Selective enrichment of probiotic and SCFA-associated gut bacteria after four months of a plant-based fermented beverage intake: a longitudinal study in healthy adults

A.C. van der Heijden[1] & D. Böhm[2]

[1] Independent Researcher, Amsterdam, The Netherlands

[2] Department of Clinical Pharmacy, Catharina Ziekenhuis Eindhoven

Abstract

Daily consumption of plant-based fermented beverages has been proposed as an accessible strategy to support gut microbial communities. Yet in-vivo human evidence for sustained effects remains limited, with most trials lasting fewer than 30 days. In this four-month single-arm study, 21 healthy adults consumed a multi-strain plant-based fermented beverage daily. Gut microbiome composition was assessed by 16S rRNA gene amplicon sequencing at baseline, one month, and four months. Analyses focused on predefined genus groups: probiotic-associated (*Lactobacillus*), SCFA-associated, and pathogenic-associated taxa, α-diversity and β-diversity. Probiotic- and SCFA-associated genera both increased significantly by four months relative to baseline and one month, with no significant change at one month. Pathogenic-associated taxa and α-diversity remained unchanged, whereas overall community composition showed a modest shift over time (β-diversity). Together, these findings suggest that sustained intake of the fermented plant-based beverage is associated with selective, progressive enrichment of probiotic- and SCFA-associated genera without loss of α-diversity or expansion of pathogenic taxa. The progressive enrichment observed across four months establishes a microbiome-level foundation and a clear rationale for future randomized controlled trials.

Keywords: Microbiome, fermentation, probiotics, short-chain fatty acids

Introduction

Fermented plant-based beverages have attracted growing interest as non-dairy sources of live microorganisms and fermentation-derived compounds (Vilela et al., 2020; Vera-Pingitore et al., 2016). The composition of the plant matrix, including fibers, polyphenols, and fermentable carbohydrates, influences the microorganisms that develop during fermentation and the compounds they produce (Deziderio et al., 2023; Makarewicz et al., 2021; Vila-Real et al., 2025). Among these fermentation-derived compounds are organic acids, exopolysaccharides, and bioactive peptides. These compounds are classified as postbiotics, which can additionally modulate the gut environment (Salminen et al., 2021). After ingestion, plant-derived compounds and fermentation products can reach the colon, where they may be further metabolized by the resident microbiome (Alharbi, 2026). Live probiotic strains, in contrast, are generally transient in the adult gut but may remain metabolically active during gastrointestinal transit (Lewandowski, 2026).

Habitual diet is among the strongest determinants of gut microbiome composition, and Western dietary patterns in particular are associated with signatures of gut dysbiosis across industrialized populations (Rinninella et al., 2023). Plant-based fermented beverages have accordingly attracted interest as accessible dietary interventions that combine live probiotic microorganisms, fermentation-derived postbiotic compounds, and matrix-derived fermentable substrates within a single food matrix (Vilela et al., 2020). Unlike isolated probiotic preparations, these products deliver multiple bioactive fractions that may interact with resident colonic communities through complementary pathways (Vila-Real et al., 2025), offering a practical route to sustained dietary support of gut microbial function in otherwise healthy adults.

A key mechanism linking dietary patterns, the gut microbiome, and host health is the microbial production of short-chain fatty acids (SCFAs). These primarily consist of butyrate, acetate and propionate and are formed through fermentation of dietary carbohydrates. Butyrate plays a central role in colonocyte metabolism and mucosal homeostasis (den Besten et al., 2013; Gasaly et al., 2021). Reduced abundance of butyrate-producing taxa has been associated with gut dysbiosis and Western dietary patterns (Gasaly et al., 2021; Ecklu-Mensah et al., 2023), making them a meaningful taxonomic target for dietary intervention. Fermentation-derived compounds present in these beverages may further support this community by providing substrates or signaling molecules that enrich native butyrate-producing taxa, potentially enhancing

fermentative capacity beyond that achieved by probiotic strains alone. On this basis, sustained consumption of complex plant-based fermented beverages has been hypothesized to support probiotic and butyrate-associated gut communities in humans.

Whether such effects are realized in vivo, and with what consistency, remains insufficiently established. Much of the mechanistic evidence is derived from in vitro studies examining probiotic survival after digestion, metabolite formation during fermentation, and fecal fermentation responses, including increased SCFA production and enrichment of beneficial bacterial genera (Vila-Real et al., 2025; Vieira et al., 2021). Human intervention studies suggest that fermented plant-based products can modulate gut microbial community structure and alter the abundance of selected taxa, although the direction, magnitude, and taxonomic specificity of these responses remain inconsistent across studies.

More specifically, daily consumption of a quinoa-based fermented beverage for 14 days increased fecal *Lactobacillus* abundance without marked changes in overall diversity (Canaviri-Paz et al., 2021). A 14-day water kefir intervention shifted community composition without altering α-diversity or *Lactobacillus* abundance, alongside increases in *Bifidobacterium*, *Prevotella*, and SCFA-associated genera such as *Roseburia* and *Blautia* (de Mel et al., 2026). In a four-week human intervention study investigating the effects of kombucha consumption, more mixed responses were observed. Modest compositional changes included enrichment of *Weizmannia coagulans* and SCFA-producing taxa, but also species-level increases in less-favorably regarded taxa and depletion of *Lactobacillus*. In that study, α-diversity declined significantly from baseline to intervention end within the kombucha group, with little evidence of increased overall diversity (Ecklu-Mensah et al., 2024). Together, these studies indicate that fermented plant-based beverages, although often grouped within a single product category, do not elicit uniform changes in the gut microbiome.

Most published human trials are relatively short in duration, commonly ranging from approximately two to eight weeks, with many lasting only 14–30 days (Canaviri-Paz et al., 2021; de Mel et al., 2026; Ecklu-Mensah et al., 2024; Otchere et al., 2025; Fraiz et al., 2025). Short interventions may capture transient microbial responses but provide limited insight into longer-term tolerability, persistence of community shifts, or sustained enrichment of probiotic and SCFA-associated taxa alongside stable diversity profiles. The effects of prolonged consumption of complex plant-based

fermented beverages on these gut microbial signatures therefore remain insufficiently described. Similarly, pathogenic-associated taxa have rarely been assessed as predefined outcomes, leaving uncertainty regarding the stability of these microbial shifts and the long-term safety-related microbiome implications of sustained intake.

Here, we evaluated daily consumption of a plant-based fermented beverage derived from a complex botanical matrix and containing eight probiotic strains, including *Lactobacillus*, over a four-month period in a prospective single-arm design. Microbiome profiles were assessed at baseline, after 1 month of consumption, and after four months. Given the limited human evidence and short duration of follow-up in previous trials, this study investigated whether sustained intake of a fermented plant-based beverage was associated with changes in gut microbial diversity and composition, including shifts in probiotic-associated taxa, SCFA-producing taxa, and pathogenic-associated taxa.

Methods

Participants

A total of 24 adults residing in Denmark were recruited using non-randomized convenience sampling from multiple geographic regions. One participant withdrew before baseline sampling and was excluded, leaving 23 participants at week 0. Of these, 21 completed the full study, including four-month follow-up. Two participants did not provide the final sample but were included in analyses up to their last completed timepoint where applicable (mean age 43.6, SD = 12.3; males = 7). Inclusion criteria were: residence in Denmark and willingness to consume the study beverage daily. Exclusion criteria were: antibiotic use prior to study initiation, and inability to complete fecal sample collection. All participants provided written informed consent prior to inclusion.

Procedure

Participants were instructed to consume a minimum of 30 mL of a plant-based fermented beverage daily throughout the intervention period (Postbiota ApS, Frederiksværk, Denmark). The fermented probiotic beverage is composed of water, organic molasses, vitamin C, and extracts from 19 organic botanicals, including anise, chamomile, ginger, oregano, peppermint, rosemary, sage, and thyme. The product contains eight probiotic bacterial strains: *Bifidobacterium lactis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactococcus lactis,* and *Streptococcus thermophilus*.

Participants were instructed not to change their habitual diet, not to alter their general lifestyle, and not to initiate new dietary supplements or other major lifestyle modifications during the study period. Compliance was supported through periodic follow-up contact every one to two weeks.

Microbiome profiling

Fecal samples were collected at baseline (week 0), 3.5 weeks (hereafter referred to as 1 month), and 4 months. Gut microbiome composition was characterized using 16S rRNA gene amplicon sequencing, with taxonomic classification performed at the genus level.

Analyses included α-diversity, β-diversity, and grouped relative abundance measures for probiotic-, SCFA-, and pathogenic-associated microbial categories. The probiotic category comprised *Lactobacillus*, as *Lactobacillus* species constituted the primary probiotic component of the plant-based fermented beverage. The pathogenic category comprised *Escherichia*, *Enterococcus* and *Pseudomonas*, which have been repeatedly associated with gastrointestinal infections, dysbiosis, and mucosal inflammation (Madhogaria et al., 2022; Maciel-Fiuza et al., 2023; Zhang et al., 2015).

The SCFA-associated category comprised short-chain fatty acid (SCFA)-associated genera. Candidate genera were selected from the Lachnospiraceae in Table 1 of Gasaly et al. (2021), which lists genera associated with butyrate production via the acetyl-CoA pathway. The acetyl-CoA pathway is considered the principal route of microbial butyrate production in the human gut, where butyrate contributes to colonocyte energy metabolism and maintenance of epithelial barrier function relevant to gut health. Genera listed in the reference table included *Eubacterium, Ruminococcus, Anaerostipes, Coprococcus, Butyrivibrio, and Clostridium XIVa*. Of these, Clostridium XIVa was not available at the corresponding taxonomic resolution and could therefore not be included. The final SCFA-associated category therefore comprised *Eubacterium, Ruminococcus, Anaerostipes, Coprococcus* and *Butyrivibrio.*

Statistics

Longitudinal changes in relative abundance across the three study timepoints (baseline, 1 month, and 4 months) were assessed using Friedman tests for participants with complete measurements at all timepoints (n = 21). Analyses were performed for summed relative abundances of predefined probiotic-, SCFA-, and

pathogenic genus groups, as well as the Shannon Index as a measure of α-diversity. Omnibus Friedman tests were conducted in MATLAB (2025b). Pairwise timepoint comparisons were performed using Wilcoxon signed-rank tests, with Benjamini–Hochberg correction applied to control for multiple comparisons. As a sensitivity analysis, all primary analyses were repeated after exclusion of extreme outliers defined as three times the interquartile range (IQR).

Beta diversity

Beta diversity was analyzed from genus-level relative abundance data using Bray–Curtis dissimilarities in RStudio (2026.04) calculated with vegan::vegdist (method = "bray"). PERMANOVA was performed with vegan::adonis2 (999 permutations), with permutations constrained within participant (permute::how) to account for the paired longitudinal design. Pairwise timepoint contrasts used the same distance matrix restricted to participants with both relevant timepoints available (baseline to 1 month: n = 23; 1 month to 4 months: n = 21). Benjamini–Hochberg correction was applied to account for multiple comparisons.

Results

Probiotic, SCFA-associated, and pathogenic genera

Probiotic-associated genera showed a significant change across the intervention period (χ^2 (2) = 29.05, p < .001). Post-hoc comparisons demonstrated significantly higher relative abundance at 4 months compared with both baseline (pFDR = .021) and 1 month (pFDR = .005), while relative abundance remained unchanged between baseline and 1 month (pFDR = .875) (Figure 1A).

Similarly, SCFA-associated genera displayed a significant change across the intervention period (χ^2 (2) = 6.00, p = .0498). Post-hoc comparisons indicated significantly higher relative abundance at 4 months compared with baseline (pFDR = .029) and compared with 1 month (pFDR = .041), whereas no difference was observed between baseline and 1 month (pFDR = .827) (Figure 1B).

No significant changes were observed for pathogenic-associated genera, χ^2 (2) = 0, p = 1.00 (Figure 1C).

Sensitivity analyses excluding extreme outliers yielded comparable results and did not alter the overall interpretation of the findings, with the overall effect across study

timepoints remaining significant for probiotic and SCFA, and non-significant for pathogenic genera.

Together, these results suggest that daily consumption of the fermented beverage was associated with increases in probiotic- and SCFA-associated genera, whereas pathogenic taxa remained stable over time.

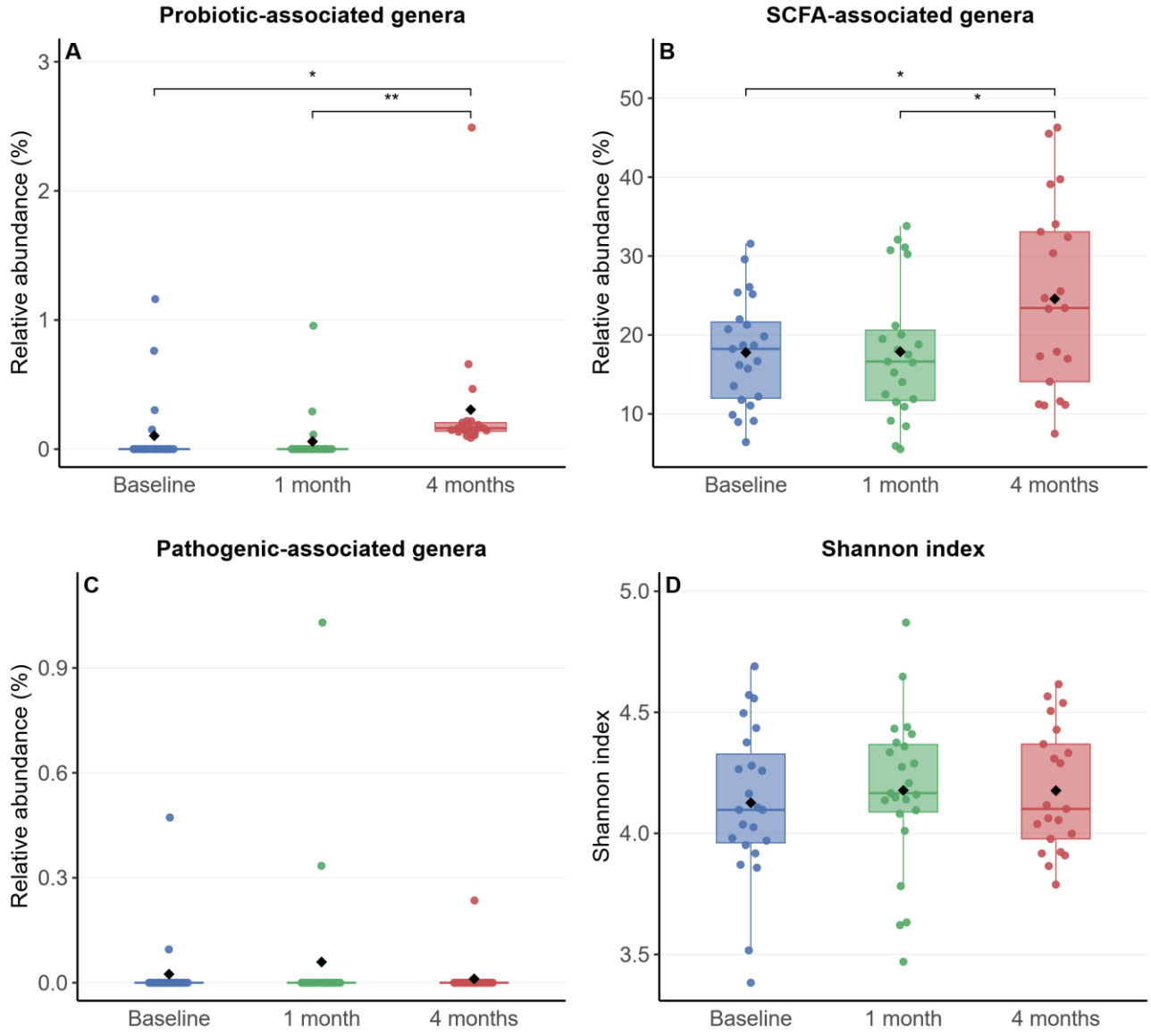

Figure 1. Longitudinal changes in probiotic-, SCFA-, and pathogenic-associated genera, and α-diversity across the intervention period. Relative abundances of probiotic (A), SCFA-associated (B), and pathogenic-associated (C) genera are displayed across baseline, 1 month, and 4 months. Probiotic-associated genera increased significantly over time ($p < .001$), with higher relative abundance at 4 months compared with baseline (pFDR = .021) and 1 month (pFDR = .005). SCFA-associated genera showed a significant effect across time ($p = .0498$), with significantly higher relative abundance at 4 months compared with baseline (pFDR = .029) and 1 month (pFDR = .041). No significant changes were observed for pathogenic-associated genera ($p = 1.00$). Shannon index (D) (α-diversity) did not change across time ($p = .276$). Points represent individual participants; boxplots indicate median and interquartile range, diamonds indicate means. * = $p < .05$, ** = $p < .01$

α- and β-diversity

A significant difference in overall microbiome composition was observed across the intervention period (R^2 = 0.028, F = 0.94, p = .004), based on Bray–Curtis beta-diversity. Pairwise comparisons showed modest differences between baseline and 1 month (R^2 = 0.015, F = 0.67, p = .052, pFDR = .052), baseline and 4 months (R^2 = 0.026, F = 1.07, p = .024, pFDR = .052), and 1 month and 4 months (R^2 = 0.026, F = 1.07, p = .041, pFDR = .052), all of which reduced to trend-level significance following Benjamini–Hochberg correction (Figure 2). Alpha diversity did not change over time (χ^2 (2) = 2.57, p = .276) (Figure 1D).

Together, these findings suggest modest time-dependent shifts in overall microbiome composition, reflected in trend-level changes in beta-diversity over the intervention period, while α-diversity was unaltered.

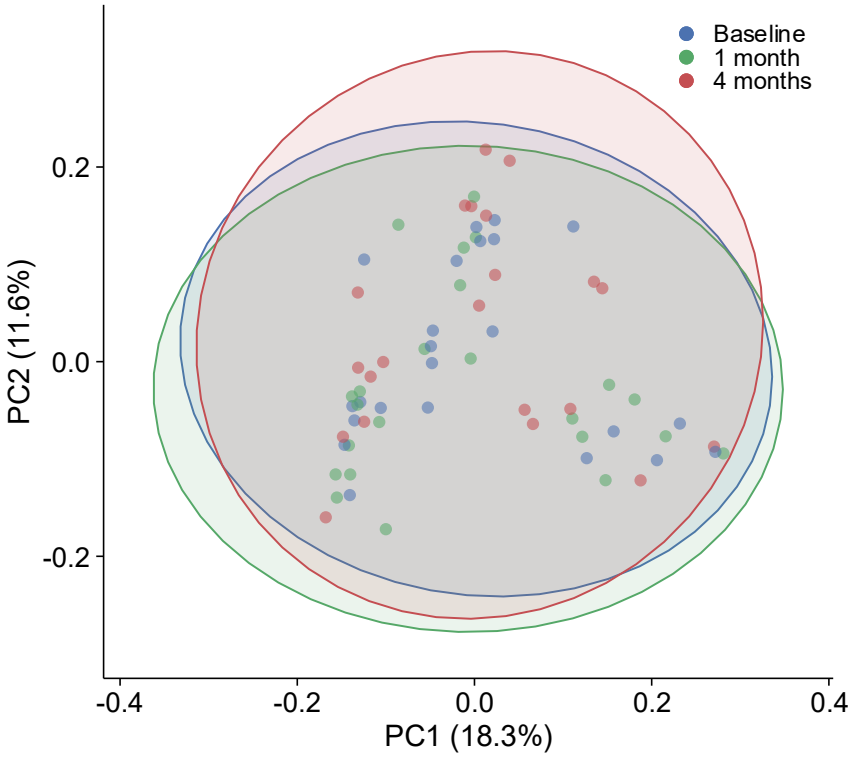

Figure 2. Principal coordinates analysis of Bray–Curtis beta-diversity across the intervention period. Principal coordinates analysis (PCoA) of Bray–Curtis dissimilarities showing overall microbiome composition across baseline, 1 month, and 4 months. A significant overall difference in beta-diversity was observed across the study period (R^2 = 0.028, F = 0.94, p = .004), with pairwise comparisons between baseline and 1 month (pFDR = .052), baseline and 4 months (pFDR = .052), and 1 month and 4 months (pFDR = .052) all reaching trend-level significance following Benjamini–Hochberg correction. Axes represent the first two principal coordinates, explaining 18.3% and 11.6% of the variance, respectively. Ellipses indicate group dispersion around timepoint centroids, and each dot represents an individual sample.

Discussion

Daily consumption of a plant-based fermented beverage over four months was associated with selective shifts in the gut microbiome of healthy adults. Probiotic-associated (*Lactobacillus*) genera increased over time, with higher relative abundance at four months than at baseline or one month. SCFA-associated genera showed a similar directional pattern. In contrast, pathogenic-associated taxa and α-diversity remained stable across the intervention, while overall compositional change (β-diversity) was detectable but modest. Together, these findings suggest that the intervention with a plant-based fermented beverage enriched functionally relevant bacterial groups without disrupting the broader resident ecosystem.

The observed increase in *Lactobacillus* relative abundance is consistent with the reported microbial composition of the drink, which contained several *Lactobacillus* species. This finding is also consistent with a quinoa-beverage trial that reported higher fecal *Lactobacillus* levels after 14 days (Canaviri-Paz et al., 2021). Notably, significant increases in *Lactobacillus* emerged at four months but not after one month, suggesting a progressive response to repeated exposure rather than an immediate transient effect of dietary passage. This gradual trajectory suggests that sustained intake may be associated with more pronounced gut microbiome modulation.

The enrichment of SCFA-associated genera at four months follows the same delayed temporal pattern, consistent with a cumulative rather than acute microbiome response. One possible explanation is that the product delivers not only live microorganisms but also postbiotic components generated during fermentation, such as exopolysaccharides, organic acids, and fermentation-derived peptides (Vila-Real et al., 2025; Vieira et al., 2021), which may provide substrates or signaling cues that selectively support native SCFA-producing taxa (den Besten et al., 2013; Gasaly et al., 2021). This dual probiotic–postbiotic mechanism could help explain why enrichment of both functional groups accumulated progressively.

While the included SCFA-producing genera are recognized primarily as butyrate producers (den Besten et al., 2013; Gasaly et al., 2021), colonic fermentation by these taxa is not limited to butyrate. Members of the genus Ruminococcus (e.g. *R. bromii*) contribute to colonic acetate pools (Laverde Gomez et al., 2019), and Coprococcus can additionally synthesize propionate via the acrylate pathway (Koh et al., 2016; Louis & Flint, 2017). Taken together, the enrichment observed across this group may reflect a broader enhancement of colonic fermentative potential rather than a butyrate-

specific effect. Direct quantification of fecal or plasma SCFA concentrations through targeted metabolomics approaches, such as gas chromatography–mass spectrometry (GC-MS) or solid-phase microextraction coupled with gas chromatography–mass spectrometry (SPME-GC-MS), would confirm whether these taxonomic shifts translate into measurable changes in metabolite production.

The broader diversity metrics should be interpreted in the context of a selective enrichment pattern. In healthy adults, the gut microbiome is characterized by considerable resilience, such that compositional shifts induced by sustained dietary intervention can occur without loss of α-diversity (Dogra et al., 2020). The stable α-diversity observed here is therefore consistent with targeted modulation of specific taxa rather than broad reorganization, a pattern also reported in comparable fermented-beverage trials including a quinoa-based beverage study and water kefir research (Canaviri-Paz et al., 2021; de Mel et al., 2026). The modest magnitude of β-diversity change reinforces this interpretation: large-scale compositional disruption is not inherently desirable, and in some trials, including a four-week intervention study with a fermented kombucha drink, broader reorganization was accompanied by enrichment of less-favorably regarded taxa alongside beneficial ones (Ecklu-Mensah et al., 2024).

The present pattern of selective enrichment alongside stable pathogenic taxa and preserved α-diversity is arguably a more favorable outcome for a healthy, non-clinical population, and strengthens the interpretation of tolerability over extended use. In previous literature, *Lactobacillus*-enriched profiles have been linked to competitive exclusion of opportunistic taxa and acidification of the colonic environment through lactate and organic acid production (Salminen et al., 2021), mechanisms commonly discussed as supportive of gut microbial health. Gains in SCFA-associated genera are directionally consistent with enhanced potential for colonic SCFA production, particularly butyrate, which contributes to colonocyte energy metabolism and epithelial barrier maintenance (den Besten et al., 2013; Gasaly et al., 2021). Taken together, these associations suggest a microbiome shift toward taxa whose functional roles are widely regarded as supportive of colonic fermentative activity and barrier-related gut health.

Several limitations should be acknowledged. The absence of a control group means that observed associations cannot be attributed causally to the intervention. Seasonal variation or changes in dietary intake or medication use over time cannot be fully

excluded as confounders. The study population consisted of healthy adults, limiting generalizability to populations with altered baseline gut microbiome composition, including patients with cancer, metabolic disorders or severe dysbiosis. Findings should not be generalized to fermented beverages as a class, as these products differ substantially in substrate chemistry, microbial consortia, and postbiotic profiles (Vilela et al., 2020; Deziderio et al., 2023). Finally, the absence of a washout or follow-up period means that the persistence of the observed shifts after cessation of intake could not be determined.

Future studies should address these gaps through randomized controlled designs with matched control arms and integration of shotgun metagenomics to resolve taxa at species and strain resolution, the latter being particularly relevant for distinguishing stable colonization from continued supplementation of resident populations. Combining longer intervention windows, as employed here, with these methodological elements and targeted SCFA metabolomics would substantially advance mechanistic understanding of how fermented plant-matrix products interact with the human gut microbiome across sustained exposure.

Conclusion

In summary, this four-month longitudinal study provides evidence that daily consumption of a plant-based fermented beverage is associated with selective and progressive enrichment of probiotic-associated and SCFA-associated genera in healthy adults, without expansion of pathogenic taxa or disruption of α-diversity. The consistency of these shifts across multiple outcome categories, emerging most clearly at four months, underscores the importance of adequate intervention duration in microbiome research. These findings establish a coherent microbiome-level rationale for this plant-based fermented beverage and provide a foundation for confirmatory randomized trials.

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