StoolFix Accurately Preserves the Gut Microbial Profile for 60 Days at Room Temperature

Introduction

Microbiome profile stabilization is crucial for ensuring exact and reproducible microbial analyses. The gut microbiome is extremely sensitive to environmental changes and improper sample handling can lead to shifts in composition due to bacterial growth, degradation, or selective survival of organisms. Room temperature stabilization methods, such as StoolFix, have become increasingly popular as they remove the need to ship and store frozen samples, which can be inconvenient and costly. StoolFix collection kits are quick and easy to use and can be safely mailed to donors to collect samples using a simple collection process.

In this study we show the ability of StoolFix to accurately capture and preserve the microbial profile of stool samples over a 60-day period at room temperature, making it an ideal choice for simple and safe microbial sample preservation. We also compare the performance side-by-side with that of an alternative collection method in order to demonstrate the efficacy of StoolFix.

Methods & Materials

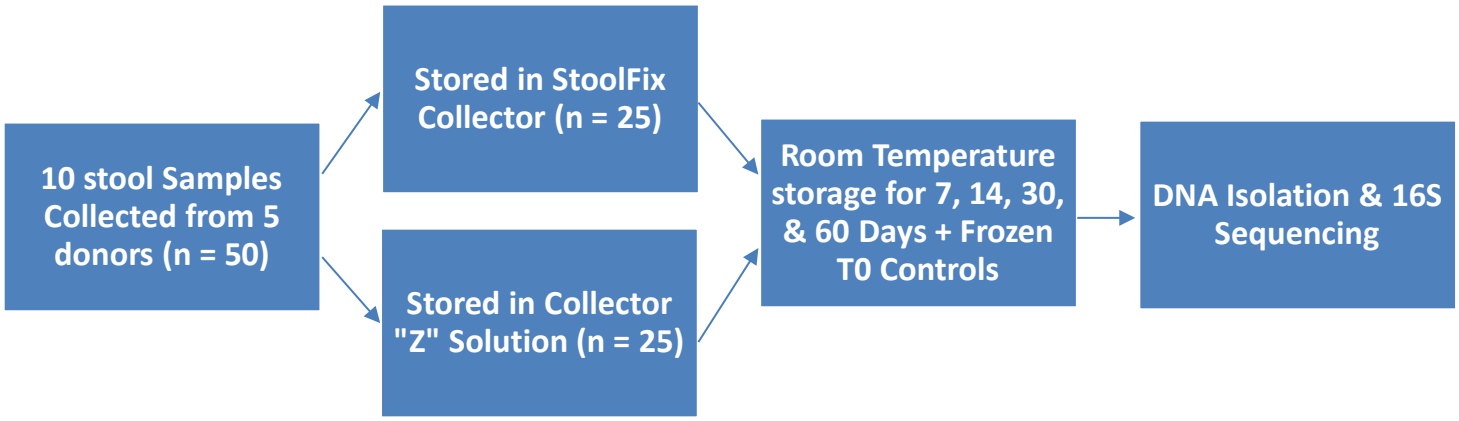

Figure 1: Flowchart of the sample collection and testing plan for StoolFix and alternative samples.

Five healthy adult donors were recruited to evaluate the stability of StoolFix kits, and twelve collection kits were mailed to each donor for sample collection (n = 50). The kits consisted of an Isohelix SK-3 swab, and a sample collection tube containing either 1ml of StoolFix preservation solution or 1ml of alternative collection solution, “Z”. Sample collection instructions and a stool collection aid were included for collection. Donors were asked to collect the samples in a single sitting, after which the swabs were placed in the preservation tubes and sealed securely. Samples were placed in provided UN3373 transport bags and mailed back to the lab within a week of collection. This method was used as it represents a real-world scenario of how samples would be collected and stored.

On return to the lab, one sample per donor collected using StoolFix and one from the alternative collection solution were immediately frozen as control samples to compare against later time points (T0 frozen control samples). The remaining samples were then incubated at room temperature (20°C-25°C) for predetermined time points (7 days, 14 days, 30 days, & 60 days). At each time point, one sample from each collection method per donor were frozen prior to DNA extraction once all samples were collected.

Following the incubation periods, each of the samples underwent DNA extraction and 16S V3-V4 amplicon generation, library preparation and sequencing using an Illumina MiSeq platform and 2 x 300bp paired end reads. Following sequencing, bioinformatic data processing of raw read data (demultiplexing, merging, filtering, and OTU/taxonomic assignment) were performed.

Using these collected data, alpha diversity (Shannon/Chao1), beta diversity (Bray-Curtis dissimilarity), and taxonomic distribution of samples were determined and visualised. The data were then used to evaluate if any statistically significant changes had occurred to the microbial profiles of donor samples over the 60-day storage period, and also if there were any differences between StoolFix and the alternative collection method.

Results & Discussion

Alpha Diversity:

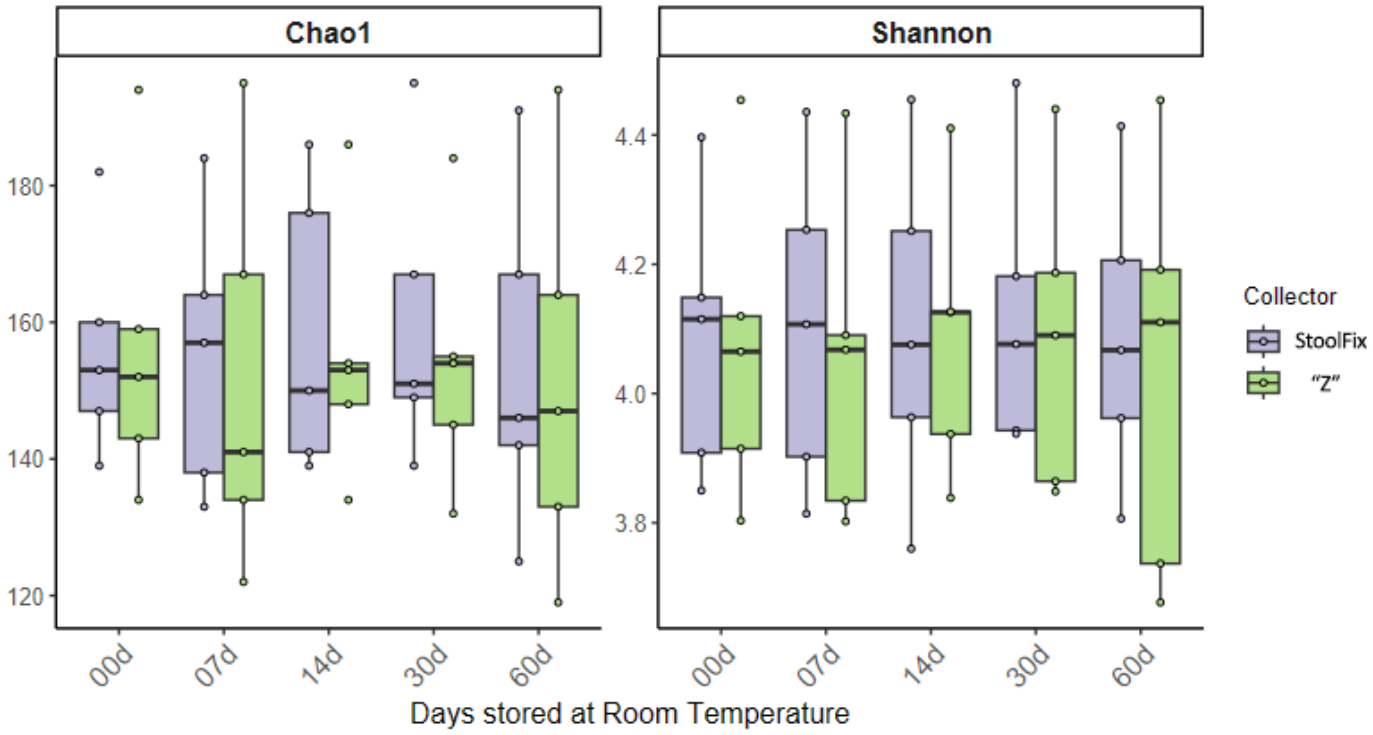

Figure 2: Boxplots of alpha diversity (Shannon & Chao1) for each set of collector samples at each time point over the testing period.

Alpha diversities of samples, as measured via Shannon index and Chao1 richness (Figure 2) were consistent between both collection methods and across progressive time points up to 60 days storage. Statistical analysis of both alpha diversity metrics did not find a significant difference between the collector groups over the stability testing period, indicating that the microbial diversity in test samples was maintained in StoolFix over the storage period.

Taxonomic Composition:

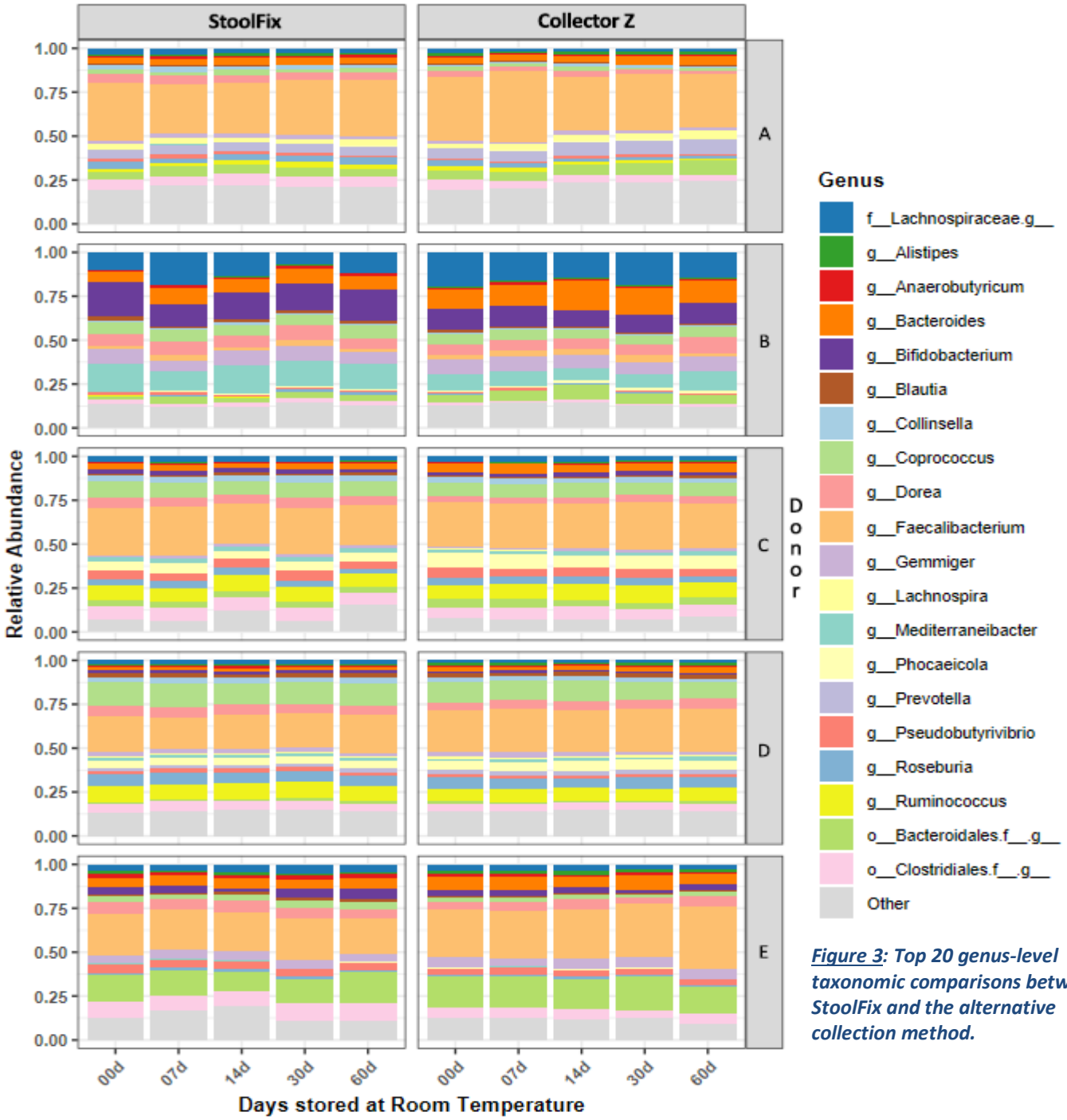

***Figure 3**: Top 20 genus-level taxonomic comparisons between StoolFix and the alternative collection method.*

Taxonomic profile analysis of samples (Figure 3) found that StoolFix samples generated similar profiles to those stored using the alternate collection method. Profiles remained consistent over time and did not see significant shifts indicative of sample dysbiosis or degradation. StoolFix maintains the accuracy of microbial profiles compared to frozen controls. In addition, potential batch effects from collecting multiple swabs from the same stool sample were minimised, demonstrating the reproducibility of StoolFix collection. Harder to lyse gram-positive bacteria, such as *Faecalibacterium & Ruminococcus* were also well represented in collected samples.

Beta-Diversity:

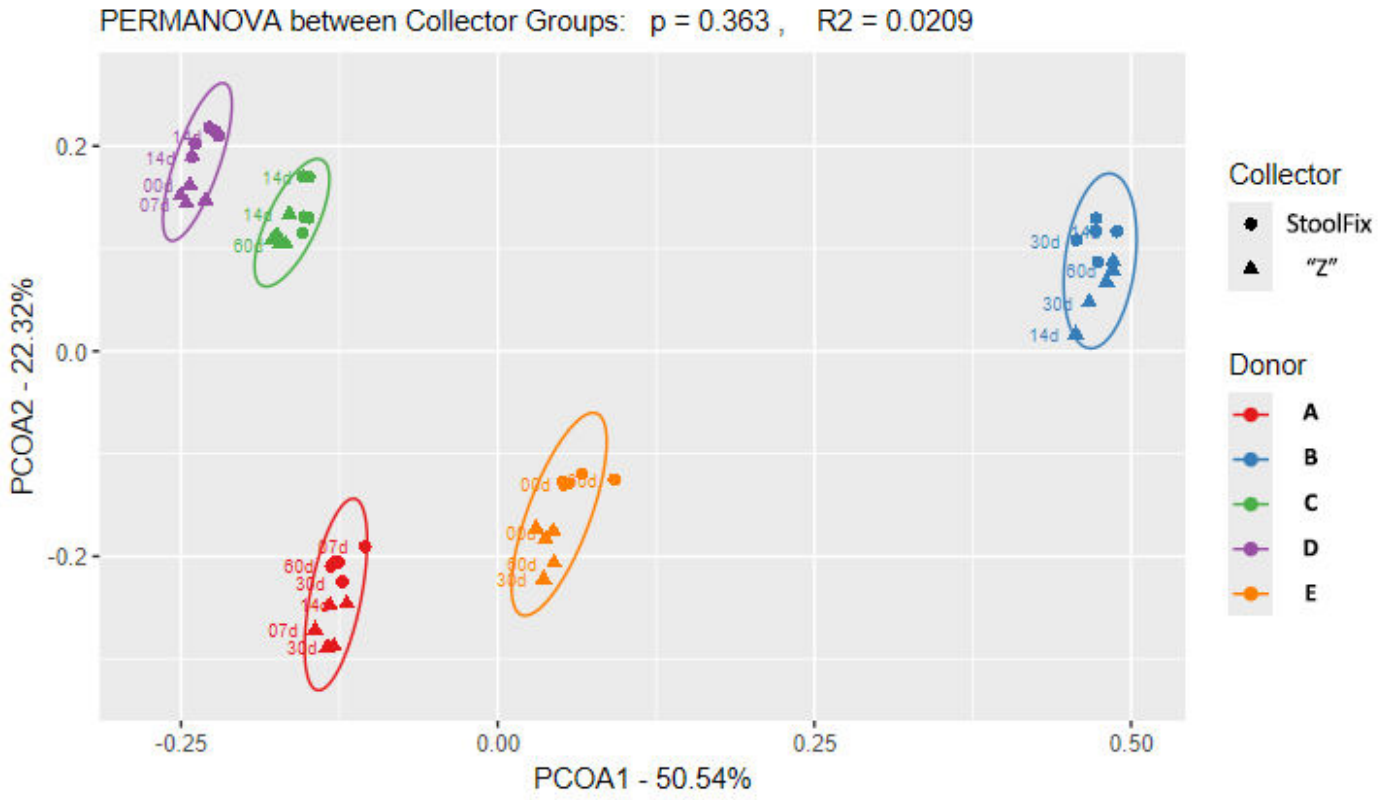

Figure 4: PCoA Beta-diversity analysis based upon Bray-Curtis dissimilarity, showing distinct clustering based upon sample donor and sample consistency over time.

Visualisation of principle co-ordinate analysis (PCoA) Bray-Curtis dissimilarity (Figure 4) revealed that samples formed distinct clusters with clear differentiation based upon sample donor. These clusters formed regardless of the collector used, and neither StoolFix nor the alternative method showed a significant drift in microbial profile over time. PERMANOVA statistical analysis comparing the two collection methods across all points did not find a significant difference in overall microbial profiles. StoolFix consistently preserves microbial samples allowing for reliable analysis.

Conclusions:

- **StoolFix accurately preserves the microbial profile stool samples for a minimum of 60 days at room temperature compared to fresh frozen samples, removing the need for costly cold-chain storage.**
- **StoolFix is comparable to alternative collection kits and methods available on the market, with comparable microbial profiles over time with paired samples.**
- **StoolFix is quick and easy to use, and unlike alternative methods features a non-toxic collection solution that can be safely mailed without risk to donors collecting at home.**