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SUPPLEMENTARY INFORMATION
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Stability of the human faecal microbiome in a cohort of adult men

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Supplementary Information for "Stability of the Human Fecal Microbiome in a Cohort of Adult Men"

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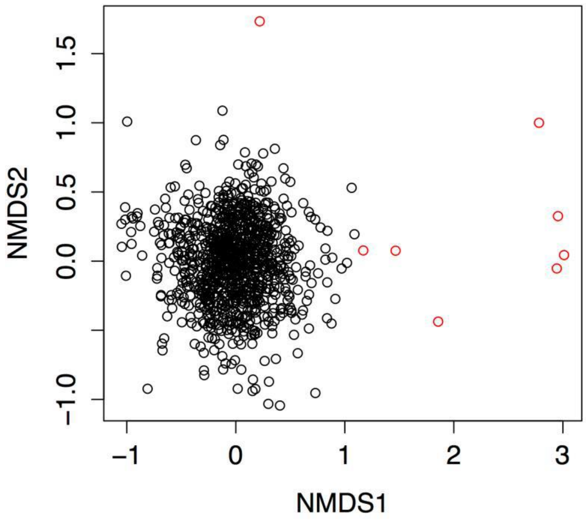
Supplementary discussion

Stool samples for this study were collected following a previously-established protocol involving delivery of sample material fixed in RNAlater to the laboratory. [1] Analyses from our pilot study demonstrated that two-day simulated shipping of RNAlater-fixed stool aliquots had only a minimal perturbative effect on the gut metagenome and metatranscriptome when compared to fresh-frozen sample collection protocols.[1] Since publication of our pilot work, other studies have confirmed no significant effect of RNAlater on community composition.[2,3] One study, however, has suggested differences in evenness in microbial taxonomic composition in stool stored in RNAlater compared to −80 °C controls.[4] Notably, variation attributable to storage method was markedly smaller than the variation explained by different sample time points. Furthermore, variation attributed to RNAlater relative to freezing at -80℃, was comparable to that introduced by sample storage in OMNIGene.Gut, another popular sample stabilization kit. Taken together, the effect of RNAlater on microbiome sample composition is local, of very small effect when significant at all, and no greater than that of any other currently used sample stabilization method.

Supplementary Figure 1: Ordination plot of taxonomic profiles (n=929). 8 outliers (samples) were removed. 4 of these came from one person who reported a colectomy at the time of participation in the study.



Supplementary Table 1. Reliability in measuring relative abundance levels over short-term (24-72 hours) and long-term (~6 months) intervals for the majority of species and gene families. Genes were most highly repeatable, followed by species, and then distantly followed by RNA pathways. An ICC of <0.40 suggests poor repeatability, 0.40 to 0.75 indicates fair to good repeatability, and ≥ 0.75 indicates excellent repeatability [49].

	Percent of features with an ICC > 0.40	
	Short-term	Long-Term
Species (n=146)	96.8%	86.8%
DNA (n=1951)	99.9%	92.8%
RNA (n=3566)	1.3%	0.79%

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