

# Gut microbe's consortium in pregnant women influenced by diet of North-east India: A metagenomic study

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## ABSTRACT

**Introduction:** Recent research highlights that the gut microbiota of pregnant women undergoes significant changes throughout pregnancy, influenced by factors such as diet, environment, and sociocultural practices. A diverse gut microbiome during pregnancy is linked to improved metabolic health and the lower risk of complications like gestational diabetes and pre-eclampsia. **Objective:** This study aimed to investigate the relationship between dietary practices of different communities of Assam and Manipur with the composition of gut microbes during pregnancy. **Methods:** A total of 18 pregnant women completed a survey assessing macronutrient consumption during the first and third trimesters. Dietary data, obtained through questionnaires, were subjected to PCoA (Principal Co-ordinate Analysis) to find similarities and dissimilarities in dietary patterns. Metagenomics analysis of stool samples was done to study the consortium of gut bacteria for the participants. **Results:** This study has demonstrated that higher intake of carbohydrates and fats during pregnancy results in a decrease in phylum such as Firmicutes and Actinobacteria. Phylum such as Gemmatimonadetes, Crenarchaeote, Fibrobacteres, and Fusobacteria dominated the gut of most participants. **Conclusion:** The relationship between gut microbiota composition and dietary habits among pregnant women in Northeast India is essential for designing effective nutritional interventions aimed at improving both maternal and infant health. Future studies should prioritize longitudinal research to better understand these connections and their impact on public health in the region, with a particular focus on identifying the specific foods and dietary patterns responsible for the observed similarities.

**Keywords:** Composition, dietary habits, gut bacteria, metagenomics, pregnant

## Introduction

Diet significantly influences the composition and function of gut microbes, which play a crucial role in human health. The gut microbiome, a complex ecosystem of trillions of microorganisms, is shaped by dietary patterns and specific food choices. Diets rich in fiber, such as Mediterranean or

plant-based diets, promote the growth of beneficial bacteria, enhancing microbial diversity and producing short-chain fatty acids that support gut health.<sup>[1,2]</sup> In contrast, diets high in sugar and saturated fats tend to favor harmful microorganisms, leading to dysbiosis associated with various health issues like obesity and metabolic disorders.<sup>[3,4]</sup> Research indicates that habitual dietary patterns have a more profound impact on gut microbiota than transient dietary changes.<sup>[5]</sup> The gut microbiome of pregnant women, similar to that of the general population, is significantly affected by their dietary habits. There are notable differences in the gut microbiome composition between the first and third trimesters of pregnancy, with these changes typically returning

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to pre-pregnancy states after childbirth.<sup>[6]</sup> During pregnancy, specific bacterial groups such as Akkermansia, Bifidobacterium, and Firmicutes increase, which are linked to heightened energy storage needs. Conversely, levels of Proteobacteria and Actinobacteria rise, providing protective effects for both the mother and fetus through pro-inflammatory mechanisms.<sup>[7,8]</sup> This study examines the eating habits of pregnant women from the north-eastern states of Manipur and Assam. Participants included individuals from five different communities within these two states, focusing on their dietary practices during pregnancy.

## Materials and Method

### Sample collection and preparation

Pregnant women from the Meitei and Naga communities in Manipur and participants from the Assamese and Bodo communities in Assam were chosen for the study. A total of 18 women aged between 19 and 35 years met the inclusion criteria based on the health history and ethnicity. A questionnaire was developed to evaluate dietary intake during pregnancy, recent changes in food consumption, and the quantity of food consumed. Additionally, it gathered information on changes in daily physical activity and cravings for specific foods. Based on the responses, a clinical characteristic chart was created for each participant. The intake of macronutrients, including carbohydrates, proteins, saturated and unsaturated fats, and total fiber, was calculated. The Body Mass Index (BMI) of each subject was then plotted against their dietary intake results.

Stool samples of the same participants were collected in sterile plastic containers of polypropylene (Himedia: PW016T, 50 mL) during the first and third trimesters of pregnancy. Sample donors were instructed to not contaminate the stool with urine and to avoid contact with the bedding while loading the sample. The samples were then transported to the laboratory and stored at  $-80^{\circ}\text{C}$ .

### Microbiota analysis by metagenomics

The metagenomic analysis involved genomic DNA extraction from the stool sample using QIAamp DNA Stool Mini Kit (QIAGEN). DNA extraction procedures and guidelines were followed in accordance with the kit instructions.<sup>[9,10]</sup> The amount of DNA extracted was measured and stored at  $-80^{\circ}\text{C}$  for metagenomic analysis using Next Generation Sequencing (NGS). Analyzing the 16S V3-V4 region in metagenomics is a common approach for studying microbial communities. The 16S rRNA gene is used as a marker gene to identify and classify bacteria and archaea. The V3-V4 region of the 16S rRNA gene is often targeted for sequencing because it provides a good balance between taxonomic resolution and sequencing cost. The V3-V4 region of the bacterial 16S rRNA gene was amplified from stool DNA using V3–V4 region-specific primers, and a 16S V3-V4 metagenome library was constructed according to the Illumina protocol.<sup>[11]</sup>

### For data quality

The raw data quality was assessed using the FastQC program. FASTQC analysis revealed suboptimal sequencing quality with the presence of poor-quality sequences. Subsequently, the low-quality sequences were eliminated, retaining only those bases with a Phred score greater than 19 for OTU picking. The integrity of the amplified sequences was checked with the help of Agarose Gel Electrophoresis.<sup>[12]</sup>

### Operational Taxonomic Unit (OTUs) picking

The task of selecting Operational Taxonomic Units (OTUs) was accomplished through the utilization of the QIIME software suite.<sup>[13,14]</sup> QIIME, which stands for Quantitative Insights Into Microbial Ecology, is a powerful and versatile tool specifically designed for the analysis of microbial community data derived from high-throughput sequencing technologies such as 16S rRNA gene sequencing or metagenomics. After pre-processing, a closed-reference OTU picking process where reads are clustered against a reference sequence database (Green\_genes\_13\_8\_97).<sup>[15-17]</sup> The reference sequences were assigned to taxonomic levels based on 97% similarity profiles.<sup>[18-20]</sup> Taxonomic assignments, from phylum down to genus, were determined for all OTUs identified in this study. These assignments were based on the complete, non-rarefied OTU table generated from the analysis, ensuring that the full diversity of the dataset was considered when characterizing the microbial community composition.

## Result

A diet chart was assessed with the guidance of a nutritionist, revealing the intake of macronutrients during the first and third trimesters among pregnant women from Assamese, Boro, Meitei, and Naga communities [Table 1]. A PCoA plot based on a distance matrix was drafted using the macronutrient consumption data showing cluster formations and notable outliers as shown in Figure 1a and b. Similarities in dietary practices are placed close together as a cluster and outliers represent a unique dietary pattern. Considering samples in clusters as closely related, and outliers as distinct samples, three samples were selected for the metagenomic study from each state.

### Bioinformatics and statistical results

A Krona plot was created individually for each sample to visually represent and explore the microbial diversity present in the dataset.

**Table 1: Macronutrient consumption during first and third trimesters of pregnancy of participants of Assam and Manipur states**

| Macronutrients | First trimester | Third trimester | P      |
|----------------|-----------------|-----------------|--------|
| Carbohydrates  | 347.11 (5.70)   | 377.44 (5.57)   | <0.001 |
| Proteins       | 88.22 (1.25)    | 88.72 (1.03)    | <0.001 |
| Total fats     | 59.11 (2.14)    | 66.89 (1.89)    | <0.001 |
| Fibers         | 34.33 (1.48)    | 37.39 (1.40)    | <0.001 |

*P*<0.05, paired *t* test

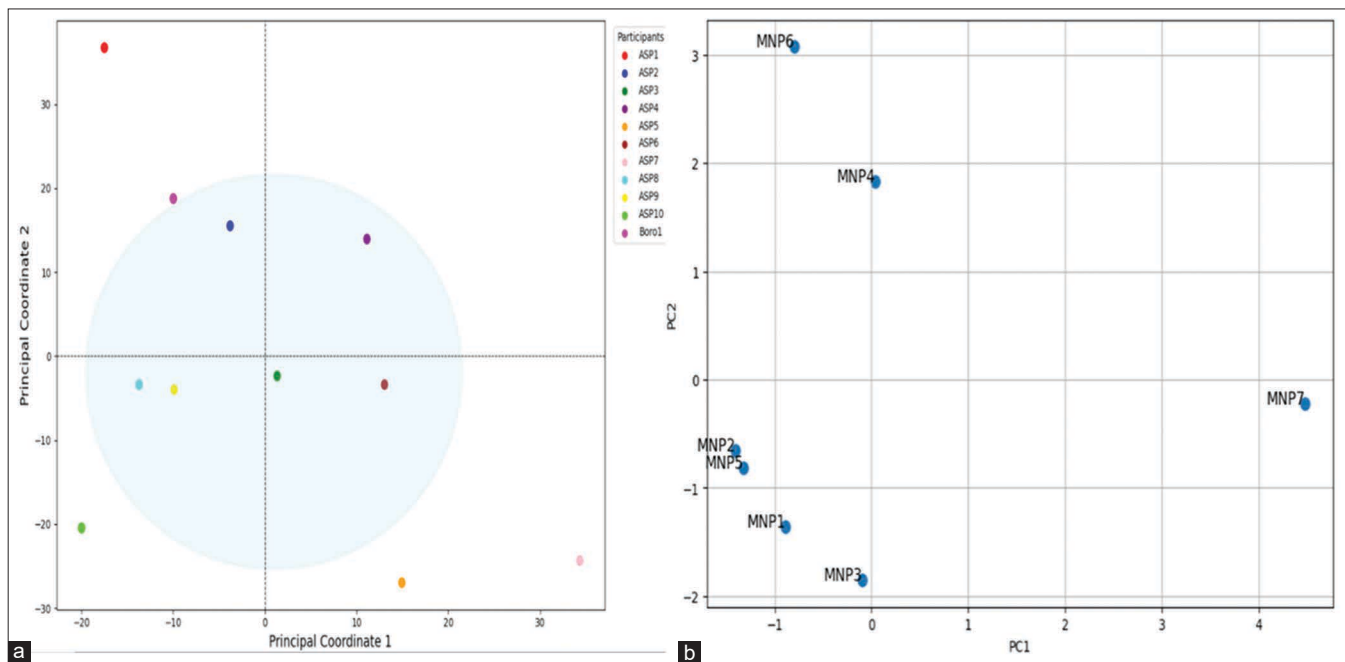
Krona plots as an effective tool helped convey complex taxonomic information of the samples in an easily interpretable and interactive manner. While participants with more consumption of carbohydrates reveal in lesser percentage of Firmicutes and Actinobacteria ranging to 2%–3% in all participants. Lactic acid bacteria like members of Lactobacillales accounting to about 30%–32%. Cases of notable outliers with BMI 35.43 shows a composition of 1% firmicutes with Lactobacillales accounting to 19%, Lactobacillaceae accounting to 12% and 65% of lactobacillales in firmicutes. Species such as *Lactobacillus iners* shows 22%, *Lactobacillus ruminis* 7%, *Lactobacillus brevis* 4%, and *Lactobacillus vaginalis* with 2%. In phylum Actinobacteria accounting for 3%, species like *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium adolescentis*, and *Bifidobacterium pseudolongum* ranges from 10%–35%. The following link provides the visuals

of Krona plot of a participant (G1) file:///E:/research%20paper%20reference/USTM-New/Krona/G1.html.

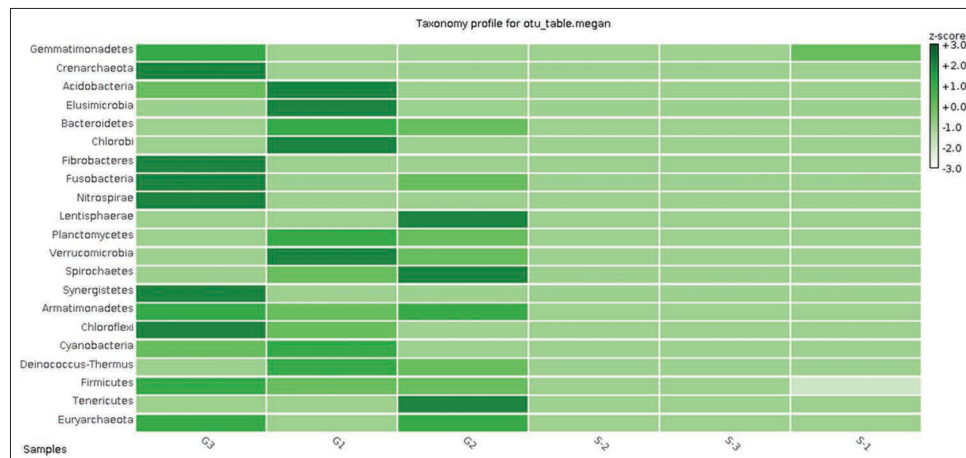
The taxonomic profile indicates that G3 is predominantly characterized by phyla such as Gemmatimonadetes, Crenarchaeota, Fibrobacteres, and Fusobacteria (as shown in Figure 2). In contrast, G1 is primarily composed of Acidobacteria, Elusimicrobia, and Verrucomicrobiota. Additionally, the bar plot at the phylum level reveals that all samples classified as G3 exhibit a significant abundance of the phylum Proteobacteria (illustrated in Figure 3).

### NSTI score analysis

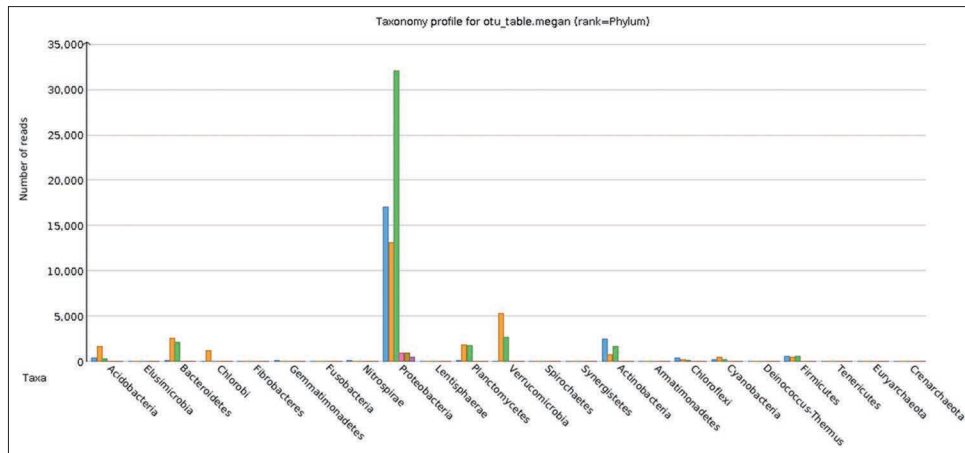
The NSTI (Nearest Sequenced Taxon Index) score is a crucial metric obtained during a QIIME analysis, which provides



**Figure 1:** PCoA plot of the nutritional data of macronutrient consumption based on distance matrix. (a) represents participants from Assam and (b) represents participants from Manipur. Notable outliers are seen in both cases



**Figure 2:** Heatmap showing taxonomy profile (rank = phylum) for the OTUs obtained from the samples



**Figure 3:** Phylum level bar plot

**Table 2: NSTI score per sample**

| Samples | Metric        | Value    |
|---------|---------------|----------|
| G-3     | Weighted NSTI | 0.111001 |
| G-1     | Weighted NSTI | 0.173516 |
| G-2     | Weighted NSTI | 0.147366 |
| S-2     | Weighted NSTI | 0.0936   |
| S-3     | Weighted NSTI | 0.093614 |
| S-1     | Weighted NSTI | 0.068301 |

insight into the comprehensiveness and accuracy of taxonomic assignments within a microbial community dataset. The NSTI score essentially quantifies the phylogenetic relatedness between the sequences in the dataset and the closest fully sequenced organisms in a reference database. Sample of Meitei community (S-1) scored least in the NSTI score analysis and G-1 of Assamese community scored the maximum [Table 2] indicating uniqueness from the reference database. This might indicate presence of more novel genes or poorly characterized taxa in cases of G1, G2 and G3 than those of S1, S2 and S3.

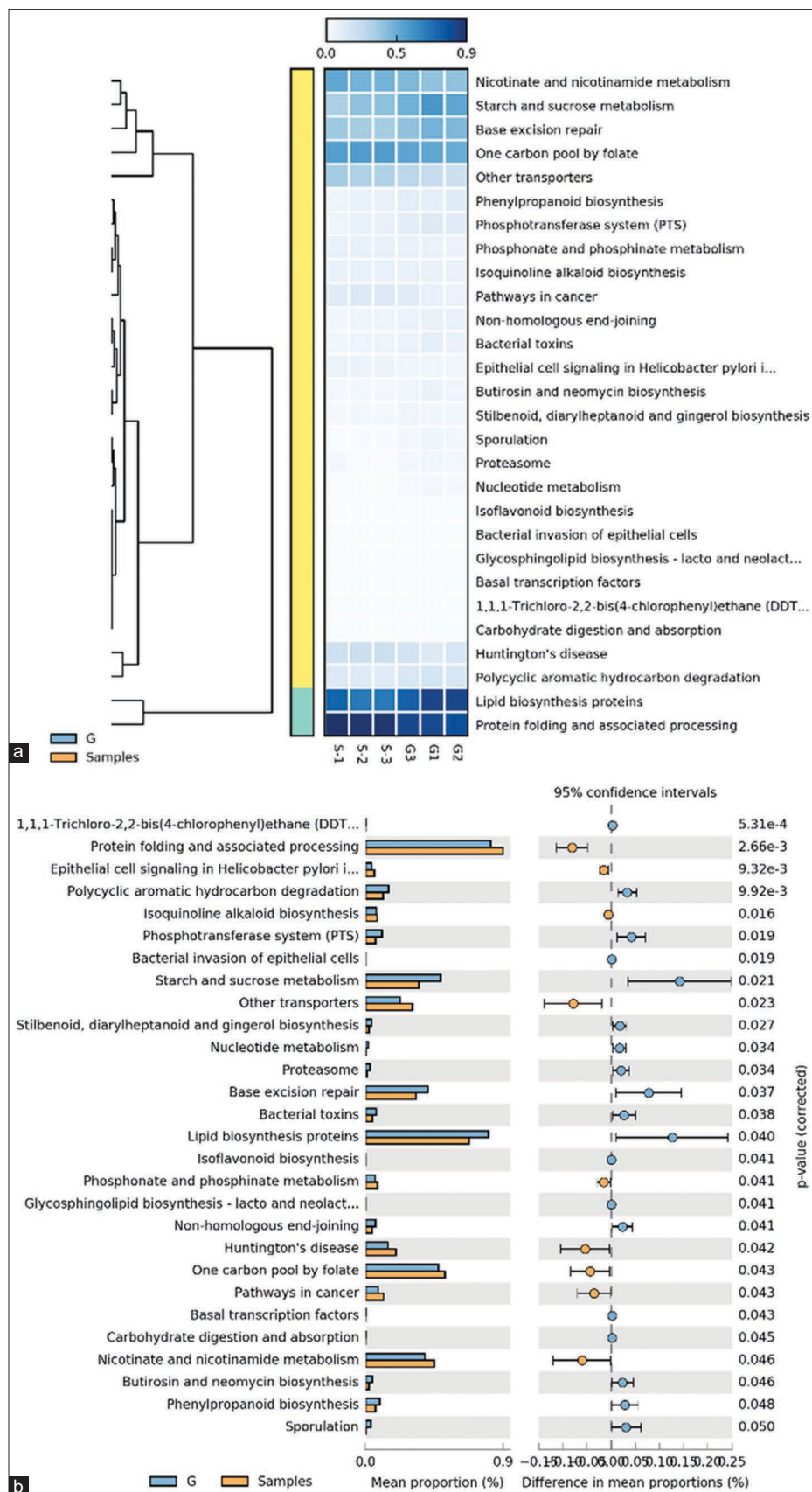
### PICRUSt analysis

The functional content of the metagenome derived from marker genes was assessed through PICRUSt analysis. This analysis indicated that samples from the G group in Assam and the S group in Manipur exhibited similarities in specific gene expressions for different metabolic pathways, including those related to lipid biosynthesis proteins, nicotinate and nicotinamide metabolism, protein folding and processing, as well as starch and sucrose metabolism. Such findings suggest a comparable composition of gut bacteria, likely influenced by similar dietary patterns among the four studied communities. This is illustrated by the extended error bar plot and STAMP heat map presented in Figure 4a and b.

### Discussion

A data-driven comparative approach was developed to investigate dietary intake patterns among participants from

various communities in Assam and Manipur, highlighting aspects that may not be reflected in traditional diet quality indices.<sup>[21,22]</sup> The resulting dietary pattern scores indicate how closely a participant's diet aligns with a specific pattern. A Principal Coordinate Analysis (PCoA) was conducted to visually examine variations in dietary intake among the participants from the diverse communities of study.<sup>[23,24]</sup> Dietary data, obtained through questionnaires, were subjected to PCoA to find similarities and dissimilarities in dietary patterns. The resulting plot revealed the formation of clusters, indicating groups of participants with comparable dietary habits, and identified outliers representing unique dietary profiles. The PCoA results suggested a notable degree of similarity in dietary practices across different communities, irrespective of their specific community affiliation or state of residence. Several factors could contribute to this observed homogeneity. Firstly, the North-eastern region of India, particularly Assam and Manipur, may share a common culinary heritage, leading to similar dietary patterns across communities.<sup>[25,26]</sup> Secondly, the availability of seasonal vegetables and other food sources, dictated by weather patterns and soil fertility, likely influences the diets of individuals throughout the region, further contributing to the convergence of dietary habits.<sup>[27,28]</sup> Understanding the interplay between gut microbiota composition and dietary practices among pregnant women in Northeast India is vital for developing targeted nutritional interventions that could enhance maternal and infant health outcomes. Future research should focus on longitudinal studies to further elucidate these relationships and their implications for public health strategies in the region and could explore the specific food items and dietary practices that contribute to the observed similarities. Investigating the role of cultural exchange, trade, and migration in shaping dietary patterns within the region could also provide valuable insights. Additionally, examining the nutritional implications of these shared dietary habits and identifying potential areas for dietary improvement would be beneficial for public health initiatives.



**Figure 4:** (a) Heatmap supporting the statistical visualization of identification of key microbial taxa, pathways, and interactions. (b) STAMP analysis with extended error bar plot showing the distribution and significance of microbial taxa and pathways across different experimental conditions with 95% confidence

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## Contribution details

The authors of this manuscript have equally contributed in the making of this review paper. The authors have equally distributed the findings of the concept, design, definition of intellectual content, literature search, data acquisition, data analysis, manuscript editing, and manuscript review.

## Conflicts of interest

There are no conflicts of interest.

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