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Galaxy Publication

Fiber-Enriched Enteral Nutrition Differentially Shapes the Gut Microbiome in Critically Ill Trauma Patients

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ABSTRACT

Disruption of the gut microbiota is a frequent occurrence among patients admitted to intensive care units. Particular shifts, including the proliferation of Enterobacteriaceae and other opportunistic pathogens (pathobionts), have been associated with worse clinical outcomes. In non-critically ill populations, administration of enteral nutrition containing prebiotic short-chain fructooligosaccharides (scFOS-EN) has been shown to help restore microbial balance. The consequences of scFOS-EN supplementation on the microbiome in the setting of critical illness, however, are uncertain and hard to forecast given the highly altered microbial ecosystem. To address this gap, we carried out a pilot randomized controlled trial (RCT) in critically ill trauma patients to investigate the influence of scFOS-EN relative to a fiber-free enteral formula (NF-EN) on the temporal evolution of the gut microbiota. This investigation was designed as a single-center, prospective, double-blind RCT. Mechanically ventilated trauma patients in the ICU were randomized to receive either scFOS-EN or an isocaloric fiber-free control formula (NF-EN). Serial stool specimens were subjected to 16S rRNA gene sequencing for detailed characterization of microbial composition. Linear mixed-effects modeling was applied to quantify changes in microbial taxa over the 10-day observation period after formula initiation. In addition, time-aware dimensionality reduction techniques were utilized to uncover individualized temporal trajectories and their links to clinical variables, while microbial interaction networks were constructed to examine interspecies relationships. Analysis included 57 stool samples obtained from 17 patients (7 assigned to NF-EN and 10 to scFOS-EN). All individuals displayed marked dysbiosis at baseline and had received broad-spectrum antibiotics. In comparison with NF-EN, scFOS-EN administration was associated with a more rapid reduction in Bifidobacterium (−0.6%/day, $p = .026$) and Firmicutes (3.5%/day, $p < .001$), accompanied by larger expansions of multiple Bacteroidaceae taxa. Notably, proliferation of the pathobiont Enterobacteriaceae (0.3%/day, $p = .003$) was observed only among patients receiving scFOS-EN. These unfavorable microbial shifts, particularly the increased Enterobacteriaceae load, were strongly modulated by antecedent and concurrent antibiotic exposure and coincided with heightened competitive interactions within the microbial community. The response of the dysbiotic gut microbiota to scFOS-EN in critically ill trauma patients is highly context-specific. Antecedent treatment with antibiotics targeting anaerobic bacteria appears to convert an otherwise potentially advantageous effect into a detrimental one. These results call into question the routine application of prebiotic interventions and emphasize the necessity for tailored nutritional approaches in the intensive care environment.

Keywords: Critical illness, Microbiome, Fiber, Enteral nutrition, ICU, Nutrition

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Introduction

The intestinal microbiota in patients admitted to the intensive care unit (ICU) experiences swift and ongoing harmful alterations, commonly termed ICU-associated dysbiosis. This imbalance is characterized by diminished

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microbial diversity, reduction of beneficial resident species, and proliferation of potentially harmful bacteria known as pathobionts [1]. Overgrowth of pathobionts, especially Enterobacteriaceae, has consistently been linked to elevated risks of hospital-acquired infections and mortality among critically ill individuals [2-4]. Given that nosocomial infections impact over 20% of ICU patients and represent a major contributor to ICU mortality [5], the development of strategies capable of limiting pathobiont proliferation and correcting ICU-related dysbiosis could yield important therapeutic advantages. Interventions that promote the growth of beneficial commensal organisms such as Bifidobacterium (Actinobacteria) and members of the Ruminococcaceae and Lachnospiraceae families (Firmicutes) may prove especially effective, as these taxa generate immunomodulatory compounds including short-chain fatty acids (SCFAs) and compete directly with pathobionts for essential resources and ecological niches [6-8]. Supporting evidence from both preclinical and clinical investigations indicates that microbiota-directed approaches, such as prebiotic supplementation, can help restrain pathobiont expansion and lower infection vulnerability [1, 6, 9-11]. Nevertheless, limited clinical research has examined the influence of such therapies on ICU-associated dysbiosis, leaving their outcomes in this environment largely undetermined.

Prebiotic fibers, including short-chain fructooligosaccharides (scFOS), escape digestion and absorption in the small intestine, thereby providing selective metabolic substrates for bacteria residing in the colon. The consequences of prebiotic administration vary according to context, with outcomes influenced by fiber composition, the metabolic capacities of existing microbial populations, and community-level dynamics such as competition and cross-feeding [12]. ScFOS represent a promising prebiotic option due to their short chain length, which facilitates efficient fermentation by beneficial Bifidobacterium species and SCFA-producing Firmicutes [13]. In patients who are not critically ill, supplementation with scFOS or scFOS-enriched enteral nutrition (scFOS-EN) has been associated with enrichment of these advantageous commensals, enhanced SCFA generation, and attenuation of systemic inflammatory responses [9-11]. However, under conditions of profound dysbiosis or nutrient scarcity, readily fermentable fibers may preferentially support the growth of Bacteroidaceae and pathobiont Enterobacteriaceae—organisms that exhibit greater resilience to environmental stressors—potentially at the cost of more vulnerable community members [14, 15]. ICU patients are subjected to multiple ecological challenges, including inadequate nutritional support, extensive antibiotic therapy, and hospital-acquired infections, all of which reshape the gut microbial landscape and promote pathobiont dominance [1, 16, 17]. These factors may substantially alter the responsiveness of the microbiota to prebiotic interventions.

Therapeutic enteral nutrition (EN) formulas containing scFOS (scFOS-EN) are already employed globally in ICUs to improve gastrointestinal (GI) tolerance [17-19]. Despite their widespread use, the effects of these and similar prebiotic strategies on microbial community dynamics in critically ill patients remain unclear. No randomized controlled trials (RCTs) have specifically evaluated microbiota alterations in response to scFOS-EN during critical illness. Although observational data from ICU cohorts suggest a favorable association between dietary fiber consumption and the presence of SCFA-producing microbes [20, 21], the two existing RCTs investigating supplemental fiber (distinct from scFOS) reported either neutral effects or decreased levels of SCFA producers [22, 23]. Importantly, these trials primarily assessed responses within selected beneficial taxa rather than the broader microbial community. In the profoundly disrupted microbial ecosystem characteristic of critical illness, clarifying how prebiotic agents such as scFOS-EN affect the proliferation and interactions among pathobionts and commensal organisms is essential.

To fill these knowledge gaps, we performed a pilot RCT that compared scFOS-enriched peptide-based therapeutic EN (scFOS-EN) with a matched fiber-free formula (NF-EN) in terms of their influence on microbial dynamics among ICU patients with severe traumatic injuries. We postulated that scFOS-EN would exert distinct effects on gut microbial trajectories throughout a 10-day treatment window, with outcomes potentially modulated by baseline microbial status and clinical variables including antibiotic exposure. Employing 16S ribosomal RNA (rRNA) gene sequencing on serial stool and oral specimens obtained after study formula initiation, we initially evaluated the consequences of scFOS-EN on overall diversity and taxon-specific dynamics. Subsequently, through sophisticated time-sensitive and network-oriented analytical methods, we demonstrate that microbial responses are interdependent and closely related to carbohydrate consumption and antibiotic administration. This practical investigation offers new understanding of intestinal microbial behavior in the ICU and indicates that, in the presence of ecological stress imposed by prior (anaerobic and aerobic) antibiotic therapy, scFOS-EN may promote the outgrowth of resilient taxa (Bacteroides) and pathobiont Enterobacteriaceae while disadvantaging less robust, potentially beneficial commensals.

Materials and Methods

This pilot, single-center, prospective, randomized controlled trial evaluated the influence of scFOS delivered via NutraFlora® (P-95, GF2-GF4) within a standard peptide-based therapeutic EN formulation (Vital AF 1.2 Cal) compared with a nutritionally equivalent fiber-free EN formula (Osmolite 1.2 Cal) on the gut and oral microbiota of critically ill trauma patients. The study protocol was registered at ClinicalTrials.gov (NCT03153397) and received approval from the Duke Health Institutional Review Board (IRB Pro00081414). Primary endpoints included alterations in the relative abundances of microbial taxa following commencement of scFOS-EN versus NF-EN; secondary measures encompassed safety parameters and clinical outcomes. The investigation conformed to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (see Additional file 1 for the complete CONSORT 2025 checklist).

Participants

This research was conducted at Duke University Hospital in Durham, North Carolina, which serves as a Level I trauma center in the southeastern United States. Screening targeted adults between 18 and 80 years of age who were admitted to the neurosurgical or surgical ICU after experiencing significant traumatic injuries. Inclusion required ongoing mechanical ventilation, dependence on enteral feeding, a projected ICU length of stay longer than 3 days, and an anticipated survival exceeding 2 days as judged by the responsible clinician. Patients were excluded for gastrointestinal tract damage, any hospitalization or antibiotic use in the 4 weeks leading up to admission, recent consumption of probiotics or prebiotic-enriched EN within the prior 7 days, history of solid organ transplantation, or pre-existing end-stage hepatic or renal failure necessitating long-term immunosuppressive treatment. Given the pilot nature of the trial, recruitment targeted approximately 10 individuals per arm to determine practicality, aligning with cohort sizes in similar previously published research [22, 23].

Intervention

Written informed consent was secured from each patient's legally authorized representative before randomization. Participants were allocated to receive either Vital® AF 1.2 Cal (delivering 1200 kcal, 75 g protein, and 111 g carbohydrate per liter, with 5.1 g of dietary fiber provided by NutraFlora® scFOS) as the scFOS-EN arm, or Osmolite 1.2 Cal, a comparable fiber-free preparation (1200 kcal, 55 g protein, 156 g carbohydrate, and 0 g dietary fiber per liter) as the NF-EN arm (both supplied by Abbott Nutrition, Chicago, IL). Full macronutrient breakdowns and the precise composition of NutraFlora® scFOS (P-95, consisting of 1-kestose (GF2), nystose (GF3), and fructosyl-nystose (GF4)) are detailed in Additional file 2. Randomization employed a computer-generated sequence concealed in sequentially numbered opaque envelopes. An unblinded coordinator prepared the assigned formula by masking its identification and handed it to the ICU nursing staff. Blinding was maintained for all other investigators and caregivers except for the dietitian, who adjusted infusion rates and supplemental protein as needed. Feeding regimens followed institutional guidelines tailored to individual requirements based on age, body mass index (BMI), respiratory support, and caloric contributions from concurrent medications [18]. No extra dietary fiber was introduced, and the sole non-formula nutritional input permitted was supplemental protein. The study formula was provided for a maximum of 10 days or until clinically discontinued. The initiation day was defined as Day 0, establishing a 10-day intervention window unless halted early due to mortality or palliative care transition. Safety monitoring involved prospective tracking of clinical events. Adverse events (AEs), including episodes of fever, tachycardia, emerging infections, and death, were logged on standardized forms per the trial protocol. Independent review confirmed that all observed AEs were unrelated to the study formulas and reflected the typical disease course in severely injured trauma patients.

Data and sample collection

Daily nutritional records encompassing EN start time, infusion rates and volumes, interruptions in feeding, and supplemental protein were obtained by reviewing electronic nursing documentation. Formula volume was summed for each 24-hour block (7 am to 7 pm or 7 pm to 7 am) starting from randomization on Day 0. Nutrient calculations for kilocalories (kcal), protein (including supplements), carbohydrates, fats, added sugars, and scFOS-derived fiber utilized the respective formula compositions and adjusted body weight (kgAdj) when BMI exceeded 30 kg/m² [19]. Averages were computed across all 24-hour intervals with active EN delivery (participant-level daily logs and summary statistics are presented in Additional file 3). Research personnel

additionally documented baseline demographics, primary diagnosis, Acute Physiology and Chronic Health Evaluation (APACHE) II scores, standard anthropometric parameters, and comprehensive records of antibiotic exposure and culture results from admission until study completion. Antibiotics were grouped according to spectrum: those active only against aerobic organisms (principally vancomycin, cefazolin, bacitracin, cefepime, and ceftriaxone) or those with additional anaerobic activity irrespective of aerobic coverage (principally piperacillin-tazobactam and metronidazole) (see Additional file 3).

Specimen acquisition occurred throughout the 10-day window by experienced study staff. Stool samples were gathered promptly upon notification of defecation (within 1 hour), whereas oral swabs from the anterior tongue were obtained on alternate days. The final analytic cohort was restricted to patients who received the allocated EN for at least 24 hours and supplied a minimum of one fecal and one oral specimen following formula commencement (modified intention-to-treat analysis).

16S rRNA amplicon sequencing

All specimens were forwarded to the Duke Microbiome Center's Microbiome Core Facility for simultaneous DNA isolation and sequencing library generation to prevent batch variation. DNA extraction from fecal and oral material utilized the Qiagen PowerSoil Pro kit processed on the automated QIAcube HT platform per manufacturer guidelines. Extracted DNA was quantified using a Qubit fluorometer and assessed for purity with a NanoDrop instrument, then standardized to 1.0 ng/µL. Amplification of the 16S rRNA gene V4 region was achieved with the 515F–806R primers equipped with Illumina adapters, Golay barcodes, and appropriate linkers in accordance with the Earth Microbiome Project protocol [24, 25]. Following purification with AMPure XP beads and Qubit quantification, libraries were equimolarly pooled and sequenced on an Illumina MiSeq system employing 2 × 250 bp paired-end reads at the Duke Sequencing and Genomic Technologies Shared Resource. Subsequent data processing and statistical evaluation were executed in R statistical software (version 2023.03.1 + 446, R Foundation for Statistical Computing, Vienna, Austria). Raw sequences underwent demultiplexing, quality control, denoising, and rarefaction to a uniform depth of 25,000 reads per sample via the DADA2 workflow [26]. Alpha diversity metrics included the Shannon index and the number of observed amplicon sequence variants (ASVs). Taxonomic annotation of ASVs relied on the Silva-138 reference database at a 99% sequence identity threshold [27].

Statistical analysis

Longitudinal analysis of diversity and composition

Primary analyses relied on multivariable linear mixed-effects models with repeated measures (LMMs) to examine trajectories of alpha diversity and taxon abundances over time. These models incorporated fixed effects for formula assignment, time (modeled continuously as days since study EN commencement), and the formula-by-time interaction, along with a random intercept per participant to accommodate within-subject correlations. Sex was added as a covariate across all models to adjust for baseline differences between groups. Linear time terms were chosen based on superior model fit according to the Akaike Information Criterion (AIC) and evaluation of residuals when testing linear, quadratic, and cubic specifications for the Shannon index. Model outputs yielded slopes (beta coefficients for time) that quantified the average daily rate of change within each treatment arm (NF-EN and scFOS-EN). The interaction term specifically tested whether temporal trends diverged between groups, revealing whether scFOS-EN was linked to accelerated increases or declines in microbial features relative to the NF-EN trajectory. Fitted models also produced participant-level predictions, such as estimated Shannon index values at Day 0 ("D0 Shannon estimates") to illustrate initial dysbiosis severity and projected Day 10 Enterobacteriaceae abundances ("predicted Enterobacteriaceae abundance") for subsequent correlative analyses. Reporting emphasized taxa meeting a mean relative abundance (r.a.) threshold of ≥ 0.1%; complete results for these and rarer taxa (< 0.01%) are available in Additional file 4. A sensitivity analysis further adjusted for potential differences in pre-enrollment ICU days. Statistical significance was defined as $p < 0.05$ for all primary models and comparisons. To support phylum-level visualizations, observed relative abundances from fecal samples were grouped into time windows (0–2 days, 2–5 days, and 5–10 days). The initial 0–2 day window was selected to capture pre-intervention baseline composition, recognizing that prebiotic effects typically emerge 36–48 hours after introduction [9]. Primary figures displayed LMM-derived univariable regression lines with 95% confidence interval (CI) shading for interpretability, alongside locally estimated scatterplot smoothing (LOESS) curves [28] applied to raw relative abundance data. All LOESS smoothing and LMM visualizations were generated in R.

Time-informed dimensionality reduction (TEMPTED)

To address irregular sampling intervals and detect groups of microbes exhibiting coordinated temporal behaviors, we implemented TEMPoral TEnsor Decomposition (TEMPTED), an unsupervised machine-learning technique developed for longitudinal microbiome datasets [29]. TEMPTED decomposes high-dimensional data into a limited set of "principal components" (PCs), where each component captures a unique temporal pattern arising from covarying microbial groups (represented by feature loadings) across individuals. Participant-specific PC scores then facilitate exploration of associations between these patterns and clinical covariates [29]. This method avoids arbitrary time binning by treating time continuously, rendering it particularly appropriate for our opportunistic sampling schedule. TEMPTED was performed on unrarefied, center-log ratio (CLR)-transformed ASV counts from participants contributing multiple samples collected at least 24 hours apart. ASVs were retained if present in ≥ 20% of samples and detected at a minimum of two time points. Analyses were conducted first on the full cohort (NF-EN + scFOS-EN) to uncover general trends and then restricted to the scFOS-EN arm to examine treatment-associated variability. Resulting participant-specific PC scores were examined for correlations (Spearman's rho or Wilcoxon rank-sum tests) with selected clinical variables at a significance level of $p < 0.05$ (variables listed in Additional file 3).

Microbial network analysis

To evaluate how formula type influenced overall ecological relationships within the microbial community, separate networks were constructed for the NF-EN and scFOS-EN groups using the MicNet toolbox [30]. Networks were built from fecal samples obtained during the days 5–10 window (using the latest sample per participant when multiple were available) to capture maximal intervention exposure while maintaining balanced group representation. ASVs were included if they achieved ≥ 0.1% relative abundance in at least 20% of samples. Network characteristics assessed included global topological features, tripartite interaction motifs (competitive [− − +] versus cooperative [+ + +]), and community clusters determined via unsupervised Louvain community detection. Pairwise microbe-microbe associations were computed with Sparse Correlations for Compositional Data (SparCC), a compositionally robust method that applies log-ratio transformations and performs well in low-diversity settings [31]. Significant correlations met an absolute threshold of > 0.4 with false discovery rate-adjusted $q < 0.1$. Network diagrams were produced using a local installation of the MicNet toolbox and GraphPad Prism (v10.1.1; GraphPad Software, San Diego, CA, USA).

Results and Discussion

Patient characteristics and clinical course

Between November 2017 and April 2021, the study enrolled and randomized 20 critically ill adults suffering from polytrauma that frequently included traumatic brain injury. Three individuals initially randomized to the fiber-free enteral nutrition arm (NF-EN) were removed within the first 24 hours owing to withdrawal of consent in one case and deviations from the study protocol by clinical personnel in two others. The final modified intention-to-treat population therefore consisted of 17 patients: 7 assigned to NF-EN and 10 assigned to the short-chain fructooligosaccharide-supplemented formula (scFOS-EN) **(Figure 1)**. In total, 57 fecal specimens and 88 oral specimens from these participants satisfied quality criteria and entered the analytic dataset. Despite the opportunistic nature of fecal collection dictated by defecation events, the number of samples was balanced between treatment arms (22 in NF-EN and 35 in scFOS-EN). All patients contributed at least one fecal sample, and all except three provided serial specimens separated by more than 24 hours.

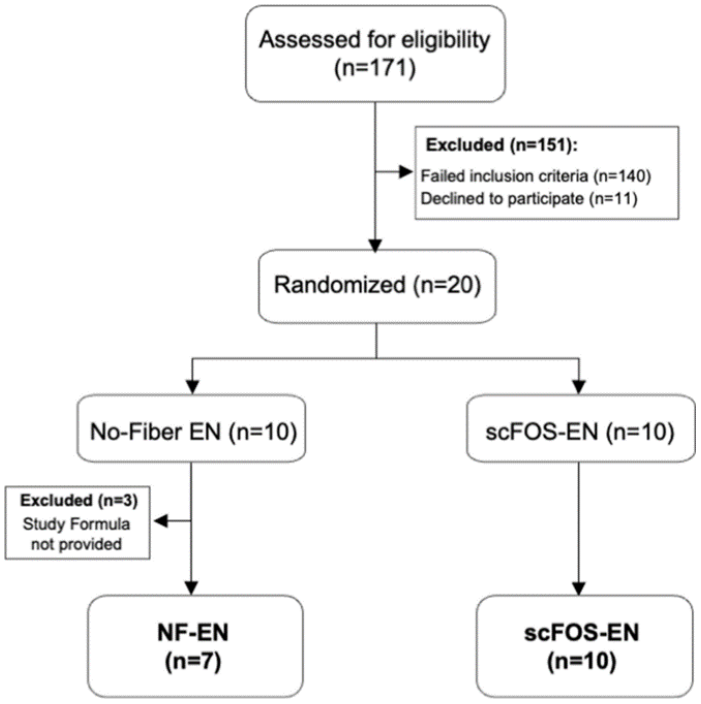


Figure 1. CONSORT flow diagram showing participant recruitment and retention throughout the randomized controlled trial.

A total of 171 critically ill trauma patients underwent eligibility screening, resulting in the exclusion of 151. Twenty patients were randomized in equal numbers to receive either fiber-free enteral nutrition (NF-EN, n = 10) or short-chain fructooligosaccharide-enriched enteral nutrition (scFOS-EN, n = 10). Three participants in the NF-EN group were withdrawn after randomization, and their samples were not processed further. The modified intention-to-treat analysis ultimately included 17 patients (7 NF-EN and 10 scFOS-EN).

Clinical and demographic features at baseline were broadly comparable between the two arms, with APACHE II scores reflecting moderate to high illness severity **(Table 1)**. The scFOS-EN group contained a larger fraction of male patients, a difference addressed through covariate adjustment in all multivariable statistical models. Study enteral nutrition was initiated at a median interval of 2 days after ICU admission. Delivered caloric loads were equivalent between groups but consistently fell short of therapeutic targets, with mean daily intake remaining below 20 kcal per kg per day (kcal/kg/day) for every participant **(Table 1)**. Fiber intake naturally diverged as designed, averaging 5.2 g per day (g/day) among scFOS-EN recipients and 0 g/day in the NF-EN arm. Consistent with the distinct macronutrient profiles of the formulas, total carbohydrate consumption was lower in the scFOS-EN group **(Table 1)**.

Table 1. Participant Characteristics

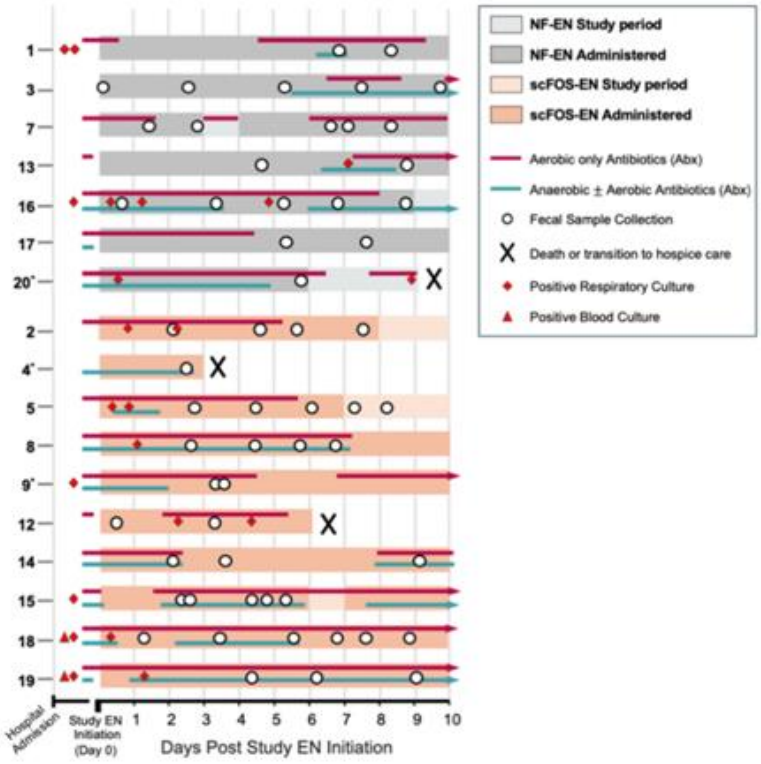
	NF-EN (N=7)	**scFOS-EN (N=10)**	**Std Mean Difference**
Patient Characteristics & Pre-Study Clinical Factors			
Age (mean (SD))	45.14 (22.21)	39.70 (16.47)	0.28
Sex = M (%)	2.00 (28.6)	8.00 (80.0)	1.21
APACHE II Score on Admission (mean (SD))	21.71 (4.89)	19.40 (6.80)	0.39
Actual weight (Kg) (mean (SD))	94.67 (20.42)	81.76 (20.17)	0.64
BMI (mean (SD))	32.42 (8.92)	26.70 (5.54)	0.77
BMI ≥ 30 (N (%))	4 (57.1)	2 (20.0)	0.83
# Days of admission prior to study EN initiation (median [IQR])	3.59 [2.03, 5.67]	2.04 [1.75, 2.62]	0.39
# Days of admission prior to any EN (median [IQR])	1.65 [1.01, 2.54]	1.52 [0.86, 1.94]	0.27
>48h of non-study EN before study (N (%))	2 (28.6)	2 (20.0)	0.2
# Days on any antibiotic (Abx) before study (N (%))	6.00 (85.7)	10.00 (100.0)	0.58

	NF-EN (N=7)	scFOS-EN (N=10)	Std Mean Difference
# Days on aerobic Abx before study (median [IQR])	2.00 [1.00, 4.00]	2.00 [1.00, 2.00]	0.22
# Days on anaerobic Abx[a] before study (median [IQR])	0.00 [0.00, 1.50]	1.50 [0.25, 3.00]	0.54
Positive respiratory culture before study (N (%))	3.00 (42.9)	4.00 (40.0)	0.06
Study Period - Nutritional Data			
# Days (full 24h) of study EN received (median [IQR])	10.00 [9.00, 10.00]	9.50 [6.50, 10.00]	0.46
Kcal prescribed per day (mean (SD))	1748.29 (189.54)	1916.80 (258.08)	0.74
# Days w>50% goal Kcal by study EN (median [IQR])	8.00 [7.00, 8.50]	5.50 [5.00, 8.25]	0.49
Weight (kg) used for daily intake (Adjusted body weight if BMI >30[b]) (mean (SD))	74.77 (16.13)	76.17 (12.79)	0.1
Average Kcal from study EN per kg per day (Kcal/kg/day[b]) (mean (SD))	17.33 (3.48)	17.11 (7.16)	0.04
Average amount of protein (g) including supplemental protein (g/kg/day[b]) (mean (SD))	1.36 (0.22)	1.23 (0.41)	0.4
Average amount of carbohydrates (g/kg/day[b]) (mean (SD))	2.28 (0.46)	1.58 (0.66)	1.24
Average amount of fat (g/kg/day[b]) (mean (SD))	0.57 (0.11)	0.77 (0.32)	0.84
Average amount of fiber (g/day) (mean (SD))	0.00 (0.00)	5.37 (1.94)	3.9
Average amount of added sugar (g/day) (mean (SD))	9.00 (2.19)	4.47 (1.62)	2.35
Study Period - Sample Collection			
# Fecal samples per patient (median [IQR])	2.00 [2.00, 5.00]	3.50 [2.25, 4.75]	0.21
ICU Days spanned by fecal sampling (median [IQR])	4.00 [2.50, 8.50]	5.00 [3.25, 6.00]	0.18
Time (in days) to 1st fecal collection (median [IQR])	3.42 [0.97, 5.33]	2.39 [1.93, 2.90]	0.48
# Fecal samples collected outside of 24h of Abx administration	1.00 (from 1 patient)	3.00 (from 2 patients)	
# Oral samples per patient (median [IQR])	6.00 [5.00, 6.00]	5.50 [5.00, 6.00]	0.04
Patient Outcomes			
# Days of study intervention period (for clinical obs data) (median [IQR])	10.00 [10.00, 10.00]	10.00 [10.00, 10.00]	0.55
ICU LOS (median [IQR])	22.96 [19.43, 26.94]	17.09 [13.32, 25.13]	0.61
Hospital LOS (median [IQR])	31.49 [26.97, 52.74]	35.94 [17.03, 52.00]	0.48
ICU LOS after Study EN start (median [IQR])	17.59 [14.60, 22.39]	15.17 [11.56, 21.08]	0.51
Hospital LOS after study EN start (median [IQR])	28.91 [23.26, 45.69]	31.02 [15.21, 45.98]	0.45
Presence of diarrhea during study period (N (%))	2.00 (28.6)	4.00 (40.0)	0.24
Average % of study days[c] on IMV (mean (SD))	92.71 (15.00)	79.00 (34.14)	0.52
Liberated from IMV during study (N (%))	2.00 (28.6)	3.00 (30.0)	0.03
Mortality 30d (N (%))	1.00 (14.3)	4.00 (40.0)	0.6
Percent of study days[c] on any Abx (mean (SD))	67% (24)	76% (21)	0.37
Percent of study days[c] on aerobic Abx (mean (SD))	60% (26)	64% (28)	0.14
Percent of study days[c] on anaerobic Abx (mean (SD))	32% (31)	46% (36)	0.41
# Positive respiratory cultures per patient during study period (median [IQR])	1.00 [0.00, 2.00]	1.00 [0.00, 1.00]	0.24

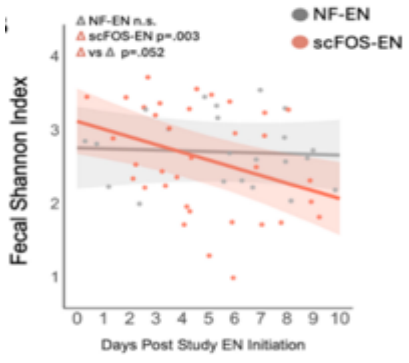
1. Acronyms: Abx antibiotics, EN enteral nutrition, IQR interquartile range, LOS length of stay, SD standard deviation
2. aAnaerobic Abx refers to those with anaerobic coverage regardless of aerobic activity (e.g. Piperacillin-Tazobactam & Metronidazole)
3. bWeight for participants with BMI ≥ 30 used Adjusted Body Weight (AdjBW = IBW + 0.4(Actual Body Weight – Ideal Body Weight)
4. cPercent of study days = (# days of [therapy] ÷ total # of days in study intervention period)100

Figure 2a displays comprehensive individual timelines for all 17 analyzed participants, aligned to the day of study EN initiation (Day 0). These timelines detail periods of formula administration (NF-EN or scFOS-EN), fecal

sampling occasions, exposure to antibiotics differentiated by aerobic-only versus combined aerobic-anaerobic spectra, results of positive blood and respiratory cultures, and key clinical endpoints such as death or transition to hospice care (complete participant-level data are presented in Additional file 3). Antibiotic administration was nearly universal: pre-study aerobic antibiotics were given to 6 of 7 NF-EN patients and all 10 scFOS-EN patients, while all participants received antibiotics during the intervention period **(Figure 2a)**. The average percentage of study days involving antibiotic therapy showed close similarity between groups for any antibiotics (67% in NF-EN vs. 76% in scFOS-EN), aerobic-only agents (60% vs. 64%), and anaerobe-active agents (32% vs. 46%) **(Table 1)**. Observed clinical outcomes were likewise comparable and underscored the severity of illness in this population. Liberation from invasive mechanical ventilation (IMV) during the study occurred in only 2 of 7 NF-EN and 3 of 10 scFOS-EN patients. Three participants died or were transitioned to hospice (1 in NF-EN and 2 in scFOS-EN). Among those who survived, post-EN ICU length of stay (LOS) extended from 9 to 37 days **(Table 1)**.



a)



b)

Figure 2. Patient-level clinical timelines and gut microbial alpha diversity dynamics.

A Swim-lane plot depicting the clinical trajectory of each of the 17 participants in the final cohort, synchronized by the day of study enteral nutrition (EN) initiation (Day 0). Horizontal lanes represent individual participants identified by unique study IDs. Displayed elements include timing of EN delivery (NF-EN or scFOS-EN), fecal sample acquisition, administration of aerobic-only versus anaerobic-inclusive antibiotics, positive blood and

respiratory culture findings, and occurrences of death or hospice transition. In-study events (Day 0 to Day 10) are scaled proportionally (24 h per day), whereas pre-Day 0 events are not to scale. B Fecal Shannon diversity trajectories across the 10-day study period for the NF-EN (grey) and scFOS-EN (orange) groups (NF-EN n = 22 samples, scFOS-EN n = 35 samples). Solid lines correspond to the average daily rate of change estimated by linear mixed-effects models. Shaded bands represent 95% confidence intervals; reported p-values indicate the statistical significance of within-group temporal change (Δ) and the difference in slopes between groups (Δ scFOS-EN vs Δ NF-EN) from the interaction term.

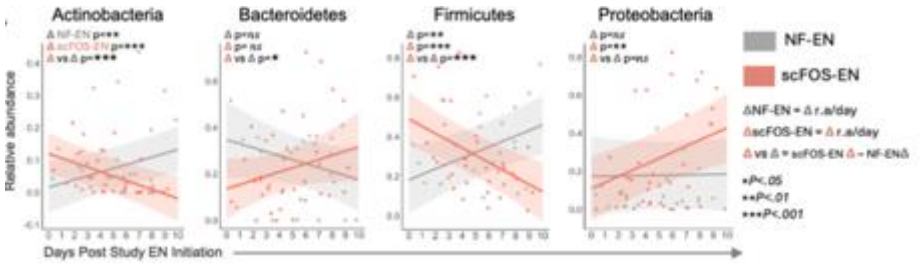
scFOS-EN is associated with declining gut microbial diversity

Multivariable linear mixed-effects models (LMMs) served as the main tool to examine how scFOS-EN influenced the intestinal microbiota. These models assessed within-arm temporal trends starting from the day of study EN initiation (Day 0) and employed the group-by-time interaction to isolate the contribution of scFOS-EN. Diversity levels in the NF-EN group stayed relatively constant during the observation period. Conversely, the scFOS-EN arm showed a clear reduction in Shannon diversity index, decreasing by −0.10 per day (95% CI [−0.17, −0.03], p = 0.003), along with a drop in observed ASV counts of −4.2 per day (95% CI [−6.6, −1.8], p = 0.0005) **(Figure 2b)**. The interaction analysis nearly reached significance, revealing that scFOS-EN was linked to an extra daily diversity decline of −0.092 compared with the NF-EN trajectory (95% CI [−0.184, 0.001], p = 0.059). This finding supports the presence of a specific effect driven by the supplemented formula.

To place these results in context, predicted Shannon index values at Day 0 (D0 Shannon estimates) were calculated from the models to represent each participant's initial microbial condition. These estimates were comparable between the NF-EN and scFOS-EN groups and ranged from 2.5 to 3.5, confirming severe dysbiosis at the outset of the intervention. Additional correlation testing indicated that lower baseline diversity correlated strongly with prolonged pre-enrollment hospital stay (r = −0.671, p = 0.004), extended pre-study antibiotic exposure (r = −0.582, p = 0.01), and detection of positive respiratory cultures (r = −0.599, p = 0.01).

scFOS-EN is associated with distinct alterations in gut microbial composition

Further evaluation focused on formula-related shifts in community structure, relying primarily on LMMs supplemented by comparisons of mean relative abundances after stratifying samples into three periods: Days 0–2 (baseline phase before appreciable prebiotic influence), Days 2–5 (initial intervention), and Days 5–10 (later intervention). Within-group LMM analyses revealed significant evolution across the four main phyla—Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria. The interaction term highlighted that scFOS-EN significantly intensified the reduction of Actinobacteria (−2.6%/day relative to NF-EN, 95% CI [−3.7%, −1.5%], p < 0.001) and Firmicutes (−6.3%/day, 95% CI [−8.8%, −3.8%], p < 0.001), while promoting greater expansion of Bacteroidetes (+3.7%/day, 95% CI [0.6%, 6.7%], p = 0.023) (full phylum results appear in Additional file 4). Inspection of average relative abundances by interval confirmed marked dysbiosis during the early baseline window (Proteobacteria comprising 5–10% and Firmicutes >40% in both arms) and illustrated the progressive phylum-level rearrangements identified statistically by the models **(Figure 3b)**.



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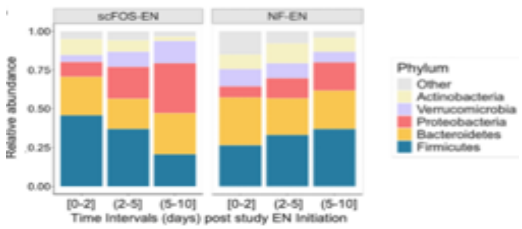


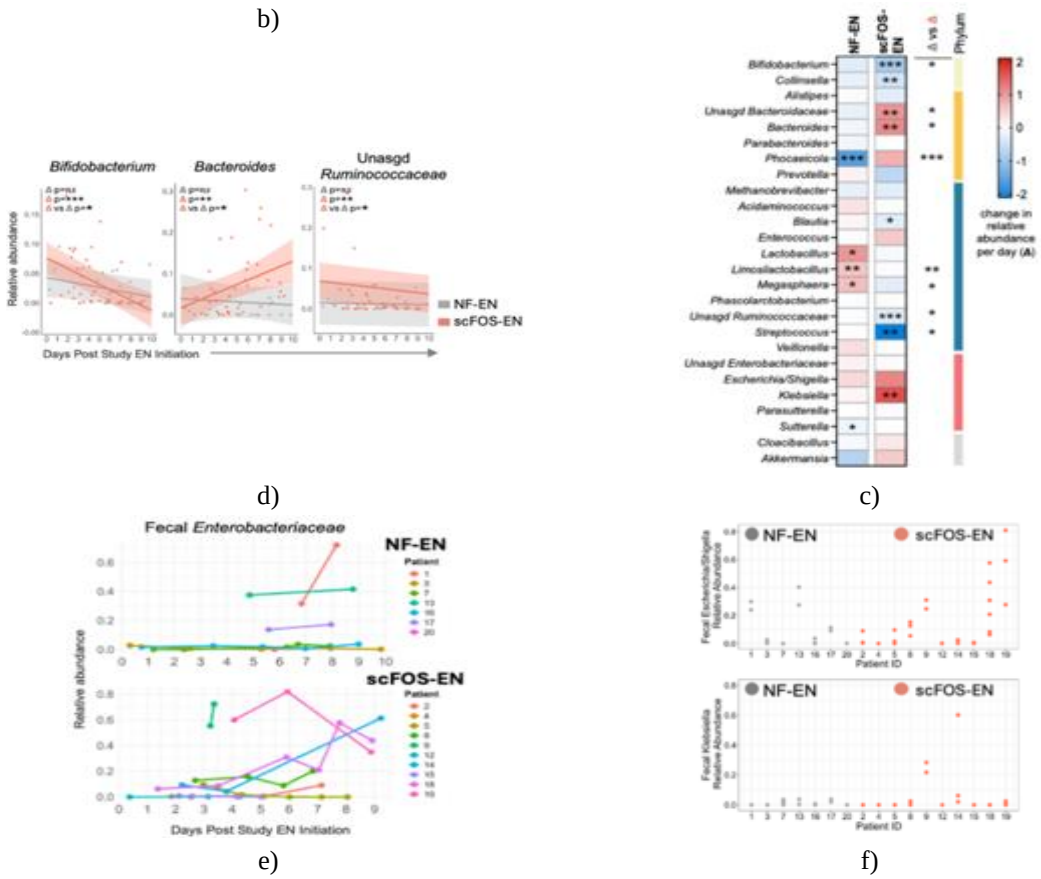


Figure 3. scFOS-EN administration is associated with significant alterations in gut microbial composition.

A Trajectories of the four dominant phyla estimated by LMMs over the 10-day study period. Lines show average slopes of relative abundance (r.a.) change for NF-EN and scFOS-EN groups, with shaded areas representing 95% confidence intervals. P-values indicate the significance of within-group temporal trends (Δ) and between-group differences in rates of change (Δ vs Δ). B Average relative abundances of major phyla across three time windows: baseline (Days 0–2) (n = 3 NF-EN samples, n = 3 scFOS-EN samples), early intervention (Days 2–5) (n = 4 NF-EN, n = 18 scFOS-EN), and late intervention (Days 5–10) (n = 15 NF-EN, n = 15 scFOS-EN). C Heatmap illustrating the modeled daily percent change in relative abundance (%Δ r.a./day) for important genera derived from LMMs. Columns present within-group trends for NF-EN, within-group trends for scFOS-EN, and the differential interaction effect (Δ vs Δ). Color intensity corresponds to the direction and strength of change (red = increase, blue = decrease). Asterisks indicate statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001). D LMM-derived trajectories for the genera Bifidobacterium, Bacteroides, and unassigned Ruminococcaceae. E–F Participant-specific trajectories showing relative abundance of the Enterobacteriaceae family and the relative contributions of Escherichia-Shigella and Klebsiella. All LMMs included repeated measures, adjusted for sex, and accounted for time from EN initiation. For visual clarity, the displayed graphs show observed abundances over time without covariate adjustment.

Genus-level analyses further identified changes in numerous taxa

Examination at the genus level uncovered temporal variations affecting a range of taxa in both treatment groups **(Figure 3c)**. However, only Phocaeicola (reduced in the scFOS-EN arm) and unassigned (U) Ruminococcaceae showed significant baseline differences. Key observations in the scFOS-EN cohort included a clear rise in the pathobiont Klebsiella (+1.7%/day, 95% CI [0.5%, 2.8%], p = 0.004) and a tendency toward increased Escherichia-Shigella (both members of the Enterobacteriaceae family) that did not reach significance. Interaction testing relative to the NF-EN group indicated that scFOS-EN produced more pronounced declines in Bifidobacterium (−0.6%/day, 95% CI [−1.1%, −0.1%], p = 0.026), U-Ruminococcaceae (−0.3%/day, 95% CI [−0.5%, 0%], p = 0.023), Limosilactobacillus (−0.6%/day, 95% CI [−1%, −0.2%], p = 0.003), Streptococcus (−2.1%/day, 95% CI [−4%, −0.2%], p = 0.035), and Megasphaera (−1%/day, 95% CI [−1.8%, −0.2%], p = 0.025). In contrast, scFOS-EN was linked to faster growth of Bacteroides (+1.3%/day, 95% CI [0.2%, 2.4%], p = 0.027), Phocaeicola

(+2.4%/day, 95% CI [1.3%, 3.5%], p < 0.001), and U-Bacteroidaceae (+1.3%/day, 95% CI [0.2%, 2.4%], p = 0.024), with a near-significant interaction for Klebsiella (+1.6%/day, 95% CI [0, 3.1%], p = 0.052) **(Figure 3d)**. All associations held after additional adjustment for the time elapsed between ICU admission and the start of study EN.

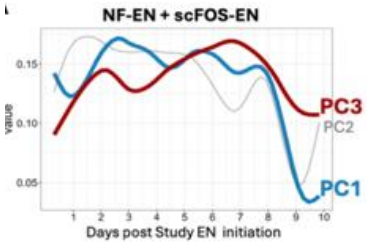
Targeted modeling of the Enterobacteriaceae family (Proteobacteria phylum) was conducted because prior ICU research has connected high levels of this family to adverse clinical outcomes [4, 32] and several of its constituents can efficiently metabolize readily fermentable fibers [33, 34]. Consistent with the genus results, scFOS-EN led to notable expansion of Enterobacteriaceae (+0.3%/day, 95% CI [1.0%, 5.0%], p = 0.003). The interaction analysis attributed an extra +2.5%/day increase in this family to scFOS-EN compared with NF-EN (95% CI [−0.2%, 5.2%], p = 0.081). This expansion appeared in the large majority of participants with serial samples. High Enterobacteriaceae burden, operationalized as ≥20% relative abundance, occurred in 29% of the NF-EN group and 50% of the scFOS-EN group **(Figure 3e)**, predominantly attributable to Escherichia-Shigella and Klebsiella **(Figure 3f)**. Projections from the models at Day 10 suggested that 90% of scFOS-EN participants would exhibit high Enterobacteriaceae burden by study conclusion, in comparison to only 29% of NF-EN participants (p = 0.04). Overall, the data show that scFOS-EN promotes unfavorable compositional shifts by favoring Bacteroidaceae taxa at the expense of Bifidobacterium and Firmicutes while also enhancing the proliferation of pathobiont Enterobacteriaceae.

scFOS-EN has little impact on oral microbial composition

Oral microbiota responses were assessed as a negative control because study formulas are delivered directly into the gastrointestinal tract, bypassing the mouth. LMM evaluation of oral specimens revealed generally modest temporal variation in both arms. The single notable difference was a small acceleration in Carnobacteriaceae-U (a typical oral organism) in the scFOS-EN group (+0.3%/day, 95% CI [0, 0.6%], p = 0.03). This pattern reinforces the conclusion that the primary microbial effects of NF-EN and scFOS-EN are confined to the gut compartment.

Linking interconnected time-dependent gut microbial responses in NF-EN and scFOS-EN to clinical features using TEMPTED

An additional objective was to investigate whether the individual taxon dynamics captured by LMMs represented coordinated shifts involving both commensal and pathobiont populations, especially in relation to antibiotic pressure and evolving clinical conditions. TEMPTED, a dimensionality reduction method tailored for irregularly timed longitudinal microbiome profiles [29], was therefore employed. The initial analysis included ASV data from participants with multiple samples (53 samples from 6 NF-EN and 8 scFOS-EN patients) **(Figure 2a)**. The first three principal components (PCs) displayed unique temporal profiles **(Figure 4a)**, of which PC1 and PC3 exhibited meaningful clinical correlations.



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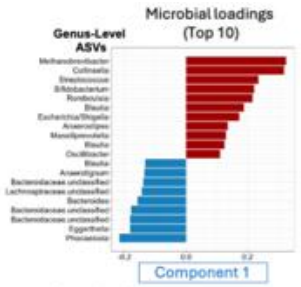

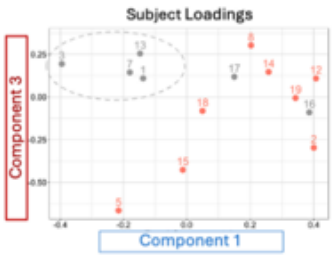

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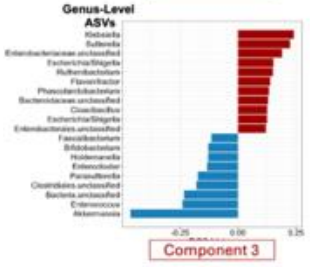

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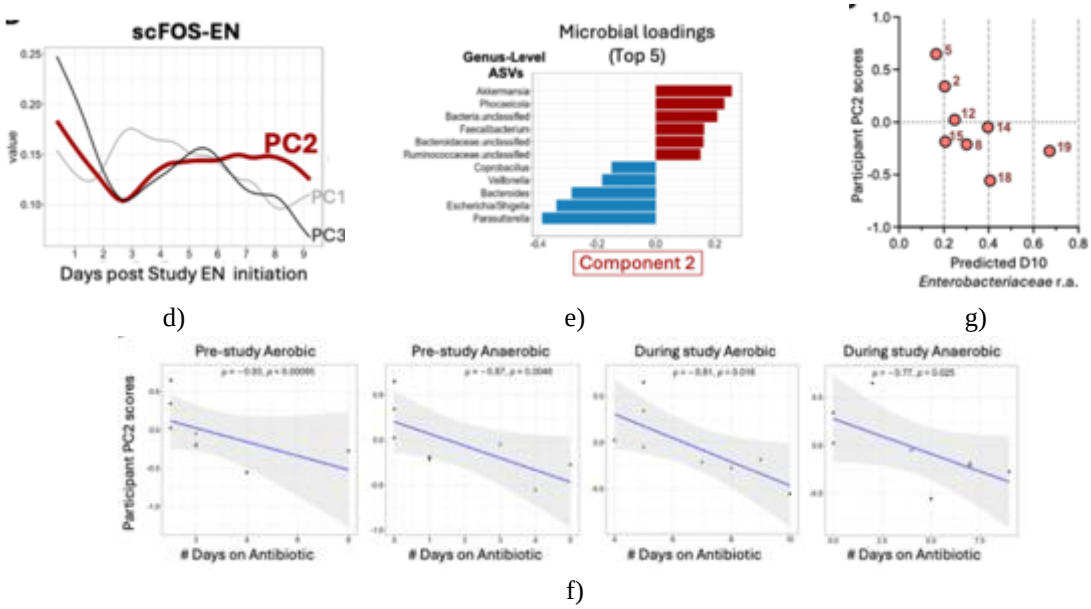

Figure 4. Time-informed dimensionality reduction reveals interconnected microbial signatures and a dichotomous response to scFOS-EN.

Outcomes of TEMPoral TEnsor Decomposition (TEMPTED), an unsupervised machine learning technique applied to longitudinal ASV-level data. A–C Findings from the analysis encompassing all participants. A Temporal loading patterns for the three leading principal components (PCs). B The ten microbial ASVs showing the strongest positive (red) and negative (blue) loadings on PC1 (infection/inflammation-related) and PC3 (carbohydrate-associated pathobiont expansion). C Participant-specific PC1 versus PC3 scores plotted with coloring by treatment assignment (NF-EN: grey, scFOS-EN: orange). NF-EN subjects lacking pre-study anaerobic antibiotic treatment are marked with grey circles. D–G Outcomes from TEMPTED restricted to the scFOS-EN participants. D Temporal loading of PC2, which differentiates beneficial (positive) from detrimental (negative) response patterns. E The five microbial ASVs with the largest positive and negative loadings on PC2. F Spearman correlation results between PC2 scores and antibiotic exposure variables, including correlation coefficients (ρ) and p-values. G Spearman correlations linking individual PC2 scores to LMM-predicted Day 10 Enterobacteriaceae abundance, highlighting the separation of participants without anaerobic antibiotic exposure (PC2 > 0) from those with exposure (PC2 < 0).

The initial principal component (PC1) displayed an early maximum, followed by relatively minor fluctuations before a pronounced drop around days 7–8 **(Figure 4a)**. This component was marked by amplicon sequence variants (ASVs) originating from genera responsive to scFOS-EN. Key positive loadings that influenced its pattern encompassed Methanobrevibacter (ASV31), Collinsella (ASV37), Streptococcus (ASV14), and Bifidobacterium (ASV39). Taxa exhibiting contrasting temporal profiles, characterized by a steep rise near days 7–8, included Phocaeicola (ASV7), Bacteroides (ASV60), and various unclassified Bacteroidaceae (ASVs 172, 176, 264) **(Figure 4b)**(top).

Participant PC1 scores demonstrated a positive association with the total count of culture events (respiratory or blood) ($r = 0.60$, $p = 0.026$) and were significantly elevated among individuals with positive respiratory cultures throughout the investigation (mean 0.234 versus −0.076, $p = 0.03$). This connection tied the microbial signature to ongoing infection and pneumonia. Conversely, principal component 3 (PC3) captured a pattern of dual peaks occurring near day 2 and day 6 **(Figure 4a)**. This trajectory corresponded to fluctuations among several Enterobacteriaceae members and additional pathobionts. Prominent positive loadings involved ASVs corresponding to Klebsiella (ASV4), Sutterella (ASV69), and Escherichia-Shigella (ASV2, ASV108). The strongest negative loadings, showing opposite dynamics, featured Akkermansia (ASV6), Enterococcus (ASV24), along with health-associated short-chain fatty acid producers such as Bifidobacterium (ASV39) and Faecalibacterium (ASV147) **(Figure 4b)**(bottom).

Associations with clinical parameters revealed that PC3 scores correlated robustly with carbohydrate consumption ($r = 0.701$, $p = 0.007$) and mean daily caloric provision ($r = 0.789$, $p = 0.001$), yet showed no link to projected Day 10 Enterobacteriaceae levels. PC3 scores also did not vary according to the presence or absence of elevated

Enterobacteriaceae loads. In summary, these observations indicate that PC3 embodies a signature of carbohydrate-stimulated pathobiont proliferation, modulated in part—though not solely—by Enterobacteriaceae taxa.

No solitary PC score exhibited statistically significant differences between the NF-EN and scFOS-EN arms. Nevertheless, plotting participant scores uncovered a notable separation when PC1 and PC3 were considered jointly **(Figure 4c)**. The majority of scFOS-EN recipients, even those lacking prior anaerobic antibiotic treatment, clustered in the upper-right quadrant of PC1/PC3 ordination space. This distribution implied enhanced prevalence of microbial configurations related to both infectious processes and carbohydrate-fueled pathobiont growth. In comparison, certain NF-EN participants—specifically those without pre-study anaerobic antibiotic administration—constituted a discrete cluster defined by elevated PC3 alongside reduced PC1 values. This pattern pointed to pathobiont proliferation independent of an infectious component **(Figure 4c)**. These results furnish mechanistic insight into the taxonomic alterations earlier detected via linear mixed models and emphasize major clinical influences, such as infection status, carbohydrate exposure, and possibly antecedent antibiotic use.

Context-specific microbial dynamics in the scFOS-EN group using TEMPTED

To more thoroughly examine variability in microbial responses within the treatment group and assess potential modulation by clinical variables including antibiotic history, TEMPTED modeling was restricted to longitudinal specimens from scFOS-EN recipients alone (32 samples obtained from $n = 8$ participants). The resulting analysis yielded three principal components with distinct microbial contributions compared to the pooled cohort **(Figures 4d–4e)**. Participant scores on PC1 and PC3 lacked ties to clinical metrics, whereas PC2 scores were positive solely in individuals without pre-study anaerobic antibiotic exposure, denoting a unique reaction to scFOS-EN (Additional file 4).

PC2 was delineated by proliferation of commensal organisms, notably Akkermansia (ASV 6), Phocaeicola (ASV 7), Faecalibacterium (ASV 147), and U-Ruminococcaceae (ASV 125), signifying outgrowth of potentially advantageous taxa. The antagonistic trajectory, however, involved expansion of the pathobiont Escherichia-Shigella (ASV2) and Bacteroides (ASV49) **(Figure 4e)**. Clinical correlations highlighted antibiotic exposure as a pivotal determinant of these divergent patterns: higher PC2 scores, indicative of enriched beneficial microbial traits, correlated negatively with pre-study anaerobic antibiotic duration ($r = -0.872$, $p = 0.01$) and aerobic antibiotic duration ($r = -0.926$, $p = 0.004$), as well as with in-study anaerobic ($r = -0.711$, $p = 0.033$) and aerobic ($r = -0.805$, $p = 0.025$) antibiotic days **(Figure 4f)**. Such relationships strongly imply that prior antibiotic-mediated disruption of the gut microbiota substantially alters the response to scFOS-EN.

Furthermore, PC2 scores (unlike other components in either analysis) aligned with positive clinical endpoints, displaying inverse correlations with the proportion of study days requiring invasive mechanical ventilation ($r = -0.764$, $p = 0.036$) and overall ICU length of stay ($r = -0.964$, $p = 0.003$). PC2 scores additionally showed a negative relationship with forecasted day-10 Enterobacteriaceae abundance ($r = -0.857$, $p = 0.01$) **(Figure 4g)** and a near-significant inverse link to high Enterobacteriaceae burden ($r = -0.764$, $p = 0.057$). These data collectively suggest that temporal rises in taxa loading negatively on PC2 (particularly Escherichia-Shigella and Bacteroides) promote Enterobacteriaceae overgrowth and contribute to extended ICU hospitalization. Hence, scFOS-EN may bolster commensal populations in relatively intact gut ecosystems, yet promote unfavorable pathobiont dominance in microbiomes previously impaired by extensive anaerobic antibiotic therapy.

Network analysis highlights increased microbial competition in scFOS-EN

In a final step, the investigation evaluated whether co-varying microbial patterns uncovered by TEMPTED corresponded to modified interspecies interactions triggered by the nutritional inputs of scFOS-EN versus NF-EN. Co-occurrence networks were therefore generated via the Micnet Toolbox at the ASV level, utilizing fecal samples from days 5–10 for each participant. This approach provided a temporal cross-section of microbial associations at the height of each nutritional intervention [30]. Samples from both groups were drawn at equivalent post-EN intervals and displayed comparable Enterobacteriaceae relative abundances, limiting potential sampling artifacts.

The two networks exhibited similar global complexity and connectivity density; however, their internal ecological organization diverged sharply. Tripartite interactions within the NF-EN network were evenly split between competitive ($- - +$) and cooperative ($+ + +$) configurations (50% each). By contrast, the scFOS-EN network was dominated by competitive interactions, which comprised approximately 75% of linkages, reflecting a markedly more antagonistic microbial environment. Features belonging to the carbohydrate-responsive pathobiont-

associated PC (PC3) formed a cohesive module in both networks (including Escherichia-Shigella (ASV2) and U-Enterobacteriaceae (ASV 120)), consistent with shared nutrient utilization strategies [30]. This module additionally incorporated Klebsiella (ASV 4) and Sutterella (ASV 69) exclusively in the scFOS-EN network, likely reflecting their enhanced co-occurrence under scFOS supplementation.

Further correlation examination indicated that Enterobacteriaceae taxa competed with commensals in both networks, yet such antagonistic relationships were substantially more extensive in the scFOS-EN network. Escherichia-Shigella (ASV 2), in particular, displayed broad negative associations with multiple taxa, encompassing Actinobacteria and Firmicutes that diminished over time according to LMM and TEMPTED results, in addition to various Bacteroidaceae representatives. These observations supply a credible ecological basis—namely, intensified resource competition—for the depletion of beneficial microbes observed in the scFOS-EN setting.

In this pilot randomized controlled trial involving critically ill trauma patients, supplementation of enteral nutrition with the prebiotic scFOS failed to produce the advantageous effects previously documented in healthier individuals [10, 11, 27]. Instead of promoting beneficial microbial groups, it coincided with a more rapid decline in Bifidobacterium and Firmicutes, proliferation of Bacteroides and additional Bacteroidaceae taxa, and possible facilitation of pathobiont Enterobacteriaceae expansion. Importantly, advanced temporal modeling demonstrated that pathobiont growth at the cost of health-associated commensals is modulated by factors including carbohydrate provision, persistent infection, and antibiotic administration. Deleterious microbial shifts in the scFOS-EN arm, notably elevated Enterobacteriaceae loads, exhibited significant associations with antibiotic exposure both prior to and throughout the intervention. Overall, these results offer compelling support for the principle that the pre-existing microbial environment determines the efficacy of prebiotic interventions in intensive care settings.

Prior investigations by our group and others, employing culture-independent sequencing methods, have established that intestinal dysbiosis in ICU patients is both severe and progressive. Typical features include depletion of short-chain fatty acid-producing Firmicutes and overgrowth of potential pathogens [3, 4, 31, 33, 35]. The present study extends this work by elucidating the influential role of enteral nutrition in directing these shifts. A comprehensive strategy was utilized to examine microbial temporal dynamics and interspecies relationships following the start of two commonly used EN preparations. The environmental challenges faced by study participants are representative of broader ICU populations: roughly 70% of such patients receive antibiotics daily [5], and insufficient caloric delivery remains common, especially in the initial week of admission [17]. Although this investigation represents, to our knowledge, the first randomized controlled trial assessing scFOS-EN in critical illness, the unfavorable microbial alterations observed are consistent with findings from other research on prebiotics in disrupted gut ecosystems. For instance, a 22-patient ICU trial of oligofructose/inulin reported reduced abundance of Faecalibacterium, an important SCFA-generating Firmicutes member [21]. Likewise, in a preclinical model of antibiotic-associated dysbiosis, FOS administration impeded microbiota recovery and promoted outgrowth of Bacteroides along with pathobionts such as Klebsiella and Escherichia-Shigella [34].

To investigate longitudinal trends in greater depth, the time-aware dimensionality reduction technique TEMPTED was applied [29]. When both study arms were analyzed jointly, TEMPTED uncovered a temporal pattern (PC3) reflecting concurrent expansion of pathobionts including Klebsiella, Sutterella, and Escherichia-Shigella, countered by beneficial commensals such as Akkermansia, Bifidobacterium, and Faecalibacterium [6]. This pathobiont-linked signature appeared widely across participants and correlated strongly with carbohydrate administration, yet not with fiber consumption. These observations are consistent with evidence that, under conditions of dysbiosis or limited carbohydrate availability, readily accessible simple carbohydrates can sustain opportunistic organisms when robust fermentative species are diminished [7, 14]. Pathobionts exhibit varying carbohydrate utilization profiles; Klebsiella and Sutterella can metabolize complex carbohydrates and extended-chain prebiotic fibers, whereas Escherichia coli preferentially consumes simpler sugars [8, 36, 37]. Such differences likely account for the co-clustering of Klebsiella and Sutterella as leading contributors to PC3, and may underlie their enhanced co-occurrence within scFOS-EN networks (absent in NF-EN networks). This pattern could also explain the LMM-identified rise in Klebsiella under scFOS-EN, even though this taxon responded to carbohydrates in both nutritional formulas.

Although PC3 scores emphasized carbohydrate-sensitive pathobionts, a specific trajectory linked to Enterobacteriaceae proliferation emerged from TEMPTED modeling confined to the scFOS-EN subgroup. Within this analysis, elevated Enterobacteriaceae burden aligned with a unique microbial configuration featuring increased prominence of Escherichia-Shigella together with particular Bacteroides and Parasutterella ASVs

(negative PC2 loadings) relative to beneficial organisms such as Akkermansia, Phocaeicola, and Faecalibacterium (positive PC2 loadings). Of particular significance, a clear divergence in PC2 temporal responses was evident according to antibiotic history. Individuals who had received pre-study anaerobic antibiotics displayed stronger representation of negative PC2 features. Moreover, the cumulative duration of both aerobic and anaerobic antibiotic therapy before and during scFOS-EN delivery emerged as relevant modifiers. This finding may clarify the absence of anticipated Bifidobacterium and SCFA-producing Firmicutes expansion despite sufficient scFOS dosing. In a microbial community depleted of these foundational commensals, hardier species are positioned to occupy the vacated niche. In nutrient-scarce conditions, specific Escherichia coli and Bacteroides strains efficiently exploit readily metabolizable carbon sources, outcompeting other residents and potentially engaging in mutual competition at later stages [15, 38]. This dynamic is corroborated by network analyses revealing heightened competitive interactions in the scFOS-EN group, especially between Enterobacteriaceae pathobionts and a range of commensal taxa, including Bacteroides. Collectively, the data portray ICU-associated dysbiosis as resembling a "scorched lawn" — impaired by restricted nutrition and extensive antibiotic exposure. Within such a depleted setting, provision of a prebiotic such as scFOS may function analogously to fertilizing weeds, enabling fast-growing, resilient organisms to prevail, suppress residual commensals, and ultimately compete among themselves for limited resources.

Despite being underpowered for clinical endpoints, the study identified associations between the negative PC2 phenotype and extended ICU length of stay as well as prolonged mechanical ventilation. These links parallel established connections between elevated Enterobacteriaceae abundance, antibiotic use [39-41], and adverse clinical trajectories reported in other ICU cohorts [4, 32, 42]. The current work contributes to this body of evidence by suggesting that scFOS-EN, when introduced following or concurrent with broad-spectrum antibiotics, may amplify microbial characteristics tied to high Enterobacteriaceae loads. It additionally underscores the relationship in ICU patients between the Enterobacteriaceae phenotype and the progressive outgrowth of Escherichia-Shigella alongside resilient Bacteroides colonizers. Synergistic proliferation of these organisms has been documented in undernourished children and specifically implicated in intestinal inflammatory processes [14].

Notably, the advantageous "PC2 positive" commensal organisms exhibited higher abundance among participants with minimal antibiotic exposure. Linear mixed models further indicated that scFOS-EN promoted accelerated growth of Phocaeicola, a prominent beneficial taxon. Collectively, these observations point toward a possible favorable impact of scFOS-EN when introduced into relatively preserved gut microbial communities. Given that antibiotic therapy is frequently unavoidable in intensive care settings, the present findings also emphasize the therapeutic potential of interventions aimed at restoring the gut microbiota, such as synbiotics. Supporting this notion, a large-scale randomized controlled trial demonstrated that the combination of Lactobacillus and FOS lowered the incidence of neonatal sepsis [8], thereby illustrating the value of combined administration approaches within compromised microbial environments.

The current study is subject to several limitations. As a pilot trial, it enrolled a modest number of participants. Consequently, it lacks sufficient statistical power to link microbial patterns directly to clinical outcomes, and the results require confirmation in a larger-scale investigation. Although residual confounding cannot be entirely ruled out, the randomized, blinded study design, coupled with consistent results obtained through multiple analytical methods (linear mixed models, network analysis, and TEMPTED), enhances confidence in the primary biological observations. Second, it is important to recognize the methodological difficulty in isolating the precise effects of scFOS amid the dominant influence of concurrent antibiotic therapy. Antibiotic use represents a major, non-randomized co-intervention in the ICU that exerts strong selective pressure on the microbiota. In a pilot cohort of limited size, this factor restricts the ability to statistically disentangle its effects from those of the nutritional intervention. Although TEMPTED modeling revealed divergent microbial trajectories according to antibiotic history, the analysis cannot fully separate the impact of scFOS from the overarching antibiotic-driven background. Thus, the intricate interaction between antibiotics and scFOS-EN should be regarded as an important observation meriting detailed examination in future, sufficiently powered studies. Third, inherent compositional differences existed between the two enteral nutrition formulas beyond the presence of scFOS. Although protein delivery was comparable, participants receiving NF-EN consumed greater quantities of added sugars. Nevertheless, the fact that numerous alterations associated with scFOS-EN also emerged as key features in TEMPTED analysis restricted to the scFOS-EN subgroup supports the interpretation that these changes were primarily driven by scFOS. Finally, the well-recognized constraints of 16S rRNA gene sequencing must be acknowledged, including its inability to measure overall microbial biomass, characterize mucosa-adherent communities, or provide direct

functional insights. Subsequent research incorporating quantitative PCR, shotgun metagenomics, and transcriptomic approaches will be necessary in larger cohorts to address these gaps.

Conclusion

In summary, the present study demonstrates that the impact of prebiotic fibers—and carbohydrates more broadly—within enteral nutrition formulations is neither uniform nor predictable, but instead highly dependent on contextual factors and the composition of the resident microbial community. In the distinctive ecological setting of the ICU gut microbiota, such context-dependence assumes particular importance and can result in unanticipated, and occasionally adverse, shifts in microbial structure. The finding that antecedent antibiotic exposure can transform the response to scFOS-EN from potentially beneficial to detrimental carries significant clinical relevance. Collectively, these results raise caution regarding the application of uniform, "one-size-fits-all" prebiotic supplementation strategies in critically ill patients and advocate for personalized, context-sensitive nutritional approaches. Future progress will depend on the identification of microbial signatures predictive of therapeutic responsiveness, along with the adoption of analytical frameworks capable of capturing temporal dynamics and microbial interactions, to facilitate the development of safe and efficacious microbiota-directed interventions in critical illness.

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