

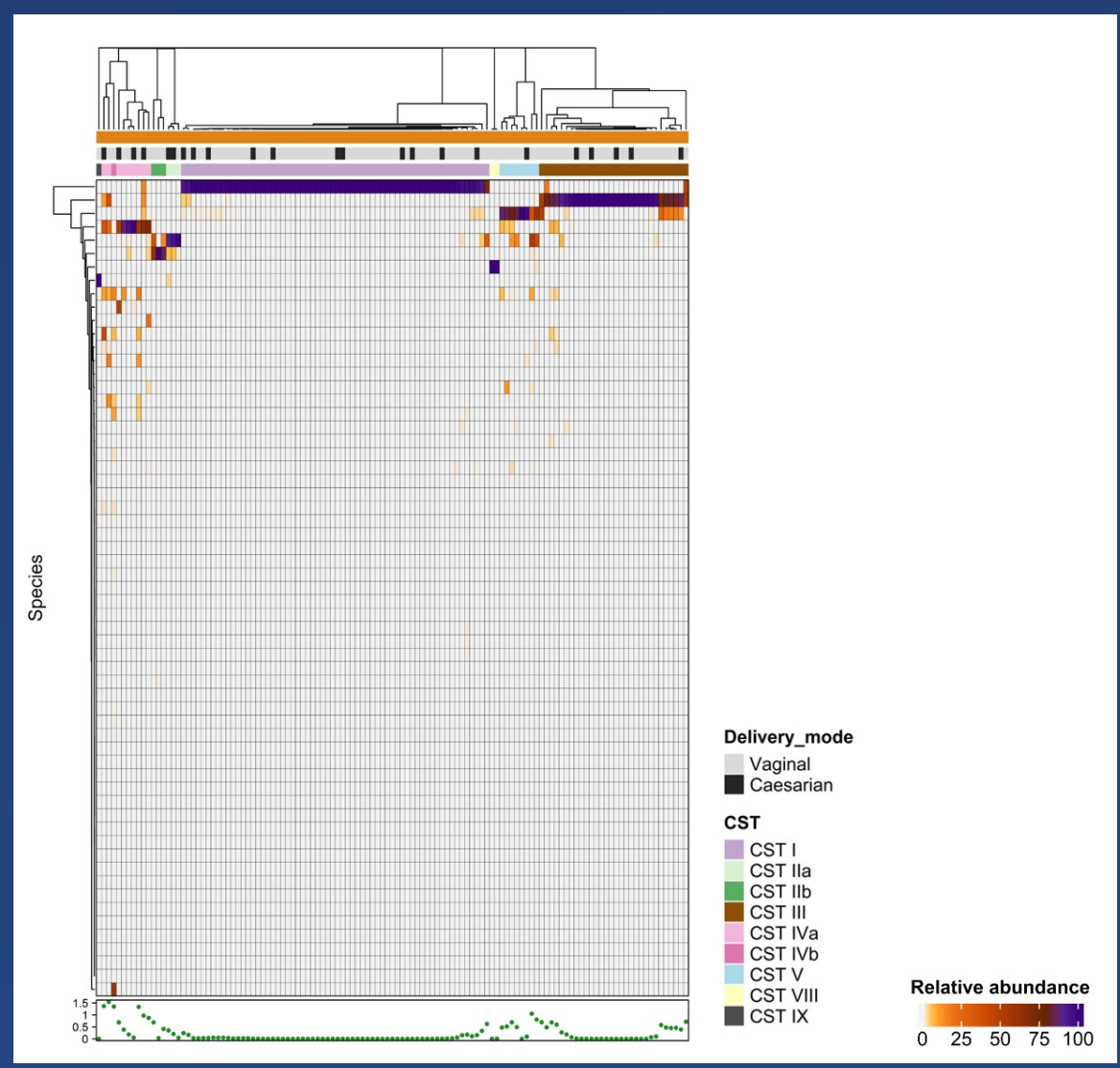
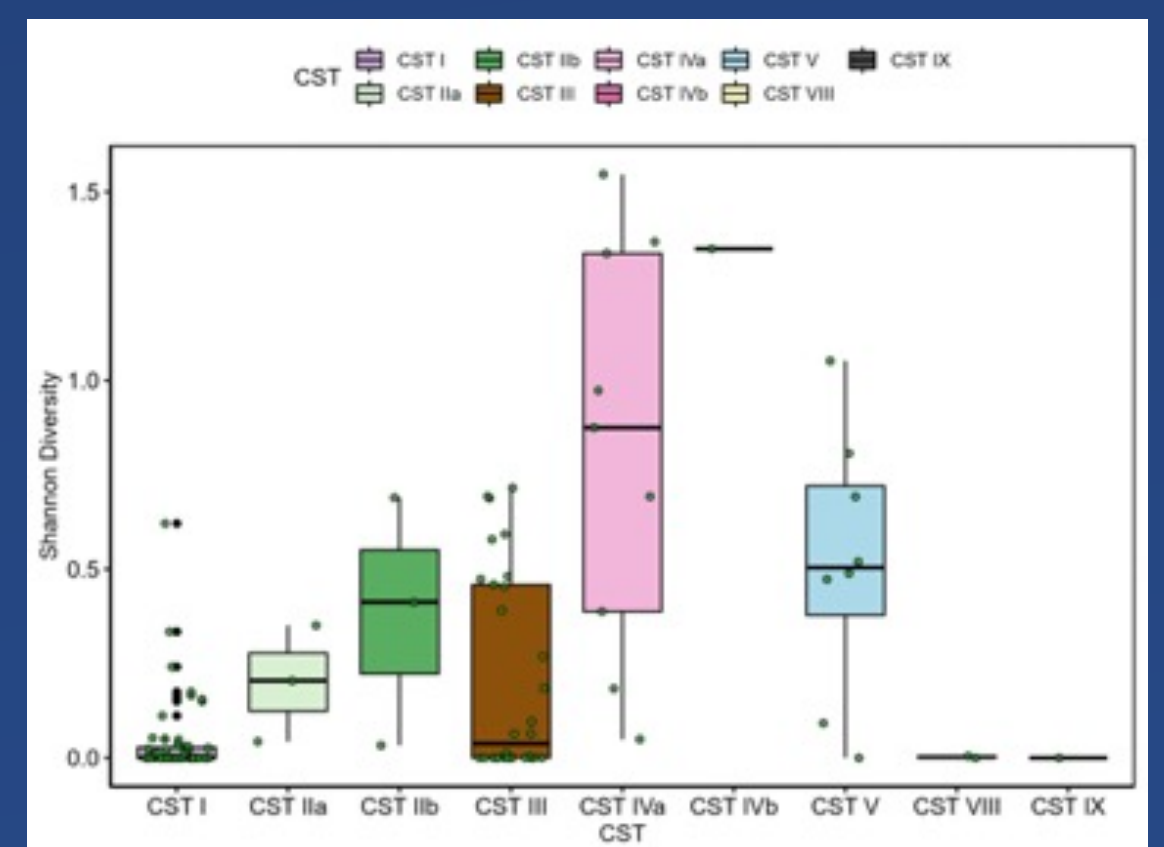


Previous Vaginal delivery, Breastfeeding, Dietary Starch, Glycaemic Load and Amino Acids have the Strongest Environmental Associations with the Vaginal Microbiome in Pregnancy:

Secondary analysis of the MicrobeMOM Randomised Controlled Trial

INTRODUCTION The vaginal microbiome is a key player in reproductive health. High quality environmental data describing associations between demographics, obstetric history, lifestyle and dietary pattern and vaginal microbiota is currently scarce. This study aimed to identify significant environmental factors that influence vaginal microbial health.

Characteristic		N	% or SD
Maternal age	In Years (mean, SD)	33.3	3.9
Parity	Nulliparous	66	55.9%
	Multiparous	52	44.1%
Previous vaginal delivery	Previous vaginal delivery	40	33.9%
	Previous caesarean delivery only	9	7.6%
Body Mass Index	Mean and SD	25.0	3.2
Ethnicity	Caucasian (European descent)	103	92.8%
	Non-Caucasian (Asian, Latino, African descent)	8	7.2%
Education status	Completed third level	102	87.9%
Smoking (	Current or previous history	46	39.0%
METS (minutes)	Per week, mean and SD	1154.8	1617.4
WHO-5 Wellbeing Score	Mean and SD	61.9	21.2
Antibiotic use	In early pregnancy	10	8.7%
Previous breastfeeding	After prior pregnancy	40	33.9%



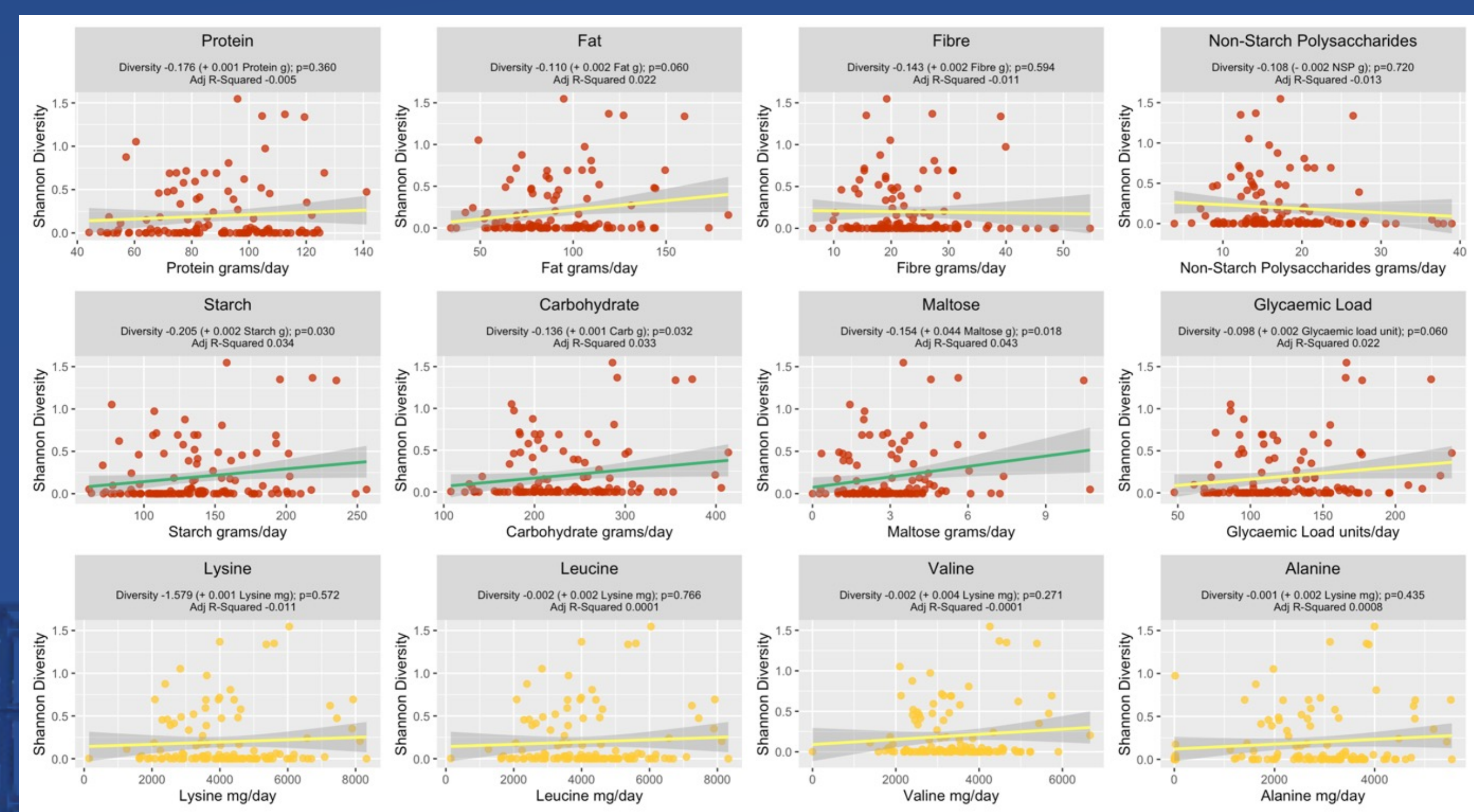
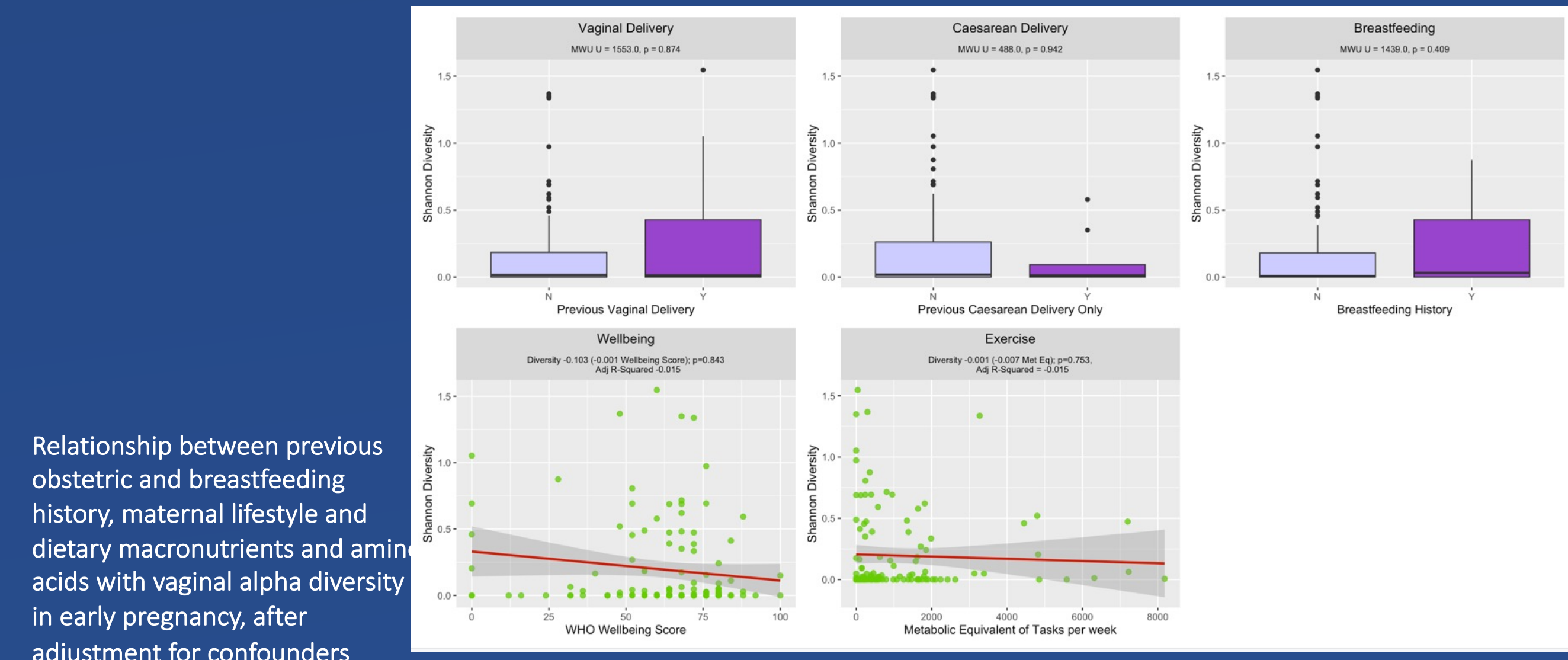
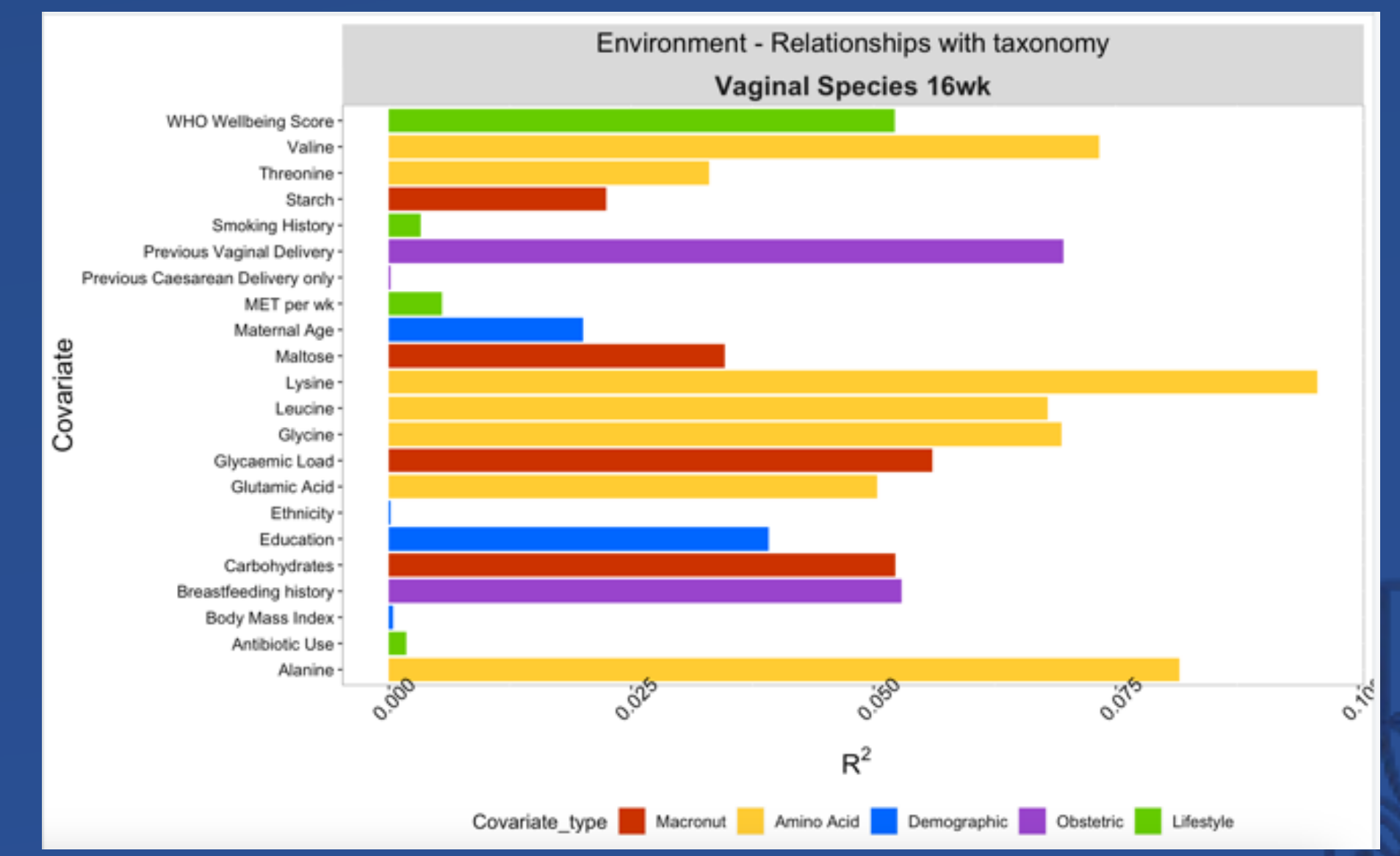
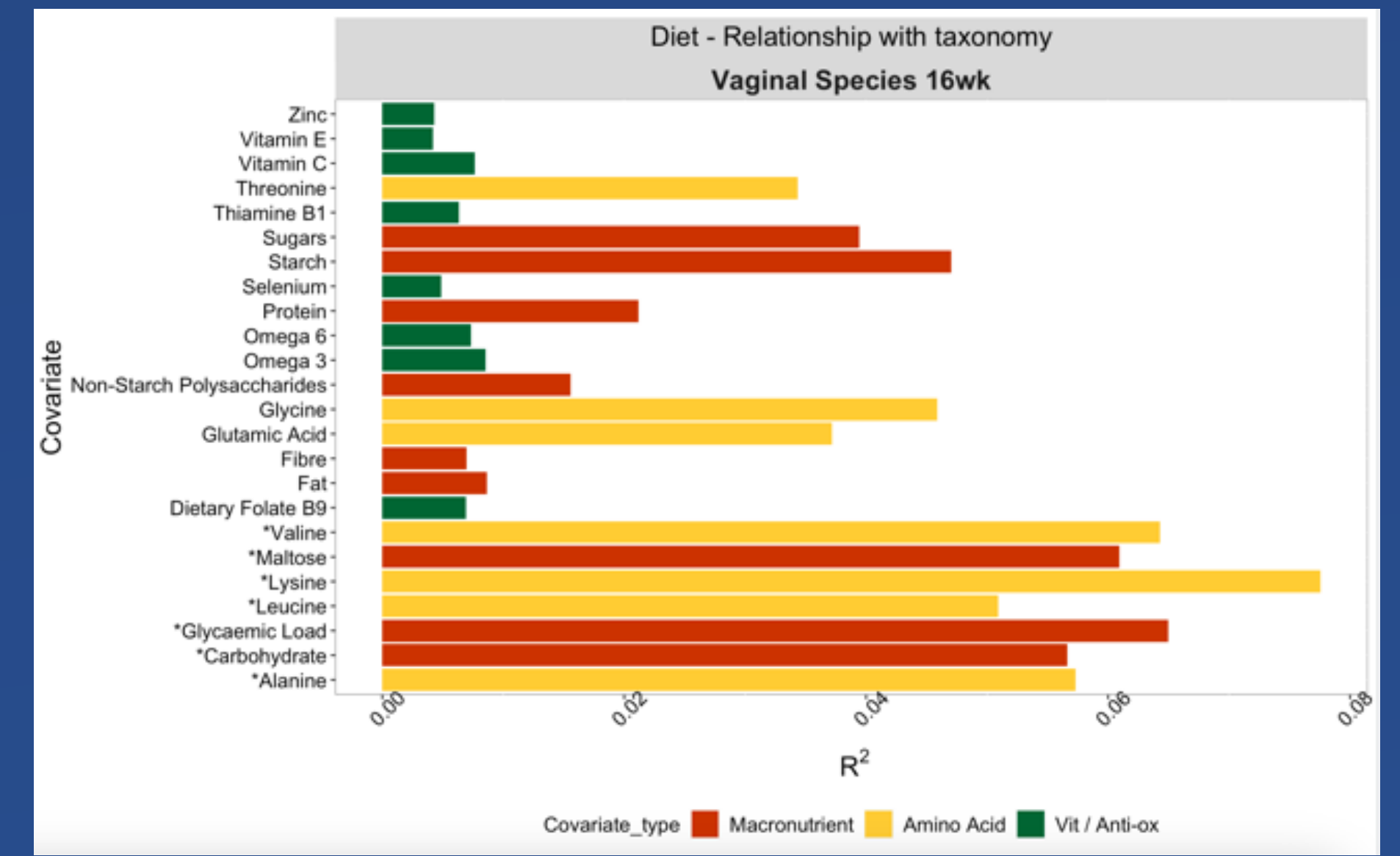
METHODS This is a secondary analysis of the MicrobeMOM randomised controlled trial (Sept 2016 - July2019). All women who had vaginal samples collected at randomisation (16weeks-gestation) were included. Shotgun sequencing of these samples was performed. Heatmapping allowed graphical assessment of vaginal species dominance. Shannon Index Alpha diversity was calculated. Baseline demographic and obstetric information was reported. Dietary data was calculated from food diaries using Nutritics software. Lifestyle factors such as wellness and exercise was recorded. Ordination of vaginal species was performed and dietary and environmental covariates were examined using envfit analysis to show effect size and significance for each covariate, comparing difference in centroids of covariate relative to total variation. Significant covariates were then examined against vaginal alpha diversity and assignment to a vaginal community state type (CST). Statistical analysis was performed employing R Statistical Software (version 4.2.2, 2022-10-31). Ethical approval was granted by the National Maternity Hospital.

RESULTS 119 participants underwent vaginal sampling at recruitment. Following metagenomic sequencing, an average of 565,192 high quality paired end reads were obtained per sample (median 350,006). Previous vaginal delivery and history of breastfeeding significantly impacted vaginal species (delivery (Adjusted R² 0.070, p=0.001;) Adjusted R² 0.053, p = 0.005, respectively), and were associated with lower assignment to CST I (p = 0.003, p = 0.005, respectively), but did not impact alpha diversity (Mann-Whitney U = 1553.0 and 488.0, p = 0.942 and p=0.874, respectively).

Dietary intake of carbohydrate, maltose and dietary glycaemic load had significant impact on vaginal microbial species (Adjusted R² 0.057, p = 0.038, Adjusted R² 0.061, p=0.033, Adjusted R² 0.065, p = 0.022). Carbohydrates, starch and maltose correlated with increased alpha diversity (+0.001 per carb g, p = 0.032; +0.002 per starch g; +0.044 per maltose g, p = 0.043). CST IV was shown to correspond to significantly higher intakes of carbohydrates and starch and higher dietary glycaemic load compared to CST I and III. Dietary intake of amino acids lysine, leucine, valine and alanine (precursors to short-chain-fatty acids) had a significant impact on vaginal species (Adjusted R² 0.077, p = 0.023, Adjusted R² 0.051, p = 0.048, Adjusted R² 0.057, p=0.028, Adjusted R² 0.064, p value = 0.018), but did not correlate with alpha diversity or assignment of CST.

No link was observed between previous caesarean section, non-starch based macronutrients (fibre and non-starch polysaccharides) or maternal exercise or wellbeing with vaginal species, diversity or CST assignment.

EnvFit Analysis showing effect size and significance for each dietary covariate (n=24) and overall environmental covariates (n=22), comparing difference in the centroids of each covariate relative to the total variation.



MAIN FINDINGS This secondary analysis of the MicrobeMOM randomised controlled trial reveals that are previous vaginal birth, history of breastfeeding, glycaemic load and dietary intake of starch and Short-Chain-Fatty-Acid-Precursor amino acids have the most significant environmental impact upon vaginal microbial composition