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Gut microbiota profiling of the population residing in Friuli-Venezia Giulia through next-generation sequencing

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Abstract

The gut microbiota is an ecological community of symbiotic and commensal microorganisms that play crucial roles in nutrient metabolism, maintaining the structural integrity of the intestinal mucosal barrier, immunomodulation, and pathogen protection. The composition of the gut microbiota varies with age, ethnicity, lifestyle, and dietary habits. Given the microbiota's growing role as a modulator of various physiological and pathological conditions, our study aimed to investigate the genetic profile of the microbiome individuals residing in the Friuli-Venezia Giulia region. We analyzed fecal swab samples from 109 individuals belonging to a general population cohort. The hypervariable V3-V4 regions of bacterial 16 S rRNA were analyzed using Next Generation Sequencing (NGS) on the MiSeq system (Illumina). The relative abundance of phyla, classes, orders, families, and species was defined using the BaseSpace 16s metagenomics app (Illumina). Firmicutes was the most represented phylum (51.1%), followed by Bacteroidetes (38.3%) and Actinobacteria (3%). At the class level, Clostridia (45.2%) and Bacteroidia (37.7%) were predominant, while Clostridiales (46.9%), Bacteroidales (26.6%), and Anaeroplasmatales (12.6%) were notable orders. Lachnospiraceae (21.9%) and Ruminococcaceae (16.2%) were the most frequent families, with *Faecalibacterium prausnitzii* (10.3%), *Bacteroides vulgatus* (4.6%), and *Bacteroides dorei* (3.5%) being prominent species. Each participant's taxa were analyzed to identify genera associated with alterations in gut microbial composition. Significant associations emerged between specific taxa of microorganisms and age, gender, anti-inflammatory drugs, tobacco consumption, and allergies. This study provides valuable insights into gut microbiota composition in a population-based cohort. The characterization of the microbiota in the Friuli-Venezia Giulia (FVG) region lays the foundation for future research into regional variations in microbiota composition and its impact on health.

Keywords Gut microbiota, Next generation Sequencing (NGS), 16s rRNA bacterial gene, Fecal swab, Regional variation

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Introduction

The gut microbiota is a highly complex and dynamic ecosystem comprising trillions of microorganisms, including bacteria, viruses, fungi, and archaea, that coexist in a symbiotic and commensal relationship with the human host [1–3]. These microorganisms are essential for numerous physiological functions. One of their primary roles is in nutrient metabolism, where they assist in the breakdown of complex carbohydrates, proteins, and lipids, producing short-chain fatty acids and other metabolites that are beneficial to the host. Additionally, the microbiota plays a critical part in maintaining the structural integrity of the intestinal mucosal barrier, a key defense against pathogens and toxins. This barrier function is crucial in preventing "leaky gut" and maintaining a healthy immune response [1, 3, 4]. Although genetic, dietary, and environmental factors are known to influence gut microbiota composition, they were not directly evaluated in this study. These dimensions will be explored in future research to better understand their contribution to interindividual variability. Beyond this, the gut microbiota is deeply involved in immunomodulation, interacting with immune cells to regulate both innate and adaptive immune responses, and it provides protection against pathogens by outcompeting harmful bacteria for resources and secreting antimicrobial substances [4, 5].

In a healthy adult, the microbiota is predominantly composed of two major bacterial phyla: Firmicutes and Bacteroidetes, though its exact composition is highly variable and subject to several factors. The age of the individual plays a significant role, with the microbiota evolving from infancy through adulthood and into old age, reflecting changes in diet, immune function, and physiology [2–4, 6]. Ethnicity can also influence microbial composition, with distinct microbial signatures observed in different populations, likely due to variations in diet, environment, and genetics [6, 7]. Additionally, lifestyle factors, such as physical activity, stress levels, and medication use (especially antibiotics), are known to affect microbiota diversity and stability. Finally, dietary habits are perhaps the most modifiable factor influencing the gut microbiome, with fiber-rich diets promoting diversity, while diets high in processed foods are linked to reduced microbial richness [2–4, 6].

In recent years, studies on the gut microbiota of populations across Italy have revealed notable differences between the microbiome compositions of individuals from the northern, central, and southern regions. These variations are primarily attributed to dietary patterns, lifestyle, and environmental factors, which differ across the country due to its geographical and cultural diversity [7–9]. The diet in the northern regions (Lombardia area) tends to be richer in animal fats, dairy products, and processed foods, which may contribute to a distinct microbial composition. Studies have shown that individuals in the north tend to have a higher ratio of Firmicutes to Bacteroidetes, a microbial signature often associated with diets higher in fats and lower in fiber [3]. The consumption of fermented foods, such as cheeses, may also influence the presence of beneficial Lactobacillus species, which play a role in maintaining gut health. In central regions, like Tuscany and Lazio, the diet often follows the more traditional Mediterranean pattern, characterized by a higher intake of fruits, vegetables, legumes, and whole grains, as well as olive oil and moderate wine consumption. This diet is associated with increased microbial diversity and a greater abundance of Bacteroidetes. Central Italians typically display a more balanced Firmicutes to Bacteroidetes ratio, which is linked to improved metabolic health and a lower incidence of obesity and inflammatory diseases [6, 8, 10]. Southern regions, such as Sicily and Calabria, maintain a diet that is also rooted in the Mediterranean tradition but often includes higher levels of plant-based foods and fresh, locally sourced ingredients. Studies indicate that individuals from the south tend to have a more diverse gut microbiota, with higher levels of Prevotella, a genus associated with the consumption of complex carbohydrates and fibers. This microbial profile is linked to better gut health and a reduced risk of chronic diseases, such as cardiovascular disease and type 2 diabetes [4–6, 11].

Overall, these regional differences in microbiota composition reflect the broader north-south gradient in dietary habits within Italy. Northern regions, with their more industrialized and high-fat diets, show lower microbial diversity, while central and southern regions, which adhere more closely to the Mediterranean diet, demonstrate higher microbial diversity and a composition more conducive to maintaining long-term health. These findings underscore the critical role of diet and regional habits in shaping the gut microbiome across different parts of Italy [8, 10].

Given the microbiota's pivotal role in modulating various physiological processes and its involvement in the pathogenesis of numerous diseases, understanding its genetic profile in specific populations is critical.

Friuli Venezia Giulia exhibits a distinctive combination of dietary, environmental, and lifestyle factors that may influence gut microbiota composition. These include a regional diet characterized by frequent consumption of fermented foods, cured meats, and maize-based products; heterogeneous environmental exposures due to the coexistence of rural and urban settings; and lifestyle patterns reflecting a convergence of Central European and Mediterranean cultural influences. However, despite its strategic location and diverse influences, there is a lack of data on the microbiota composition in the local population.

Nowadays, high-throughput sequencing has revolutionized microbiome research, particularly with the targeted sequencing of the 16 S rRNA gene [12–14]. This gene is an ideal target for bacterial classification due to its hypervariable regions (V1-V9), which allow for precise taxonomic resolution. Extensive reference databases, such as SILVA, Greengenes and RefSeq further enhance the accuracy of taxonomic assignments [15, 16].

Advances in Next Generation Sequencing (NGS) and metagenomics offer even greater insights into microbial ecosystems [14, 15, 17, 18].

Investigating the Friuli Venezia Giulia region's microbiome using a targeted 16 S rRNA amplicon sequencing could provide valuable insights in understanding of microbial diversity within Italy.

Our study focuses on the gut microbiome of a study cohort of individuals residing in the Friuli-Venezia Giulia region. This population offers a unique opportunity to examine how regional factors, such as local dietary patterns, environmental conditions, and genetic diversity, may shape the microbiome's composition and functionality, providing insights into its role in health and disease.

Materials and methods

Enrollment and sample collection

In this study, fecal swabs were collected from subjects residing in the Friuli Venezia Giulia region using designated collection tubes (Copan's FecalSwab®). Samples were stored at 4 °C for up to 24 h after collection, then frozen at − 20 °C until DNA extraction. Extracted DNA samples were subsequently stored at − 80 °C until further analysis. Ethics Committee approval was obtained by Regional Ethics Committee of Friuli Venezia Giulia, Italy (CEUR) (CEUR-2022-Os-137; file no. 132083, August 18, 2022). All participants provided written informed consent prior to enrollment. The study was conducted in accordance with ethical standards and applicable regulations.

To ensure confidentiality, each sample was anonymized, and a unique identification code was assigned to each subject, in accordance with current ethical standards and data protection guidelines, including the Declaration of Helsinki as required by CEUR.

Beside the sample collection, participants completed a questionnaire detailing their dietary habits and lifestyle choices (Supplementary file 1). This questionnaire was labeled with the same unique code to safeguard anonymity and privacy throughout the study. In the anonymized questionnaire, the following clinical information was requested: sex (if female, specify pregnancy status and week of pregnancy if applicable), age, ethnicity, weight (kg), height (cm), use of antibiotics, use of anti-inflammatory drugs, use of probiotics and/or prebiotics, use of proton pump inhibitors, and presence of symptoms such as constipation, diarrhea, alternating constipation/diarrhea, abdominal bloating, anxiety, depression, and abdominal pain, along with their frequency. Regarding lifestyle, we assessed regular physical activity, average sleep hours per night (5–6 h, 7–8 h, and 9–10 h), frequency of alcohol consumption, frequency of tobacco use, and dietary habits (specific diets such as veganism, vegetarianism, lactose-free, gluten-free, or other). Additionally, a list of current or past medical conditions was provided, including menstrual cycle irregularities, anxiety/depression, heart disease, type 2 diabetes, thyroid disorders, Crohn's disease, neoplasms, irritable bowel syndrome, fatigue, and allergies.

Bacterial DNA extraction

Bacterial DNA was extracted from fecal samples obtained from 109 subjects using the QIAamp® PowerFecal® DNA Kit (Qiagen; RRID: SCR_020984). This kit is designed specifically for isolating high-quality microbial DNA from stool samples. The protocol begins with mechanical and chemical lysis, which breaks down bacterial cell walls, followed by the removal of PCR inhibitors commonly found in fecal matter (such as bile salts, complex polysaccharides, and humic substances). The DNA is then purified through silica membrane spin columns, ensuring maximum yield and purity of the bacterial DNA. The extracted DNA was quantified and assessed for quality using spectrophotometry (e.g., NanoDrop) and gel electrophoresis before further processing.

Library preparation and sequencing

The hypervariable V3-V4 regions of the bacterial 16 S rRNA gene were selected for amplification because these regions provide enough variability to distinguish between different bacterial taxa [14, 19]. The 16 S rRNA gene is highly conserved among bacterial species, making it a target for microbial identification through sequencing [12]. The V3–V4 region of the 16 S rRNA gene was amplified using fusion primers composed of Illumina overhang adapter sequences and locus-specific sequences. The forward primer consisted of the overhang sequence 5′- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′ followed by the locus-specific sequence 5′-CCTACGGGNGGCWGCAG-3′. The reverse primer consisted of the overhang sequence 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′ followed by the locus-specific sequence 5′-GACTACHVGGGTATCTAATCC-3′. The PCR program consists in an initial denaturation step at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The program ends with a final extension at 72 °C for 5 min, and the reaction is then held at 4 °C (https://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/che

mistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf).

After PCR amplification, the amplicons were cleaned to remove residual primers and reagents using magnetic bead-based purification. Next Generation Sequencing (NGS) was performed on the Illumina MiSeq platform, using a targeted metagenomics sequencing. Adapter sequences required for Illumina sequencing were added to the amplicons through a secondary PCR. The final libraries were quantified using qPCR or fluorimeter (e.g., Qubit) and pooled to ensure even sequencing coverage across all samples.

Data analysis

Data from sequencing runs were processed through the BaseSpace 16s metagenomics app (Illumina), a cloud-based bioinformatics tool designed for taxonomic classification of 16 S rRNA gene sequencing data, utilizing a pipeline that includes read preprocessing, quality filtering, and taxonomic assignment through a k-mer-based Naive Bayes classifier derived from the Ribosomal Database Project (RDP). The app compares sequencing reads against a curated reference database, Greengenes 13_8; confidence threshold = 0.7, to assign taxonomy with a confidence threshold, generating outputs of relative abundances at various taxonomic levels [16]. Relative abundances are calculated as the proportion of classified reads per taxon relative to the total classified reads in each sample. The app does not perform copy number normalization for the 16 S gene.

To account for differences in sequencing depth across library batches, taxonomic summaries at lower resolution (particularly order and species level) were initially inspected separately by sequencing run, whereas intermediate taxonomic levels (family and genus) were also summarized across the full cohort to provide more stable population-level estimates. Therefore, comparisons across taxonomic ranks should be interpreted with caution when tables are presented using different aggregation strategies.

Table 1 Demographic and clinical characteristics of the study population ($N = 103$). Data are presented as median (Q1, Q3) for continuous variables and number (%) for categorical variables

Characteristic	$N = 103$[1*]
Female gender	59 (57%)
Age	47 (34, 58)
Weight	72 (58, 82)
Height	1.70 (1.64, 1.78)
BMI	24.5 (20.8, 26.3)
Antibiotics	4 (3.9%)
Anti-inflammatories	19 (18%)
Proton pump inhibitors	4 (3.9%)
Probiotics/Prebiotics	11 (11%)
Disorders	54 (52%)
Hours of sleep	
1	51 (50%)
2	48 (47%)
3	4 (3.9%)
Alcoholic consumption	69 (67%)
Tobacco	15 (15%)
Pathologies	44 (43%)
Physical activity	29 (28%)
Allergies	18 (17%)

[1] n (%); Median (Q1, Q3)

*Demographic and questionnaire data were available for 103 of the 109 participants included in the study

Statistics

Population-based ranges, expressed as percentages, are presented as median and interquartile range (IQR). Univariable zero-inflated beta regression models were performed to estimate the associations between microbial taxa and various lifestyle factors, accounting for the high proportion of zeros in microbial abundance data. Benjamini-Hochberg false discovery rate (FDR) correction was applied in order to control for multiple testing. All analyses were performed by the STATA 18 statistical software and R (gamlss package), with statistical significance set at $p < 0.05$.

Results

Population-based ranges of gut microbiota composition for microbial taxa

In microbiome research, understanding the reference ranges of microbial taxa (such as phyla, classes, orders, families, genera and species) is essential for characterizing a balanced and healthy microbial community [11, 20]. Establishing baseline values for each taxonomic level helps researchers identify deviations linked to various diseases or conditions, enabling a more precise assessment of microbial imbalances. These ranges serve as a reference for comparing microbiome compositions across different populations and experimental groups, thereby facilitating the study of microbiome-related health impacts. In our study, reported values represent population-based ranges of gut microbiota composition observed within this cohort and should not be interpreted as clinical reference ranges. Table 1 summarizes the main characteristics of the 103 participants included in the study. The cohort consisted of 59 females, representing 57% of the sample. The median age was 47 years (interquartile range [IQR]: 34 to 58), with a median weight of 72 kg (IQR: 58 to 82) and median height of 1.70 m (IQR: 1.64 to 1.78). The median body mass index (BMI) was 24.5 (IQR: 20.8 to 26.3). Medication use was reported for a minority of subjects, with 4 (3.9%) taking antibiotics, 19 (18%) on anti-inflammatories, and 4 (3.9%) using proton pump inhibitors. Probiotics or prebiotics

were used by 11 participants (11%). About half of the participants reported some form of disorders (54, 52%), while 44 individuals (43%) reported pathologies. Regarding lifestyle factors, 69 participants (67%) reported alcohol consumption, 15 (15%) used tobacco, and 29 (28%) engaged in physical activity. Allergies were reported by 18 participants (17%). Sleep duration was categorized, with 51 (50%) reporting one hour, 48 (47%) two hours, and 4 (3.9%) three hours.

Distribution of gut microbiota composition at the phylum level in our population-based cohort

The relative abundances of the top 10 bacterial phyla found in our samples, together with their respective standard ranges, expressed as percentages, are reported in Table 2. The two principal phyla are Firmicutes and Bacteroidetes, which together comprise the majority of the bacterial community, with Firmicutes at 51.107% and Bacteroidetes at 38.337%. These percentages fall within their respective ranges (42.452–62.530 for Firmicutes and 26.809–47.572 for Bacteroidetes), indicating that these are the primary constituents of a typical gut microbiome, commonly seen in healthy individuals. Other phyla, such as Actinobacteria (2.945%) and Proteobacteria (2.536%), are present at much lower levels within a significant range. Verrucomicrobia, Acidobacteria, Tenericutes, and many others, are present in minimal amounts, generally below 0.1%, reflecting the diversity and complexity of the microbial community. These lower-abundance phyla may play specialized roles. Overall, this distribution suggests a balanced microbial community, where Firmicutes and Bacteroidetes dominating. This pattern is valuable for establishing a baseline in studies examining gut health, microbial diversity, and disease states.

Population-based ranges of gut microbiota composition at the class level

The relative abundances of bacterial classes, with percentages and their respective population-based ranges are reported in Table 3. The two most prevalent classes were Clostridia and Bacteroidia, which together form the bulk of the microbial community observed here. Clostridia constitutes 45.244% of the sample, with a range of 37.327–55.205%, while Bacteroidia is present at 37.742% (reference range of 26.059–47.305%). These two classes play essential roles in digestion, metabolism, and maintaining a balanced microbial environment [21]. Members of the class Clostridia are known to play a key role in the fermentation of dietary fibers and the production of short-chain fatty acids (SCFAs), which are essential for gut homeostasis and host metabolism [21, 22]. Other bacterial classes, such as Actinobacteria (2.87%) and Negativicutes (1.775%), appear in lower abundances and are often involved in metabolic processes and gut health maintenance. The remaining classes, such as Erysipelotrichia, Bacilli and Proteobacteria (e.g., Beta-, Gamma-, Delta-, Alpha-proteobacteria), are present at minimal levels, generally under 1%. Their roles are context-dependent, often associated with specific functions or environmental responses. For example, certain Proteobacteria classes can increase in cases of dysbiosis,

Table 2 Relative abundances of top 10 bacterial phyla found in 109 samples

Median (IQR)	*N* = 109
Firmicutes %	51.107 (42.452–62.530)
Bacteroidetes %	38.337 (26.809–47.572)
Actinobacteria %	2.945 (1.626–5.049)
Proteobacteria %	2.536 (1.312–3.926)
Verrucomicrobia %	0.087 (0.012–0.857)
Acidobacteria %	0.058 (0.043–0.093)
Armatimonadetes %	0.036 (0.017–0.091)
Tenericutes %	0.020 (0.011–0.056)
Synergistetes %	0.020 (0.008–0.046)
Cyanobacteria/Chloroplast %	0.015 (0.010–0.023)

Table 3 Relative abundances of top 10 bacterial classes found in 109 samples

Median (IQR)	*N* = 109
Clostridia %	45.244 (37.327–55.205)
Bacteroidia %	37.742 (26.059–47.305)
Actinobacteria %	2.87 (1.63–5.11)
Negativicutes %	1.775 (1.147–3.099)
Erysipelotrichia %	0.902 (0.546–2.042)
Bacilli %	0.892 (0.492–1.451)
Betaproteobacteria %	0.739 (0.263–1.119)
Gammaproteobacteria %	0.455 (0.210–1.491)
Deltaproteobacteria %	0.312 (0.153–0.604)
Alphaproteobacteria %	0.095 (0.043–0.361)

Population-based ranges of gut microbiota composition at the order level

Several key trends in the relative abundances of bacterial orders are illustrated in Table 4. Samples were divided into four separate sequencing runs to increase sensitivity at the order and species level by achieving higher coverage per sample. Without this approach, accurately assessing orders and species-level variability would not have been possible. Because lower-level taxonomic assignment may be more sensitive to sequencing depth and run-specific variation, some summaries are shown by sequencing run, whereas others are presented for the entire cohort. This reporting strategy was adopted to maximize robustness of the descriptive analysis; however, direct comparison across taxonomic levels should be interpreted cautiously. Clostridiales and Bacteroidales are the most prominent bacterial orders, representing 46.91% and 26.56%, respectively. These orders are typically dominant

Table 4 Relative abundances of top 10 bacterial orders found in 109 samples

Median (IQR)	Run 1 *N* = 9	Run 2 *N* = 12	Run 3 *N* = 24	Run 4 *N* = 64
Clostridiales %	43.434 (37.844–43.793)	50.217 (46.318–54.935)	56.530 (43.739–65.397)	44.167 (36.510–51.180)
Bacteroidales %	41.930 (39.430-50.278)	28.080 (24.186–34.185)	29.128 (15.471–40.594)	42.134 (33.789–49.027)
Selenomonadales %	2.412 (1.821–3.823)	1.860 (1.428–2.393)	1.766 (1.141–3.280)	1.692 (1.075–3.117)
Coriobacteriales %	1.178 (1.004–1.640)	3.210 (1.857–34.185)	1.270 (0.987–2.474)	1.209 (0.531–2.579)
Erysipelotrichales %	0.774 (0.254–1.126)	1.437 (0.705–2.310)	1.240 (0.639–2.617)	0.884 (0.517–1.858)
Bifidobacteriales %	0.718 (0.045–1.634)	3.750 (1.331–7.240)	0.561 (0.191–1.467)	0.832 (0.307–1.932)
Lactobacillales %	0.626 (0.215–0.849)	1.088 (0.658–1.318)	1.093 (0.427–2.162)	0.648 (0.381–1.242)
Burkholderiales %	1.579 (0.656–2.903)	0.863 (0.576–1.299)	0.882 (0.480–1.195)	0.586 (0.204–0.959)
Desulfovibrionales %	0.420 (0.274–0.540)	0.294 (0.132–0.371)	0.161 (0.062–0.506)	0.223 (0.080–0.411)
Enterobacteriales %	-	0.348 (0.126–1.458)	0.467 (0.175–1.362)	0.181 (0.034–0.531)

Table 5 Relative abundances of top 10 bacterial families found in 109 samples

Median (IQR)	*N* = 109
Lachnospiraceae %	21.975 (17.126–29.788)
Ruminococcaceae %	16.175 (12.157–22.791)
Bacteroidaceae %	15.370 (3.001–24.936)
Porphyromonadaceae %	2.735 (0.896–5.365)
Prevotellaceae %	1.988 (0.350-11.176)
Coriobacteriaceae %	1.365 (0.713–2.746)
Rikenellaceae %	1.267 (0.480–2.827)
Eubacteriaceae %	0.920 (0.482–1.546)
Bifidobacteriaceae %	0.857 (0.300-2.061)
Veillonellaceae %	0.697 (0.376–1.860)

Table 6 Relative abundances of top 10 bacterial genus found in 109 samples

Median (IQR)	*N* = 109
Eubacterium %	0.863 (0.454–1.302)
Collinsella %	0.807 (0.271–1.979)
Bifidobacterium %	0.751 (0.328–1.938)
Dorea %	0.744 (0.451–1.033)
Clostridium IV %	0.722 (0.273–1.399)
Prevotella %	0.687 (0.130-14.318)
Barnesiella %	0.627 (0.081–1.362)
Rummeliibacillus %	0.597 (0.001–1.446)
Eisenbergiella %	0.565 (0.246–1.185)
Oscillibacter %	0.537 (0.246–1.185)

in the human gut microbiome, playing crucial roles in gut health through processes like dietary fiber fermentation and short-chain fatty acid production [23, 24]. Other notable bacterial orders include Anaeroplasmatales (12.62%), Coriobacteriales (3.10%), Enterobacteriales (1.53%), Bifidobacteriales (1.39%), and Rhodospirillales (1.34%). Although less abundant, these orders contribute to the diversity and functionality of the microbiome. For instance, Bifidobacteriales is recognized for its positive impact on gut health, particularly in aiding digestion and modulating immune responses.

Population-based ranges of gut microbiota composition at the family level

Data regarding family highlights Lachnospiraceae a key family, representing the 21.975% (Table 5).

Other notable families include Ruminococcaceae (16.175%) and Bacteroidaceae (15.370%), which play essential roles in fiber degradation and overall gut health.

Lower-abundance families such as Porphyromonadaceae (2.735%), Prevotellaceae (1.988%), Coriobacteriaceae (1.365%), and Rikenellaceae (1.267%) contribute to the microbial diversity, with specialized metabolic functions that support the overall ecosystem. The presence of families like Bifidobacteriaceae (0.857%) and Streptococcaceae (0.481%) also reflects beneficial bacteria associated with gut health and immune modulation [25, 26]. Bifidobacteria are widely recognized for their beneficial role in gut health, including modulation of the immune system and maintenance of intestinal barrier function [26]. Short-chain fatty acids, produced through microbial fermentation, are key mediators of host–microbiota interactions and play important roles in energy metabolism and immune regulation [25, 26].

Other rare families, including Lactobacillaceae (0.029%) and Leuconostocaceae (0.063%), are present in traces. These families can have specific health impacts, such as fermentation and vitamin synthesis. The diversity across these microbial families suggests a balanced gut microbiota composition, indicative of a healthy and stable microbial community that supports various digestive and immune functions.

Population-based ranges of gut microbiota composition at the genus level

Data reported in Table 6 presents median fractions (with IQR) for bacterial genera, with their distribution across our 109 subjects. *Eubacterium* shows the highest median abundance at 0.863%. Similarly, *Collinsella* has a median of 0.807%.

Other genera, such as *Bifidobacterium* (median of 0.751%) and *Dorea* (median of 0.744%), display moderate abundances. *Clostridium IV* (median of 0.722%) *and*

Prevotella (median of 0.687%) also contribute to the gut microbiota of the reference population.

Barnesiella (median of 0.627%) and *Rummeliibacillus* (median of 0.597%) are less abundant. *Eisenbergiella* and *Oscillibacter*, with medians of 0.565% and 0.537% respectively, are present at lower levels.

Summary plots illustrating gut microbiota composition across different taxonomic levels, from phylum to genus, are available in Supplementary File 1.

Population-based ranges of gut microbiota composition at the specie level

The ten most frequent bacterial species across 4 different sequencing Runs (Run1, 2, 3, 4), are illustrated in Table 7 (median and interquartile range (IQR)). *Faecalibacterium prausnitzii* shows the highest values, followed by *Bacteroides vulgatus*, *Bacteroides dorei* and *Bacteroides uniformis*.

Gemmiger formicilis, *Fusicatenibacter saccharivorans*, *Collinsella aerofaciens*, *Blautia wexlerae*, *Blautia luti* and *Ruminococcus bromii* are detected only in low fractions.

Results are shown either pooled across all samples or stratified by sequencing run depending on taxonomic resolution and data robustness.

Correlations with dietary habits and lifestyle

The composition of the gut microbiota is closely influenced by various lifestyle factors, such as diet, physical activity, medication use, and sleep patterns, which can significantly shape microbial diversity and abundance [3, 4, 6]. We decided to explore the correlations between Friuli Venezia Giulia's population characteristics, collected through questionnaires, and the microbial phyla identified in the previous analysis.

The heatmap shown in Fig. 1 presents the regression coefficients (β) between various covariates and the relative abundance of different bacterial phyla. Each colored cell represents the direction and strength of the association between individual factors (age, sex, BMI, use of antibiotics, anti-inflammatories, proton pump inhibitors, probiotics/prebiotics, physical activity, tobacco use, hours of sleep, and allergies) and the 10 most representative bacterial phyla found.

No significant associations between host covariates and bacterial phyla remained statistically significant. The only labeled in the heatmap was a negative association between the number of hours of sleep and the relative abundance of Proteobacteria (β = −0.0693), suggesting a slight decrease in this phylum in association with shorter sleep duration.

Analyzing the relationship between individual characteristics and the relative abundance of specific bacterial classes, several factors, such as age, BMI, use of tobacco, individuals' disorders and lifestyle habits, emerge as significant predictors. As shown in Fig. 2, both positive and negative associations are evident across the FVG population dataset. For instance, Gammaproteobacteria are positively associated with BMI (β = 0.0331), while negatively associated with physical activity (β = -0.0762), suggesting that metabolic factors may strongly influence this class. Bacilli show a significant negative association with tobacco (β = -0.2599), and a positive correlation with BMI. Other associations include Negativicutes, which are positively linked to disorders (β = 0.0241) and negatively to physical activity (β = -0.0793), indicating a complex interplay between dietary supplements and host physiology.

These findings provide class-level insight into how host traits and exposures shape the microbiota composition, supporting further stratified analyses at lower taxonomic ranks.

At the family level, no associations of strong biological significance were identified (Fig. 3). While the heatmap highlighted two associations, a positive correlation with disorders (β = 0.075) and a negative correlation between Veillonellaceae and pathologies (β = −0.0782), both showed modest effect sizes, and their biological relevance remains uncertain.

Furthermore, considering bacterial genera, *Rummeliibacillus* positively associated with disorders (β = 0.6151),

Table 7 Relative abundances of top 10 bacterial species found in 109 samples

Median (IQR)	Run 1 *N* = 9	Run 2 *N* = 12	Run 3 *N* = 24	Run 4 *N* = 64
Faecalibacterium prausnitzii %	6.609 (6.016–10.174)	10.383 (9.001–11.789)	7.481 (4.078–13.063)	8.673 (5.295–12.693)
Bacteroides vulgatus %	3.000 (1.882–7.538)	4.641 (1.009–7.477)	2.303 (1.000-5.230)	1.789 (0.600-5.423)
Bacteroides dorei %	5.446 (1.772–7.359)	3.497 (1.135–8.510)	1.496 (0.663–3.346)	1.682 (0.835–4.602)
Bacteroides uniformis %	4.344 (1.092–5.558)	3.138 (1.274–5.834)	1.759 (0.714–2.756)	1.667 (0.687–3.535)
Gemmiger formicilis %	2.107 (1.394–2.562)	3.233 (2.215–4.453)	2.670 (0.918–3.912)	2.091 (1.182–3.655)
Fusicatenibacter saccharivorans %	1.599 (0.724–1.962)	2.971 (2.024–5.255)	1.419 (1.136–2.389)	1.504 (0.794–2.310)
Collinsella aerofaciens %	-	2.542 (1.316–3.708)	1.006 (0.226–1.703)	-
Blautia wexlerae %	-	3.100 (1.667–4.402)	1.437 (0.956–2.591)	1,385 (0.692–2.533)
Blautia luti %	-	1.840 (1.341–3.680)	0.996 (0.493–1.426)	-
Ruminococcus bromii %	-	-	2.469 (0.410–3.562)	1.222 (0.028–3.291)

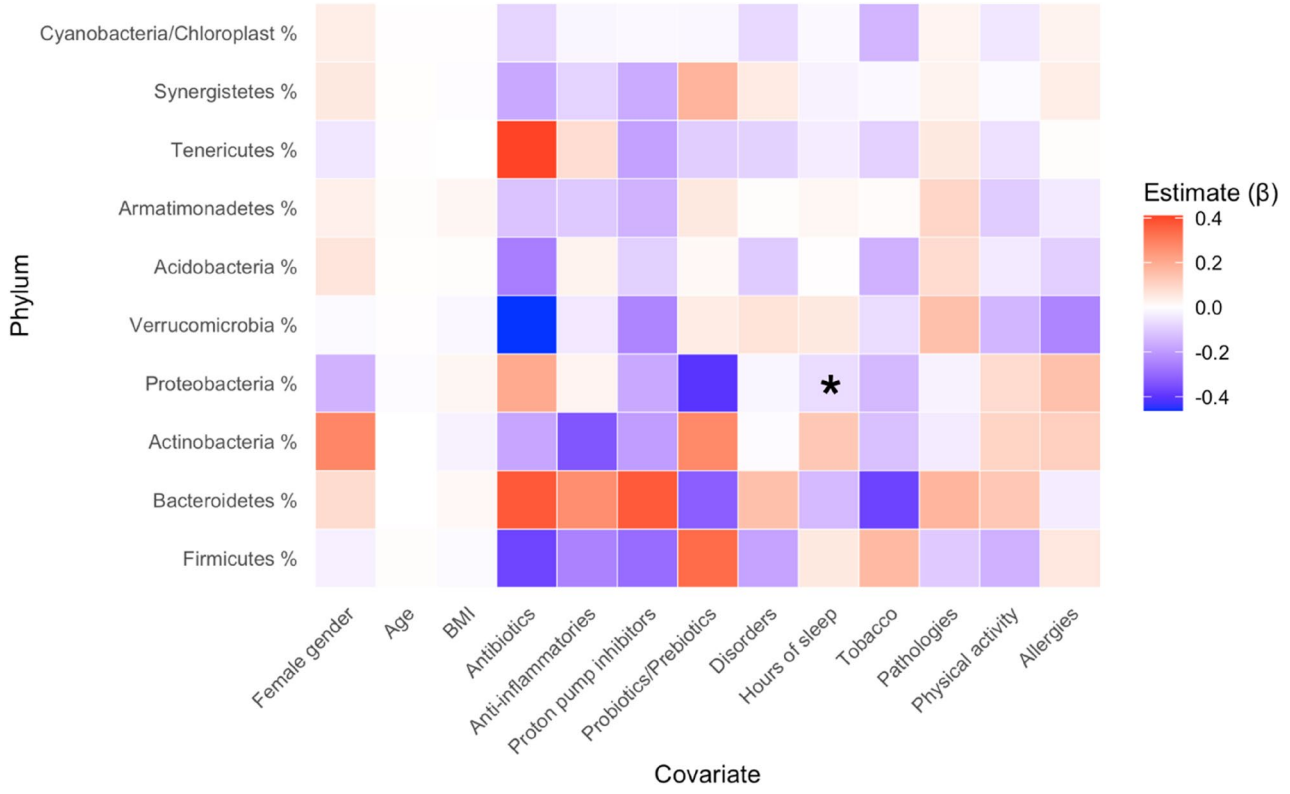

Fig. 1 Associations between host covariates and bacterial phylum relative abundance estimated using univariable zero-inflated beta regression models. Colors represent effect estimates (β). Statistically significant associations after FDR correction (adjusted $p < 0.05$) are indicated by an asterisk (*)

Barnesiella showed a negative association with anti-inflammatory use (β = −0.1233), while *Oscillibacter* was positively associated with pathologies (β = 0.0521). Additionally, Bifidobacterium displayed a negative association with age (β = −0.0005) and a very small negative effect estimate with BMI (β = −0.0669) (Fig. 4).

Discussion

The presented study enabled the identification of population-based ranges of gut microbiota in individuals residing in the Friuli Venezia Giulia (FVG) region, offering for the first time a comprehensive analysis of bacterial composition and its associations with demographic, clinical, and lifestyle factors. The cohort included 109 adult volunteers, with a median age of 47 years and a median BMI of 24.5, at the limit of the upper normality value. Medication use was limited (11% used probiotics), while health issues were reported by approximately half of participants. Alcohol consumption was common (67%), 15% smoked, and 28% reported engaging in regular physical activity. Allergies were declared by 17%.

The gut microbiota, a highly complex and dynamic ecosystem composed of trillions of microorganisms, is essential for numerous physiological functions, including nutrient metabolism and immune regulation [2, 3, 5].

In the FVG cohort, the dominant bacterial phyla were Firmicutes and Bacteroidetes, consistent with global profiles in healthy individuals and similar to those reported in other Italian regions [8, 20, 27–30].

A limitation of this study is the lack of normalization for 16 S rRNA gene copy number, as the Illumina BaseSpace 16 S Metagenomics pipeline does not include this correction. Given that bacterial taxa can vary substantially in their 16 S rRNA gene copy numbers, taxa with higher copy numbers may be overrepresented, potentially introducing bias in relative abundance estimates and their biological interpretation. An additional limitation concerns the presentation of taxonomic summaries across sequencing runs. While some taxonomic ranks were summarized across the full cohort and others were displayed separately by run, this reflects an effort to balance interpretability with taxonomic robustness. Nevertheless, this reporting framework may reduce immediate comparability across tables and should be considered when interpreting hierarchical taxonomic patterns.

No statistically significant associations were observed between clinical, lifestyle factors and phylum level, a part from the modest negative association between Proteobacteria and sleep duration, that suggests that shorter sleep may favor the expansion of potentially pro-inflammatory taxa [31–33]. Interestingly, while other Italian

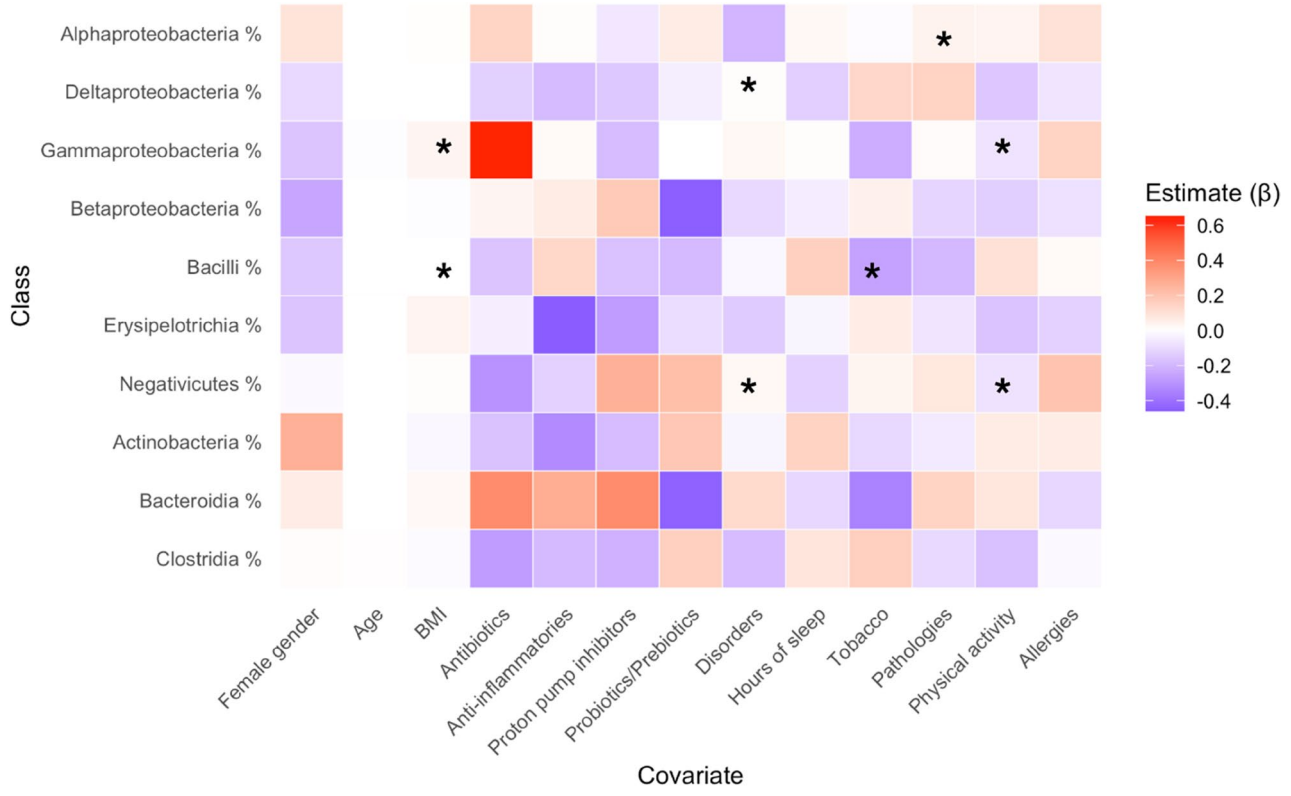

Fig. 2 Associations between host covariates and bacterial class relative abundance estimated using univariable zero-inflated beta regression models. Colors represent effect estimates (β). Statistically significant associations after FDR correction (adjusted $p < 0.05$) are indicated by an asterisk (*)

studies from regions such as Lazio and Tuscany reported significant correlations between lifestyle and bacterial phyla [8, 10, 20], such associations were absent in the FVG dataset, suggesting that regional variability and behavioral factors may influence microbiota composition.

Our findings are consistent with previous studies describing gut microbiota composition in European and Italian populations, where Firmicutes and Bacteroidota are typically the dominant phyla [34–36]. Previous studies have reported regional variability in gut microbiota composition within Italy, with differences observed between northern, central, and southern populations [27]. These differences have been associated with variations in diet, lifestyle, and environmental factors.

At finer taxonomic resolution, several associations aligned with findings from other Italian cohorts. For example, the class-level analysis revealed relevant host-microbiota relationships. Gammaproteobacteria were positively associated with BMI and negatively with physical activity [37, 38], which found increased levels of this class in individuals with obesity and metabolic syndrome [2, 6, 8, 20, 37]. Bacilli displayed a negative association with smoking [39, 40], in line with data reporting that tobacco use is associated with lower levels of lactic acid bacteria [38].

Negativicutes were positively associated with self-reported disorders and negatively with physical activity. These trends support the hypothesis that a sedentary lifestyle and underlying health conditions favor the proliferation of inflammation-associated taxa [41–43].

Consistent with previous studies [44–47], Bifidobacterium showed an inverse correlation with age and BMI, confirming its recognized role as a beneficial genus sensitive to both aging and metabolic alterations [43–47]. Furthermore, evidence from northern Italy, particularly in Lombardy, suggests that diets rich in animal fats and low in fiber are associated with reduced microbial diversity and increased metabolic risk [8, 20]. Central and southern Italian populations adhering more closely to the Mediterranean diet demonstrate a more balanced Firmicutes: Bacteroidetes ratio and improved metabolic profiles [7, 8, 10, 20].

The traditional diet in FVG, shaped by Austrian and Slovenian culinary influences, also appears to impact microbiota structure. Participants with diets rich in complex carbohydrates and fiber (from fresh, locally sourced foods) showed increased, but not significant, levels of Prevotella, a genus involved in fermenting dietary fiber and producing short-chain fatty acids (SCFAs), key metabolites for gut health and immune modulation [32, 48–50]. Finally, a negative correlation between

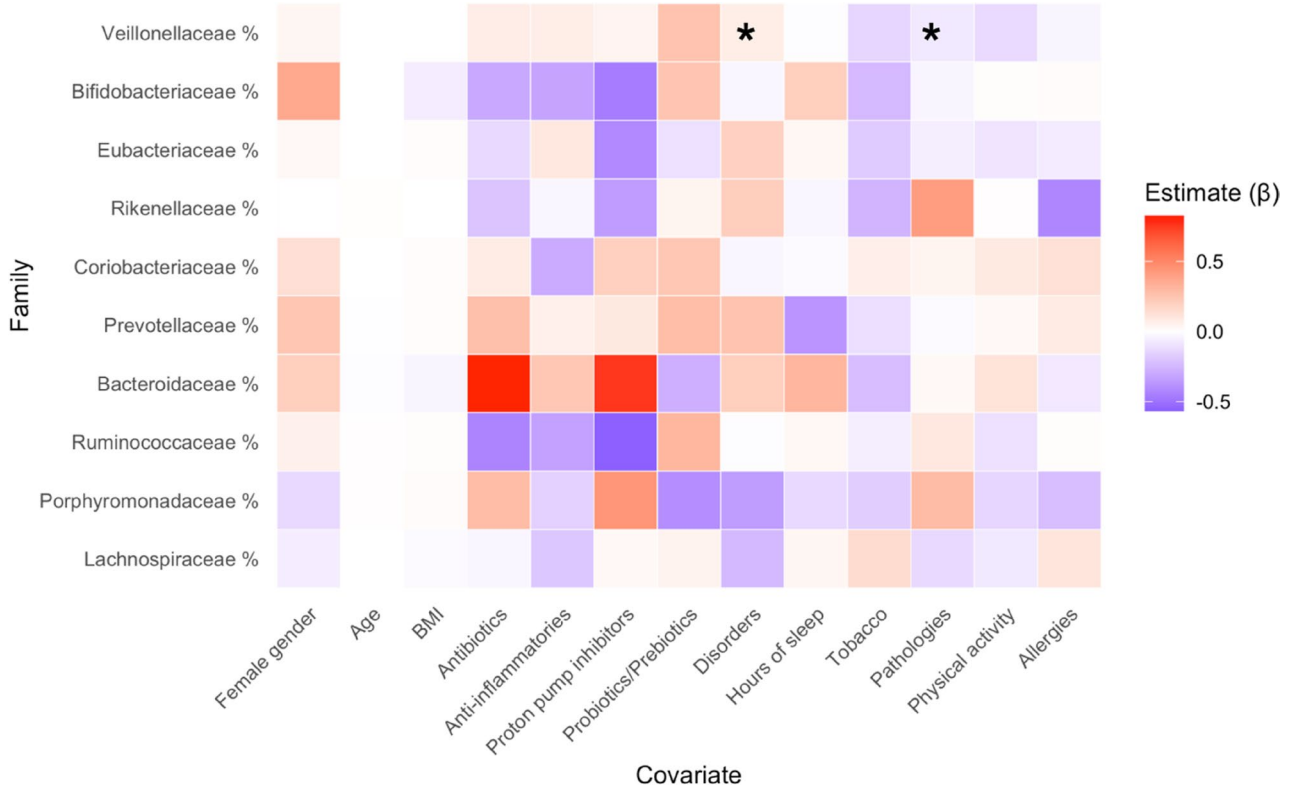

Fig. 3 Associations between host covariates and bacterial family relative abundance estimated using univariable zero-inflated beta regression models. Colors represent effect estimates (β). Statistically significant associations after FDR correction (adjusted $p < 0.05$) are indicated by an asterisk (*)

Barnesiella and anti-inflammatory use (β = −0.1233) emerged. Recent evidences suggest that certain species within the genus may enhance the production of anti-inflammatory cytokines or suppress pro-inflammatory pathways. Anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), can alter gut microbiota composition, potentially leading to a decrease in *Barnesiella* abundance [34, 51].

Overall, the microbial community observed in the FVG population is consistent with profiles of a putative healthy gut, dominated by Clostridiales and Bacteroidales. This composition supports a balanced microbial ecosystem essential for maintaining digestive and immune functions.

The observed patterns may reflect the combined influence of dietary habits, cultural background, and environmental exposures, which are known to shape gut microbiota composition across populations [34, 52].

The NGS approach we employed, targeting the hypervariable V3–V4 regions of the bacterial 16 S rRNA gene [6, 12–15, 17]. This method also supports future investigations into clinical associations, disease-specific shifts in microbiota, and personalized interventions. The scalability of the approach ensures efficient processing of large sample cohorts at sustainable costs [18].

Our results showed region-specific host–microbiota interactions that partially overlap with findings from other Italian studies but also underscore the geographic and cultural heterogeneity shaping the human microbiome. This variability reinforces the importance of conducting standardized, multicenter studies across Italy to establish robust population-based ranges of gut microbiota composition and disentangle the complex interplay between host traits and microbial composition.

While higher taxonomic ranks provide a general overview, they may obscure subtle but biologically significant associations. Our findings emphasize the need for deeper resolution analyses to capture specific microbial signatures to obtain a more refined understanding of microbiota-based health assessment.

Due to the cross-sectional nature of this study, the observed relationships between host factors and gut microbiota should be interpreted as associations rather than confirmed interactions. Moreover, although our findings reflect patterns within this cohort, further comparative studies are needed to determine whether these observations are truly region-specific. However, methodological heterogeneity across studies, including differences in sequencing targets, platforms, and bioinformatic pipelines, poses a significant challenge to direct data comparison and highlights the need

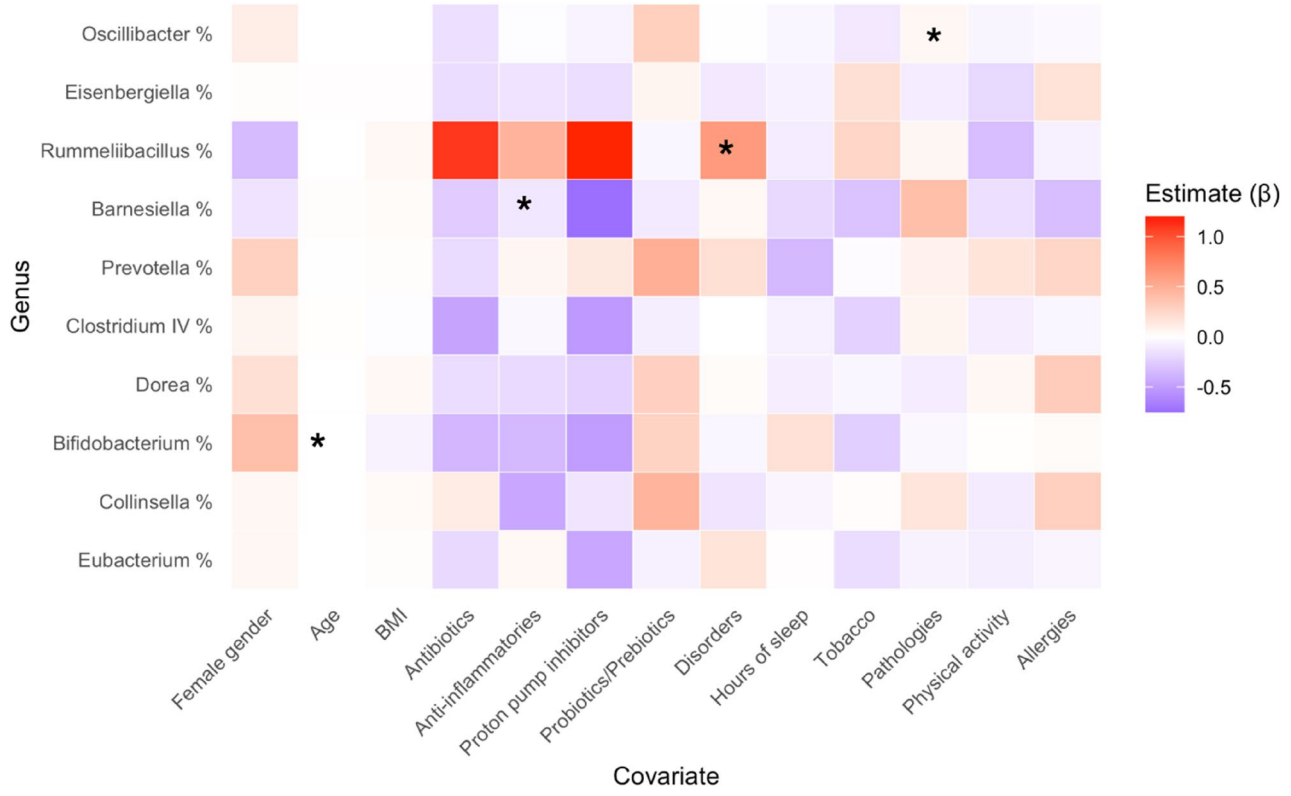

Fig. 4 Associations between host covariates and bacterial genus relative abundance estimated using univariable zero-inflated beta regression models. Colors represent effect estimates (β). Statistically significant associations after FDR correction (adjusted $p < 0.05$) are indicated by an asterisk (*)

for harmonized protocols to ensure consistency and reproducibility.

Conclusions

This study provides, for the first time, valuable insights into the gut microbiota composition of individuals residing in the Friuli Venezia Giulia region, establishing representative population-based ranges of gut microbiota composition for key bacterial taxa and uncovering significant associations between lifestyle factors and microbial diversity. By comparing these results with data from other Italian regions, it is evident that geographic and lifestyle differences significantly impact gut health. These insights highlight the potential of targeted lifestyle interventions to enhance gut health and prevent microbiota-related disorders.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-026-05117-1.

Supplementary Material 1

Supplementary Material 2

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a preliminary abstract has been published on https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1208565.

Clinical Trial Number
This study was approved by the Regional Ethics Committee of Friuli Venezia Giulia, Italy (CEUR FVG), and was conducted as part of the MICROBIntest-2022 protocol (Ethics Committee approval No. CEUR-2022-Os-137), in accordance with the approved protocol (file No. 132083, August 18, 2022).

Authors' contributions
M.B. and M.D.M., conceptualization, methodology data analysis and curation, writing—review and editing; N.G., B.K., G.V., S.M. and M.B., methodology; C.P. R.C. and M.B., B.M., methodology and interpretation of results; M.B, C.P. C.T., and F.C. writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Data availability
The authors confirm that all relevant data are included in the articleand materials are available on request. Sequencing data have been deposited in the NCBI Short Read Archive (SRA) under BioProject ID PRJNA1208565 and are available at the following link: [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1208565]. Custom scripts used for statistical analysis are available at: [https://github.com/Mariadmm96/microbiome-gamlss].

Declarations

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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