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Exploring the influence of urbanization on gut mycobiota through dietary habits in Burkina Faso

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Abstract

Background The phenomenon of urbanization is associated with significant shifts in lifestyle and dietary habits, which can impact the composition of gut microbiota. While variations in gut bacterial communities between rural and urban residents are documented, changes in fungal communities (mycobiota) remain underexplored. This study investigates the impact of urbanization-related dietary shifts on the gut mycobiota in a sub-Saharan African context (Burkina Faso). We analyzed the gut mycobiota composition of individuals from households of rural and semi-urbanized areas, and that of wealthy families living in the capital city (Ouagadougou). We compared the gut mycobiota of three Burkinabè cohorts with that of a cohort of Italian families, as representative of a Western urban lifestyle. Using high-throughput sequencing, we characterized gut mycobiota composition and diversity, assessing changes in accordance with the different dietary patterns and lifestyle.

Results Our findings revealed modifications in gut mycobiota composition along the rural-to-urban gradient. A significant reduction in the alpha-diversity of the gut mycobiota was observed in the cohorts residing in the urban setting compared to those living in rural and semi-urbanized areas. Members of rural households exhibited greater fungal richness and diversity compared to those in urban families, including affluent families in the capital city. Furthermore, we identified 33 fungal amplicon sequence variants (ASVs) significantly associated with the different lifestyle and dietary patterns related to the studied areas.

Conclusion The household-level survey of rural and urban settings in Burkina Faso highlighted the impact of urbanization-related dietary shifts on gut mycobiota diversity and composition. The observed loss of fungal diversity and the significant reduction of fungal taxa associated with a rural lifestyle are potential indicators of the shift from a rural to an urban context. In agreement with the *hygiene hypothesis*, these findings lay the foundation for further studies aiming at investigating the effect that these microbial losses will have on human health, similarly to those already observed for gut bacterial communities.

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Introduction

The human gut microbiota, comprising bacteria, fungi, archaea, and viruses, plays a crucial role in influencing host metabolism, immune function, and overall health [1, 2]. The microbiota composition is shaped by different factors, such as diet, lifestyle, and geographic location, which significantly influences microbial community structure. Among environmental factors, urbanization is known to be highly impactful, as it is associated with changes in lifestyle, socioeconomic status, and dietary habits. This transition often leads to a dietary shift from non-processed, fiber-rich and fermented food to hypercaloric diets high in fats, animal proteins, and processed foods, characteristic of industrialized dietary habits [3, 4]. Although these changes are common features of non-traditional dietary habits, a “universal urban diet” cannot be defined, as dietary habits are strongly linked to specific cultural and geographic contexts [5–7]. Overall, compared to isolated rural environments, urban areas provide access to a wider range of processed and preservative-enriched products, which are often consumed alongside local traditional foods, shaping unique local dietary patterns. However, the increasing influence of Western dietary habits is contributing to significant alterations in gut microbiome community structure [3–5]. Dietary shifts have been shown to reduce gut microbiota diversity, particularly by reducing beneficial bacteria involved in fiber fermentation, leading to a consequent decrease in short-chain fatty acid (SCFAs) production [3–5, 8–11]. Higher socioeconomic conditions often coincide with enhanced hygiene practices, increased antibiotic use, and food sterilization, all of which adversely affect the gut microbiota by reducing or eliminating potentially beneficial microorganisms [3, 4, 8]. The *hygiene hypothesis*, first proposed by Strachan in 1989, suggests that reduced microbial exposure in early life impairs the proper development of the host immune system. This idea has since evolved into the ‘missing microbes’ concept, which offers a framework for understanding how the depletion of ancestral microorganisms—driven by industrialized lifestyles and practices—contributes to the rise of non-communicable diseases [12, 13].

The exact role of the fungal component of the microbiota, i.e. mycobiota, within this scenario has not been clarified yet. However, increasing evidence highlights its role in shaping host immunity and influencing health and disease outcomes [14]. Fungal colonization of the human gastrointestinal tract is established at birth through maternal transmission and is further shaped by environmental exposure and dietary intake [15–18]. Notably,

the early development of gut fungal communities plays a crucial role in the maturation of the host immune system, contributing to immune competence during early life [19–23]. Studies on adult gut mycobiota have identified two key findings: the existence of a core mycobiota [24], consisting of a relatively stable set of fungal species, and a dynamic fungal community that varies in response to dietary patterns and environmental factors [6, 25, 26]. Among fungal inhabitants of the gut, yeasts represent the predominant component, with *Candida*, *Malassezia* and *Saccharomyces* being the most detected genera. In addition, other frequently identified yeasts include *Aspergillus*, *Clavispora*, *Cyberlindnera*, *Debaryomyces*, *Galactomyces*, *Geotrichum*, *Hanseniaspora*, *Meyerozyma*, *Pichia*, *Torulaspora*, *Yarrowia*, and *Zygosaccharomyces* [27].

Globally, fermented products serve as an important dietary source of beneficial yeast strains, particularly those belonging to the genera *Saccharomyces*, *Candida*, *Pichia*, *Debaryomyces*, *Kluyveromyces* and *Hanseniaspora*. These fungi have been associated with potential health benefits for humans, including modulation of the gut microbiota, enhancement of immune function, production of bioactive compounds (e.g., B-vitamins and antioxidants), and improved nutrient bioavailability [28, 29]. In Africa, these yeasts are widely present in traditional fermentation processes involving both plant and animal-derived raw materials. A comprehensive survey conducted by Johansen et al. [30] analyzing 43 traditional fermented foods from sub-Saharan Africa identified *S. cerevisiae* as the predominant yeast species in 77% of the sampled products, followed by *Pichia kudriavzevii* (anamorph *Candida krusei*) at 60%, *Candida tropicalis* at 47%, and *Kluyveromyces marxianus* (anamorph *Candida kefyri*) at 44%. As part of the ongoing urbanization process, the adoption of new dietary habits and the subsequent replacement of traditional foods result in the loss of associated beneficial microorganisms, which contribute to gut health and nutrition [31].

This study aims to investigate the effect of the urbanization gradient and related diet and living conditions on the intestinal mycobiota of three populations living in rural, semi-urbanized, and urban areas in Burkina Faso. Gut fungal communities of these cohorts have been compared with the ones of an Italian cohort, representing a population fully characterized by Western urban habits [32]. Our findings reveal a significant influence of dietary habits and lifestyle on the decreasing gradient of fungal diversity in the three Burkinabè cohorts according to the level of urbanization. Variations in fungal diversity were

also observed within family members in rural and semi-urbanized areas, highlighting a shift towards less diverse fungal profiles during the rural-to-urban transition.

Materials and methods

Study populations

The enrollment took place in Burkina Faso, specifically in the Boulkiemdé province, spanning from February 2019 to January 2020. This study included healthy individuals from 30 households and families from three different Burkinabè cohorts, comprising 147 individuals ranging in age from less than 1 to 73 years old. As detailed in our previous work [32], these households were carefully chosen from two distinct locales with different urbanization levels: rural villages and the semi-urbanized area of Nanoro town, and high-income families from the urban area of the capital city, Ouagadougou. Households belonging to the same environmental context represent well-defined social units, where members share similar diets, water sources, and exposure to environmental contaminants. Studying mycobiota composition at the household level allows us to assess variations within the same geographic area, making it possible to disentangle the effects of family-specific factors from those of the broader environment.

Participants' enrollment was performed during the dry season with the support of the Nanoro Health and Demographic Surveillance System (HDSS), a surveillance organization established by the Clinical Research Unit of Nanoro (CRUN). Data on lifestyle, dietary habits, socio-economic conditions, hygiene, sanitation, health status at the time of enrollment and previous illnesses, were collected in a database by means of a questionnaire already analyzed by Casari et al. 2022 [32]. The term 'urbanization' refers to the different lifestyle-related profiles and to the different dietary patterns mentioned in Supplementary Table 1, associated with the four different areas.

Cohorts description

To improve clarity and standardize terminology, the cohorts will hereafter be referred to using acronyms that denote their level of urbanization: Rural villages as BF-Rural, Nanoro as BF-Semiurban, Ouagadougou as BF-Urban, and Italy as IT-Urban.

(i) BF-Rural ($n = 51$ individuals).

Ten polygamous households, composed by one father, at least two mothers and up to four children, were randomly selected from the rural villages of Boulpon, Poessi and Godo, located in Boulkimele province, with respective populations of 4400, 2200 and 894 (Burkinabè government inforoute communale Archived 2007-03-23 at the Wayback Machine). Residents rely on subsistence farming

and small livestock rearing (e.g., chickens and goats) in close contact with humans. Housing consists of clusters of huts built using soil, wood, and straw. These villages lack electricity, sewage systems and water treatment infrastructures; water is collected from public reservoirs, streams or wells. These households are isolated and in poor contact with urban areas. Their diet is traditional Burkinabè and predominantly plant-based (i.e., local cereals, legumes, and herbs) with minimal Western influence. Access to healthcare, antibiotics and sanitation is limited resulting in high risk of infectious diseases and malnutrition.

(ii) BF-Semiurban ($n = 51$ individuals).

This cohort included ten polygamous or monogamous families, each comprising one father, one or two mothers, and up to four children. Participants resided in Nanoro, a semi-urbanized area with a population estimated between 33'000 and 34'000 [33]. People live in groups of small brick houses with limited access to electricity. Water is collected from public wells. Similar to rural areas, sewage systems and water treatment plants were absent. Their diet is still traditional Burkinabè and plant-based, but also occasionally including meat and dried fish, and limited processed and Western-like foods. Despite improved food access, this cohort remained at risk of malnutrition and infectious diseases.

(iii) BF-Urban ($n = 45$ individuals).

Ten monogamous families, each with one mother and father and up to four children were, selected among wealthy urban families from the capital city of Ouagadougou. These families resided in concrete or brick buildings with access to electricity, sewage infrastructure and private sources of drinkable water. While the diet remained predominantly Burkinabè, it included a broader range of foods typical of an industrialized dietary pattern, including sugar rich beverages, canned goods, and highly processed foods rich in preservatives. These families have access to healthcare and sanitation infrastructure, thereby markedly reducing the risk of infection and malnutrition.

(iv) IT-Urban ($n = 143$ individuals).

An additional cohort of 45 monogamous families, each consisting of two parents and up to four children, was enrolled from the urban areas of Florence (Tuscany, Italy) to represent an urban population with Western-like diet and lifestyle. All families in the Italian cohort were of Caucasian ethnicity, with the exception of one family of Japanese descent and one of mixed Caucasian/South American descent. Compared to the Burkinabè

cohorts, Italian participants had greater access to industrialized and processed foods, and followed diets higher in carbohydrates, animal protein and fat, but lower in fiber and whole grains. Participants resided in areas with well-developed infrastructure, including electricity, centralized water distribution, sewage systems, and waste management services. Access to public and private healthcare services was consistent and widespread, with availability of routine medical care, vaccinations, and antibiotic treatments. These conditions contribute to a low burden of infectious diseases and overall high standards of hygiene and sanitation. Their lifestyle and dietary habits reflect a fully urbanized Western context.

For further details regarding the human cohorts see Casari et al. 2022 [32].

Inclusion and exclusion criteria of study participants

Inclusion criteria were standardized across cohorts and required: (i) family units composed of at least two parents (one biological father and one or more mothers) and up to four children; and (ii) residence within a defined geographic area (urban, semiurban, or rural). For the Burkinabè cohort, participants were also required to belong to the Mossi ethnic group. Italian families were selected from urban areas within the province of Florence, Italy. All individuals underwent a health screening prior to enrollment. Participants were excluded if they presented with acute illness or infections at the time of recruitment, or had experienced fever (>38.5 °C) within the previous 72 h. Individuals with chronic gastrointestinal disorders known to alter gut microbiota composition, such as Crohn's disease or ulcerative colitis, were also excluded. Specifically, 8 children with Crohn's disease and 12 with ulcerative colitis were removed from the Italian cohort. To avoid technical bias, two samples with undetectable fungal content (namely samples 'IT-IBD-21-F1' and 'F304N') were excluded from all analyses. Detailed information on health status, medical history, vaccinations, dietary habits, and socio-economic background was collected through standardized questionnaires and reported in Supplementary Table 1. For Burkinabè participants, data were collected by trained local staff using written questionnaires in French or local languages, or through interviews in cases of illiteracy. Italian participants completed self-administered written questionnaires. Both questionnaires assessed food consumption patterns, including intake of traditional, processed, and industrial foods, with weekly frequency estimates. Portion sizes were assessed using reference images (small, medium, large) in the Burkinabè cohort. Dietary assessment included a semi-quantitative food

frequency questionnaire (FFQ) and a 24-hour recall, used to estimate daily intake of energy and macronutrients (carbohydrates, fiber, simple sugars, fats, vegetable and animal proteins). Full methodological details are available in our previous study [32].

After applying all inclusion and exclusion criteria, the final dataset consisted of 122 participants from the IT-Urban cohort, 45 from the BF-Urban, 50 from the BF-Semiurban, and 51 from the BF-Rural cohort, used for downstream statistical analysis.

Ethics

In Burkina Faso, the study was carried out in accordance with the recommendations of the National Ethics Committee of Burkina Faso that granted ethical clearance (reference number 2018-8-104). For the Italian families, the study was approved by the Ethics Committee of Meyer Children Hospital, Florence, Italy (reference number 187/2018). Adult participants gave their informed consent. Parents or primary caregivers gave consent on behalf of children under the age of 18. Confidentiality was ensured for data and sample collection by assigning each participant a unique identification code.

Sample collection and DNA extraction

For each participant, a stool sample was collected by a commercial sterile collection tube supplied with RNAlater (Thermo Fisher Scientific), then stored at -80 °C to preserve nucleic acids. The DNeasy PowerSoil Pro Kit (Qiagen) was used to extract total DNA from 250 mg (wet weight) of each fecal sample according to the manufacturer's instructions. The Qubit 4 Fluorometer (Thermo Fisher Scientific) and the 1x dsDNA High Sensitivity Assay Kit were used to assess DNA concentration before the downstream analyses.

ITS1 amplification, library preparation and sequencing

For each DNA sample, the fungal internal transcribed spacer 1 (ITS1) was amplified using a specific primer set for the ITS1 rDNA region (ITS1f: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS2r: 5'-GCTGCGTTCTTCATCGATGC-3') [34], with overhang Illumina adapters. Library preparation was performed according to the Fungal Metagenomic Sequencing Demonstrated Protocol (Document # 1000000064940 v01). Sequencing was performed on the Illumina MiSeq platform (Illumina) with the V3 chemistry 600 cycle PE300 protocol at the Department of Biology, University of Florence, Italy.

Amplicon sequence variants assemblage

Both primer sequences were removed by using cutadapt version 1.15 [35] in paired-end mode. If a primer sequence was not found, the entire sequence was discarded along with its pair to reduce possible

contamination. The raw sequences were processed using DADA2 pipeline version 1.14.1 [36] to infer amplicon sequence variants (ASVs). Low quality reads were filtered using the “filterAndTrim” function with a maximum number of expected error thresholds of two for forward and reverse read pairs and a minimum cut-off length of 70 bp. Error rate estimation (“learnErrors” function) and denoising (“dada” function) with default parameters were performed. Denoised reads were merged using the “mergePairs” function, discarding those with any mismatches and/or an overlap length shorter than 20 bp. Chimeric sequences were also removed (“removeBimeraDenovo” function) and taxonomic classification was produced by using DECIPHER package version 2.14.0 against the Warcup database for fungal ITS1 [37]. Amplicon sequences with a 100% identity with references were tentatively classified at species level [38]. The number of reads retained after each qualitative step of the DADA2 pipeline was reported in Fig. S1. After DADA2 quality filtering, all sequence variants not classified as Fungi and/or with no fungal counts were removed from the dataset to properly perform the downstream analyses.

Statistical analysis

Fungal diversity and nutritional intake assessment

Statistical analyses were performed in R environment version 4.2.2 [39]. Sequencing depth was inspected by rarefaction curves generated by using the “ggrare” function of “ranacapa” package version 0.1.0 [40]. This analysis was conducted only to assess whether the observed diversity had been sufficiently sampled; all subsequent analyses were performed on non-rarefied counts. Mean relative abundance was calculated using “microbiomeutilities” package version 1.0.17 [41]. Variations in fungal diversity (beta diversity analysis) were inspected using “vegan” package version 2.6.4 [42]. In detail, samples distribution was displayed by Principal Coordinate Analysis (PCoA) using the “cmdscale” function of the “stat” package performed on distance matrices based on Bray-Curtis diversity index and Jaccard similarity coefficient to infer quantitative and qualitative differences, respectively. The data were normalized before multidimensional analysis removing singletons within the sample dataset and then transformed in relative abundances to reduce coverage bias among samples. Permutational multivariate analysis of variance using distance matrices (adonis PERMANOVA) was performed to inspect differences between sample groups by using the “adonis2” function of “vegan” package. Adonis PERMANOVA was tested on a multiple factor formula (formula = ~Area * Family member + Age + Sex) considering the interaction between area and family member variables due to the crossed design experiment. The Age was first tested as a covariate (continuous variable) by using the “adonis2” function

of “vegan” package, highlighting a significant effect (adonis PERMANOVA: $R^2 = 0.005$, $p = 0.049$). Therefore, in accordance with the study purpose the Age variable was added in the model formula stratifying it by using the following ranges: 0–5 (up to, but not including, 6 years), 6–17 (up to, but not including, 18 years) and ≥ 18 years of age. Pediatric subjects were grouped into the 0–5 year category considering the differences in dietary habits, lifestyle, physiological growth and sexual maturation between Burkinabè and Italian children and adolescents, and after testing for differences in beta diversity between the 0–2 (up to, but not including, 3 years) and 3–5 (up to, but not including, 6 years) age categories observing no significant differences between these two groups (adonis PERMANOVA: $R^2 = 0.032$, $p = 0.408$). Pairwise comparisons on variance among geographical areas were assessed using pairwise adonis PERMANOVA by using the “pairwise.adonis” function from “pairwiseAdonis” package version 0.4 [43] adjusting the resulting p-values with Holm correction method. The same model formula was used to inspect differences in alpha-diversity metrics, first fitted the model formula using linear model (“lm” function of “stats” R package) then inspected by analysis of variance ANOVA type II from “car” package version 3.1.0 [44]. When performing the statistical analyses, we grouped the environmental variables associated to the four different living conditions of the studied populations in four qualitative classes described as “areas”, and tested the dietary variables associated to the different urbanization conditions according to the quantitative intake of macronutrients, animal proteins, vegetable proteins, total proteins, carbohydrates, sugars, fats and dietary fibers. Covariation between nutritional data (amounts of macronutrients represented by the percentages of carbohydrates, simple sugar, total protein, animal protein, vegetable protein, fats, and gr/1000 Kcal of fibers) and beta diversity was assessed by fitting the nutritional parameters onto the Principal Components Analysis (PCA) by using the “envfit” function in “vegan” package setting a number of permutations of 1000. PCA was built on VST (variance stabilizing transformation) scaled abundance matrix by using DESeq2 package version 1.38.3 [45] after single counts removal and estimated using the “rda” function of the “vegan” package. To assess differences on variance related to the family degree only, thus excluding the geographic area effect, the adonis PERMANOVA was also performed on multiple factors formula (formula = ~Family member + Age range + Sex) after separating the dataset according to four geographical areas. All multivariate analyses were conducted with a number of permutations of 1000 and the r-squared values from adonis PERMANOVA were reported to indicate the amount of variance explained. The dispersion among sample group centroids was computed using the “betadisper” function

of “vegan” package and the differences in dispersion were tested using the analysis of variance (ANOVA) and Tukey post-hoc test (TukeyHSD function from “stats” R package) to inspect significant pairwise contrasts.

Differences in nutritional parameters among sample groups were assessed by Wilcoxon test by using the “wilcox_test” function from the “rstatix” package version 0.7.2 adjusting the resulting p-values with Benjamini-Hochberg correction method [46].

ASV-level characterization across study cohorts

After singleton removal, the differential abundance analysis was performed using the likelihood-ratio test (LRT) method by the “DESeq” function from the “DESeq2” package. LRT was used to test the significance of change in deviance between a full model (Area + Family member) and reduced model (Family member) provided in the model formula. Statistically significant counts ($\alpha=0.05$) were scaled using VST then clustered using “pheatmap” package version 1.0.12 [47]. Differences in alpha-diversity metrics and taxa relative abundances were assessed using the “ggpubr” package [45]. Prevalence and total abundance were calculated for each area separately. We set the prevalence threshold of 30 (i.e. ASVs present in at least 30% of samples) and total abundance threshold of 10'000 counts to select the most representative ASVs in the entire dataset (Fig. S5a) and each cohort separately (Fig. S5b). The ASVs selected were resumed with the related summary statistics (prevalence and total abundance) and deepest taxonomic assignment in Table S2. We also refer to the ASVs, selected within the thresholds described above, shared between cohorts as “core membership”.

Figures were produced by using “ggplot2” package version 3.4.2 [48] and edited using open-source graphics editor Inkscape (<http://inkscape.org/>).

Results

Changes in fungal diversity depending on geographical area and family member

Beta diversity analysis was assessed to describe distribution and similarity between sample groups according to different geographical areas and family structure of Burkinabè and Italian family cohorts. By Principal Coordinates Analysis (PCoA) based on distance matrices achieved by quantitative (Bray-Curtis) and qualitative (Jaccard) indexes (Fig. 1a and Fig. S7a), multidimensional analyses showed a clear separation of the Italian families from the three Burkinabè cohorts, regardless of the different level of urbanization of Burkinabè households (Fig. 1a and Fig. S7a). Differences in beta diversity were assessed with PERMANOVA, showing that “area” (IT-Urban, BF-Urban, BF-Semiurban, and BF-Rural) together with its interaction with “family member” (father,

mother, or child) were the only significant factors affecting fungal distribution (Table S4). Fungal communities were thus shaped according to both the area of origin and the family member within the same area. Beta-dispersion analysis confirmed significance for “area” and “area x family member” interaction (ANOVA, $p < 0.001$ for both Bray-Curtis and Jaccard indexes). However pairwise tests (Tukey post-hoc) highlighted that this was driven only by a limited number of comparisons (17 for Bray-Curtis, 14 for Jaccard, on a total of 66 combinations) (Table S5). Pairwise PERMANOVA showed significant differences in fungal diversity between all areas except Ouagadougou vs. Nanoro (Fig. 1c, S7c). The higher variance was produced from comparison between rural villages and Italian cohorts, reflecting higher mycobiota dissimilarity. To assess differences between the “family members” within each area, a separate analysis was performed by using the same approach described above. No significant differences were found among family members in Italy and Ouagadougou (Table S7, Fig. 1b). However, in BF-Semiurban and BF-Rural, family structure influenced fungal diversity, with significant differences between children and mothers in BF-Semiurban ($R^2: 0.06$, Holm p-adj: 0.02) and fathers and mothers in BF-Rural ($R^2: 0.07$, Holm p-adj: 0.03) (Table S7).

Differences in alpha-diversity measures (Observed, Shannon index and Inverse Simpson index) among different areas were also inspected. Analysis of variance (ANOVA type II) showed that all diversity metrics were significantly influenced by the area variable (Table S8). The pairwise comparisons (Wilcoxon test with Benjamini-Hochberg correction method) showed that the alpha-diversity metrics were overall higher in the cohort with the lowest level of urbanization (i.e. family from BF-Rural), as shown by Wilcoxon test (Fig. 1d and Table S9). Pairwise comparison showed significant differences in the total number of observed ASVs among all cohorts, except for the comparison between cohorts from BF-Urban and BF-Semiurban (Fig. 1d and Table S9). Shannon index metrics showed significant differences in fungal diversity between IT-Urban cohorts and the three Burkinabè cohorts, and between BF-Rural and families from BF-Urban capital city (Fig. 1d and Table S9). Moreover, by using the Inverse Simpson index, we found statistically significant differences in fungal diversity between the IT-Urban cohort and BF-Rural, and between the IT-Urban cohort and the BF-Semiurban cohort, respectively (Fig. 1d and Table S9). These results highlight the impact of urbanization on fungal diversity indicating the presence of a gradient with increasing values in accordance with the reduction of the level of urbanization.

Each geographical cohort presented different dietary habits, resulting in different intake of the main nutrients considered, i.e., total kcal per day, fiber intake (grams

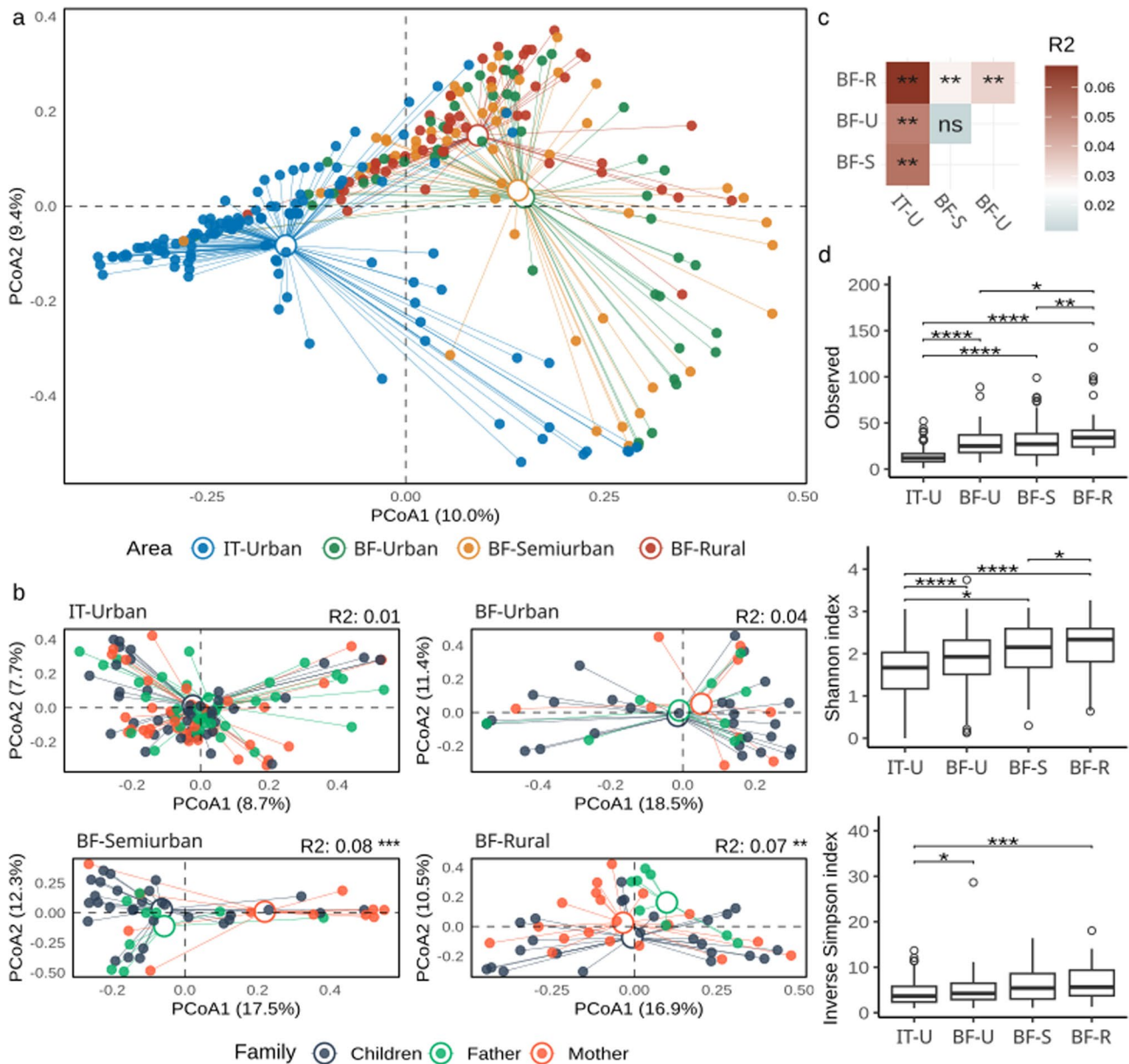


Fig. 1 Sample distribution among cohorts and impact of geographical areas and family members. Principal Coordinates Analysis (PCoA) based on a Bray-Curtis distance matrix depicted the distribution of the four different cohorts according to the areas. Sample groups were depicted using color scheme in the legend (Area). Samples are represented by using solid-colored points whereas white-filled points represent centroids (a). PCoA based on a Bray-Curtis distance matrix, for each single cohort, shows the distribution of the samples according to the family members. Sample groups were depicted using color scheme in the legend (Family). Samples are represented by using solid-coloured points whereas white-filled points represent centroids. R-squared values and significance from adonis PERMANOVA were reported above each panel. Significant effects were highlighted using asterisks (**, $p < 0.01$; ***, $p < 0.001$) (b). Heatmap displayed statistical significance for each comparison from pairwise adonis PERMANOVA (Holm correction method) based on a Bray-Curtis distance matrix for each group combination. R-squared values from pairwise adonis PERMANOVA are reported from lower value (blue scale) to higher value (red scale) and significant comparisons were reported using asterisks (ns, $p > 0.05$; **, $p < 0.01$) (c). Comparison of alpha-diversity measures among cohorts according to the different levels of urbanization by using Wilcoxon test (Benjamini-Hochberg correction method). Statistically significant effects are reported using asterisks (*, p -value < 0.05 ; **, p -value < 0.01 ; ***, p -value < 0.001 ; ****, $p < 0.0001$) and the four different cohorts according to the different areas are mentioned in figures by using the following label code, IT-U: IT-Urban, BF-U: BF-Urban, BF-S: BF-Semiurban, BF-R: BF-Rural (d)

per 1000 kcal), percentages of animal proteins, vegetable proteins, total proteins, total carbohydrates, total sugars, and total fats (specific comparisons between cohorts are reported in Fig. S2 and Fig. S3). Therefore, we performed

environmental fitting analysis (*envfit*) to assess whether the nutritional parameters described above drive variation in the fungal communities. This analysis revealed significant associations between gut mycobiota variation

and seven out of eight nutritional parameters tested (Fig. 2a-b, Table S10), suggesting that differences in macronutrient intake parallel observed geographic shifts in fungal composition. Among the tested variables, the relative intake of animal and vegetable proteins emerged as the strongest drivers of fungal community structure, based on the square correlation coefficient. Figure 2c illustrates the distribution of daily macronutrient intake across the different cohorts, supporting the trends depicted in the envfit analysis. Specifically, PCA-based environmental fitting showed that increased intake of total protein, animal protein, sugars, and fats, correlated strongly with the Italian cohort, while higher consumption of fiber (per 1000 kcal), vegetable proteins and carbohydrates characterized the rural cohort and influenced its distinct fungal profile (Fig. 2a).

Differences in fungal abundances follow an urbanization gradient

We performed prevalence-abundance estimation to depict the most representative ASVs (ASVs present in at least the 30% of the samples within the dataset and with minimum total reads of 10'000) within the four different cohorts. To deepen the taxonomic variability associated with each cohort, we highlighted the most representative ASVs following prevalence and occupancy thresholds (Table S2). In accordance with the threshold parameters, the analysis showed that ASV_1 (*Candida sp VVT 2012*) was a part of the most representative core membership of IT-Urban and BF-Urban cohorts (i.e. shared in all samples within the Italian dataset, according to the prevalence threshold), whereas ASV_2 (*Pichia*) was representative of core membership in the Burkinabè cohorts only (i.e. shared in all three Burkinabè groups but not in the

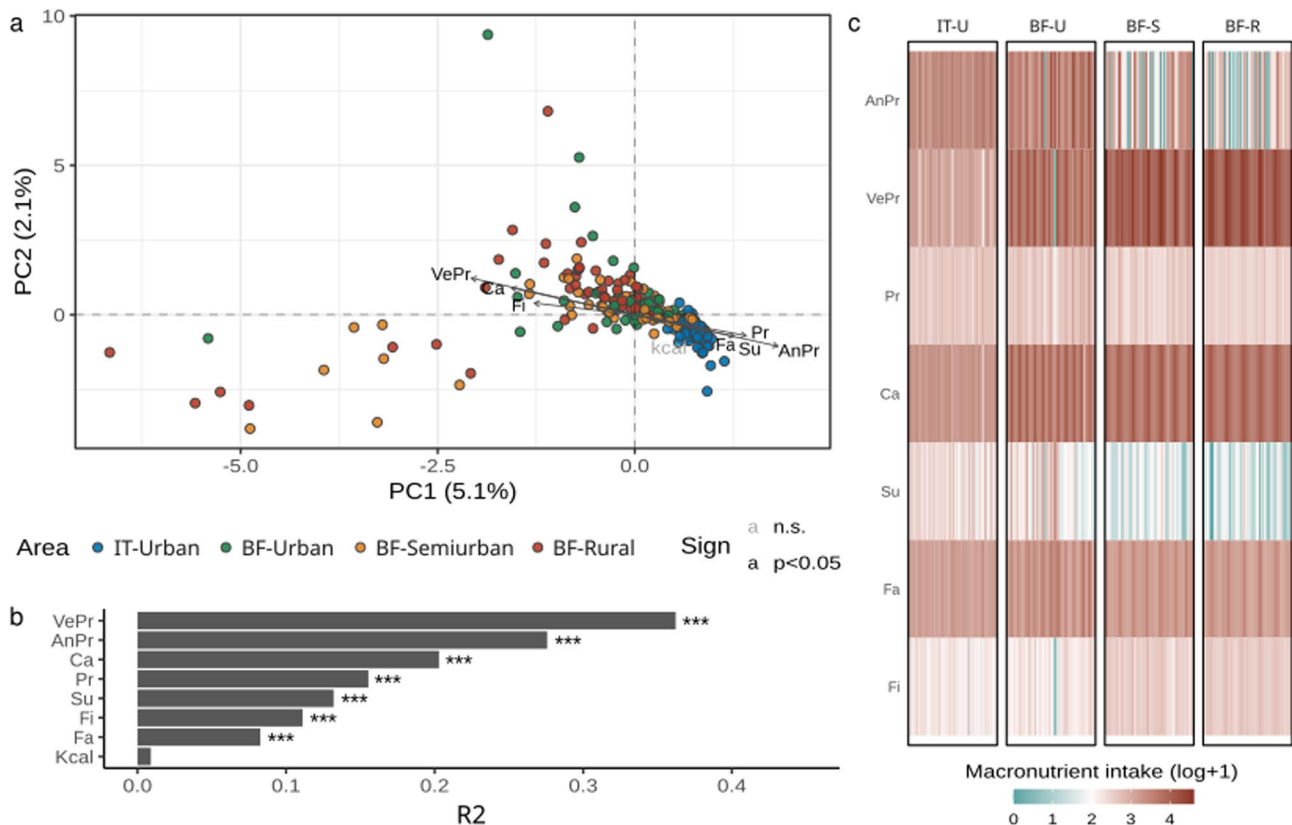


Fig. 2 Beta diversity and environmental variables fitting. Principal component analysis (PCA) based on VST scaled abundance. Environmental variables (nutritional data) were used as factors to display correlation with the ordination configuration by envfit analysis. Arrows highlight the correlation of the environmental variables with the principal components, and color pattern shows the four different geographical areas (a). Histograms report the goodness of fit statistic, i.e., the squared correlation coefficient of envfit (R-squared value) by each covariate in the model, and asterisks represent the significant covariates (***, p-value < 0.001) (b). Heatmap reports the distribution of the significantly fitted (see envfit) environmental variables (Macronutrient intake) across the four different geographical areas. Macronutrient intake is expressed as log + 1 of the percentage, except for the fibers expressed as log + 1 of the grams per 1000 kcal. Macronutrients intake values are reported using color gradients in the legend (blue scale for low values and red scale for higher values) (c). The environmental variables tested are reported in the figure by using the following label code: percentages of vegetable proteins (VePr), percentage of animal proteins (AnPr), percentage of total carbohydrates (Ca), percentage of total proteins (Pr), percentage of total sugars (Su), fiber intake expressed in grams per 1000 kcal (Fi), percentage of total fats (Fa) and kcal per day (kcal). The four different geographical areas compared were reported by using the following label code, IT-U: IT-Urban, BF-U: BF-Urban, BF-S: BF-Semiurban, BF-R: BF-Rural

Italian cohort, according to the prevalence threshold) and ASV_6 (*Epicoccum sorghi*) as core memberships of BF-Semiurban and BF-Rural cohorts (Fig. S5 and Table S2). This highlighted how the specific and wide representative ASVs within the dataset were also shared among cohorts in accordance with the level of urbanization.

To inspect differences in fungal distribution within sample groups, we performed a log-likelihood ratio test (LRT) using DESeq2. The analysis showed a total of 33 ASVs differentially abundant among the family cohorts (Table S11). These ASVs represented 1.99% of the total ASVs profiled in the whole fungal communities (33 out of the 1655 identified ASVs), and 59.9% of the total fungal abundance (mean abundance within the sample dataset: 50.5% and standard error: 1.83%). Sequence variant clustering (by Ward D2 Linkage method) was performed to observe the distribution of the 33 identified ASVs among the four cohorts. A total of 8 different ASVs (Fig. 3) was more abundant in the Italian cohort, compared to the Burkinabè cohorts. The Italian pattern includes 3 ASVs assigned to 3 different fungal species (ASV_29: *Rhodotorula mucilaginosa*, ASV_71: *Candida sake*, ASV_239: *Verticillium leptobactrum*) and 5 ASVs assigned to 4 fungal genera (ASV_88 and ASV_154: *Eupenicillium*, ASV_77: *Debaryomyces*, ASV_55: *Penicillium*, ASV_58: *Eurotium*). Burkinabè cohorts depicted 25 different ASVs, which included 9 fungal species (ASV_6: *Epicoccum sorghi*, ASV_10: *Candida mesorugosa*, ASV_12: *Candida tropicalis*, ASV_33: *Aspergillus flavus*, ASV_34: *Kluyveromyces marxianus*, ASV_70: *Cryptococcus flavus*, ASV_83: *Aspergillus penicillioides*, ASV_99: *Malassezia restricta*, ASV_134: *Cyberlindnera fabianii*) and 10 fungal genera (ASV_2, ASV_7 and ASV_18: *Pichia*, ASV_3: *Candida*, ASV_9: *Eurotium*, ASV_20: *Trichosporon*, ASV_30: *Davidiella*, ASV_50 and ASV_82: *Aspergillus*, ASV_74: *Gibberella*, ASV_480: *Rhizopus*, ASV_646: *Plectosphaerella*). Other ASVs with different taxonomic assignments were also found (Fig. S8 and Table S11). All significant ASVs and the related taxonomic annotations were reported in Table S11.

Pairwise comparison by using the Wilcoxon test (Benjamini-Hochberg correction method) (Fig. 4 and Fig. S8; Table S12 and Table S13) showed that the relative abundance of the ASVs selected by LRT analysis, follows a gradient according to the rural-to-urban transition pattern. In particular, the species level variants *C. mesorugosa*, *C. flavus*, *C. fabianii*, *E. sorghi*, *K. marxianus*, and *M. restricta* were more represented in the rural villages cohort, and their abundance progressively decrease according to the increase in the urbanization level (Fig. 4). We also identified fungal species that are more, or almost exclusively, associated to the Italian cohort compared to the Burkinabè cohorts, such as *C. sake*, *R. mucilaginosa* and *V. leptobactrum* (Fig. 4). Variations

in fungal relative abundances as a function of the rural-to-urban transition were also visible in the non-species assigned ASVs identified by LRT and reported in Fig. S8. Some of these, such as the fungal genera *Aspergillus*, *Candida* and *Pichia*, showed significantly higher relative abundances in the Burkinabè cohorts than in the Italian one (Fig. S8). Conversely, the genera *Penicillium* and *Eupenicillium* were markedly associated with the Italian cohort and almost absent in the Burkinabè cohorts (Fig. S8).

Relative abundances of significant ASVs identified at Species level (by loglikelihood ratio test of DESeq2) displayed according to the four different areas. Differences in the relative abundance were tested by using the Wilcoxon test (Benjamini-Hochberg correction method). Each cohort was depicted following the color pattern in the legend. Significant comparisons were displayed using asterisks (*, p-value < 0.05; **, p-value < 0.01; ***, p-value < 0.001).

Samples were displayed according to the color pattern legend, based on belonging to the area and family members. Relative abundance values reported on the y-axis range from 0 to 1 corresponding to the percentage range of 0 to 100%, respectively.

Discussion

Impact of urbanization-driven dietary shifts on gut mycobiota composition

This study explores how changes in dietary habits associated with different degrees of urbanization affect gut mycobiota composition across three cohorts from Burkina Faso, with a comparative perspective including an Italian urban population. Our findings reveal a distinguishable pattern of decreasing fungal diversity along a rural-to-urban gradient, suggesting that dietary shifts associated with different environmental settings impact the gut mycobiota composition. The highest fungal diversity was observed in individuals from rural households, while a significant reduction was observed in urban populations. These results are consistent with findings from highly urbanized Chinese cohorts [3, 6, 7], supporting the hypothesis that the degree of urbanization, often accompanied by industrialized and processed food consumption, high sanitation and medication level, alters gut fungal ecology. These trends also align with observations from studies conducted in different African countries [5, 7], who examined adults and/or children, and highlighted geography and nutrition as key determinants of gut fungi and microeukaryotes structure.

Within our study, beta diversity analysis further confirmed that geographical location and family structure significantly influence mycobiota composition. Since the cohorts consisted of groups of people who are united by ties of partnership and parenthood, including both

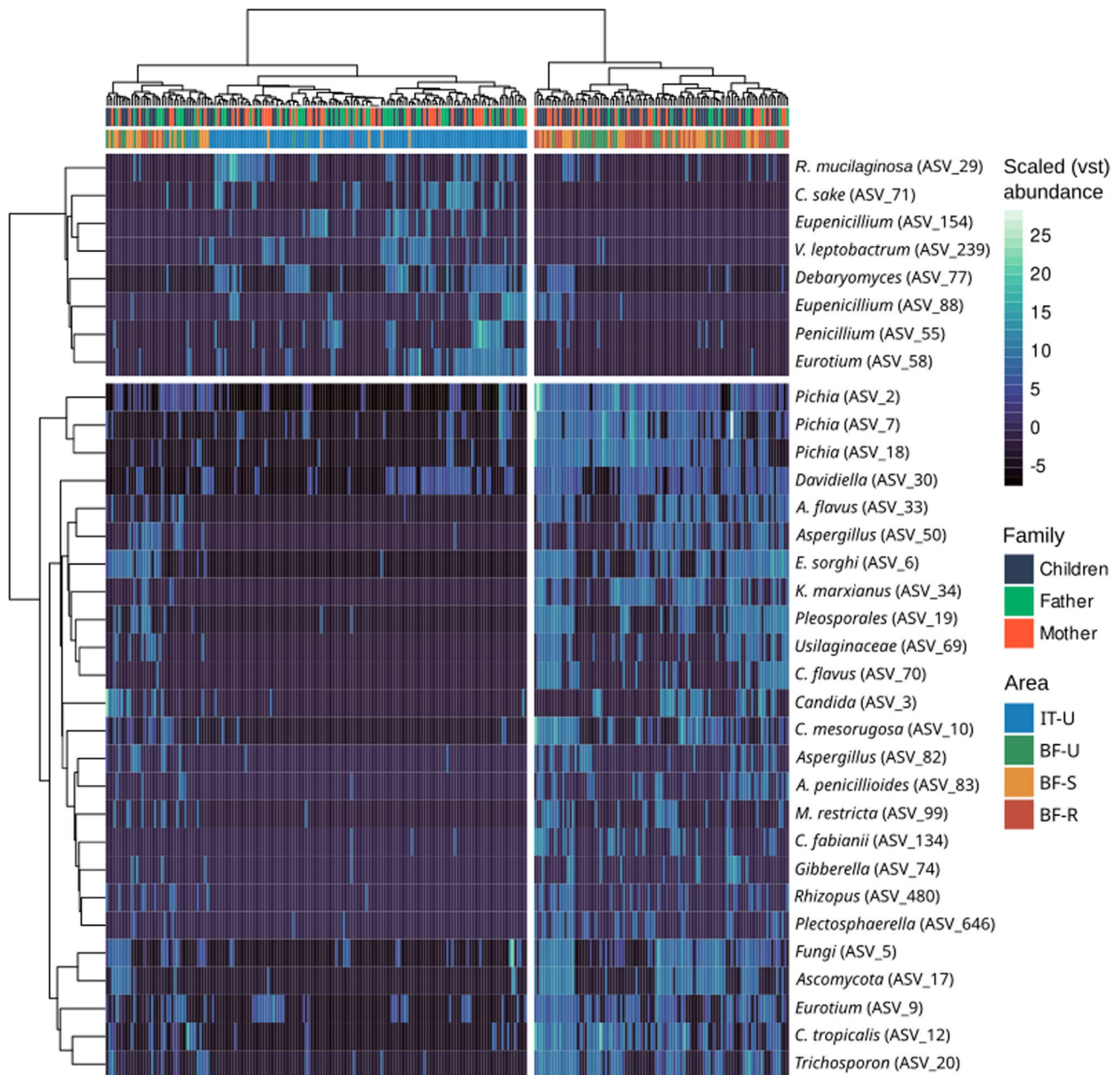


Fig. 3 Sequence variants clustering distributed among the studied cohorts. Amplicon sequence variants whose abundances significantly differed within the dataset (loglikelihood ratio test of DESeq2) were clustered according to their variance-stabilized abundance. Euclidean distances were used as measure in the cluster dendrogram, as displayed in the rows and columns. Ward D2 linkage was used as a method to produce hierarchical clusters. Significant ASVs with the most reliable taxonomic assignment were reported on the heatmap rows, while sample distribution within the clustering was reported on the columns. Different geographical areas and Family groups were reported by using the following abbreviations (Geographical area; IT-U: IT-Urban, BF-U: BF-Urban, BF-S: BF-Semiurban, BF-R: BF-Rural. Family; C: Children, F: Father, M: Mother)

adults and children, we evaluated whether age affected mycobiota composition. No significant age-related differences were found. However, our primary goal was to assess differences in mycobiota composition in cohorts characterized by different family units (polygamous and non-polygamous) and living in various environmental settings. Notably, differences among family members were more pronounced in rural (BF-Rural) and

semi-urbanized (BF-Semiurban) households, particularly between mothers and children from BF-Semiurban and between mothers and fathers from BF-Rural. These intra-family differences were further explained by cultural and behavioral dynamics. In BF-Semiurban households, the greater dissimilarity between mothers and children may reflect lifestyle transitions, where children attend school and adopt more urbanized behaviors (e.g., different diets,

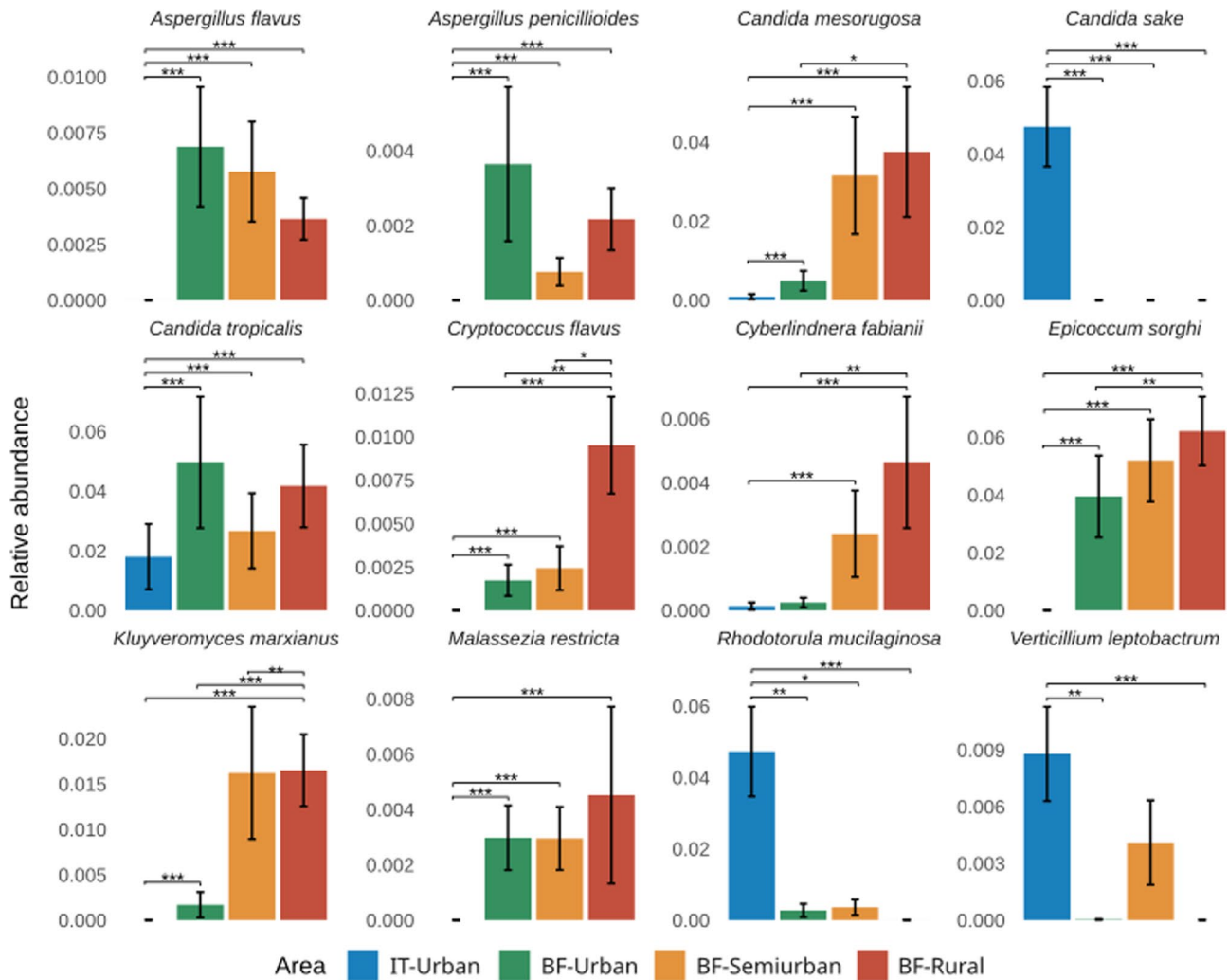


Fig. 4 Pairwise comparisons of the significant abundances at fungal species level

reduced contact with natural environments), while mothers tend to maintain traditional roles. In BF-Rural households, the pronounced differences between mothers and fathers likely stem from a gender-based division of labor: women are primarily engaged in domestic work and food preparation, whereas men are more involved in agricultural and livestock activities. These distinct daily routines expose individuals to different microbial reservoirs, shaping their individual mycobiota profiles. Moreover, it is not uncommon for adult men and women in these settings to consume different portions or even different foods. Even in the absence of substantial dietary differences, gender roles may contribute to intra-household variation through differential exposure to food sources and environmental microbes. The low intra-family differences observed in urban settings, where family members shared more similar mycobiota profiles, probably reflect reduced environmental exposure, and more uniform lifestyle and dietary habits along the urbanization gradient.

In rural and semi-urban areas, individuals are more frequently exposed to a variety of environmental fungal sources (e.g., soil, plants, animals, untreated water) and often engage in distinct daily activities that differently shape their fungal communities. Burkinaabè traditional diets rich in naturally fermented foods, along with reduced sanitation interventions, may further contribute to a more heterogeneous mycobiota among cohabiting individuals. Furthermore, differences in age, sex and household roles may play a role, particularly in traditional rural environments. In contrast, the urban lifestyle, characterized by widespread use of antimicrobials, standardized diets and more hygienically controlled living conditions, may lead to reduced environmental fungal exposure and increased homogeneity of mycobiota within families. The combination of environmental and behavioral factors thus appear to drive the intra-family diversity observed.

Key fungal taxa and their relevance in the rural-to-urban gradient

Several sequence variants (ASVs) showed variations in abundance according to different levels of urbanization associated with different geographical areas. In particular, ASVs related to the *Aspergillus* genus were significantly more represented within the Burkinabè groups, likely due to environmental exposure [49] and local differences in food storage methods [50]. Although the fungal variants assigned to *Aspergillus* are potential etiological agents in several disease conditions, it does not necessarily represent a pathogenic microorganism in the Burkinabè populations. Indeed, a recent study reported a beneficial role of *Aspergillus* in *Clostridioides difficile* infections (CDI) in decreasing the risk of CDI [51]. Therefore, the association of *A. penicillioideus* with healthy individuals rather than intestinal infections, as CDI, was also corroborated by findings of its abundance following fecal transplantation in CDI subjects [52]. *C. tropicalis*, also prevalent in the Burkinabè cohorts, is often associated with traditional sub-Saharan fermented foods, especially in the cereal-based ones, which are also characterized by the presence of anti-nutritional factors such as phytate. *C. tropicalis* isolated from different fermented beverages were found to have high extracellular phytase activity [53], suggesting a possible role of the yeast in promoting nutrients bioavailability. However, despite the positive contribution that this yeast can make within fermentation, the pathogenic potential found in clinical isolates of *C. tropicalis*, as well as the opportunistic nature of yeasts belonging to the *Candida* genus, must be considered [54]. *C. mesorugosa*, *C. flavus*, *C. fabianii*, *E. sorghi*, *K. marxianus*, and *M. restricta* were highly represented in the BF-Rural cohort and progressively declined depending on the increase in degree of urbanization. Many of these species are linked to traditional diets, particularly fermented foods [55–59], which serve as a dietary source of beneficial yeasts [28–30]. The prevalence-abundance analysis in accordance with the LRT analysis corroborated the role of *E. sorghi* as a representative species of areas with a lower degree of urbanization (BF-Semiurban and BF-Rural). The wide occurrence of *E. sorghi* within low urbanized areas can be explained by contamination mechanisms; in fact, a natural fungal contamination in food consumed in Durban, South Africa was described by Olagunju et al. [60]. *M. restricta*, classically associated with skin mycobiota, is also widely described as a component of the intestinal microbiota [61, 62]. The decline of these taxa in urban populations suggests that reduced consumption of fermented foods, increased sanitation, and greater antibiotic use may contribute to altered gut mycobiota composition [11–13]. We also identified prevalent environmental fungal species such as *R. mucilaginosa*, which is almost exclusively associated with the

Italian cohorts compared to the Burkinabè cohorts. In humans, *R. mucilaginosa* is described as a transient commensal microorganism found on the skin, nails, and in the gastrointestinal, urinary, and respiratory tracts [63]. Yeasts of this genus are deemed opportunistic pathogens, primarily impacting immunocompromised individuals [64].

Conclusion, limitations and future perspectives

The observed reduction in fungal diversity in urban populations, and in general the differences in early microbial exposure [65, 66], is crucial in shaping the host immune system. Traditional rural lifestyles, which often involve greater exposure to environmental microbes, could promote immune tolerance and protection. In agreement with the *hygiene hypothesis*, our findings lay the foundation for further studies aiming at investigating the effect that these microbial losses will have on human health. Therefore, our results underscore the need for thorough exploration of both environmental and gut fungal communities in Burkina Faso and newly developing urban regions to uncover the mechanisms underlying the connection between fungi and human health.

Some limitations of this study should be taken into account when interpreting the results. The average age of the participants differed between populations, which reflects the practical constraints often encountered in recruiting age-matched cohorts in cross-continental studies, moreover ensuring an appropriate enrollment for the all family members. Although age was included as a variable in the analyses, its potential influence on mycobiota composition cannot be entirely ruled out. Additionally, intrinsic differences in environmental exposures, dietary habits, and fungal detection sensitivity between Burkina Faso and Italian populations may affect the observed mycobiota changes. Individual-level hygiene and sanitation conditions were not directly assessed, as they are difficult to quantify in a standardized manner; however, these factors were taken into account as part of the broader environmental and geographical context characterizing each cohort.

Furthermore, families living in rural and semi-urban areas of Burkina Faso often follow a polygamous structure, unlike those in urban areas of both Burkina Faso and in Italy, where monogamy is more common. Due to this variability, it was not feasible to account for family relatedness, which may influence the development of the gut mycobiota.

These factors are inherent to studies of this nature and highlight the complexity of comparing microbial communities across diverse human populations.

Future perspective to integrate the results here shown should aim to include mycobiota characterization of both environmental sources, including soil, water and air, as

well as dietary sources, in order to establish a detailed map of fungal transmission and its dynamics within different environmental and dietary contexts. A shotgun metagenomics approach will increase the reliability of fungal species detection, despite being technically challenging due to the scarce amount of fungal cells compared to the bacterial cells in human samples [38]. Such efforts would provide a more holistic and robust understanding of fungal ecology and its impact on the host in the rural-to-urban transition.

Abbreviations

IT-U	IT-Urban
BF-U	BF-Urban
BF-S	BF-Semiurban
BF-R	BF-Rural
VePr	vegetable proteins
AnPr	percentage of animal proteins
Ca	percentage of total carbohydrates
Pr	percentage of total proteins
Su	percentage of total sugars
Fi	fiber intake expressed in grams per 1000 kcal
Fa	percentage of total fats

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-025-04278-9>.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.
Supplementary Material 8.
Supplementary Material 9.
Supplementary Material 10.
Supplementary Material 11.
Supplementary Material 12.
Supplementary Material 13.
Supplementary Material 14.
Supplementary Material 15.
Supplementary Material 16.
Supplementary Material 17.
Supplementary Material 18.
Supplementary Material 19.

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Authors' contributions

D.C. and P.L. conceived the work; S.R., N.M., M.D.P., C.D.F. and D.C. wrote the manuscript; S.R. and B.C. produced the metagenomic libraries and sequencing, N.M. performed amplicon sequence variants inference and statistical analysis, G.B. contributed to amplicon sequence variants inference, A.G., S.C., E.B. and A.D.B. managed and analyzed nutritional diaries data, S.D., B.K., K.D. and H.T. coordinated the enrollment and sample collection in Burkina Faso. Q.D.M., D.C., P.L., S.R., N.M., M.D.P., C.D.F., B.C., G.B., A.G., S.C., E.B., A.D.B., S.D., B.K., K.D., and H.T. revised and approved the manuscript.

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Data availability

The ITS1 raw sequences data has been deposited to the European Nucleotide Archive (ENA) under the accession code PRJEB59322. All results from statistical analyses were mentioned by writing in the main text, represented as figures in the main text or reported as supplementary tables and figures. Further information can be provided upon request to the corresponding author.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the principles of the Declaration of Helsinki. In Burkina Faso, the study was carried out in accordance with the recommendations of the National Ethics Committee of Burkina Faso that granted ethical clearance (reference number 2018-8-104). For the Italian families, the study was approved by the Ethics Committee of Meyer Children Hospital, Florence, Italy (reference number 187/2018). Adult participants gave their informed consent. Parents or primary caregivers signed off on children under the age of 18. Confidentiality was maintained for data and sample collection by assigning each participant an identifying code.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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