

16S rRNA V4 primers and PCR conditions

Primer 515F-OH1 GTGACCTATGAACTCAGGAGTCGGACTACNVGGGTWTCTAAT

Primer 806R-OH2 CTGAGACTTGACATCGCAGCGTGYCAGCMGCCGCGGTAA

PCR1: 94°C for 3 minutes; 20 cycles at 94°C for 45 seconds each; 55°C for 1 minute; 72°C for 90 seconds. Final step at 72°C for 10 minutes. Amplicons were diluted 1 in 10 before PCR2 (with reaction conditions as above, except an increase to 25 cycles).

Table S1: Baseline Characteristics of participants in the BRISC microbiome sub-study (shotgun metagenomic samples only).

	Iron N=99	MNPs N=109	Placebo N=111
Union			
Bhulta	28/99 (28.3%)	32/109 (29.4%)	40/111 (36.0%)
Golakandail	30/99 (30.3%)	35/109 (32.1%)	38/111 (34.2%)
Rupganj	41/99 (41.4%)	42/109 (38.5%)	33/111 (29.7%)
Household Food Secure status*	81/99 (81.8%)	82/106 (77.4%)	88/109 (80.7%)
Age extra food in addition to breastfed (months)	6.0 (5.0-6.0)	6.0 (5.0-6.0)	6.0 (5.0-6.0)
Hemoglobin concentration (g/L) venous, mean (SD)	109.8 (10.7)	110.9 (8.8)	110.3 (9.8)
Anemia venous†	42/99 (42.4%)	45/105 (42.9%)	49/110 (44.5%)
Ferritin (ug/L)	24.6 (11.6-32.8)	24.7 (15.4-46.7)	24.8 (13.8-38.7)
Iron deficient‡	33/98 (33.7%)	26/102 (25.5%)	31/105 (29.5%)
Iron deficient anemia venous¶	23/98 (23.5%)	15/102 (14.8%)	24/105 (22.9%)
Hepcidin – (ng/mL), median (IQR)	29.5 (19.0-51.7)	31.2 (15.4-56.9)	32.8 (18.0-57.2)
Low hepcidin (hepcidin <10 ng/mL)	7/92 (7.6%)	12/97 (12.4%)	11/101 (10.9%)
C-reactive protein (mg/L)	0.95 (0.38-2.75)	0.80 (0.31-3.14)	0.84 (0.34-3.20)

	Iron	MNPs	Placebo
	N=99	N=109	N=111
Inflammation--	14/98 (14.3%)	18/102 (17.6%)	18/105 (17.1%)
Length/height-for-age z-score	-1.31 (0.96)	-1.28 (1.02)	-1.25 (0.97)
Stunted	23/99 (23.2%)	28/108 (25.9%)	22/111 (19.8%)
Weight-for-age z-score	-0.55 (0.96)	-0.67 (1.06)	-0.46 (0.90)
Underweight**	6/99 (6.1%)	8/108 (7.4%)	6/111 (5.4%)
Weight-for-length/height z-score	0.34 (0.92)	0.17 (1.01)	0.42 (0.98)
Wasting††	1/99 (1.0%)	2/108 (1.9%)	1/111 (0.9%)

Data are n/N (%) unless stated otherwise. MNPs = Micronutrient powders; SD = Standard Deviation; IQR = Interquartile range (25th to 75th percentile).

*Household food security was assessed and defined using the Household Food Insecurity Access Scale.

†Anaemia was defined as venous haemoglobin <110 g/L.¹

‡Iron deficiency was defined as ferritin level <12 µg/L or <30 µg/L if C-reactive protein was >5 mg/L).¹

¶Iron deficiency anaemia was defined as concurrent iron deficiency and anaemia.

--Inflammation was defined as C-reactive protein >5 mg/L.

||Stunting was defined as length-for-age z-score <-2.²

**Underweight was defined as weight-for-age z-score <-2.²

††Wasting was defined as weight-for-length z-score <-2.²

Figure S1: CONSORT Diagram of the BRISC Microbiome Sub-study.

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SUPPLEMENTARY FIGURE 1

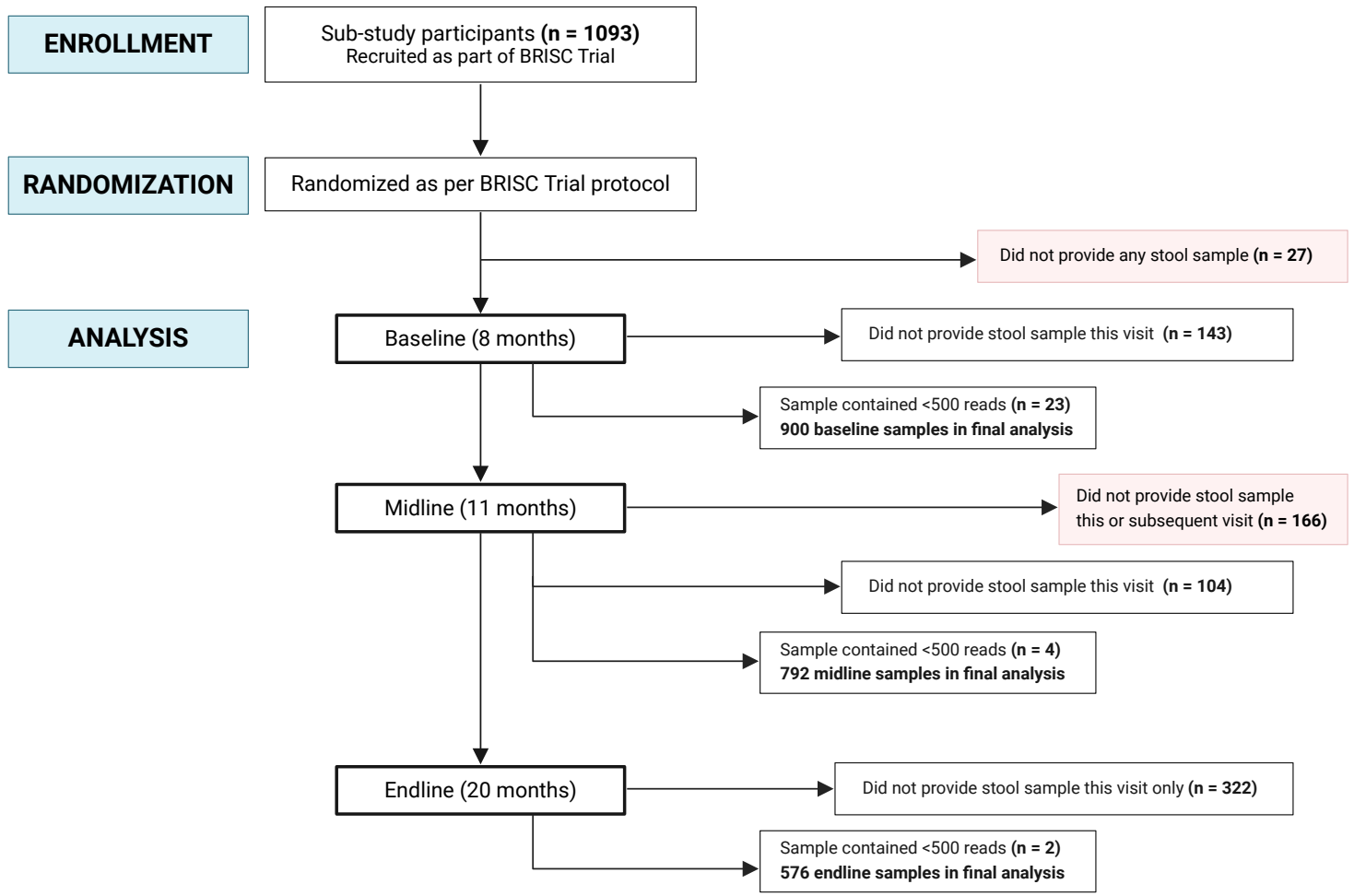
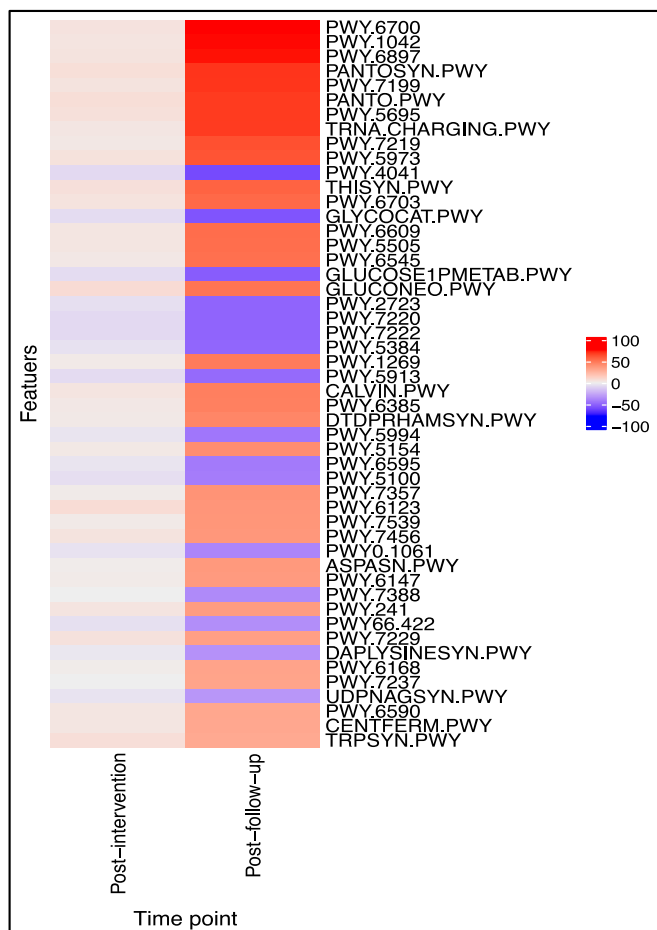


Figure S2: Heatmap showing changes in abundance of genes relating to microbiome functional profile by sampling time point.

Heatmap demonstrating relative differences in abundance of genes relating to microbiome functional profile by sampling time point across all trial arms, with baseline as reference. The 50 features exhibiting the greatest relative change in abundance (increase – in red – or decrease – in blue – from baseline) are listed. Abundance is expressed as $-\log_{10}(\text{FDR-adjusted p-value}) * \text{sign}(\log_2\text{-fold change})$ as per the heatmap calculation used by *MaAsLin2*. (n = 255 at baseline, n = 231 at post-intervention and n = 313 at post-follow-up time points) (Shotgun metagenomic data). Source data for this figure are provided in the Source Data file.

SUPPLEMENTARY FIGURE 2



Abbreviation	Pathway
PWY-6700	Queuosine biosynthesis
PWY-1042	Glycolysis IV (plant cytosol)
PWY-6897	Thiamin salvage II
PANTOSYN-PWY	Pantothenate and coenzyme A biosynthesis I
PWY-7199	Pyrimidine deoxyribonucleosides salvage
PANTO-PWY	Phosphopantothenate biosynthesis I
PWY-5695	Urate biosynthesis/inosine 5-phosphate degradation
TRNA-CHARGING-PWY	tRNA charging
PWY-7219	Adenosine ribonucleotides de novo biosynthesis
PWY-5973	cis-vaccenate biosynthesis
PWY-4041	γ -glutamyl cycle
THISYN-PWY	Superpathway of thiamin diphosphate biosynthesis I
PWY-6703	preQ0 biosynthesis
GLYCOCAT-PWY	Glycogen degradation I (bacterial)
PWY-6609	Adenine and adenosine salvage III
PWY-5505	L-glutamate and L-glutamine biosynthesis
PWY-6545	Pyrimidine deoxyribonucleotides de novo biosynthesis III
GLUCOSE1PMETAB-PWY	Glucose and glucose-1-phosphate degradation
GLUCONEO-PWY	Gluconeogenesis I
PWY-2723	Trehalose degradation V
PWY-7220	Adenosine deoxyribonucleotides de novo biosynthesis II
PWY-7222	Guanosine deoxyribonucleotides de novo biosynthesis II
PWY-5384	Sucrose degradation IV (sucrose phosphorylase)
PWY-1269	CMP-3-deoxy-D-manno-octulosonate biosynthesis I
PWY-5913	TCA cycle VI (obligate autotrophs)
CALVIN-PWY	Calvin-Benson-Bassham cycle
PWY-6385	Peptidoglycan biosynthesis III (mycobacteria)
DTDPRHAMSYN-PWY	dTDP-L-rhamnose biosynthesis I

Abbreviation	Pathway
PWY-5994	Palmitate biosynthesis I (animals and fungi)
PWY-5154	L-arginine biosynthesis III (via N-acetyl-L-citrulline)
PWY-6595	Superpathway of guanosine nucleotides degradation (plants)
PWY-5100	Pyruvate fermentation to acetate and lactate II
PWY-7357	Thiamin formation from pyrithiamine and oxythiamine (yeast)
PWY-6123	Inosine-5-phosphate biosynthesis I
PWY-7539	6-hydroxymethyl-dihydropterin diphosphate biosynthesis III (Chlamydia)
PWY-7456	Mannan degradation
PWY0-1061	Superpathway of L-alanine biosynthesis
ASPASN-PWY	Superpathway of L-aspartate and L-asparagine biosynthesis
PWY-6147	6-hydroxymethyl-dihydropterin diphosphate biosynthesis I
PWY-7388	Octanoyl-[acyl-carrier protein] biosynthesis (mitochondria, yeast)
PWY-241	C4 photosynthetic carbon assimilation cycle, NADP-ME type
PWY66-422	D-galactose degradation V (Leloir pathway)
PWY-7229	Superpathway of adenosine nucleotides de novo biosynthesis I
DAPLYSINESYN-PWY	L-lysine biosynthesis I
PWY-6168	Flavin biosynthesis III (fungi)
PWY-7237	Myo-, chiro- and scillo-inositol degradation
UDPNAGSYN-PWY	UDP-N-acetyl-D-glucosamine biosynthesis I
PWY-6590	Superpathway of Clostridium acetobutylicum acidogenic fermentation
CENTFERM-PWY	Pyruvate fermentation to butanoate
TRPSYN-PWY	L-tryptophan biosynthesis

Figure S3: Subgroup analysis of adherence to trial intervention by trial arm.

A: Violin plot presenting taxonomic alpha diversity – measured by Shannon and inverse Simpson indices – grouped by trial arm and restricted to participants with $\geq 70\%$ adherence to trial intervention (16S rRNA data). **B.** Volcano plot illustrating differential abundance at the genus level by trial arm of the same adherence subgroup as measured immediately post-intervention (16S rRNA data). **C.** Volcano plot illustrating differential abundance at the species level by trial arm of the same adherence subgroup as measured immediately post-intervention (Shotgun metagenomic data). **D.** Volcano plot illustrating differential abundance of functional pathways by trial arm of the same adherence subgroup as measured immediately post-intervention (Shotgun metagenomic data). For **B-D** \log_2 -fold change is shown on the x-axis and the $-\log_{10}$ (FDR-adjusted p-value) on the y-axis. The horizontal red line indicates an FDR-adjusted p-value 0.05. Source data for Figure S3B-D are provided in the Source Data file.

SUPPLEMENTARY FIGURE 3

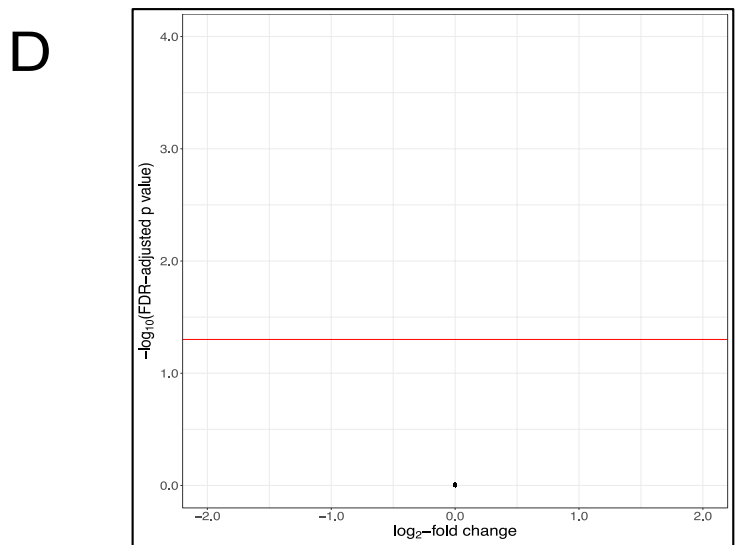
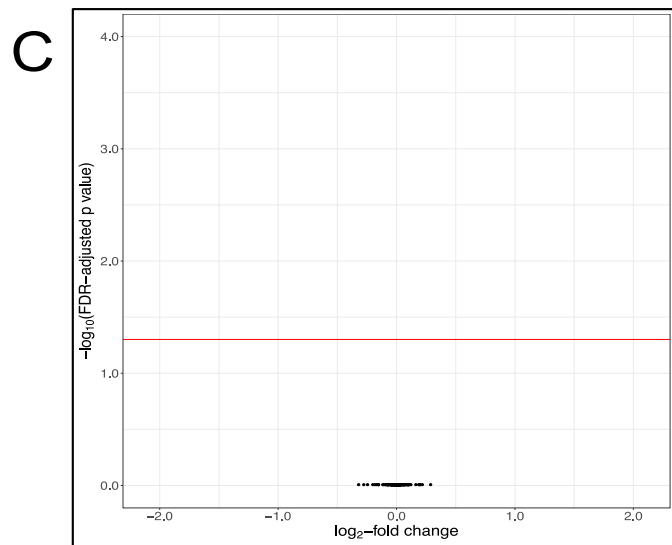
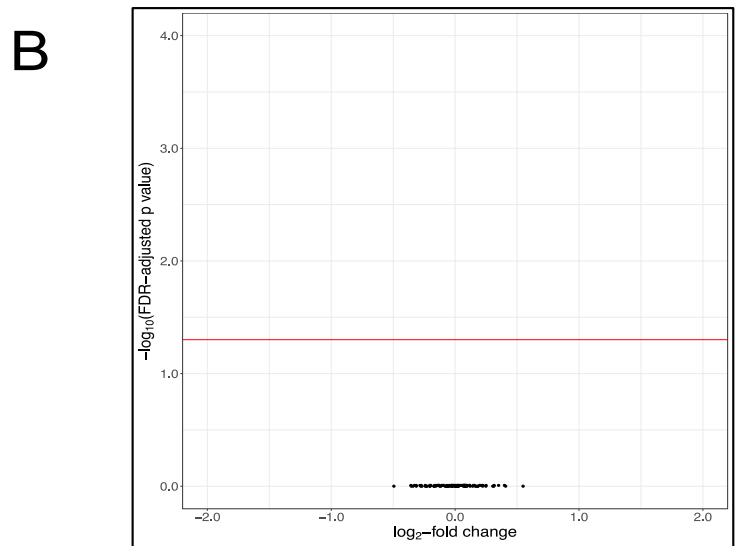
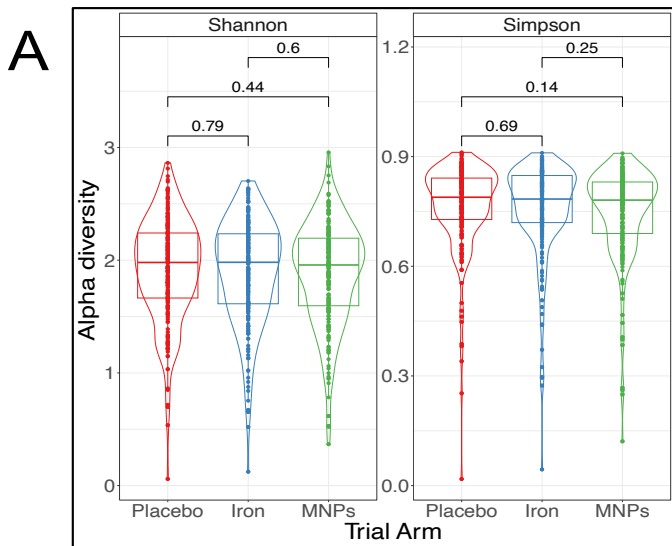
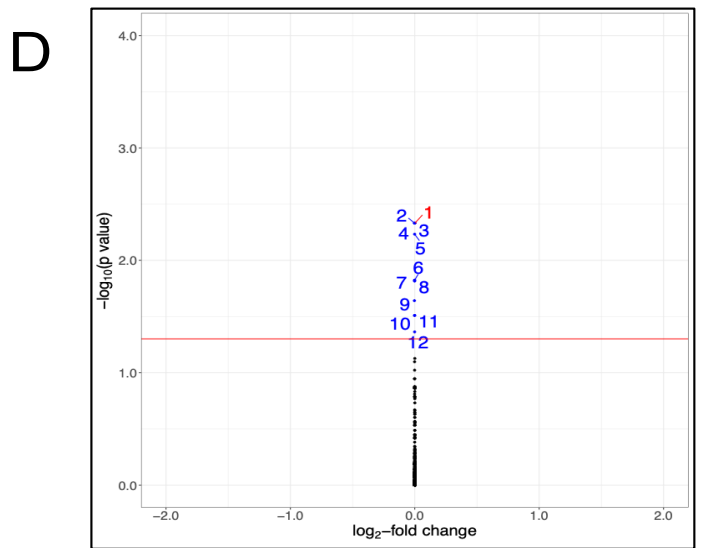
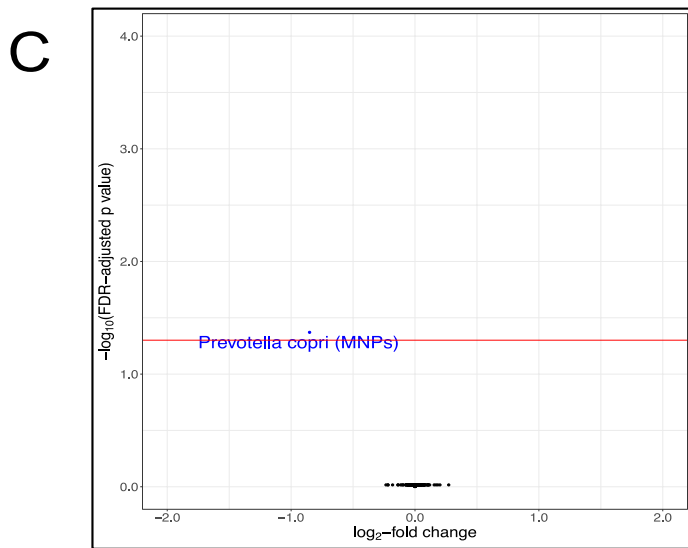
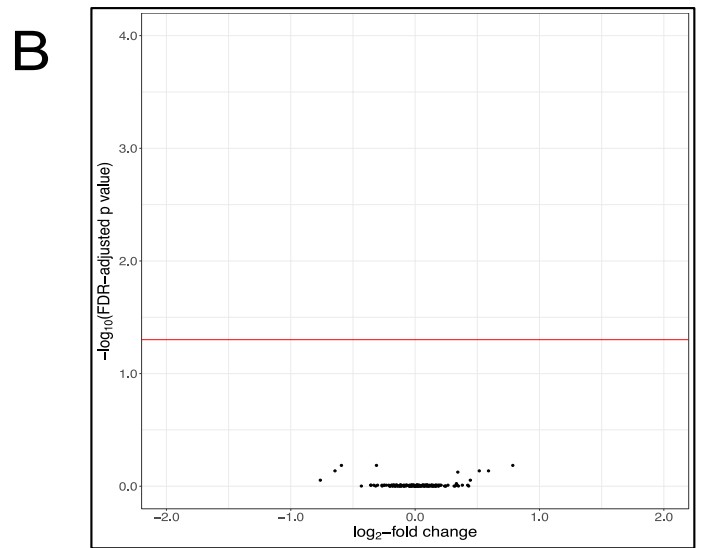
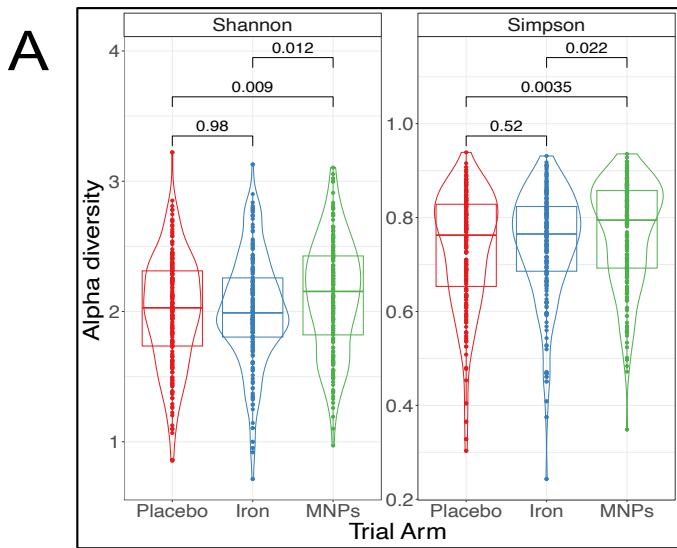


Figure S4: Alpha diversity and differential abundance analysis by trial arm at the follow-up time point.

A: Violin plot presenting taxonomic alpha diversity – measured by Shannon and inverse Simpson indices –by trial arm and measured at the post-follow-up time point (16S rRNA data). **B.** Volcano plot presenting differential abundance at the genus level by trial arm and measured at the post-follow-up time point (16S rRNA data). **C.** Volcano plot illustrating differential abundance at the species level by trial arm and measured at the post-follow-up time point (Shotgun metagenomic data). **D.** Volcano plot presenting differential abundance of functional pathways by trial arm and measured at the post-follow-up time point (Shotgun metagenomic data) (1. Pentose phosphate pathway (non-oxidative branch), 2. CMP-3-deoxy-D-manno-octulosonate biosynthesis I, 3. PWY.5154, 4. Thiamin salvage II, 5. Superpathway of L-aspartate and L-asparagine biosynthesis. 6. 6-hydroxymethyl-dihydropterin diphosphate biosynthesis III (Chlamydia), 7. Flavin biosynthesis I (bacteria and plants), 8. 6-hydroxymethyl-dihydropterin diphosphate biosynthesis I, 9. dTDP-L-rhamnose biosynthesis I, 10. L-isoleucine biosynthesis III, 11. Urate biosynthesis/inosine 5'-phosphate degradation, 12. Superpathway of branched amino acid biosynthesis). Log₂-fold change is shown on the x-axis and the -log₁₀(FDR-adjusted p-value) on the y-axis. The horizontal red line indicates an FDR-adjusted p-value 0.05. Source data for Figure S4B-D are provided in the Source Data file.

SUPPLEMENTARY FIGURE 4



Reference

1. World Health Organization. Iron deficiency anaemia: assessment, prevention and control — a guide for programme managers. (World Health Organization, Geneva, 2001).
2. de Onis, M., Onyango, A.W., Van den Broeck, J., Chumlea, W.C. & Martorell, R. Measurement and standardization protocols for anthropometry used in the construction of a new international growth reference. *Food Nutr Bull* **25**, S27-36 (2004).