

Early postoperative microbiota transplant therapy is associated with coordinated microbial, metabolic, and immune recovery dynamics following intestinal surgery

Alexander Troester

University of Minnesota

Julia Frebault

University of Minnesota

Max Hill

University of Minnesota

Parthasarathy Rangarajan

University of Minnesota

Sonja Boatman

University of Minnesota

Harika Nalluri-Butz

University of Minnesota

Amy J. Thorsen

University of Minnesota

Paolo Goffredo

University of Minnesota

Wolfgang B. Gaertner

University of Minnesota

Byron Vaughn

University of Minnesota

Abigail J. Johnson

Riddet Institute, NEW ZEALAND

Levi Teigen

University of Minnesota

Adam Herman

University of Minnesota

Alexander Khoruts

University of Minnesota

Geetha Saarunya

University of Minnesota

Sabarinathan Ramachandran

University of Minnesota

Christopher Staley

University of Minnesota

Cyrus Jahansouza

jahan023@umn.edu

University of Minnesota

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Abstract

Background

Microbiota disruption following colorectal surgery contributes to impaired gastrointestinal recovery and postoperative inflammation. Perioperative interventions essential for surgical safety, including mechanical bowel preparation and antibiotic prophylaxis, profoundly suppress microbial community diversity and fermentative metabolic activity, creating a window of ecological vulnerability during the early postoperative period. Despite growing recognition of the microbiome's role in postoperative physiology, including its contributions to epithelial repair, immune regulation, and gastrointestinal motility, no standardized strategies exist to restore gut microbial composition and function after surgery. Here, we evaluated the safety, feasibility, and exploratory biological effects of early postoperative microbiota transplant therapy (MTT) following colorectal surgery.

Results

In this phase I clinical study, twelve patients undergoing sigmoid colectomy for diverticular disease or colon cancer received MTT via nasojejunal feeding tube on postoperative day 2 and were compared with matched non-MTT patients from a prior cohort undergoing identical surgery. Longitudinal microbiome, metabolomic, and immune profiling identified sustained donor-attributed microbial community similarity at 16S resolution and recovery-associated increases in fecal short-chain and branched-chain fatty acids by postoperative day 14, consistent with restoration of microbial metabolic activity. Exploratory integrated microbiome-metabolite modeling identified a dominant recovery-associated latent axis reflecting shared variation between microbial community structure and metabolite profiles across the postoperative period. Longitudinal immune profiling within MTT recipients demonstrated temporally structured immune remodeling characterized by early innate immune shifts followed by later adaptive immune transitions consistent with immune resolution. Cross-omic integration revealed time-stratified associations linking microbial and metabolic recovery trajectories with staged immune remodeling. No adverse events were attributed to MTT.

Conclusions

Early postoperative MTT was feasible, well tolerated, and associated with exploratory microbial, metabolic, and within-recipient immune recovery dynamics following colorectal surgery. Together, these findings suggest that postoperative recovery may involve temporally aligned microbial, metabolic, and immune dynamics, and that MTT may serve as a useful biological perturbation model for investigating host-microbiome interactions during surgical recovery. Prospective controlled studies are needed to determine whether these observations are MTT-specific and whether microbiome restoration strategies can improve clinical outcomes such as reduced ileus, surgical site infection, or anastomotic complications.

BACKGROUND

Postoperative complications such as ileus, surgical site infection, and anastomotic leak remain major drivers of morbidity, prolonged hospitalization, and healthcare costs following colorectal surgery, despite widespread adoption of Enhanced Recovery After Surgery protocols and standardized perioperative care. [1–3] Although these complications are traditionally considered distinct clinical entities, emerging evidence suggests they share overlapping biological drivers linked to disruption of the gut ecosystem and its downstream effects on inflammation, mucosal repair, and immune regulation.[4] Postoperative ileus, for example, is associated with impaired epithelial recovery and altered gut–brain signaling, processes influenced by microbial metabolites and host–microbiota–immune interactions.[3, 5] Similarly, specific microbial taxa have been implicated in the pathogenesis of surgical site infection and anastomotic leak, including *Enterococcus faecalis*, which promotes collagen degradation, and *Streptococcus* species, which are enriched in postoperative infections and disrupted gut communities. [6–8] Together, these observations identify the gut microbiome and its interaction with host immune responses as central and potentially modifiable determinants of surgical recovery and suggest that postoperative recovery reflects an active process of gut ecosystem reconstitution, raising the possibility that microbiome restoration could be investigated as a strategy to support postoperative gut ecosystem recovery after surgery.

The gut microbiome plays an important role in intestinal healing, immune homeostasis, and gastrointestinal motility, in large part through the production of short-chain fatty acids (SCFAs) that support epithelial integrity, regulate innate and adaptive immune function, and influence enteric neuroimmune signaling.[6, 9–13] However, perioperative interventions essential for surgical safety, including mechanical bowel preparation and antibiotic prophylaxis,[14, 15] can profoundly disrupt microbial community structure and lead to profound suppression of microbial metabolic activity, including loss of fermentative capacity and SCFA production.[16–18] This postoperative dysbiosis is characterized by depletion of SCFA-producing taxa and expansion of opportunistic pathobionts such as *Enterococcus* and *Streptococcus*, patterns that have been associated with delayed gastrointestinal recovery, sustained inflammation, and impaired immune resolution.[6–8] Across human and animal studies, this perioperative ecological collapse has been linked to impaired epithelial healing, weakened colonization resistance, and disrupted neuroimmune signaling critical for gut motility and function.[6, 13, 16, 17, 19–25] Despite growing recognition of the microbiome’s role in postoperative physiology, there are currently no standardized clinical strategies aimed at restoring gut microbial composition, metabolic function, and immune recovery after surgery.

Microbiota transplant therapy (MTT) has demonstrated the capacity to induce durable microbial engraftment and restore metabolic function in other settings of severe dysbiosis, most notably in *Clostridioides difficile* infection.[26–33] These studies provide biologic precedent for microbiome restoration strategies; however, the safety, timing, and biological effects of MTT during the immediate postoperative period following colorectal surgery, and how such intervention reshapes systemic immune responses following surgical injury, remain unknown. Importantly, restoration of gut microbial

communities may not only support reestablishment of fermentative metabolism but also modulate systemic immune responses linked to postoperative inflammation, tissue repair, and recovery, given the established role of microbiota-derived signals in immune programming and resolution. [4, 23]

In this Phase I clinical study, we evaluated the feasibility and biological effects of administering MTT during the early postoperative period following colorectal surgery. MTT was delivered via nasojejunal feeding tube on postoperative day 2, after perioperative antibiotic exposure, leveraging the microbiota-depleted gut as a receptive niche for engraftment.[27, 34] Longitudinal multi-omic profiling was used to explore microbial, metabolic, and immune recovery-associated dynamics following postoperative MTT administration. Together, these findings support the concept that postoperative recovery may involve temporally aligned microbial, metabolic, and immune processes, and suggest that MTT may serve as a mechanistically informative biological probe for investigating microbiome-directed approaches to surgical recovery.

METHODS

Ethics statement

All research was conducted in accordance with the Declaration of Helsinki and relevant ethical regulations. This study was approved by the Institutional Review Board (study ID 00019579) and the Cancer Protocol Review Committee (SURG-2023-29833) at the University of Minnesota. Written informed consent was obtained from all participants prior to enrollment. The trial was registered at ClinicalTrials.gov (NCT06370884). The United States Food and Drug Administration authorized study conduct under an Investigational New Drug application (IND 28152). The investigational microbiota product was provided through the University of Minnesota Microbiota Therapeutics Program (MTP).[35]

Donor Material and Microbiota Product Overview

MTP-101LR consists of cryopreserved microbiota purified from the stool of healthy donors. Donors underwent comprehensive medical history review, physical examination, and serial laboratory testing to exclude infectious diseases, gastrointestinal disorders, metabolic and autoimmune conditions, and risk factors for colonization with multidrug-resistant organisms. Screening included serologic, molecular, and culture-based testing for enteric pathogens and antimicrobial-resistant organisms at defined intervals, consistent with previously standardized fecal microbiota preparation protocols.[35] MTP-101LR was manufactured in accordance with protocols detailed in IND 15071 in a Good Manufacturing Practices facility at the University of Minnesota. Donor microbiota were purified from stool using sequential filtration and washing steps and cryopreserved as a liquid suspension. Each unit contained 7.5×10^{11} bacteria in 35 mL phosphate-buffered saline containing 10% glycerol as a cryoprotectant.[36]

Clinical Study Design and MTT Administration

This single-center, open-label Phase I study evaluated the safety, feasibility, and biological effects of MTT in patients undergoing sigmoid colon resection with primary anastomosis for diverticulitis (n = 9) or sigmoid colon adenocarcinoma (n = 3) at the University of Minnesota Fairview Medical Center between February and November 2024 registered prospectively at ClinicalTrials.gov (NCT06370884). The study was conducted as an exploratory single-arm investigation; the original protocol included a parallel non-MTT arm, which was replaced by matched historical controls from a prior prospective cohort to maximize efficiency and minimize patient burden.

Eligible participants were adults aged 21–75 years scheduled for elective surgery. Exclusion criteria included inflammatory bowel disease, neutropenia (absolute neutrophil count $< 0.5 \times 10^9$ cells/L), prior solid organ or bone marrow transplantation, current immunosuppressive therapy, active chemotherapy or radiation, ostomy creation, pregnancy, or limited life expectancy. This was an open-label study, and no randomization or blinding was performed. No formal power calculation was performed, as the study was designed to assess safety, feasibility, and recovery-associated biological signals rather than clinical efficacy.

All patients received standardized mechanical bowel preparation with oral antibiotics and perioperative intravenous antibiotics per institutional protocol. Oral antibiotics included neomycin and metronidazole, and perioperative intravenous regimens were selected based on allergy status. Given known antibiotic elimination half-lives,[37] MTT was administered 48–52 hours after the final intravenous antibiotic dose, coinciding with anticipated near-complete antibiotic clearance in patients with normal renal function.

On the day of administration, the thawed microbiota suspension was delivered via a post-pyloric nasojejunal feeding tube, followed by saline flush. The feeding tube was subsequently removed, and postoperative diet advancement proceeded per routine clinical criteria.

Sample Size and Analysis Population

The study was designed as an exploratory phase I investigation focused on safety, feasibility, and characterization of recovery-associated biological signals rather than clinical efficacy. The original protocol included a planned parallel non-MTT comparator arm; however, due to limited accrual and logistical considerations, enrollment was completed as a single-arm treated cohort (n = 12). Exploratory comparisons were subsequently performed using a matched historical non-MTT cohort derived from prior prospective studies conducted at the same institution under similar perioperative protocols.[16, 17] Use of historical comparator samples and associated analyses was approved by the Institutional Review Board. No formal power calculation was performed. Safety analyses were descriptive and included all treated participants.

Sample Collection

Fecal samples were collected within two weeks prior to surgery (baseline), during the early inpatient postoperative period, and on POD 14, 30, 90, and 180. Samples were stored at – 20°C following collection and transferred on ice for long-term storage at – 80°C until analysis.

Peripheral blood samples were collected at baseline and on POD 1, 2, 3, 14, 30, 90, and 180. Aliquots were reserved for longitudinal immune profiling, and routine clinical laboratory testing was performed at each time point. Analyses were performed on all available samples at each timepoint; no imputation was performed, and sample sizes are reported per comparison throughout. Stool sample completeness exceeded 95% across all scheduled postoperative timepoints, with one MTT recipient missing stool samples at POD30 and POD90. Immune profiling samples were available from all MTT recipients across all scheduled blood collection timepoints.

Fecal DNA Extraction and 16S rRNA Gene Sequencing

DNA was extracted from fecal samples using a standardized commercial kit optimized for inhibitor removal. The V4 hypervariable region of the 16S rRNA gene was amplified using the 515F/806R primer set and sequenced using dual-indexed 2 × 300 bp paired-end reads on the Illumina MiSeq or Element AVITI platforms, as previously described.[38] Negative extraction and amplification controls were included in all sequencing runs and did not yield detectable amplicons. Raw data were stored in the Sequence Read Archive under BioProject accession number SRP533899.

Fecal Short-Chain and Branched-Chain Fatty Acid Quantification

Fecal short-chain and branched-chain fatty acids were quantified using gas chromatography–mass spectrometry (GC–MS) as previously described.[39, 40] Quantified metabolites included acetate, propionate, butyrate, valerate, isobutyrate, isovalerate, and 2-methylbutyrate. Detailed sample preparation, calibration, and quality control procedures are provided in the Supplementary Methods.

Microbiome Bioinformatics and Statistical Analysis

Amplicon sequence data were processed using mothur [41] following a previously published pipeline.[42] Reads were quality-filtered, aligned to the SILVA reference database,[43] preclustered,[44] and screened for chimeras.[45] Operational taxonomic units were assigned using OptiClust,[46] and taxonomic classification was performed against the Ribosomal Database Project database.[47] A detailed pipeline description is provided in the Supplementary Methods.

Alpha diversity was assessed using the Shannon index.[48] Beta diversity was calculated using Bray–Curtis dissimilarity[49] and visualized by principal coordinate analysis.[50] Community differences were evaluated using analysis of similarity (ANOSIM) with Bonferroni correction for multiple comparisons.[51] SourceTracker was used to estimate donor similarity using Bayesian inference.[52] SourceTracker

estimates donor-attributed community similarity at 16S taxonomic resolution and does not establish strain-resolved donor engraftment.

Immune profiling and Feature Construction

Whole-blood samples were stabilized[53] immediately after collection and profiled by spectral flow cytometry using dedicated innate and adaptive antibody panels[54, 55]. After standard preprocessing, including quality filtering to remove debris, doublets, and dead cells, followed by arcsinh transformation, major immune lineages were identified by hierarchical gating and subjected to unsupervised clustering using FlowSOM, with UMAP used for visualization of phenotypic structure. For each sample, immune features were defined as cluster frequencies (proportion of parent lineage) for downstream modeling. To focus on longitudinal immune remodeling rather than baseline abundance, immune inputs were expressed as within-subject changes (Δ frequencies) across defined postoperative contrasts (innate: POD1→POD3; adaptive: POD1→POD30). Cluster solutions were reviewed using marker-expression heatmaps and stability across samples and batches prior to downstream analyses. Batch effects across flow cytometry runs were assessed using clustering stability metrics and marker-expression distributions prior to downstream integration. Detailed antibody panels, gating strategies, quality control procedures, and clustering workflows are provided in the Supplementary Methods. Immune profiling was performed only in MTT recipients. Accordingly, immune analyses reflect within-cohort longitudinal immune changes and do not represent direct between-group treatment comparisons.

Multi-omics Integration and Network Analyses

To integrate microbiome composition, fecal metabolite dynamics, and immune remodeling, we applied a two-stage multi-omics framework combining latent-factor modeling and shared-embedding network analysis. Partial least squares (PLS) regression was used to derive shared latent axes linking genus-level microbiome structure with SCFA profiles.[56–59] Exploratory cross-omic co-variation was further evaluated using a shared-embedding correlation network (SECN) framework based on low-rank embeddings and sparse correlation networks.[60–63] These analyses were designed to characterize recovery-associated co-variation patterns within available longitudinal samples and do not establish causal or MTT-specific mechanistic coupling between microbial, metabolic, and immune features.

Microbiome genus composition and fecal SCFA profiles were first integrated using PLS regression to derive latent axes capturing maximal cross-omic covariance (e.g., Z2). Immune remodeling features (cluster-frequency Δ values) were subsequently incorporated as a third data layer within the SECN framework, in which patient-aligned microbiome, SCFA, and immune-delta matrices were embedded into a shared low-rank space to construct sparse, time-stratified cross-omic correlation networks. This approach enabled identification of phase-specific immune–microbe–metabolite coupling, baseline-adjusted lead–lag associations between innate and adaptive immune changes, and connector nodes bridging all three biological layers.

Time-ordered immune coordination was assessed using baseline-adjusted lead–lag analyses with partial Spearman correlations and multiple-testing correction.[61, 62, 64] Network edges were retained if they satisfied predefined effect-size thresholds ($|p| \geq 0.30$), false discovery rate correction using the Benjamini–Hochberg procedure ($q \leq 0.10$), and sparsification criteria designed to limit network density and stabilize inference under small-n conditions. Connector-node analyses were performed to identify features bridging microbiome, metabolite, and immune layers, with enrichment testing performed using Fisher's exact test.[65] Lead–lag analyses were used to distinguish synchronous co-variation from temporally ordered cross-omic associations. Because the study was exploratory and observational and sampling density was limited, these analyses were interpreted as evidence of time-ordered coordination rather than causal direction.[61, 62, 64] Full methodological details are provided in the Supplementary Methods.

RESULTS

Safety, Feasibility, and Cohort Characteristics

Twelve patients who consented to receive MTT were compared with 12 non-MTT controls drawn from two previously published prospective cohort studies of patients undergoing identical sigmoid colectomy at the same institution.[16, 17] All enrolled participants successfully received the planned MTT intervention and completed longitudinal follow-up. Baseline demographic and perioperative characteristics were generally comparable between groups (Table 1). All patients received standardized mechanical bowel preparation with preoperative oral antibiotics followed by perioperative intravenous antibiotics tailored to tolerance and allergy history.

Table 1

Patient Demographics and Clinical Characteristics. Perioperative IV antibiotic selection differed by allergy/tolerance. Continuous variables are presented as mean $\pm$ standard deviation. Categorical data are shown as counts and percentages. MTT = Microbiota Transplant Therapy.

Male sex, n (%)	MTT (n = 12)	Non-MTT Controls (n = 12)
	4 (33)	8 (66)
Age, years	54.0 $\pm$ 13.1	53.0 $\pm$ 11.3
BMI, kg/m²	28.2 $\pm$ 5.2	29.7 $\pm$ 4.4
Preoperative albumin, g/dL	4.3 $\pm$ 0.3	3.9 $\pm$ 0.3
Charlson comorbidity index	2.6 $\pm$ 1.4	1.4 $\pm$ 1.4
Smoking status, n (%)		
Active smoker	1 (8)	2 (17)
Former smoker	6 (50)	4 (33)
Non-smoker	5 (42)	6 (50)
Chronic narcotics, n (%)	1 (8)	1 (8)
History of diabetes mellitus, n (%)	1 (8)	2 (17)
Metformin	1 (8)	1 (8)
Preoperative bowel prep, n (%)		
Polyethylene glycol and Magnesium citrate	12 (100)	12 (100)
Preoperative oral antibiotics, n (%)		
Neomycin + metronidazole	12 (100)	12 (100)
Perioperative intravenous antibiotics, n (%)		
Cefazolin+ metronidazole	11 (92)	3 (25)
Clindamycin + gentamicin	1 (8)	0 (0)
Ciprofloxacin + metronidazole	0 (0)	4 (33)
Cefotetan	0 (0)	2 (17)
Ertapenem	0 (0)	3 (25)
Disease pathology, n (%)		
Sigmoid diverticulitis	9 (75)	9 (75)
Sigmoid colon cancer	3 (25)	3 (25)

Male sex, n (%)	MTT (n = 12)	Non-MTT Controls (n = 12)
	4 (33)	8 (66)
Postoperative complications, n (%)		
None	11 (92)	11 (92)
Anastomotic leak/sepsis	-	-
Urinary tract infection	-	-
Surgical site infection	-	-
Deep organ space infection	-	-
Pneumonia	-	-
Ileus requiring nasogastric tube	1 (8)	1 (8)
Anemia requiring transfusion	-	-
Acute renal failure	-	-
Deep venous thrombosis	-	-
Return of bowel function, days	1.7 ± 0.8	2.2 ± 1.3
30-day hospital readmission, n (%)	1 (8)	0 (0)

Postoperatively, one control patient developed small bowel ileus requiring nasogastric decompression. One patient in the MTT group experienced gastric ileus with preserved colonic function, which was similarly managed with nasogastric decompression and resolved with recovery of gastric function. No treatment-attributed serious adverse events were observed. Although the study was not powered for clinical endpoints, MTT recipients demonstrated numerically earlier return of bowel function compared with matched non-MTT controls (mean 1.7 ± 0.8 vs 2.2 ± 1.3 days; $P = 0.28$). Postoperative antibiotic exposure during the 6-month follow-up period is summarized in the Supplementary Materials.

Durable Microbial Engraftment Following MTT

Prior to MTT, recipient gut microbiota shared limited similarity with the donor community (median 19.6%, IQR 7.7–30.6%). By postoperative day (POD) 14, donor similarity increased to 51.0% (IQR 44.1–58.2%; $P < 0.001$) and remained significantly elevated at POD30 (51.4%, IQR 40.0–66.5%; $P < 0.001$), POD90 (68.2%, IQR 37.1–83.5%; $P < 0.001$), and POD180 (36.9%, IQR 17.6–56.9%; $P < 0.001$) (Fig. 1), consistent with durable microbial engraftment following MTT.

Consistent with these compositional shifts, beta-diversity analyses demonstrated sustained divergence in community composition between MTT recipients and non-MTT controls beginning at POD14. Alpha diversity declined postoperatively in both groups; however, MTT recipients exhibited higher alpha

diversity at POD14, though this difference did not reach statistical significance, consistent with differences in microbial recovery dynamics (Fig. S1).

Early-Recovery Taxa and Reduced Pathobiont Expansion Following MTT

MTT was associated with distinct taxonomic shifts compared with non-MTT controls (Fig. 3). By POD14, MTT recipients exhibited significantly higher relative abundances of *Akkermansia* ($P = 0.018$) and *Collinsella* ($P = 0.004$), with both taxa remaining elevated at POD30 ($P = 0.003$ and $P = 0.022$, respectively). In contrast, relative abundances of *Bacteroides* and *Phocaeicola* were not affected by MTT (Fig. S2A).

Canonical butyrate-producing taxa, including *Blautia*, members of the *Ruminococcaceae*, and *Lachnospiraceae* (Fig. S2B), declined postoperatively in both groups. Although between-group differences at POD14 and POD30 did not reach statistical significance, relative abundances of these taxa were consistently higher among MTT recipients ($P \geq 0.070$). *Streptococcus*, a taxon commonly associated with postoperative dysbiosis and reduced fecal SCFA levels, was lower in the MTT group at POD14, though this difference did not reach statistical significance ($P = 0.070$; Fig. S2C).

Collectively, these taxonomic patterns suggest that MTT is associated with early recovery of putative recovery-associated taxa alongside reduced postoperative expansion of pathobionts, consistent with microbial community structures supportive of downstream functional recovery. We next examined whether these compositional shifts were accompanied by recovery of microbial metabolic function.

Recovery-Associated Differences in Short-Chain and Branched-Chain Fatty Acids Following MTT

SCFAs (acetate, propionate, butyrate, and valerate) declined sharply after surgery in both groups. At baseline, acetate, propionate, and valerate concentrations were similar between cohorts, whereas butyrate levels were significantly lower in the MTT group ($P = 0.037$). Despite these baseline differences, postoperative trajectories demonstrated rapid convergence between groups. The early overshoot of SCFA levels in MTT recipients is consistent with recovery-associated differences in microbial metabolic output rather than baseline group bias. By POD14, concentrations of all measured SCFAs were significantly higher in MTT recipients compared with non-MTT controls (acetate $P = 0.0025$, propionate $P = 0.0003$, butyrate $P = 0.0018$, valerate $P = 0.0069$), consistent with accelerated early recovery of microbial metabolic function (Fig. 4, Table S1).

Branched-chain fatty acids (BCFAs; 2-methylbutyrate, isobutyrate, and isovalerate), which are derived from branched-chain amino acid fermentation, were significantly lower in the MTT group at baseline ($P = 0.008$, $P = 0.018$, and $P = 0.011$, respectively; Table S2). By POD14, each BCFA was significantly higher in MTT recipients compared with non-MTT controls (2-methylbutyrate $P = 0.010$, isobutyrate $P = 0.0049$,

isovalerate $P = 0.0086$), consistent with accelerated recovery of both carbohydrate- and protein-derived microbial fermentation products following surgery.

Integrated Modeling Identifies Microbiome–Metabolite Recovery Signatures

To understand how the gut microbiome and its metabolic activity recover together during the perioperative period, we jointly analyzed bacterial composition and SCFA concentrations at three timepoints: before MTT (baseline), POD2, and POD14. To identify shared patterns linking microbial shifts with metabolic output, we applied PLS modeling, which extracts latent axes capturing dominant shared variation between high-dimensional datasets.

Across all perioperative timepoints, a single dominant latent axis (Z2) consistently linked microbial community structure with SCFA output, representing the strongest shared microbe–metabolite covariance. This pattern was not evident at baseline, where microbial composition showed limited association with SCFA levels. By POD2, early associations between microbial shifts and SCFA production began to emerge (Fig. S4), and by POD14, Z2 was strongly associated with increases across both unbranched and branched SCFAs (Fig. S5).

Z2 was positively loaded by fermentative genera, including *Parvimonas*, *Peptostreptococcus*, *Butyricicoccus*, and *Intestinibacter*, and inversely associated with *Staphylococcus* and *Massiliimalia* (Fig. 5, Table S3). These findings suggest that microbial reconstitution becomes progressively coupled to metabolic output during postoperative recovery. Because microbiota-derived metabolites influence systemic immune responses, we next examined whether MTT was associated with remodeling of the postoperative immune landscape.

Innate Immune Remodeling Following MTT at POD3

Having identified a progressively structured microbe–metabolite recovery trajectory, we next examined whether early postoperative immune dynamics showed parallel changes within the innate immune compartment. POD3 samples from MTT recipients demonstrated marked shifts in innate immune populations compared with POD1 samples (gating strategy shown in Fig. S6A). A 0.43-fold decrease was observed in CD62L[−]CD16[−] immune-modulatory neutrophils.[55] In contrast, increased frequencies were detected in multiple neutrophil subsets, including CD63[−]CD66b⁺ granule-releasing neutrophils (1.53-fold), CD63[−]CD66b[−] granule-releasing neutrophils (2.57-fold), CD11b⁺CD62L[−] neutrophils (1.79-fold), CD11b⁺CD62L⁺ activated migrating neutrophils (2.23-fold) (Fig. 6A), and CD49d⁺CD54[−] rare neutrophils (6.24-fold).[55]

Increased frequencies were also observed among immature and phenotypically distinct neutrophil populations, including banded neutrophils (10.06-fold), A1 GRIM neutrophils (11.13-fold), CD62L⁺CD16⁺ neutrophils (12.77-fold), and GRIM intermediate neutrophils (71.77-fold) (Fig. 6A). In parallel, POD3 samples demonstrated increased frequencies of myeloid-like myeloid-derived suppressor cells (MDSCs) (1.67-fold) and early natural killer (NK) cells (2.71-fold) compared with POD1 samples (Fig. 6B). No

significant changes were observed in the major gated monocyte and dendritic cell compartments at this time point (Fig. 6B; gating strategy shown in Fig. S6B and S6C), although unsupervised clustering identified shifts in specific monocyte and dendritic cell phenotypic clusters (Fig. 6G–I).

Neutrophil Phenotypic Remodeling Following MTT

Unsupervised clustering analysis of neutrophils identified nine distinct phenotypic clusters with unique marker expression profiles (Fig. 6D). These clusters included populations with marker-expression patterns consistent with quiescent phenotypes (CD62L^{high}, CD11b^{low}), degranulation-associated states (CD66b^{high}),^[66] activated phenotypes (CD62L^{low}),^[67] hyper-adhesive features (CD54^{high}),^[68] as well as clusters characterized by banded, A1 GRIM, and CD62L⁺CD16⁺ phenotypes.

Dimensionality reduction using UMAP showed shifts in neutrophil composition at POD3 compared with POD1 samples from MTT recipients. Quantitative analysis of cluster proportions (Fig. 6E) and marker-expression heatmaps (Fig. 6F) demonstrated reduced abundance of cluster c0, characterized by low expression of CD66b and CD62L. This reduction was accompanied by increased proportions of clusters c2 and c4, defined by CD62L⁺CD16⁺ marker expression.^[67]

Monocyte and Dendritic Cell Remodeling Following MTT

Unsupervised clustering of monocytes and dendritic cells identified nine clusters with distinct phenotypic profiles (Fig. 6G). Comparative analysis of cluster proportions (Fig. 6H) and marker-expression heatmaps (Fig. 6I) demonstrated increased frequency of the C5 cluster at POD3 relative to POD1 samples from MTT recipients. This cluster was characterized by a CD11b⁺CD14⁺HLA-DR^{low/-} myeloid-derived suppressor cell phenotype. This shift was accompanied by reduced frequencies of dendritic cell clusters expressing CD141 and BDCA3,^[69] including clusters C1, C2, and C3.

Adaptive Immune Remodeling at POD30 Following MTT

Having observed early innate immune shifts following MTT, we next examined whether these changes were followed by coordinated remodeling of the adaptive immune compartment during later postoperative recovery (gating strategy shown in Fig. S7). Immune profiling of POD30 MTT samples revealed a reduction in terminally differentiated and effector T cell populations compared with POD1 MTT baseline samples. These included CD8⁺ terminal effector T cells (0.52-fold), total CD8⁺ effector T cells (0.61-fold), CD4⁺ effector subsets (0.63-fold), and Th1 cells (0.69-fold) (Fig. 6C). In contrast, naive CD4⁺ T cells increased by 2.36-fold, whereas early and intermediate CD4⁺ effector subsets increased by 2.73-fold and 16.57-fold, respectively. Regulatory T cells (Tregs) increased by 1.69-fold (Fig. 6C).

Among B cell populations, plasmablast frequencies increased by 2.11-fold (Fig. S8). Substantial increases were additionally observed in $\gamma\delta$ T cell subsets, including CD197⁺CD45RA⁻ (3.07-fold) and CD197⁺CD45RA⁺ (4.01-fold) populations, ^[70] as well as in unswitched memory B cells (3.70-fold) in POD30 MTT samples relative to POD1 MTT baseline samples.

Unsupervised Clustering Reveals Adaptive Immune Reorganization

Unsupervised clustering analysis of Tregs identified seven phenotypically distinct clusters (Fig. 6J). Quantitative analysis demonstrated expansion of the C2 and C6 Treg clusters at POD30, characterized by increased expression of CXCR4 (CD185, C2) and CCR4 (CD194, C6), markers associated with migratory immune phenotypes. This expansion was accompanied by reduced abundance of the naïve/resting-like Treg C0 cluster, characterized by lower expression of CD28 and CD196 (Fig. 6K and Fig. 6L).

Cross-Omic Integration Reveals Time-Stratified Associations

Given these parallel microbial, metabolic, and immune trajectories, we next examined whether these systems demonstrated coordinated associations at the level of individual patients. To accomplish this, we integrated microbiome composition, SCFA concentrations, and immune-cluster frequency changes using a Shared-Embedding Correlation Network (SECN) framework. This approach uses low-rank patient embeddings to summarize shared cross-domain variation and constructs sparse correlation networks to stabilize inference in small-n longitudinal datasets. Rather than evaluating each dataset independently, this framework enabled identification of structured cross-omic associations across systems and time.

When relationships were examined across perioperative windows, a time-stratified pattern emerged. Early after surgery, innate-associated immune features showed stronger statistical coupling with gut microbial and metabolite shifts. At later timepoints, adaptive-associated immune features exhibited relatively stronger cross-domain associations (Fig. 7A). This temporal pattern paralleled the independently observed immune remodeling sequence, in which innate transitions preceded adaptive changes. Together, these findings suggest that microbial and metabolic recovery trajectories are temporally aligned with staged systemic immune remodeling, with distinct immune compartments predominating during different phases of postoperative recovery.

Lead-Lag Analysis Identifies Time-Ordered Innate-Adaptive Associations

To evaluate whether early innate immune changes were temporally aligned with subsequent adaptive remodeling, we performed lead-lag analyses linking innate deltas at one timepoint to adaptive deltas at the subsequent timepoint while adjusting for baseline adaptive levels. This framework introduces temporal structure into cross-domain associations and enables evaluation of time-ordered coordination in a constrained observational setting without implying causality.[61, 62, 64]

Across both POD0→POD1 and POD1→POD14 intervals, structured innate→adaptive association networks were identified (Table 2). Among adaptive targets, CD4 T cells (n = 388), CD8 T cells (n = 326),

and B cells (n = 238) demonstrated the highest in-degree connectivity. Among innate sources, monocytes (n = 348), neutrophils (n = 348), and NK/NKT populations (n = 256) showed the strongest out-degree contributions, consistent with temporally structured coupling between innate and adaptive compartments. Although overall edge counts were comparable across windows due to predefined sparsification criteria, the identity and sign of retained edges differed between phases, indicating window-specific association structure.

Table 2

Lead–Lag Innate-to-Adaptive Immune Edge Counts by Time Window. For each lead–lag window (POD0→POD1 and POD1→POD14), the table reports the number of significant innate → adaptive edges that pass effect-size ($|p| \geq 0.30$), BH-FDR control ($q \leq 0.10$), and sparsification criteria. Values are derived from the baseline-adjusted (partial Spearman) analysis, in which innate deltas at the lead timepoint predict adaptive deltas at the subsequent timepoint after adjusting for adaptive baseline at the lead timepoint.

Lead–lag window	Positive correlations (n)	Negative correlations (n)	Total edges (n)
POD0→POD1	267	209	476
POD1→POD14	267	209	476

We next assessed whether cross-domain association strength varied across perioperative stages. Median absolute correlation magnitude ($|p|$) increased from 0.354 at POD0 to 0.393 at POD1 and declined to 0.314 at POD14. $|p|$ was significantly higher at POD1 compared with POD0 (paired Wilcoxon $P = 0.035$) and POD1 compared with POD14 ($P = 0.0013$), consistent with transiently increased cross-domain coordination during the immediate postoperative period.

Connector-Node Analysis Identifies Multimodal Bridges Across Microbial, Metabolic, and Immune Layers

To characterize features bridging modalities, we identified three-view connector nodes, defined as nodes with neighbors in all three layers (immune, microbe, and SCFA). Connector backbones were examined using induced subgraphs to highlight compact cross-omic motifs linking immune remodeling to gut recovery features (Fig. 7B–C). Notably, three-view connectivity was concentrated among non-immune features (Fisher's exact test OR = 0.28, 95% CI 0.12–0.63, $P = 7.97 \times 10^{-4}$; Table 3), suggesting that microbial and SCFA components serve as the predominant cross-layer bridge elements under the current sparsification and significance thresholds, with a subset of immune features participating in these multimodal links.

Table 3

Immune Versus Non-immune Feature Representation Among Three-view Connector Nodes. Counts are shown for immune and non-immune features that qualify as three-view connectors (nodes with at least one significant edge to immune, microbial, and SCFA features) versus non-connectors. Immune features were significantly depleted among connector nodes compared with non-immune features (Fisher's exact test OR = 0.28, 95% CI 0.12–0.63, $P = 7.97 \times 10^{-4}$), indicating that cross-omic bridge roles are preferentially carried by microbial and metabolite features under the current sparsification thresholds.

Non-immune features	Non-connector nodes	Connector nodes	Row total
	44	38	82
Immune features	50	12	62
Column total	94	50	144

DISCUSSION

In this single-center, open-label phase I study, early postoperative MTT was feasible to administer and no treatment-attributed serious adverse events were observed among 12 treated participants undergoing colorectal surgery. Exploratory longitudinal profiling identified donor-attributed microbial community similarity at 16S resolution, recovery-associated fecal metabolite changes, and temporally structured within-recipient immune dynamics following MTT administration. Together, these findings support further investigation of microbiome restoration strategies during postoperative recovery.

A central observation of this study is the dissociation between early recovery of microbial metabolic function and the slower recovery of canonical SCFA-producing taxa. Early after MTT, enrichment of *Akkermansia* and *Collinsella* was observed despite incomplete recovery of classical butyrate-producing genera such as *Blautia*, *Ruminococcaceae*, and *Lachnospiraceae*. Notably, recovery of SCFA levels occurred despite lower baseline levels in MTT recipients, suggesting that metabolic recovery may have been linked to microbial network reconstitution rather than baseline compositional differences. Although *Akkermansia* and *Collinsella* are not primary SCFA producers, both occupy key upstream positions in gut metabolic networks.[71–73] *Akkermansia* resides in the mucus layer and degrades mucin into oligosaccharides and organic acids that serve as substrates for downstream fermentative taxa,[71, 72] whereas *Collinsella* ferments host- and diet-derived carbohydrates into lactate, formate, and other intermediates that support pathways leading to butyrate and propionate production.[73, 74] Early expansion of these taxa after MTT may therefore condition the gut metabolic environment and provide substrates that support SCFA synthesis prior to full reconstitution of downstream fermentative producers.[75]

This network-based interpretation is supported by the observation that SCFA recovery occurred despite only modest, non-significant differences in the abundance of canonical butyrate-producing taxa between MTT recipients and non-MTT controls at POD14 and POD30. SCFA concentrations represent emergent properties of microbial ecosystems, integrating substrate availability, cross-feeding efficiency, and

metabolic activity rather than reflecting simple taxonomic abundance.[76] Early enrichment of upstream suppliers may therefore enhance SCFA output by stimulating downstream fermentative pathways and supporting functional recovery within the microbial community. These findings highlight that functional metabolic readouts can capture recovery dynamics that are not evident from abundance-based microbial analyses alone.[76, 77]

Consistent with this interpretation, integrated microbiome–metabolite modeling identified a dominant recovery-linked latent axis (Z2) that connected microbial community structure with recovery of both unbranched and branched SCFAs. At baseline, Z2 showed weak and inconsistent coupling to SCFA levels. Alignment with fermentative metabolites emerged by POD2 and strengthened substantially by POD14, at which point Z2 was strongly associated with increases across all measured SCFAs. Taxa with strong positive loadings on Z2 included fermentative genera associated with SCFA production, whereas taxa inversely associated with Z2 included organisms less aligned with fermentative metabolism. In contrast, a separate latent axis representing baseline community structure showed minimal association with metabolic recovery. Together, these findings suggest that latent-axis modeling can capture system-level microbial dynamics associated with postoperative metabolic recovery.

Beyond the gut ecosystem, longitudinal immune profiling within MTT recipients demonstrated dynamic changes across the systemic immune compartment. Early after transplantation, POD3 samples demonstrated increased heterogeneity consistent with coordinated innate immune remodeling across neutrophil subsets. Expansion of granule-releasing and CD11b⁺ migratory neutrophils is consistent with enhanced degranulation and tissue trafficking. Enrichment of banded, GRIM intermediate, and A1 GRIM neutrophils further suggests emergency granulopoiesis and accelerated neutrophil maturation under systemic inflammatory stress.[18, 78–83] Importantly, these inflammatory features occurred alongside expansion of immunomodulatory neutrophil populations and myeloid-derived suppressor cells, suggesting that innate immune activation was balanced by emerging regulatory programs rather than progressing toward uncontrolled inflammation. [81, 84, 85] The concomitant increase in early natural killer cells further supports coordinated activation across innate immune compartments.[86] Together, this immune profile reflects a regulated response balancing antimicrobial defense, tissue protection, and compensatory immune restraint during early postoperative recovery. Notably, these innate immune changes occurred in temporal alignment with microbial engraftment and recovery of fermentative metabolism, raising the possibility that immune remodeling may parallel recovery of gut microbial function following surgery.

At later timepoints, immune remodeling shifted toward patterns suggestive of adaptive immune reconstitution and recovery. By POD30, MTT recipients showed contraction of terminally differentiated effector and Th1 T-cell populations alongside expansion of naïve and early or intermediate CD4⁺ effector subsets, consistent with evolving immune reconstitution and preserved differentiation capacity.[87, 88] Enrichment of $\gamma\delta$ T-cell populations is consistent with enhanced innate-like immune surveillance and bridging between innate and adaptive immunity.[89–91] Concurrent increases in plasmablasts and unswitched memory B cells are also consistent with active humoral responses and B-cell–associated

tissue repair processes.[92–94] Together, these adaptive immune changes occurred in temporal alignment with microbial engraftment and metabolic recovery and may reflect coordinated recovery-associated immune dynamics following surgery.

This study has several limitations. First, the open-label design and use of matched historical controls limit causal inference and may introduce batch or calendar-time effects across sequencing, metabolomic, and immune analyses. Although conservative sparsification and stability-selection strategies were applied to mitigate false discovery in multi-omic integration, residual technical variation cannot be fully excluded. Second, immune phenotyping was performed only in MTT recipients, whereas control patients contributed microbiome and metabolite data. Accordingly, the immune trajectories described here reflect longitudinal remodeling within the treated cohort and cannot be directly compared with immune responses during standard postoperative recovery. Third, sampling windows were not fully synchronized across stool and blood modalities, limiting precise inference regarding temporal relationships between microbial, metabolic, and immune changes. Microbiome and metabolomic sampling also began at POD 14 for the primary postoperative window, restricting evaluation of very early microbial dynamics following surgery. Finally, all patients received microbiota derived from a single donor microbiota product. Future studies incorporating multiple donors and larger prospective cohorts will be required to determine the generalizability of the coordinated recovery dynamics described here. Additionally, the study was designed and conducted as an exploratory phase I investigation, and integrated multi-omic analyses were exploratory and not prospectively specified; findings should therefore be interpreted as hypothesis-generating rather than confirmatory.

CONCLUSIONS

In summary, early postoperative MTT was feasible and well tolerated in this small single-center phase I cohort. Exploratory longitudinal profiling identified recovery-associated microbial, metabolite, and within-recipient immune trajectories that were temporally structured across the postoperative period (Fig. 8). Together, these observations suggest that postoperative recovery may involve coordinated dynamics spanning microbial, metabolic, and immune systems, and that MTT may serve as a useful biological perturbation model for investigating host–microbiome interactions during surgical recovery. Controlled prospective studies will be required to determine whether these observations are MTT-specific and whether microbiome restoration strategies can translate into improved clinical outcomes such as reduced ileus, infection, or anastomotic complications.

Declarations

Ethics approval and consent to participate:

All research conducted in this study complied with relevant ethical regulations. The protocol was approved by the Institutional Review Board (study ID 00019579) and the Cancer Protocol Review Committee (SURG-2023-29833) at the University of Minnesota. Written informed consent was obtained

from all participants prior to enrollment. The trial was registered at ClinicalTrials.gov (NCT06370884). The United States Food and Drug Administration approved the investigational product under an Investigational New Drug application (IND 28152).

Consent for publication:

Not applicable.

Competing Interests:

WBG is a consultant and advisory board member for Intuitive Surgical, Coloplast, Steel Therapeutics, BD, and Applied Medical. The remaining authors declare that they have no competing interests.

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Author Contribution

AT: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft, Writing – review and editing
JF: Investigation, Visualization, Writing – original draft, Writing – review and editing
MH: Investigation, Visualization, Writing – original draft, Writing – review and editing
PR: Methodology, Investigation, Writing – review and editing
SB: Investigation, Writing – review and editing
HNB: Investigation, Writing – review and editing
AT: Investigation, Writing – review and editing
PG: Investigation, Writing – review and editing
WG: Investigation, Writing – review and editing
BV: Investigation, Writing – review and editing
AJ: Investigation, Writing – review and editing
LT: Investigation, Writing – review and editing
AH: Investigation, Writing – review and editing
AK: Conceptualization, Methodology, Investigation, Visualization, Writing – review and editing
GS: Methodology, Investigation, Visualization, Writing – original draft, Writing – review and editing
SR: Methodology, Investigation, Visualization, Writing – original draft, Writing – review and editing
CS: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft, Writing – review and editing
CJ: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft, Writing – review and editing

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Data Availability

Sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive under accession number SRP533899. De-identified immune profiling and metabolomic datasets are available from the corresponding author upon reasonable request and subject to institutional data use agreements and participant consent restrictions. The microbiota transplantation product used in this study was manufactured and supplied by the University of Minnesota Microbiota Therapeutics Program and is not publicly available.

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Figures

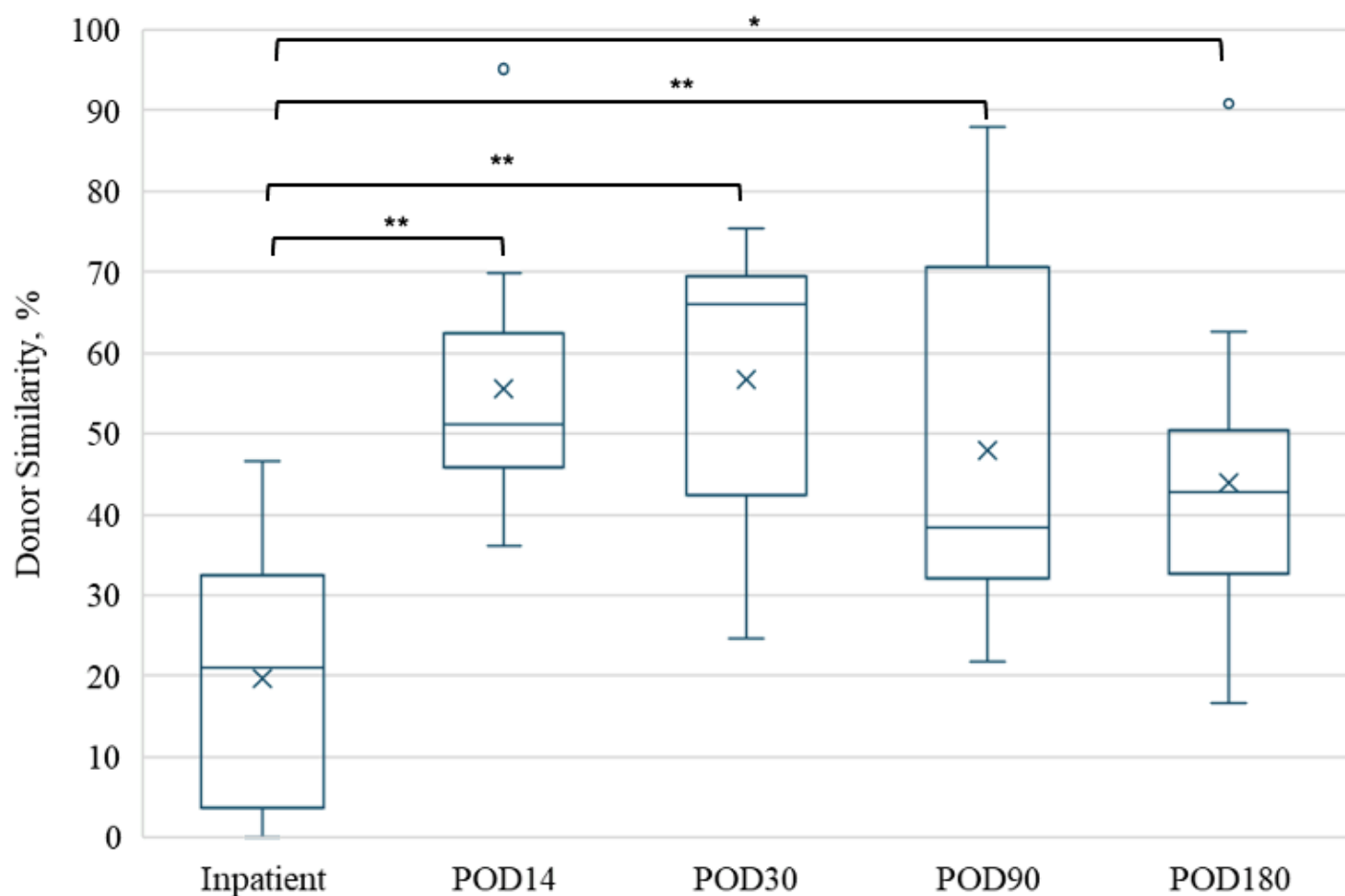


Figure 1

Microbiota Transplant Therapy (MTT) Promotes Durable Microbial Engraftment. Engraftment was assessed as the percentage similarity between the fecal microbiota of transplanted patients and that of healthy donors using SourceTracker. Donor similarity increases by POD14 and remains elevated through 6 months.

* denotes statistical significance at $P < 0.05$; ** denotes statistical significance at $P < 0.005$

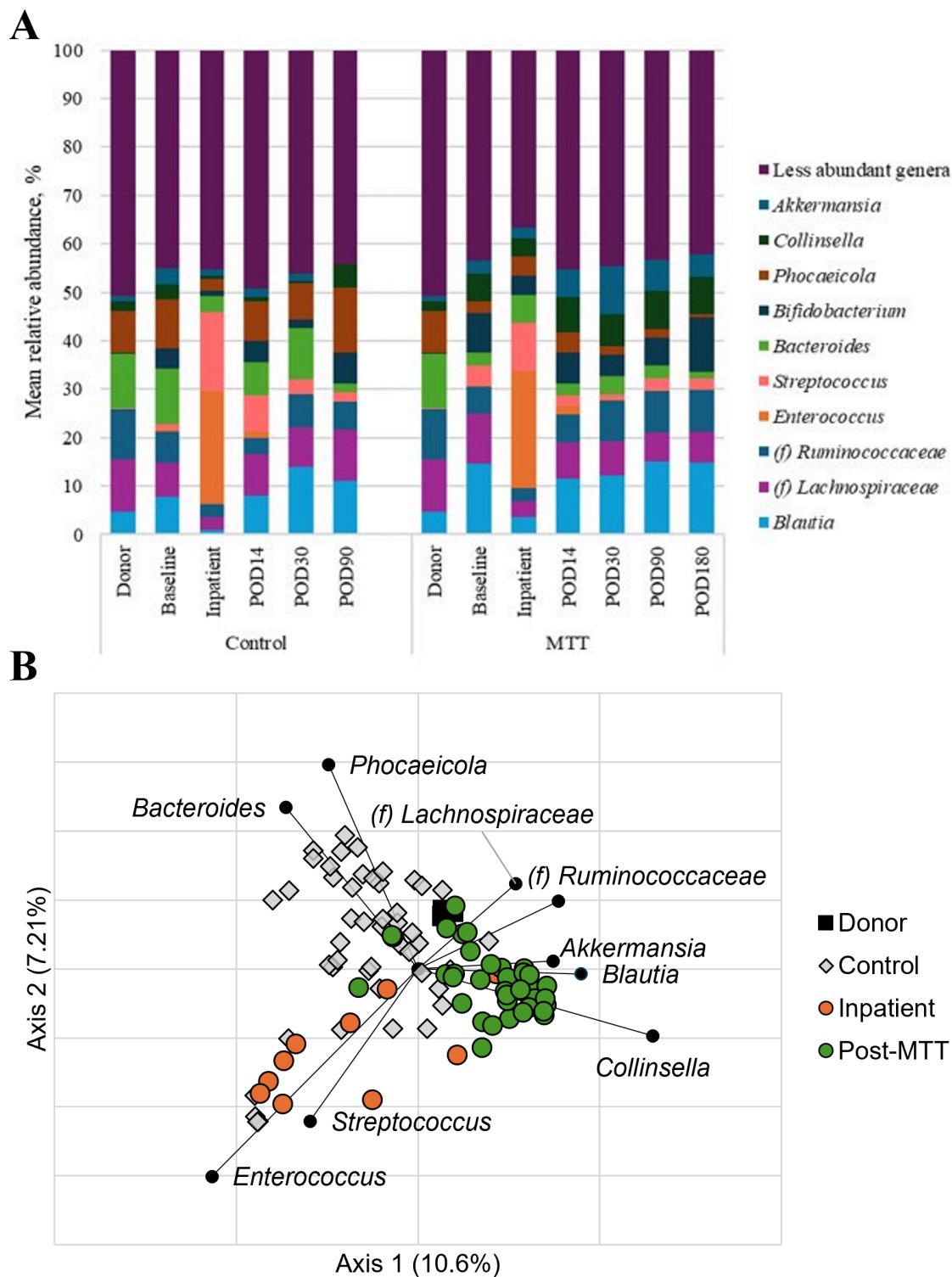


Figure 2

MTT Alters Microbial Diversity and Community Structure After Surgery.

(A) Relative abundance of predominant taxa in non-MTT controls and MTT recipients across perioperative timepoints, demonstrating postoperative collapse followed by divergent community

recovery trajectories. Predominant taxa represent the most abundant genera across all samples, collapsed across timepoints for visualization.

(B) Principal coordinate analysis (PCoA) of Bray–Curtis distances showing fecal microbial community structure in control patients (all timepoints) and MTT recipients before (orange circles) and after (green circles) transplantation ($r^2 = 0.37$). Samples from MTT recipients diverge away from both inpatient and baseline samples.

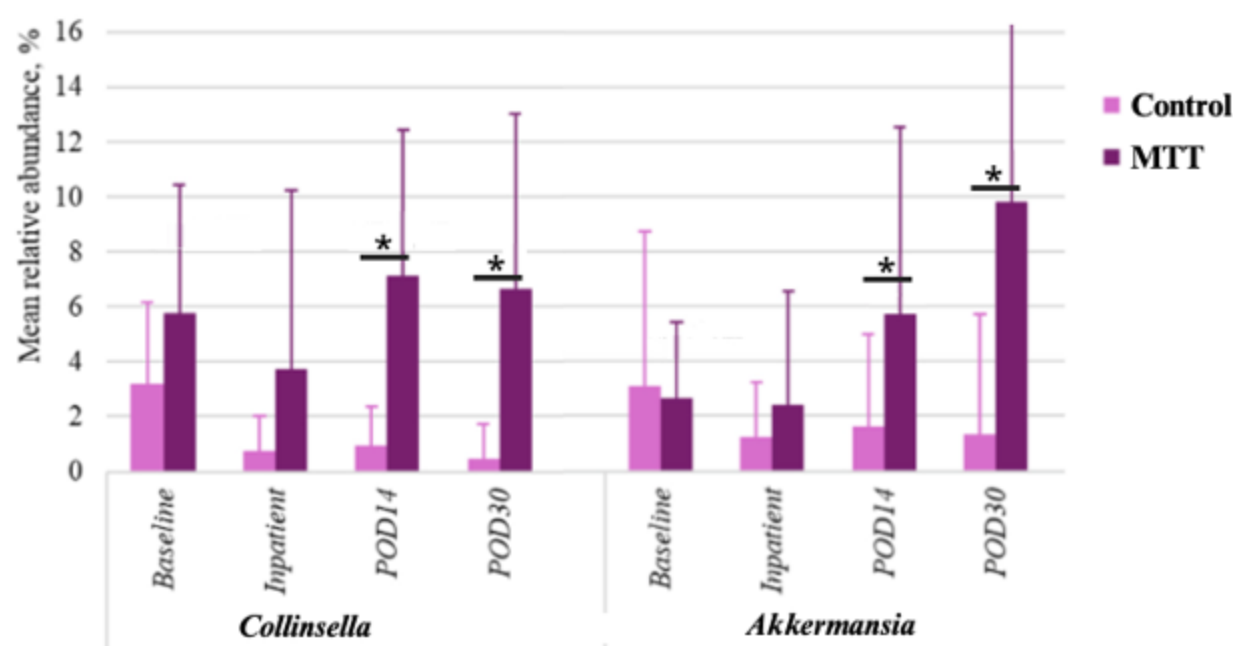


Figure 3

MTT Modulates Keystone Commensal Genera Following Surgery. Mean relative abundance ($\pm$ SD) of *Collinsella* and *Akkermansia* over time in control and MTT-treated patients. MTT is associated with increased abundance of both genera by POD14, with enrichment evident through POD30.

* $P < 0.05$ for between-group comparisons at indicated time points

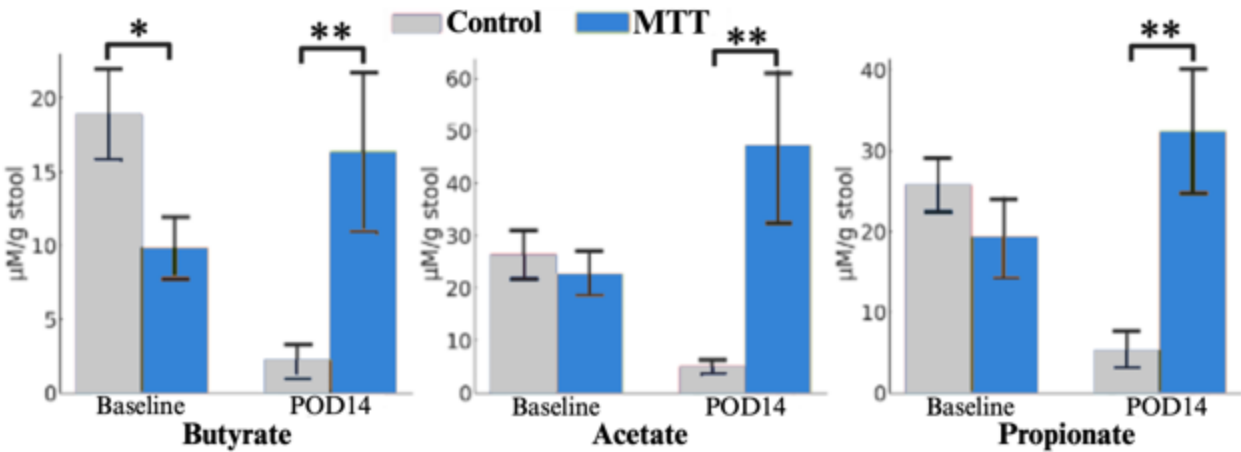


Figure 4

MTT is Associated with Accelerated Recovery of Fecal Short-Chain Fatty Acids (SCFAs) After Surgery.
 Fecal concentrations (mean ± SD) of butyrate, acetate, and propionate at baseline and POD14 in patients receiving MTT and non-MTT controls. SCFA levels decline after surgery in non-MTT controls and are significantly higher in MTT recipients by POD14, consistent with recovery of microbial fermentative function.

P* < 0.05; *P* < 0.005 for between-group comparisons at POD14

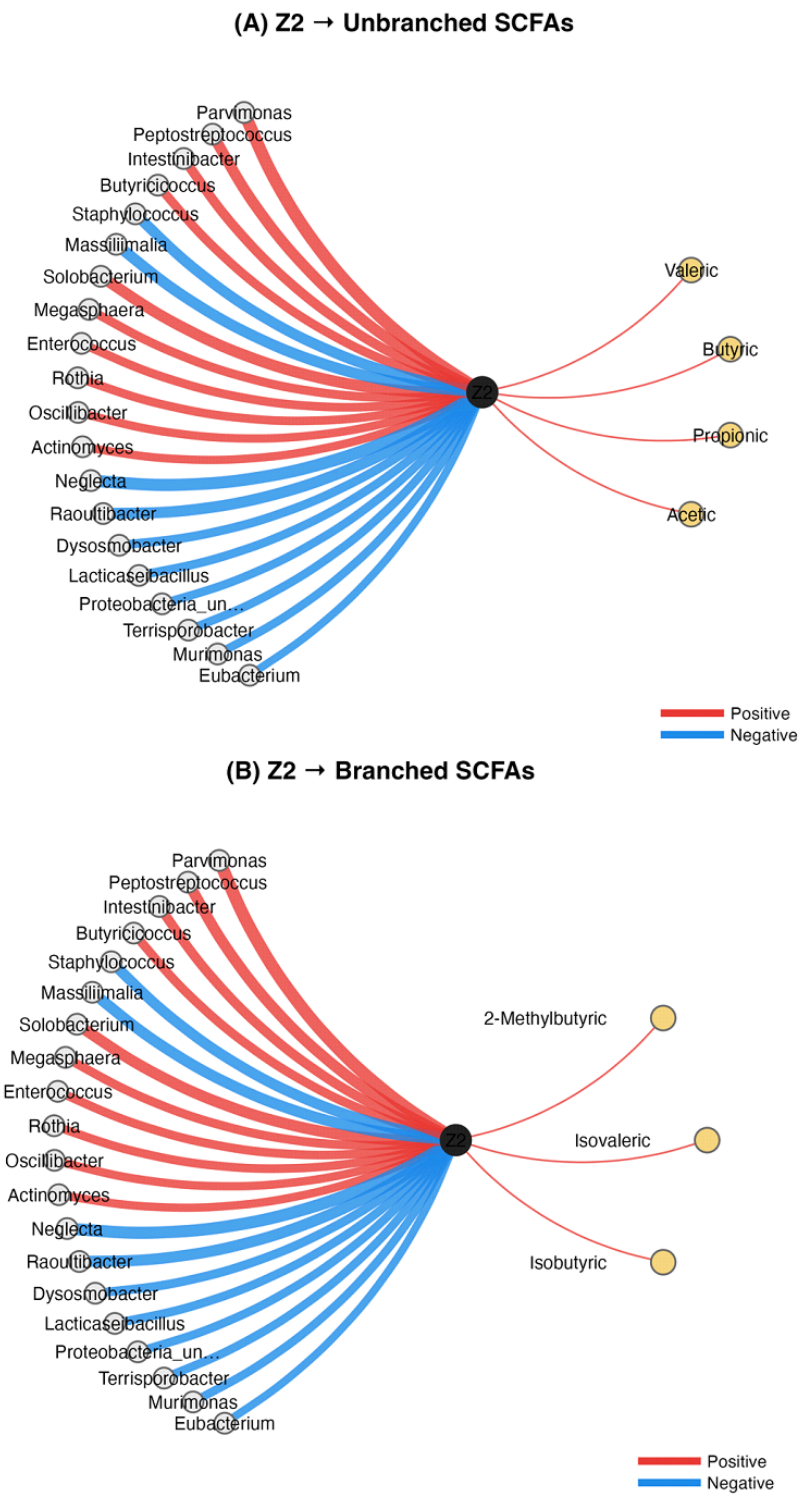


Figure 5

Multi-omic Integration Identifies a Recovery-linked Latent Axis Connecting Microbial Reconstitution with SCFA Restoration.

(A) Network linking microbial genera with strong loadings on the latent factor Z2 to unbranched short-chain fatty acids (SCFAs; acetate, propionate, butyrate, and valerate).

(B) Network linking Z2 to branched-chain fatty acids (BCFAs; isobutyrate, isovalerate, and 2-methylbutyrate).

Z2 represents the second latent component derived from partial least squares (PLS) integration of genus-level microbiome composition and SCFA profiles. Edges represent stable positive (red) or negative (blue) associations between microbial taxa and metabolites. Genera with strong positive loadings on Z2 include *Parvimonas*, *Peptostreptococcus*, *Intestinibacter*, and *Butyricicoccus*, whereas *Staphylococcus* and *Massiliimalia* show negative loadings. These relationships indicate that microbial community reconstitution becomes progressively coupled to fermentative metabolic output during postoperative recovery.

quantified by spectral flow cytometry. These changes reflect increased neutrophil heterogeneity consistent with coordinated innate immune remodeling early after surgery.

(B) Fold change from baseline (POD1 MTT baseline samples) in monocyte and innate immune subsets at POD3 following MTT, including myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells, and dendritic cell populations. No significant changes were observed in conventional monocyte or dendritic cell subsets at this time point.

(C) Fold change from baseline (POD1 MTT baseline samples) in adaptive immune subsets at POD30 following MTT, including CD4⁺ and CD8⁺ effector T cells, early and intermediate CD4⁺ effector populations, and regulatory T cells (Tregs), demonstrating later adaptive immune remodeling consistent with immune resolution.

(D) Uniform Manifold Approximation and Projection (UMAP) visualization of neutrophil populations at POD1 and POD3 following MTT, colored by unsupervised cluster assignment.

(E) Differences in neutrophil cluster proportions at POD3 relative to POD1 following MTT, expressed as changes in the proportion of each cluster relative to the total neutrophil population.

(F) Heatmap showing normalized marker expression profiles defining neutrophil clusters, including markers associated with activation, degranulation, adhesion, migration, and maturation.

(G) UMAP visualization of monocyte and dendritic cell populations at POD1 and POD3 following MTT, colored by unsupervised cluster assignment.

(H) Differences in monocyte and dendritic cell cluster proportions at POD3 relative to POD1 following MTT, expressed as changes in the proportion of each cluster relative to the total myeloid population.

(I) Heatmap showing normalized marker expression profiles defining monocyte and dendritic cell clusters, including features associated with antigen presentation and myeloid suppression.

(J) UMAP visualization of adaptive immune populations at POD1 and POD30 following MTT, colored by unsupervised cluster assignment.

(K) Differences in adaptive immune cluster proportions at POD30 relative to POD1 following MTT, expressed as changes in the proportion of each cluster relative to the total CD4⁺ or CD8⁺ T cell populations, as indicated.

(L) Heatmap showing normalized marker expression profiles defining adaptive immune clusters, including markers associated with effector, regulatory, and memory phenotypes.

Unsupervised clustering and dimensionality reduction analyses (D–L) corroborate and extend frequency-based findings (A–C) by revealing coordinated shifts in immune phenotypic states across early innate

and later adaptive phases. Data are presented as mean $\pm$ SD. Statistical significance was assessed using paired within-MTT recipients (POD1 vs POD3; POD1 vs POD30); ns indicates not significant; * $P < 0.05$.

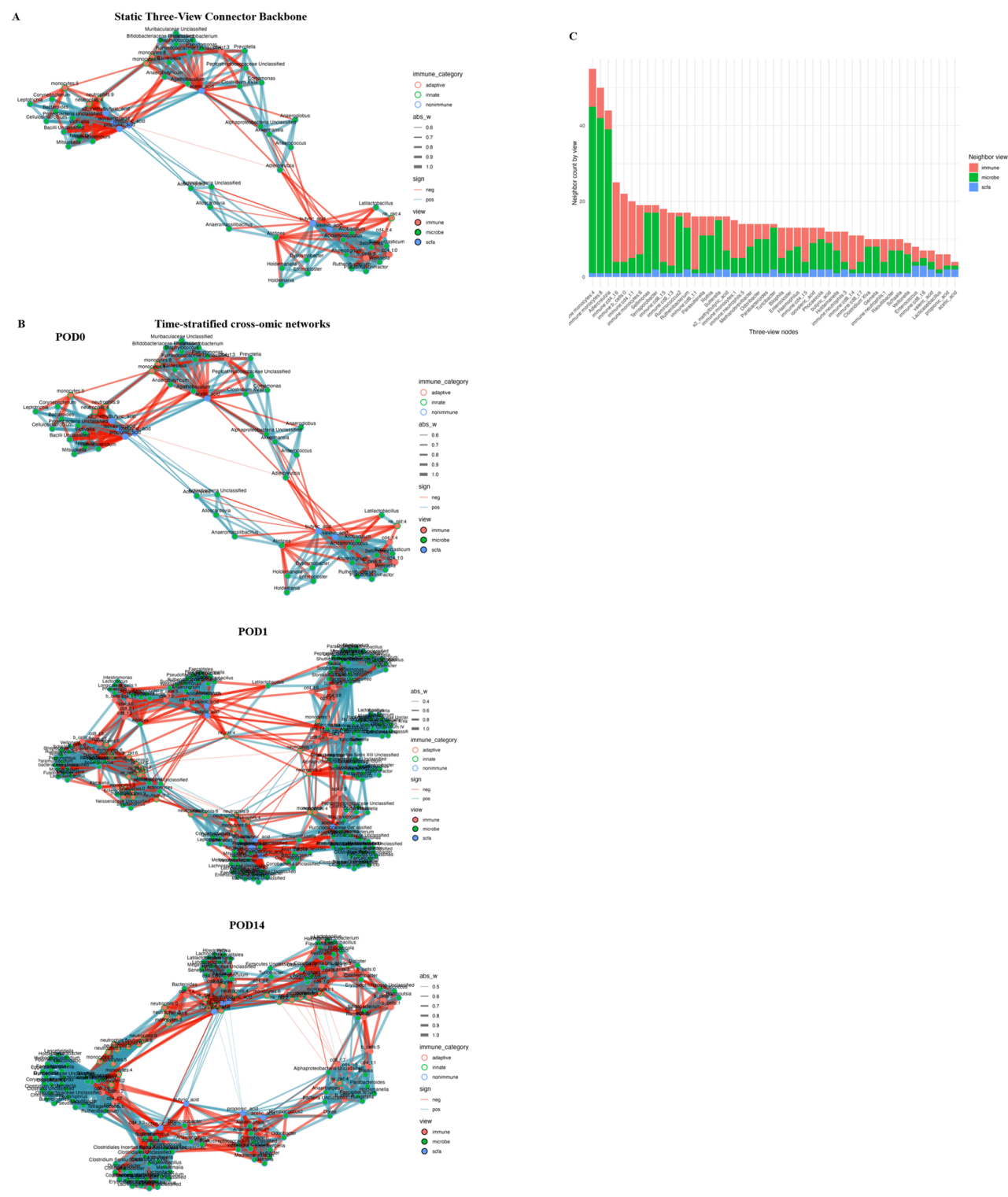


Figure 7

Cross-omic Integration Reveals Multimodal Connector Architecture and Time-Stratified Network Reorganization During Postoperative Recovery.

(A) Three-view Connector Backbone. Nodes represent immune clusters, microbial genera, and SCFAs that participate in statistically supported associations across all three modalities. The induced subgraph highlights compact cross-omic motifs linking gut microbial recovery features with systemic immune remodeling.

(B) Time-stratified cross-omic networks reconstructed at POD0, POD1, and POD14 demonstrate dynamic reorganization of multimodal associations during postoperative recovery. Early networks show stronger coupling between microbial and metabolite features and innate immune populations, whereas later networks exhibit relatively greater coordination involving adaptive immune features.

(C) Composition of connector-node neighborhoods. Stacked bar plots show the number of first-order neighbors for each three-view connector node stratified by modality (immune, microbe, SCFA), illustrating heterogeneous cross-layer connectivity across systems.

Node colors indicate modality, edge color indicates correlation sign (positive or negative), and edge thickness reflects association strength.

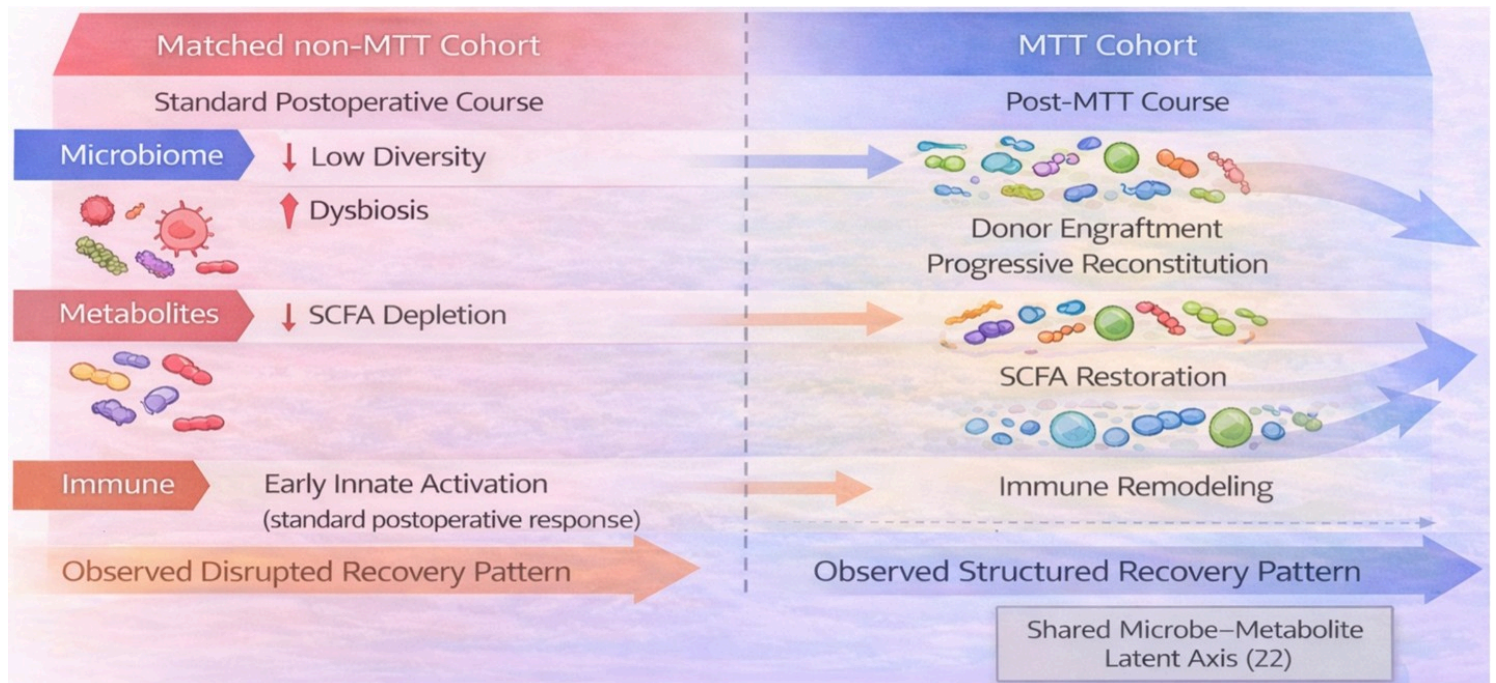


Figure 8

Conceptual Model of Coordinated Recovery Following MTT. Colorectal surgery induces perioperative ecological perturbation marked by microbial community collapse, depletion of SCFAs and BCFAs, and disruption of immune homeostasis. Administration of MTT on postoperative day 2 introduces targeted microbial reconstitution during a window of ecological vacancy. Longitudinal profiling demonstrates temporally ordered coupling between durable microbial engraftment, restoration of microbial metabolic function, and immune reprogramming spanning early innate and later adaptive phases. Across these measurements, Z2 captures a recovery-linked latent axis reflecting coordinated structure in the microbial and metabolite data that is temporally aligned with immune remodeling.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterials.docx](#)