housed mice, and the fecal bacterial community of control Jackson and Taconic mice that never received antibiotics, pooled by week (days pooled: [0,7), [7,14), [14,21]).

Transfer of specific bacterial taxa differ between methods of transplantation

Changes in the taxonomic composition of the bacterial community were profiled throughout the course of the experiment. In line with previous results, the microbiota of control communities was relatively stable over time (**Figure 3A**). Bacilli and Gammaproteobacteria noticeably increased in relative abundance in mice that received the antibiotic cocktail by day 3 (**Figure 3A**), although the overall bacterial load (**Figure 1B**) and richness remained low (**Figure 1C**).

By day 9, two days after beginning microbial transfer, co-housed mice were significantly enriched for Taconic ASVs belonging to the *Tannerellaceae*, *Prevotellaceae*, and *Muribaculaceae* families, as well as for *Lactobacillaceae* ASVs that were not statistically associated with the original Jackson or Taconic communities, relative to mice that did not receive transplantation (**Figure 3B**). Mice that received cecal or fecal transplant were also enriched for these *Lactobacillaceae* ASVs at day 9, along with *Enterobacteriaceae* ASVs that were not associated with either supplier, relative to mice that did not receive a transplant. *Bacteroidaceae* ASVs associated with the original Taconic communities were also enriched at day 9 in mice that received fecal or cecal transplants. The *Enterobacteriaceae* ASVs were significantly enriched in mice receiving cecal transplant compared to co-housed mice, while co-housed mice were significantly enriched for *Muribaculaceae* ASVs.

Taconic-associated ASVs from the *Muribaculaceae* family were significantly enriched at day 15 in all mice that received a microbiota transfer, regardless of transplantation method, compared to mice that did not receive transplantation (**Figure 3C**). Taconic *Bacteroidaceae* ASVs were significantly enriched in mice that received a cecal transplant while Taconic-associated *Akkermansiaceae* ASVs were significantly enriched in mice that received a fecal transplant relative to the 'no transfer' group. Taconic-associated *Akkermansiaceae* ASVs were also significantly enriched in mice receiving fecal transplant compared to co-housed mice. Jackson-associated *Erysipelotrichaceae* ASVs were the first to recover in antibiotic treated mice that did not receive a transplant (**Figure 3C**). By day 15, these bacteria were significantly enriched relative to mice that were co-housed, or mice that received a fecal or cecal transplant.

By day 21, significant differences in bacterial taxa enrichment were observed across groups. Taconic *Staphylococcaceae* ASVs were significantly enriched in the 'no transfer' group relative to co-housed mice and also enriched in the fecal transplant group relative to co-housed mice. Taconic *Lactobacillaceae* ASVs were enriched in the 'no transfer' group relative to co-housed mice. Notably, *Christensenellaceae* ASVs, which were not associated with either supplier, were significantly enriched in the 'no transfer' group compared to all transplantation groups. Additionally, several Jackson-associated taxa, including *Ruminococcaceae*, *Lachnospiraceae*, *Erysipelotrichaceae*, *Clostridiaceae*, and *Acholeplasmataceae*, were enriched in the 'no transfer' group relative to all transplant groups. These findings highlight that by day 21, the 'no transfer' group retained significant enrichment of Jackson-associated and non-supplier-specific taxa, while *Taconic*-associated taxa remained prominent in transplanted groups. The fecal transplant group exhibited a distinct enrichment pattern, including higher levels of *Staphylococcaceae*, relative to co-housed mice.

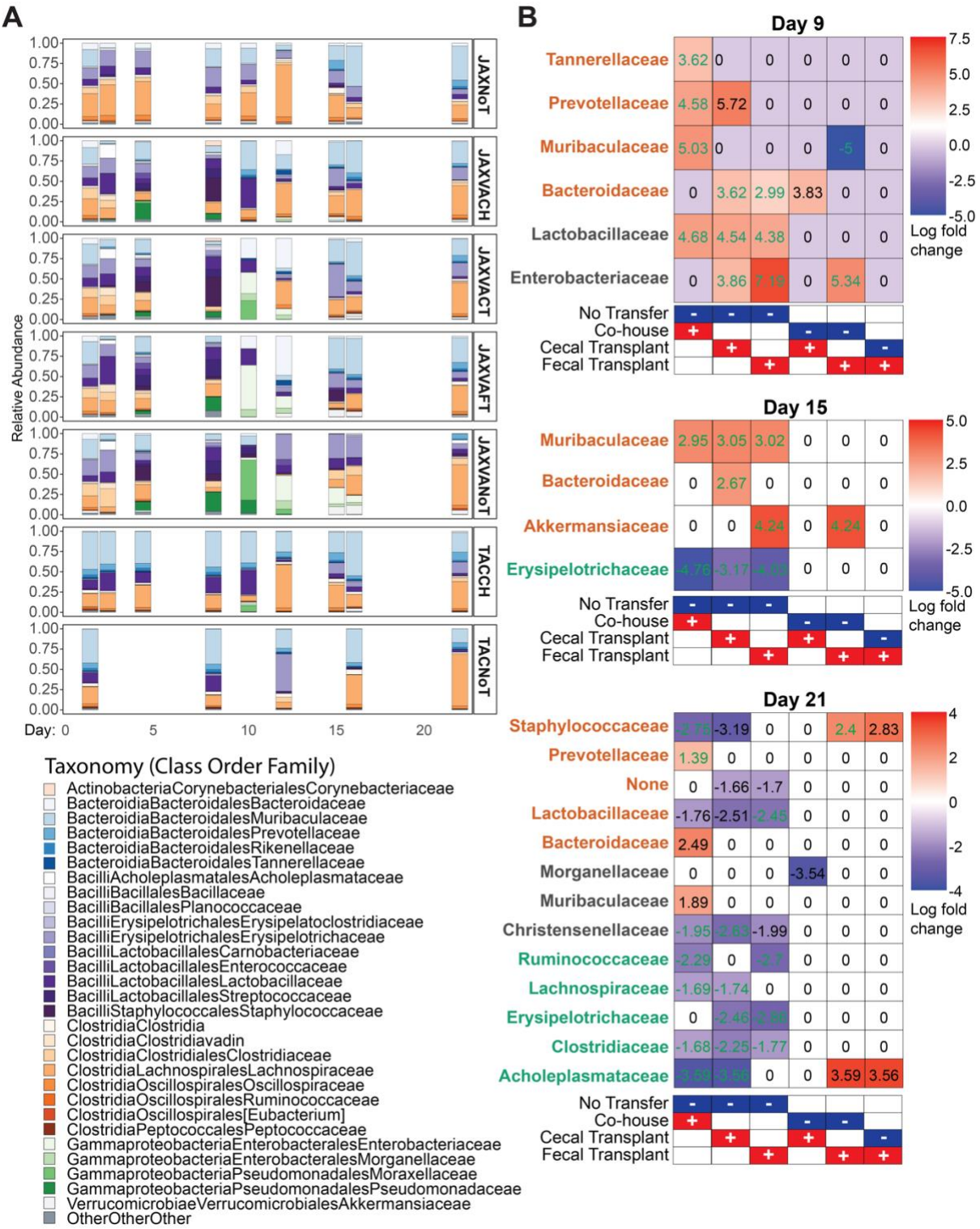


Figure 3. Specific bacterial taxa are enriched across transplantation methods (A) Relative abundance of bacterial families throughout the course of the experiment in each of the treatment groups. (**B**) Bacterial families associated with original bacterial communities of mice from Taconic (orange), Jackson (green) or neither (black) that were significantly enriched or depleted for the comparisons shown below each plot as determined using ANCOM-BC2. Panels show results from days 9, 15, and 21. Log fold change values in green were significant after applying a stringent mixed directional FDR control.

Bacterial diversity and community composition differ across gastrointestinal sites but not the method of transfer

At the end of the experiment, bacterial diversity and community composition were assessed across different gastrointestinal sites: cecal, ileal, and stool samples. Observed bacterial diversity varied significantly across these sites and treatment groups, with lower richness in ileal contents and mice that did not receive microbiota transfer following antibiotic treatment (**Figure 4A**). Bacterial richness in the stool of mice that received transplantation trended higher than Jackson controls and lower than Taconic controls, suggesting incomplete transfer of bacterial taxa across all three methods, although richness was significantly higher than in mice that were treated with antibiotics without transplantation. Principal coordinate analysis of Unweighted UniFrac distances further illustrated the community composition differences, with samples clustering according to treatment groups and gastrointestinal sites (**Figure 4B**). Axis 1 and Axis 2 explained 19.8% and 15.6% of the variance, respectively. The distinct clustering patterns indicated differences in bacterial community composition between the treatment groups. Jackson mice that received antibiotics and no subsequent microbial transplantation clustered together in the upper right quadrant. Ileal and stool samples from mice that received a microbiota transfer resembled control communities with samples from the same gastrointestinal site clustering together. To compare community compositions across treatments, PCoA was performed on samples subset by the gastrointestinal site (**Figures 4C-E**). JAX mice that received antibiotics, but no microbial transplant harbored distinct microbial communities. Mice that received transplantation tended to cluster together, with differences between mice that received transplants or Taconic co-housed donors and untreated Jackson controls being most pronounced in cecal and ileal contents (**Figures 4D, E**). Additionally, the ileal bacterial communities of mice that received fecal or cecal transplants clustered closely together and were distinct from either co-housed group (**Figure 4E**), a difference that was not as pronounced in stool or cecal samples (**Figures 4C, D**). These findings provided insight into the dynamics of the re-establishment of bacterial communities at gastrointestinal sites across methods of microbiota transplantation.

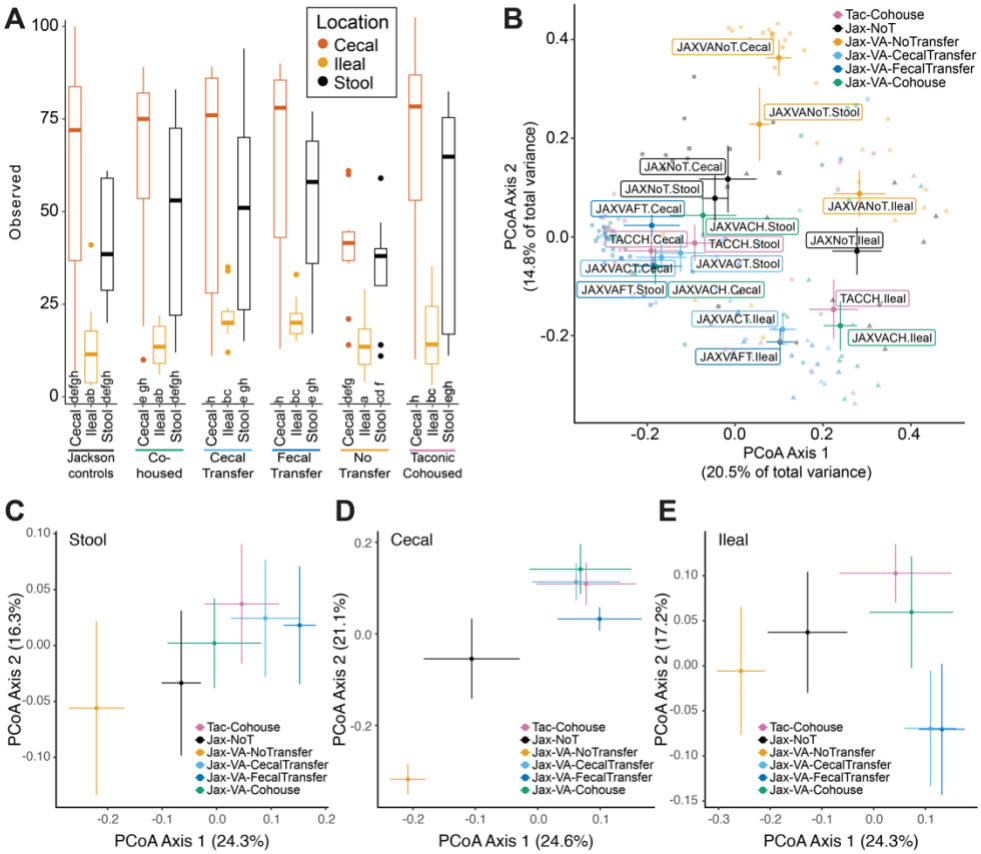
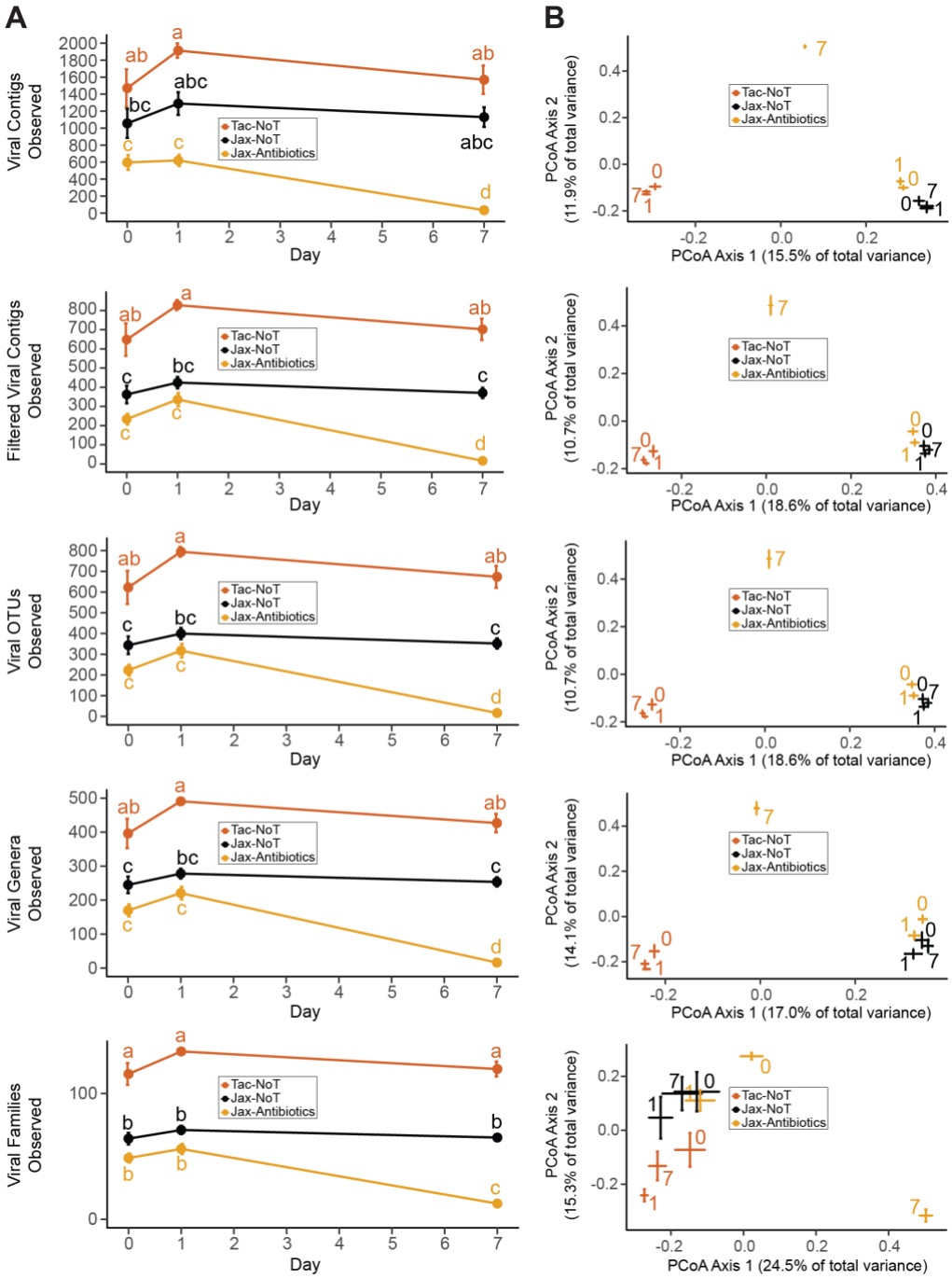


Figure 4. Bacterial diversity and community composition across different body sites and treatment groups (**A**) Bacterial richness, the number of bacterial taxa observed in cecal, ileal, and stool samples across different groups, including Jackson controls, co-housed, cecal transfer, fecal transfer, no transfer, and Taconic controls. (**B**) Plot of Principal Coordinate Analysis (PCoA) of Unweighted UniFrac Distances illustrating bacterial community composition from cecal, ileal, and stool samples, across each method of transplantation, including Jackson controls and cohoused Taconic donors. PCoA of Unweighted UniFrac Distances of samples on Day 21 across treatment groups from (**C**) stool, (**D**) cecal, or (**E**) ileal contents.

Viral Community Dynamics Post-Antibiotics

We next sought to characterize the viral response to antibiotic treatment, given the significant reduction in bacterial load and diversity observed over the first week of the experiment (**Figures 1B, C**). Short-read shotgun sequencing of DNA purified from viral-like particles was performed to evaluate the impact of antibiotic treatment on viral communities. Viral richness (the number of unique contigs assembled from shotgun sequencing data) from days 0 to 7 was compared between Taconic and Jackson controls and Jackson mice that received antibiotics during the first week of the experiment. Read counts were normalized across sequencing libraries to mitigate batch effects. The results showed a significant reduction in viral richness following antibiotic administration, with richness dropping from 596 ± 84 (mean

± SE) unique viral contigs on day 0 to 34 ± 2 (mean ± SE) on day 7 (**Figure S2A**). The same pattern was observed when contigs were subset to only those with clear viral signatures, and when aggregated into clusters representing viral species (OTUs), genera, and families (**Figure S2A**). The reduction in viral richness highlights the susceptibility of viral populations to disruptions in the bacterial community, likely due to their dependence on bacterial hosts. A marked shift in viral community composition, driven primarily by the antibiotic treatment, was observed in PCoA of Jaccard distances (**Figure S2B**). The separation along axis 1 was predominantly between antibiotic-treated and untreated groups, underscoring the substantial impact of antibiotics on viral community composition. Again, similar results were obtained when considering only high-quality viral contigs, or different taxonomic ranks (**Figure S2B**).



Supplemental Figure 2. One-week treatment with antibiotic cocktail significantly alters fecal viral richness and community composition. (**A**) Viral richness and (**B**) Principal Coordinate Analysis of Jaccard distances calculated from contig read counts of unique viral contigs observed per mouse, throughout the first week of the experiment via shotgun metagenomics sequencing of viral-like particles from feces. Panels represent data before (i) and after contigs had been stringently filtered and grouped into clusters representing ii) strains, iii) species/OTUs, iv) genera, and v) families. In A, the dot indicates the mean richness for that group, error bars (± SE), in B, the error bars (± SE) intersect at the centroid of all samples collected for each group of mice on the day indicated by the number on the plot. In A, the letters next to each point indicate significant groupings, those with the same letter were not significantly different from one another by two-way ANOVA followed by Tukey's HSD post-hoc test for pairwise comparisons with an alpha of 0.05.

Viral Community Dynamics Post-Transplantation

To assess the impact of different microbiota transfer methods on viral communities in antibiotic-treated mice, we conducted a comparative analysis of viral richness and community composition following the various transplantation approaches described previously. Viral communities were sampled from days 7 to 14 to capture the reestablishment of viral populations post-transplantation.

Viral richness, as measured by the number of unique viral contigs observed, did not show a uniform increase across all groups immediately after the cessation of antibiotics. By day 9, the co-housed mice (Jax-Antibiotics-Cohouse) exhibited a more rapid increase in viral richness compared to the other groups, and their viral richness was not significantly different from that of mice from Taconic (**Figure 5A**). Viral richness remained relatively low in mice that received fecal or cecal transfers, or no transfer at this timepoint. Viral richness increased in all groups by day 11, although all groups that received transplantation had lower viral richness than Taconic donors, this difference was not significant. Viral richness in mice that did not receive a microbial transplant (Jax-Antibiotics-NoTransfer) remained significantly lower and only marginally increased by day 11. By day 14, viral richness levels in all transfer groups converged and became similar to those in untreated controls, indicating successful colonization.

We then evaluated the impact of the transplantation methods on viral community composition, visualized using Principal Coordinate Analysis (PCoA) of Jaccard distances. The results showed a gradual alignment of the viral communities in fecal and cecal transplanted mice (Jax-Antibiotics-FecalTransfer and Jax-Antibiotics-CecalTransfer) with those of the Taconic donor community profiles between days 9 and 11, with full convergence observed by day 14 (**Figure 5B**). Notably, viral communities in co-housed mice (Jax-Antibiotics-Cohouse) rapidly converged with the donor profiles by day 9, indicating that co-housing facilitated a more efficient and quicker viral transfer. On the other hand, viral communities in mice that did not receive any transplant (Jax-Antibiotics-NoTransfer) showed a delayed recovery, remaining

distinct from both donor and control profiles until the final day of sampling, gradually approaching the composition of the original Jackson community by day 14.

These findings suggest that the method of microbiota transplantation plays a crucial role in the reestablishment of viral communities. Co-housing was the most effective approach for rapid and complete viral transfer, while fecal and cecal transplants also facilitated successful viral colonization, albeit at a slower pace. Mice that did not receive a microbial transplant exhibited a delayed and incomplete recovery of viral communities, highlighting the importance of external microbial inputs for restoring gut virome diversity and composition after antibiotic treatment.

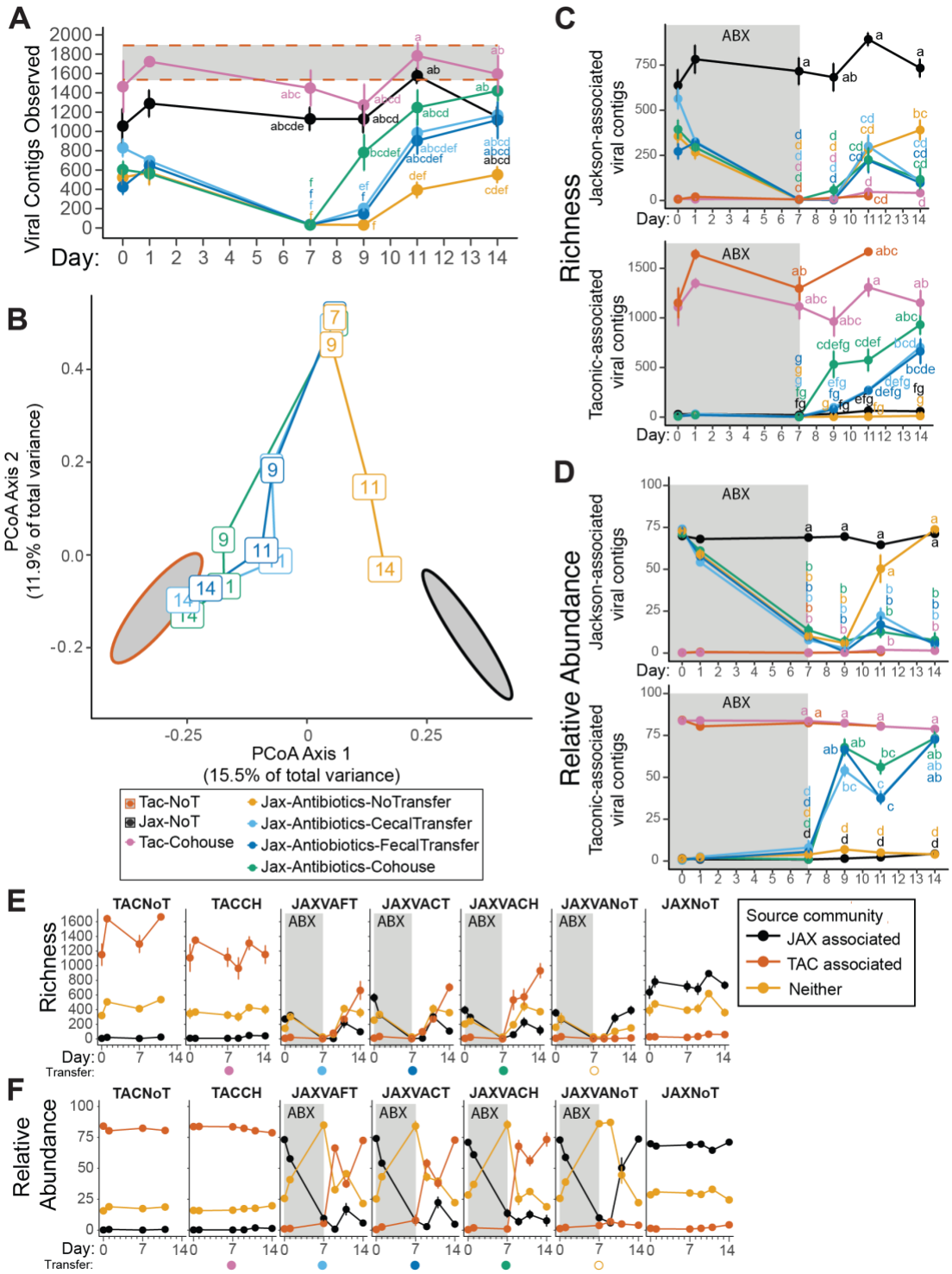


Figure 5. Cohousing of mice reconstitutes the viral microbiota more rapidly than fecal or cecal transplant (**A**) Viral richness from days 7 through 14 of the experiment shows the rapid recovery of viral diversity following transplantation. The letters next to each point indicate significant groupings, those with the same letter were not significantly different from one another by two-way ANOVA followed by Tukey's HSD post-hoc test for pairwise comparisons with an alpha of 0.05 (**B**) Principal Coordinate Analysis of Jaccard distances calculated from contig read counts across two weeks of the experiment illustrate the change in community composition following antibiotic treatment and microbiota transplantation. Ellipses (95% confidence) represent the baseline fecal viral communities of untreated Jackson (Jax-NoT) and Taconic (Tac-NoT) mice. (**C**) Richness and (**D**) relative abundance of viral contigs defined as (i) Jackson or (ii) Taconic-associated throughout the course of the

experiment. The letters next to each point indicate significant groupings, those with the same letter were not significantly different from one another by two-way ANOVA followed by Tukey's HSD post-hoc test for pairwise comparisons with an alpha of 0.05. The (**E**) richness and (**F**) relative abundance of viral contigs associated with mice from Jackson, Taconic, or neither, over the first two weeks of the experiment.

Transfer of Taconic-associated viruses to Jackson mice

To track the movement of viruses between Taconic and Jackson mice, viral contigs were defined as being associated with mice from Taconic, Jackson, or neither, based on their differential prevalence and abundance in samples collected over the first week of the experiment from mice that did not receive antibiotics (n = 34 Taconic samples, 50 Jackson samples). Of the 6,249 viral contigs, 2,535 were defined as Taconic-associated while 1,129 were defined as Jackson-associated.

The richness and relative abundance Jackson-associated viruses significantly decreased in response to antibiotic treatment (**Figures 5C-F**). Throughout the experiment, Taconic and Jackson control mice maintained steady viral richness and relative abundance (**Figures 5C-F**), with Taconic mice harboring a greater number of unique viral genome fragments compared to Jackson controls (**Figures 5C, E**).

Following microbiota transplantation, the richness and relative abundance of Taconic-associated viruses in co-housed Jackson mice reached levels similar to Taconic donors by day 9, with no significant differences observed between these groups (**Figures 5C,D**). Additionally, by day 9, two days post-transplantation, the relative abundance of Taconic-associated viruses in mice that received a fecal transplant was not significantly different from the donor community (**Figure 5D**), although the viral community remained significantly less diverse (**Figure 5C**). However, by day 11, four days after transplantation, the relative abundance of Taconic-associated viruses decreased in mice that received fecal or cecal transplants, falling to levels significantly lower than those of Taconic donors (**Figures 5D, F**). In contrast, the relative abundance of Taconic-associated viruses in co-housed mice remained relatively stable during this period (Figure 3F). This drop in the relative abundance of Taconic-associated viruses in the fecal and cecal transplant groups

was accompanied by a slight resurgence of Jackson-associated viruses on day 11 (**Figures 5D, F**). However, this rebound was not sustained, except in Jackson mice that did not receive any transplantation (JAXVANoT), where Jackson-associated viruses persisted (**Figures 5D, F**).

Despite only receiving a single gavage of fecal or cecal contents, the number of unique viral genome fragments in transplanted mice continued to increase, with detectable levels of Taconic-associated viruses rising even after one week (Figures 3C,E). Although increasing, the richness of Taconic-associated viruses in Jackson mice that received cecal or fecal transplants remained significantly different from that of Taconic donors one week after gavage, on day 14 of the experiment (**Figures 5C, E**).

Meanwhile, viral diversity in Jackson mice that received antibiotics, and no subsequent transplantation (JAXVANoT) remained depleted throughout the experiment (through day 14), even though the relative abundance of Jackson-associated viruses returned to levels similar to those observed in untreated Jackson controls and day 0 samples by day 11, four days after cessation of antibiotics (**Figures 5C-F**). This suggests a long-lasting impact of antibiotic treatment on the viral diversity in mice that did not receive microbial transplants, despite the partial recovery of Jackson-associated viral abundance.

These results highlight the complexity of viral community dynamics following microbiota transplantation, with co-housing proving to be the most effective method for the rapid restoration of Taconic-associated viral communities. Although fecal and cecal transplants also facilitated the colonization of Taconic viruses, the process occurred more slowly and less consistently, particularly in terms of viral richness and abundance.

Discussion

This study builds upon and expands existing literature on microbiota transplantation by providing a detailed comparison of co-housing, fecal transplantation, and cecal transplantation as methods for reconstituting bacterial and viral communities in antibiotic-treated mice[22,23,31,37]. These findings are particularly relevant for improving experimental methods and enhancing the reproducibility of studies relying on mouse models to investigate

host-microbe interactions. Understanding the strengths and limitations of each transplantation approach is critical for optimizing experimental designs and ensuring robust results in microbiome research.

Mouse models are a cornerstone of microbiome research, offering a manipulatable platform for studying the effects of microbial communities on host physiology. Transplantation of donor microbiota into recipient mice allows researchers to examine how microbial composition influences health and disease, yet there remains a surprising lack of systematic comparisons of commonly used transfer methods. This study addresses this gap by assessing the efficiency, dynamics, and ecological outcomes of three widely used microbiota transfer techniques.

Our results demonstrate that co-housing is the most effective method for microbiota transfer, achieving rapid and complete reconstitution of both bacterial and viral communities. This efficiency likely arises from sustained microbial exchange through natural behaviors such as coprophagy and direct contact, which mirror ecological interactions in natural settings. These findings align with previous studies showing that co-housing facilitates the transfer of donor-associated microbial taxa more robustly than single-dose gavage methods (e.g., fecal or cecal transplantation)[31,38]. Importantly, the rapid reconstitution of microbial diversity and functionality observed in co-housed mice underscores its utility in experimental designs requiring time-sensitive microbiota establishment.

Fecal and cecal transplantation, while less efficient than co-housing in the short term, ultimately achieved comparable microbial diversity and functional outcomes by day 21. These methods may still hold value in experimental scenarios where co-housing is impractical, such as those requiring strict separation of donor and recipient populations or where there are insufficient numbers of donor mice. However, the slower pace of microbial establishment with these methods highlights the importance of considering time frames and endpoints when designing experiments. Our findings emphasize the need for researchers to select transplantation methods that align with the specific goals of their studies to optimize reproducibility and minimize variability.

Mice that did not receive microbial transplantation exhibited delayed and incomplete recovery of their microbiota, with bacterial and viral communities remaining distinct from donor or untreated controls even after 21 days. Although incomplete, with reduced microbial richness, the recovery observed highlights the resiliency of the gut microbiota. Without transplantation, the limited natural recolonization observed in our study suggests that native microbial recovery is insufficient to restore microbiota diversity to pre-treatment levels. These findings are particularly relevant for studies investigating the long-term impacts of microbiota depletion or the role of microbial diversity in shaping host outcomes, as the choice to include transplantation can significantly influence experimental results.

The bacterial and viral dynamics observed in this study were closely interdependent, reflecting the ecological relationships between bacteriophages and their bacterial hosts. Co-housed mice exhibited rapid establishment of donor-associated bacterial taxa, such as *Muribaculaceae* and *Tannerellaceae*, alongside their associated phages. This synchronized reconstitution underscores the importance of ecological interactions in microbial recovery and suggests that co-housing provides a more naturalistic environment for microbial community establishment. Notably, temperate phages demonstrated greater resilience to antibiotic treatment and were more effectively transferred during co-housing, consistent with their genomic integration into host bacteria and replication during bacterial colonization.

The site-specific variation in microbial recovery observed across gastrointestinal regions further highlights the complexity of microbiota reconstitution. While co-housing facilitated the most uniform recovery across sites, the distinct differences in microbial diversity and composition between ileal, cecal, and fecal samples reinforce the importance of sampling multiple gastrointestinal regions to fully capture the dynamics of microbiota transplantation. These findings are consistent with previous work demonstrating anatomical differences in microbial communities and their functional capacities.

From an experimental perspective, our study provides critical insights into best practices for microbiota transplantation in mouse models. Co-housing emerges as the most effective method for achieving rapid and complete microbial transfer, making it a valuable

approach for experiments requiring a robust and naturalistic establishment of donor microbiota. However, researchers should weigh the logistical and experimental constraints of co-housing, such as the need for sustained contact between donor and recipient animals, against its benefits. Fecal and cecal transplantation, while slower to achieve microbial reconstitution, remain valuable alternatives for experiments with shorter time frames or constraints on co-housing feasibility. The results of this study underscore the importance of tailoring transplantation methods to the specific requirements of individual studies to maximize reproducibility and reliability.

The results also emphasize the growing need for systematic evaluations of transplantation methods across different experimental contexts. Future studies should investigate how factors such as donor-recipient compatibility, microbial complexity, and host genotype influence the outcomes of microbial transplantation. Additionally, while this study focused on bacterial and viral communities, future work should incorporate other microbial taxa, such as fungi and archaea, to provide a more comprehensive understanding of microbiota dynamics. Advances in functional and longitudinal metagenomic analyses, coupled with improved animal models, will further enhance our ability to study the mechanisms underlying microbiota establishment and its implications for host health and disease.

By providing a systematic comparison of microbiota transplantation methods, this study contributes to the refinement of experimental approaches in microbiome research and highlights the ecological and functional dynamics underlying microbial reconstitution. These insights are essential for improving the reproducibility and translational potential of microbiota studies, ultimately advancing our understanding of the microbiome's role in health and disease.

Materials and Methods

Mice

WT C57BL/6J (stock no. 000664) mice were purchased from Jackson Laboratories (JAX) and C57BL/6N (stock #B6), murine pathogen free health standard, mice were purchased from Taconic Biosciences (TAC) and maintained at Washington University School of Medicine under specific-pathogen-free conditions according to University guidelines. Female mice, aged six to eight weeks, were exclusively used in all experiments to facilitate cohousing experiments. Experimental mice were co-housed with up to two mice of the same sex per cage with autoclaved standard chow pellets and water provided *ad libitum*.

Cages of female mice were treated at 6 weeks of age with an antibiotic cocktail VNA [1 g/L ampicillin, 1 g/L neomycin, 0.5 g/L vancomycin (Sigma, St. Louis, MO)] in drinking water for 7 days.

16S rRNA gene Illumina sequencing

Phenol:chloroform-extracted DNA from fecal pellets was used for both 16S rRNA gene qPCR and sequencing. SYBR green qPCR for the 16S rRNA gene was performed with 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 805R (5'-GACTACCAGGGTATCTAATCC-3') primers to detect the V4 hypervariable region.

Primer selection and PCRs were performed as described previously [14]. Briefly, each sample was amplified in triplicate with Golay-barcoded primers specific for the V4 region (F515/R806), combined, and confirmed by gel electrophoresis. PCR reactions contained 18.8μL RNase/DNase-free water, 2.5μL 10X High Fidelity PCR Buffer (Invitrogen), 0.5μL 10 mM dNTPs, 1μL 50 mM MgSO4, 0.5μL each of the forward and reverse primers (10 μM final concentration), 0.1μL Platinum High Fidelity Taq (Invitrogen) and 1.0μL genomic DNA. Reactions were held at 94°C for 2 min to denature the DNA, with amplification proceeding for 26 cycles at 94°C for 15s, 50°C for 30s, and 68°C for 30s; a final extension of 2 min at 68°C was added to ensure complete amplification. Amplicons were pooled and purified with 0.6x Agencourt Ampure XP beads (Beckman-Coulter) according to the manufacturer's instructions.

The final pooled samples, along with aliquots of the three sequencing primers, were sent to the Center for Genome Sciences (Washington University School of Medicine) for sequencing by the 2x250bp protocol with the Illumina MiSeq platform.

The resulting FASTQ files were processed using the DADA2 pipeline (v1.28.0) implemented in Nephele (version 2024_Jan_16, commit: 71b6a72)[32]. Initial quality assessment was conducted to determine appropriate truncation and filtering parameters. Reads were trimmed to remove low-quality bases and sequencing primers and truncated based on quality scores to ensure high-confidence base calls[39,40]. Specifically, reads were truncated at the position where the quality score dropped below a threshold (default: 4)[41,42]. Reads with expected errors exceeding a set threshold (default: 5) were discarded to further enhance data quality. Forward and reverse reads were merged, requiring a minimum overlap of 12bp and allowing for zero mismatches in the overlapping region to ensure accurate reconstruction of the target amplicon[43,44]. Chimeric sequences were identified and removed using the consensus method to prevent false-positive operational taxonomic units (OTUs).

Taxonomic classification was performed using the Ribosomal Database Project (RDP) classifier, with a minimum bootstrap confidence threshold of 40 for taxonomic assignments and the SILVA v138.1 database[45]. Additionally, a phylogenetic tree was constructed using MAFFT for multiple sequence alignment and FastTree2 for tree building, facilitating downstream phylogenetic analyses[46,47]. The output included an amplicon sequence variant (ASV) table, taxonomic assignments, and phylogenetic relationships, providing a comprehensive dataset for subsequent diversity and composition analyses. All analyses were conducted using default parameters unless otherwise specified, adhering to best practices for 16S rRNA gene sequence processing to ensure accurate and reproducible results.

Bacterial ecological and distance analyses

Bacterial community composition and ecological metrics were assessed using both alpha and beta diversity analyses. Alpha diversity metrics, including observed richness and Shannon diversity, were calculated to evaluate within-sample diversity. Beta diversity was assessed using unweighted and weighted UniFrac distances to capture phylogenetic

differences between samples. These analyses were implemented using the 'phyloseq' and 'vegan' R packages, which offer comprehensive tools for ecological and diversity analyses. Principal coordinate analysis (PCoA) was performed to visualize patterns in beta diversity across treatment groups and time points[48,49]. Pairwise comparisons between groups were conducted using PERMANOVA with Bonferroni corrections to identify statistically significant differences (alpha = 0.05). These analyses provided insights into the ecological shifts in bacterial communities associated with different transplantation methods and recovery stages. All figures were generated in R using ggplot2[50].

Identifying bacterial taxa associated with each vendor

Bacterial taxa specific to each vendor were identified using amplicon sequence variants (ASVs) derived from 16S rRNA gene sequencing. Taxa were classified as Jackson-associated or Taconic-associated based on their differential prevalence and abundance in fecal samples collected from untreated mice during the first week of the experiment (days 0-7). Samples were limited to those without antibiotic treatment to accurately capture baseline community differences. Differential abundance analyses were conducted using the DESeq2 pipeline, with ASVs assigned taxonomy using the SILVA database. Significant differences were determined using an adjusted p-value threshold of 0.01 and a combined log2(fold change) cutoff of ±4. Prevalence was calculated as the proportion of samples in which a given ASV was detected within each vendor group. To incorporate abundance and prevalence, a combined fold-change metric was calculated by summing the log2(fold change) from differential abundance analysis and the log2(prevalence ratio) between the two groups. Statistical and prevalence-based analyses were compared to confirm associations, and taxa that did not meet these criteria were categorized as non-supplier-specific.

Predicting functional capacity of bacterial communities

Functional capacities of bacterial communities were inferred from 16S rRNA gene sequencing data using the PICRUSt2 (v. 2.5.3) pipeline. ASVs were mapped to predicted genes and metabolic pathways using the MetaCyc database. Differentially abundant pathways were identified using DESeq2 with an alpha threshold of 0.05. Comparisons were made

between antibiotic-treated recipient mice (co-housed, fecal transplantation, or cecal transplantation groups) and control communities from untreated Jackson and Taconic mice. Functional convergence over time was assessed across treatment groups to evaluate the recovery of microbial metabolic potential.

Taxonomic changes following transplantation

Changes in bacterial taxonomic composition were assessed longitudinally using 16S rRNA gene sequencing. ASVs were classified into taxonomic families, and relative abundances were tracked over time in recipient mice across different transplantation methods (co-housing, fecal, and cecal). Differentially enriched taxa were identified using ANCOM-BC2 (v. 2.6.0), and significant changes were determined using Kruskal-Wallis tests with Bonferroni corrections for multiple comparisons (alpha = 0.05). Taxonomic dynamics were visualized using stacked bar plots and principal coordinate analyses (PCoA) of unweighted UniFrac distances.

Virome Illumina sequencing and analysis.

Viral communities were characterized using short-read Illumina sequencing of viral-like particles (VLPs) extracted from fecal samples. DNA was purified from VLPs, and libraries were prepared using standard protocols. Sequencing was performed on an Illumina platform, generating paired-end reads. Reads were assembled into contigs using Flye, which is optimized for accurate assembly of viral genomes from metagenomic data. Viral contigs were identified using stringent criteria for viral hallmark genes and annotated taxonomically using Cenote-Taker2, a tool designed for comprehensive identification and annotation of eukaryotic and prokaryotic viruses. Contigs were clustered into operational taxonomic units (OTUs) representing viral strains, species, and higher taxonomic ranks. Viral richness and relative abundances were quantified for each treatment group over time, providing insights into virome dynamics and recovery across experimental conditions.

Viral ecological and distance analyses

Viral community composition and ecological dynamics were assessed using alpha diversity metrics (e.g., richness) and beta diversity measures (e.g., Jaccard distances). These

analyses were implemented using the ‘phyloseq’ and ‘vegan’ R packages, which provide robust tools for microbial ecological analyses. Principal coordinate analysis (PCoA) was conducted to visualize temporal changes in viral community structure across treatment groups. Pairwise comparisons between groups were performed using PERMANOVA with Bonferroni corrections for multiple comparisons (alpha = 0.05). Viral communities were further classified as Jackson-associated, Taconic-associated, or non-supplier-specific based on their prevalence and abundance in control communities. Ecological metrics were integrated with bacterial data to explore host-virus interactions during microbiota transplantation. Source discriminatory viral contigs were defined using the combined fold-change metric described for bacteria above.

Acknowledgments: We acknowledge all members of the Baldridge laboratory for helpful discussions as well as member of June Round's laboratory.

Funding: This work was supported by the National Institutes of Health (NIH) grants R01AI139314 and R01AI173360 (M.T.B.), and the Crohn's and Colitis Foundation Litwin IBD Pioneers Award #1065897 (M.T.B.). E.A.K. was supported by NSF grant DGE-1745038/DGE-2139839 and NIH grant F31AI167499. J.S.W. was supported by NIH T32AI007172.

Author contributions: J.S.W performed data analysis generated figures and wrote the manuscript. M.E.S., H.M., S.A., M.H., E.A.K., L.S. and L.F. performed the experiments. R.R. and M.T.B. analyzed the results. M.E.S., H.M., S.A., and M.T.B. designed the project. L.A.C.C and A.R contributed analysis of viral data. M.T.B. contributed to the writing of the manuscript. All authors read and edited the manuscript.

Competing interests: We declare no competing interests.

Data and materials availability: The data from this study are tabulated in the main paper and supplementary materials. All reagents are available from M.T.B. under a material transfer agreement with Washington University.

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