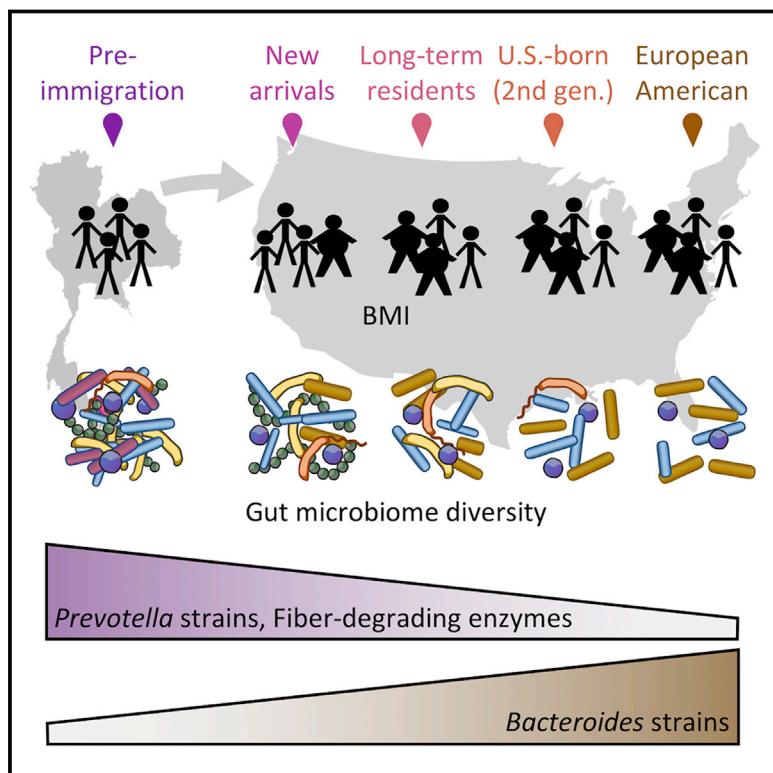


US Immigration Westernizes the Human Gut Microbiome

Graphical Abstract



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In Brief

Migration from a non-western nation to the United States is found to be associated with a loss in gut microbiome diversity and function in a manner that may predispose individuals to metabolic disease.

Highlights

- US immigration is associated with loss of gut microbiome diversity
- US immigrants lose bacterial enzymes associated with plant fiber degradation
- *Bacteroides* strains displace *Prevotella* strains according to time spent in the USA
- Loss of diversity increases with obesity and is compounded across generations



US Immigration Westernizes the Human Gut Microbiome

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SUMMARY

Many US immigrant populations develop metabolic diseases post immigration, but the causes are not well understood. Although the microbiome plays a role in metabolic disease, there have been no studies measuring the effects of US immigration on the gut microbiome. We collected stool, dietary recalls, and anthropometrics from 514 Hmong and Karen individuals living in Thailand and the United States, including first- and second-generation immigrants and 19 Karen individuals sampled before and after immigration, as well as from 36 US-born European American individuals. Using 16S and deep shotgun metagenomic DNA sequencing, we found that migration from a non-Western country to the United States is associated with immediate loss of gut microbiome diversity and function in which US-associated strains and functions displace native strains and functions. These effects increase with duration of US residence and are compounded by obesity and across generations.

INTRODUCTION

Previous work has established that diet and geographical environment are two principal determinants of microbiome structure and function (De Filippo et al., 2010; Febinia, 2017; Gomez et al.,

2016; Kwok et al., 2014; Obregon-Tito et al., 2015; Rothschild et al., 2018; Schnorr et al., 2014; Yatsunenko et al., 2012). Rural indigenous populations have been found to harbor substantial biodiversity in their gut microbiomes, including novel microbial taxa not found in industrialized populations (Clemente et al., 2015; Gomez et al., 2016; Obregon-Tito et al., 2015; Schnorr et al., 2014; Smits et al., 2017; Yatsunenko et al., 2012). This loss of indigenous microbes or “disappearing microbiota” (Blaser and Falkow, 2009) may be critical in explaining the rise of chronic diseases in the modern world. Despite the frequent migration of people across national borders in an increasingly interconnected world, little is known about how human migration affects the human microbiome.

The United States hosts the largest number of immigrants in the world (49.8 million or 19% of the world’s total immigrants and approximately 21% of the US population) (United Nations, 2017). Epidemiological evidence has shown that residency in the United States increases the risk of obesity and other chronic diseases among immigrants relative to individuals of the same ethnicity that continue to reside in their country of birth, with some groups experiencing up to a 4-fold increase in obesity after 15 years (Goel et al., 2004; Lauderdale and Rathouz, 2000). Refugees, in particular, appear to be more vulnerable to rapid weight gain (Henev et al., 2014; Hervey et al., 2009), with Southeast Asian refugees exhibiting the highest average increases in body mass index (BMI) after relocation to the United States (Careyva et al., 2015). The Hmong, a minority ethnic group from China who also reside in Southeast Asia, make up the largest refugee group in Minnesota (22,033 total refugees as of 2014) (Minnesota Department of Health, 2017; Pfeifer and



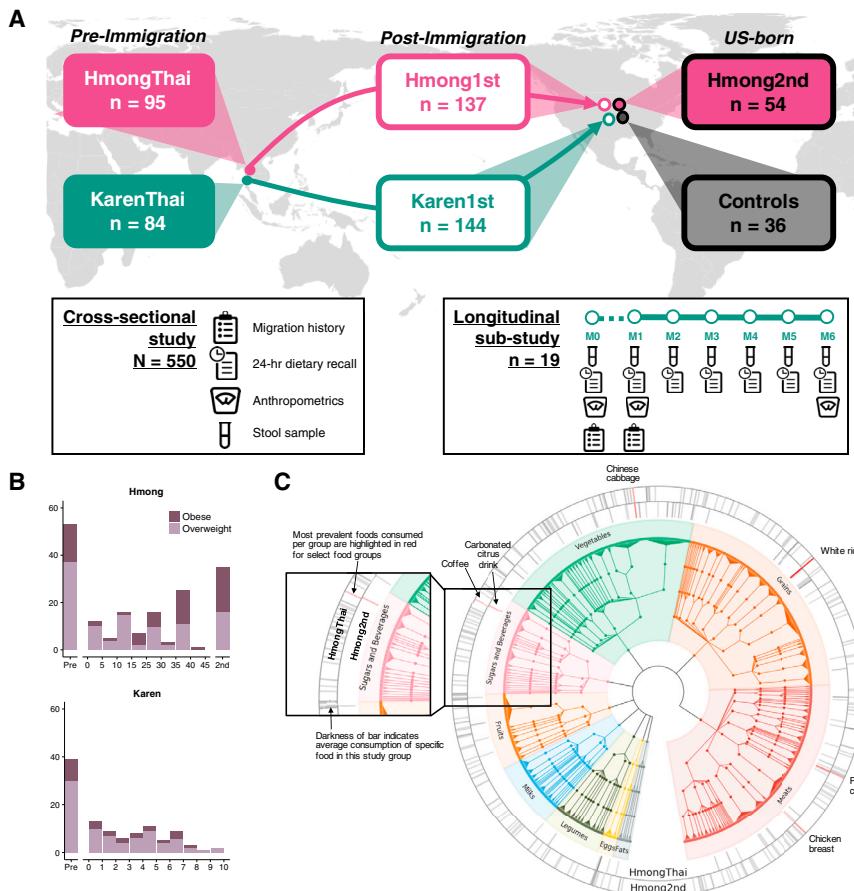


Figure 1. Assembly of a Multi-generational Asian American Cohort

(A) Experimental design for cross-sectional and longitudinal cohorts. See also Figure S1A.

(B) Ratios of overweight-to-obese individuals across sample groups and over time in the United States separated by ethnicity due to differences in time in years.

(C) HmongThai (n = 43) and Hmong2nd (n = 41) (ages 20–40) diet diversity displayed on a tree that groups related foods together. Bars denote unique foods, with darkness of the bar showing prevalence of foods reported averaged within HmongThai or Hmong2nd. Items highlighted in red denote the most prevalent vegetables, sweets and beverages, grains, and meats reported within sample groups. Full descriptions of foods highlighted in red: coffee, brewed, regular; carbonated citrus fruit drink; Chinese cabbage or bok choy family, raw; rice, white, no salt or fat added; pork chop, broiled, baked, or grilled, lean only eaten; chicken breast, roasted, skin not eaten. See also Figure S1B.

Thao, 2013). The Karen, an ethnic minority from Burma, have been arriving in large numbers in more recent years (Minnesota Department of Health, 2017). Overweight status and obesity rates are highest among Hmong and Karen compared to other Asian ethnic groups in Minnesota (Arcan et al., 2014; Franzen and Smith, 2009; Mulasi-Pokhriyal et al., 2012; Dawson-Hahn et al., 2016). Western diet acculturation, previous exposure to food insecurity, and physical inactivity have been identified as contributing factors (Franzen and Smith, 2009; Mulasi-Pokhriyal et al., 2012), although they do not fully explain risk of obesity.

The gut microbiome plays a critical role in host metabolism and is heavily influenced by an individual's long-term diet (Hildebrandt et al., 2009; Wu et al., 2011) and can also respond quickly to dramatic dietary changes (David et al., 2014; Turnbaugh et al., 2009a). Thus, the gut microbiome offers an important window into the consequences of diet and lifestyle changes associated with human migration. To study the short- and long-term impacts of migration on the microbiome, we measured gut microbiomes and dietary intake from Hmong and Karen immigrants and refugees (henceforth referred to as immigrants) in cross-sectional and longitudinal cohorts undergoing relocation to the United States stratified by BMI (high ≥ 25 and low < 25). A first-generation immigrant group (foreign-born US residents) included individuals with duration of US residence ranging from a few days to more than 40 years, allowing us to test for

changes in the gut microbiome associated with long-term US residence. We included second-generation Hmong immigrants (born in the United States to first-generation Hmong immigrants) to determine whether the effects of US immigration were compounded across generations by birth in the United States. Finally, we followed a unique longitudinal cohort of 19 Karen refugees for up to 9 months beginning immediately before or after arrival in the United States to measure the short-term effects of US immigration.

RESULTS

Assembly of a Multi-generational Asian-American Immigrant Cohort

We recruited 514 healthy Hmong and Karen female individuals (aged 18–78; see STAR Methods for full exclusion criteria) who either (1) were living in Thailand (HmongThai, KarenThai; n = 179), (2) were born in Southeast Asia and had moved to the United States (Hmong1st, Karen1st; n = 281), or (3) were born in the United States and whose parents were born in Southeast Asia (Hmong2nd; n = 54) (Figure 1A). We also recruited healthy European American female individuals to serve as US controls (controls; n = 36) (Figure 1A). We limited our study to women based on insight from our Hmong community advisory board that substantially more Hmong women than men were relocating to United States. Participants in each sample group were recruited into lean or overweight/obese BMI class stratifications ($BMI < 25$ or $BMI \geq 25$, respectively) (Table S1). In 2016 and 2017, we recruited eligible individuals throughout the Minneapolis-St. Paul metropolitan area in Minnesota and at two locations in Thailand: a rural village in Chiang Mai province (Khun Chang Khian) and a refugee camp in Tak province (Mae La) (Figure S1A).

Bilingual-bicultural research teams collected migration and medical histories (Table S2), anthropometrics (weight, height, waist circumference), 24-hr dietary recalls, and single stool samples from all participants. Karen participants who were about to leave Thailand for the United States or who had arrived in the United States within 2 months were invited to participate in a longitudinal sub-study in which 24-hr dietary recalls and stool samples were collected monthly for 6 months (Figure 1A). We collected a total of 673 stool samples comprising 531 single- and 142 multiple-time-point collections. Consistent with the previously observed high rate of obesity in US immigrants (see Introduction), obesity prevalence relative to overweight status in our cohort increased after a decade in the United States in the Hmong1st group (Chi-squared test statistic = 5.23, $p = 0.022$) (Figure 1B). There was not a sufficient number of Karen subjects with long-term US residence to test for changes in prevalence of obesity.

To be able to associate gut microbiome variation with dietary intake, we collected 24-hr dietary recalls from all participants and analyzed macronutrient content using the United States Department of Agriculture (USDA) SuperTracker food record system (Britten, 2013; United States Department of Agriculture) and published literature. We utilized the hierarchical format of food codes derived from the USDA's Food Nutrient and Database for Dietary Studies (FNDDS) to categorize foods into a tree structure where more closely related foods were grouped together (Figure 1C). These groupings allowed us to share statistical strength across closely related foods to complement dietary analysis of macronutrients, much in the way that phylogenetic beta-diversity analysis complements taxonomy-based profiles of microbiomes. Foods reported by participants that were not found in any USDA database ($n = 72$; Table S3) were manually inserted into the hierarchical food tree, allowing us to account for all foods reported by all participants. We confirmed our ability to discriminate between the Karen1st, Hmong1st, and Hmong2nd group diets using tree-based distances (Figure S1B), identifying a stark increase in the variety of foods eaten by second-generation Hmong relative to Hmong in Thailand (Figure 1C) (t test of phylogenetic diversity, $p = 4.828 \times 10^{-10}$).

US Immigration Is Associated with Loss of Native Gut Microbiome Species

We performed amplicon-based sequencing of the 16S rRNA gene V4 region on 550 stool samples (one sample per participant). Principal-coordinates analysis (PCoA) of unweighted UniFrac distances (Lozupone et al., 2011) revealed that Hmong and Karen harbor distinct gut microbial compositions regardless of country of residence, yet their microbiomes converge toward European American microbiomes after relocating to the United States (analysis of similarities [ANOSIM] $R = 0.25$, $p = 0.001$), with second-generation Hmong and European American microbiomes sharing nearly identical cluster centroids (Figure 2A). Interestingly, all US immigrant groups had higher interindividual variation than their Thai counterparts (t test Hmong1st versus HmongThai, $p = 1.2 \times 10^{-12}$; Hmong1st versus HmongThai, $p = 6.5 \times 10^{-4}$; Karen1st versus KarenThai, $p = 4.9 \times 10^{-37}$). The first-generation immigrants with the most perturbed microbiomes (most distant tertile from Thai groups) had both higher

age (t test, $p = 0.0013$) and longer time (t test, $p = 0.00079$) in the United States than those with the least perturbed microbiomes (least distant tertile from Thai groups).

Microbial diversity and richness were highest in Thailand and decreased with each generation of residence in the United States (Tukey's honest significant difference [HSD], $p < 0.01$; Figure 2B). As in other studies (Sze and Schloss, 2016; Turnbaugh and Gordon, 2009), we found that lower phylogenetic diversity was associated with obesity across all major study groups (unbalanced two-way ANOVA, $p = 0.0044$; Figure 2B), even after stratification by ethnicity (Tukey's HSD, $p < 0.01$; Figure S2A). Furthermore, we observed a consistent loss of certain native bacterial operational taxonomic units (OTUs) among first-generation Hmong (Figure 2C). Although 7 of the 10 most prevalent OTUs found in HmongThai were also found at similar levels in Hmong1st, others such as *otu1812* (*Faecalibacterium prausnitzii*) incurred a 45% loss in prevalence (Fisher's exact test, false discovery rate [FDR]-corrected $q = 3.05 \times 10^{-14}$) (Table S4). Prevalence-abundance curve analysis showed that many OTUs that were highly prevalent (>75% prevalence) in Thai-resident individuals had both decreased abundance and prevalence in first-generation US residents (paired t test, area under the prevalence-log-abundance curve, HmongThai versus Hmong1st, $p < 2.2 \times 10^{-16}$) (Figure 2D). 28 OTUs incurred at least a 50% loss in prevalence among first-generation Hmong, with more than half of them belonging to the genus *Prevotella* (Table S4).

Bacteroides Strains Displace *Prevotella* Strains across Generations in the United States

The Western-associated genus *Bacteroides* increasingly displaced the non-Western-associated genus *Prevotella* across generations in the United States (Figure 3A). The ratio of *Bacteroides* to *Prevotella* was lowest in Thailand-resident individuals, highest in US-born European Americans, and increased in a stepwise fashion from first-generation Karen to first-generation Hmong to second-generation Hmong (unbalanced two-way ANOVA, resident continent $p = 3.4 \times 10^{-13}$, birth continent $p = 0.00085$, ethnicity $p = 5.5 \times 10^{-12}$). This progression corresponded with the time that these groups had spent in the United States.

Using deep shotgun metagenomics on 55 samples (mean 22,406,875 reads per sample) from Hmong in Thailand, newly arrived Karen, long-term (>30 years) US resident Hmong, and controls, we profiled strain-level variation within *Bacteroides* and *Prevotella*. We aligned shotgun metagenomic sequences against all 256 *Bacteroides* genomes and 153 *Prevotella* genomes in RefSeq version 87 (O'Leary et al., 2016), retaining any strains with at least 50% genome coverage in at least one sample. We found that US controls had varied *Bacteroides* strain profiles, while those with *Prevotella* had only a single strain of *P. copri* (Figure 3B). Conversely, Thailand-based individuals carried up to four strains of *Prevotella*, with low abundance and generally low genomic coverage of *Bacteroides* strains possibly due to lack of related strains in the database. Long-term US-resident Hmong displayed an intermediate profile, carrying a variety of *Bacteroides* strains and, in several individuals, multiple *Prevotella* strains. Prevalence-abundance curves for the

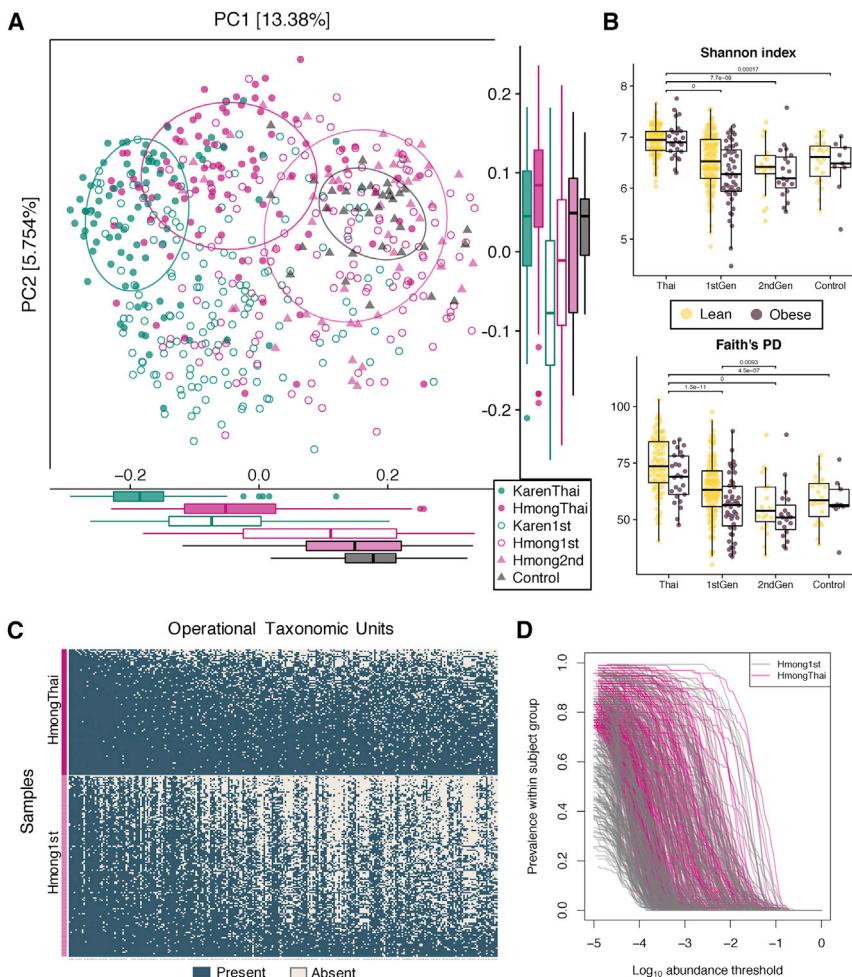


Figure 2. Loss of Diversity and Native Bacterial Taxa with Time Spent in the United States

(A) PCoA of unweighted UniFrac distances between bacterial communities of cross-sectional participants revealed that phylogenetic variation was differentiated by sample group (ANOSIM R = 0.25, p = 0.001). 95% standard error ellipses are shown around Hmong and Karen in Thailand, second-generation Hmong, and controls.

(B) Alpha diversity of obese and lean individuals across sample groups in Shannon's diversity index and Faith's phylogenetic distance (PD). P values denote significantly different groups using pairwise tests of sample groups without stratification by BMI (Tukey's HSD, p < 0.01). Microbiome diversity is significantly lower in obese individuals across all sample groups (unbalanced two-way ANOVA analysis with BMI class and sample group as covariates, p = 0.0044). See also Figure S2A.

(C) Prevalence of OTUs in HmongThai and Hmong1st, with OTUs sorted by prevalence in HmongThai and samples sorted by richness within sample group. OTUs shown are found in at least 75% of HmongThai samples (see Table S4 for taxonomic assignments, mean group prevalence, and statistics).

(D) Prevalence-abundance curves of all OTUs present in at least 75% of HmongThai samples, plotted separately for the Hmong1st and HmongThai sample groups. See also Figure S2B.

Bacteroides and *Prevotella* strains with the largest change in overall prevalence between HmongThai and Hmong1st showed marked loss of *Prevotella* strains accompanied by an expansion of pre-existing low-abundance *Bacteroides* strains following US immigration (Figure S2B).

US Immigrants Lose Enzymes Associated with Plant Fiber Degradation

We identified differences in functional pathways (Abubucker et al., 2012) between HmongThai and long-term US-resident Hmong1st (>30 years residence) using shotgun metagenomics data (ANOVA, FDR-corrected q < 0.10; Figure S3A). First-generation Hmong harbored microbiomes with increased capacity for sucrose degradation, glycerol degradation, glucose/xylose degradation, and glucose fermentation to lactate, potentially related to increased consumption of more sugary foods, although most sucrose and glucose would not be expected to reach the lower gastrointestinal (GI) tract (Dahlqvist and Thomson, 1963). In HmongThai, we found an enrichment of pathways relating to the degradation of complex carbohydrates, including β -(1,4)-mannan degradation and starch degradation (Flint et al., 2012). In order to better understand the substrates degraded by these

pathways that are either lost or below the detection limit in US immigrants, we assembled the shotgun data into scaffolds and annotated carbohydrate-degrading enzymes (CAZymes) (Lombard et al., 2014; Yin et al., 2012). We found significant shifts in abundance of 58 CAZymes across the HmongThai, Hmong1st, and control groups (Kruskal-Wallis test, FDR-corrected q < 0.05; Figure 3C), including three β -glucan-targeting glycoside hydrolases (GH17, GH64, and GH87) that were highly abundant in the Thailand group but almost entirely unobserved in the US groups. Loss of these glycoside hydrolases may be associated with loss of dietary fiber sources that promote persistence of the organisms that harbor these enzymes, reducing the ability of the microbiota to degrade these dietary fibers.

These three glycoside hydrolases predominantly originated from *P. copri* (42 ± 11.1%; Figure S3B), supporting the hypothesis that loss of *Prevotella* strains following US immigration drove loss of plant-fiber degradation capability. We also observed a loss of GH5 and GH26 glycoside hydrolases from HmongThai to Hmong1st and US controls, which indicates a loss of cellulose and β -mannan and possible xyloglucan degradative potential. Beta-mannans are present in seeds, kernels, and corms, such as palm (Subrahmanyam et al., 1956), coconut (Kooiman, 1971), and konjac (Pangrui et al., 2015), and xyloglucan is found most abundantly in tamarind (Mishra and Malhotra, 2009); these, interestingly, are food ingredients prevalent in Southeast Asia.

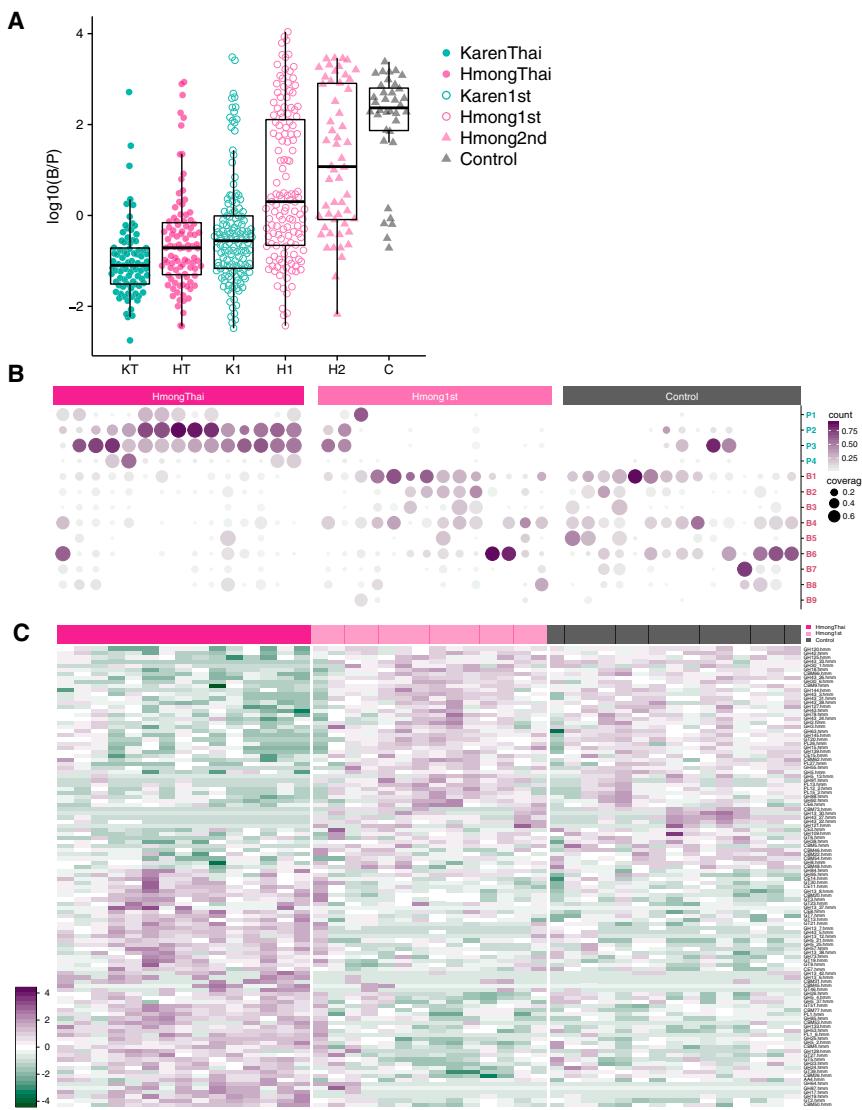


Figure 3. Bacteroides and Prevotella Strain Diversity and Abundances

(A) Log-transformed ratio of *Bacteroides* to *Prevotella* (B/P) relative abundances. US residence, US birth, and ethnicity were all significantly associated with B/P ratio (unbalanced two-way ANOVA, $p = 3.4 \times 10^{-13}$, $p = 0.00085$, $p = 5.5 \times 10^{-12}$, respectively). KT, KarenThai; HT, HmongThai; K1, Karen1st; H1, Hmong1st; H2, Hmong2nd; C, controls.

(B) Coverage and relative abundance of *Bacteroides* and *Prevotella* strains in 44 samples across HmongThai, Hmong1st (who have lived in the United States for more than 30 years), and controls. Strains with genomic coverage >50% in at least one sample were included. Hierarchical clustering of strains and samples within group is based on relative abundances. Strains with genome coverage of <1% within a person are considered not present (not plotted). See Table S5 for strain names.

(C) CAZymes with significantly different relative abundances across HmongThai, Hmong1st (who have lived in the United States for more than 30 years), and controls (Kruskal-Wallis test, FDR-corrected $q < 0.05$). See also Figure S3.

The loss of glycoside hydrolases for degrading cellulose, a plant cell-wall component, was another indication that the microbiota of post-immigration individuals had lost some of their ability to degrade plant-derived fibers (El Kaoutari et al., 2013). These findings parallel previous findings in a mouse model demonstrating that the microbiomes of mice deprived of dietary fiber lost the capability to produce certain glycoside hydrolases (Sonnenburg et al., 2016).

Dietary Acculturation Partly Explains Microbiome Acculturation

We observed significant differences across study groups in the consumption of macronutrients commonly associated with a Western diet: sugars, fats, and protein (unbalanced two-way ANOVA, $p < 0.01$; Figures 4A and S4). There were no significant associations between fiber content and the microbiome, possibly due to the many uncharacterized polysaccharides present in different foods. PCoA of diet-based unweighted UniFrac

(Lozupone et al., 2011) revealed distinct separation by sample group (ANOSIM $R = 0.29$, $p = 0.001$; Figure 4B) and a gradient of dietary acculturation along PC1 (Table S6). First- and second-generation Hmong had similar food choice profiles (Figure 4B), while US controls shared few foods with other groups and consumed almost 10-fold less white rice than other groups (Figure S5). Although the microbiomes of US control and second-generation Hmong clustered together (Figure 2A), their diets did not (Figure 4B).

Overall dietary profile was significantly associated with overall microbiome profile across individuals (Procrustes test, $p = 0.001$, $n = 999$ permutations) (Figure S5C), but constrained ordination of the microbiome by the first five principal coordinates of diet variation revealed that diet explained a relatively small fraction (16.8%) of the total variation explained in the microbiome PCoA (Figure 4C). Thus, we found that diet likely was not the sole contributor to the observed gut microbiome changes in our cohort, although it is possible that dietary variation explains substantially more microbiome variation in this cohort than we are able to determine due to our limited knowledge of precise polysaccharide and other nutrient content of the foods and due to complex individualized diet-microbiome interactions.

Gut Biodiversity Decreases According to Duration of Residence in the United States

After finding that US residence was associated with a major shift in dominant taxa in the microbiome (Figure 3A), we decided to

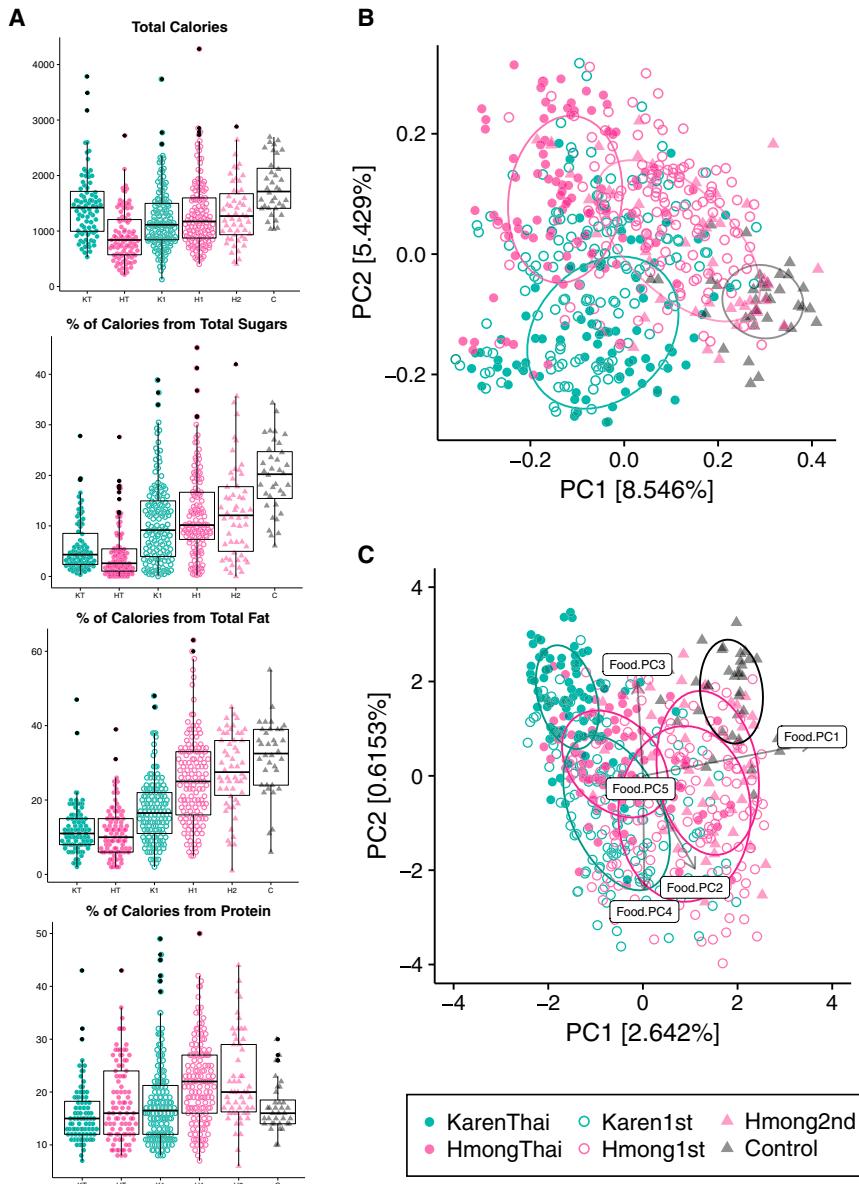


Figure 4. Dietary Acculturation Partially Explains Microbiome Variation

(A) Comparison of macronutrient consumption across sample groups. Ethnicity is significantly associated with calories ($p = 3.4 \times 10^{-5}$), sugars ($p = 0.00023$), fat ($p = 1.3e-07$), and protein ($p = 3.2 \times 10^{-07}$), whereas US residency is associated with sugar ($p = 1.3 \times 10^{-16}$), fat ($p = 7.1 \times 10^{-24}$), and protein consumption ($p = 5.7 \times 10^{-05}$), and birth continent is only associated with fat consumption ($p = 0.0081$) (unbalanced two-way ANOVA). HT, HmongThai; KT, KarenThai; H1, Hmong1st; K1, Karen1st; H2, Hmong2nd; C, Controls. See also Figure S4.

(B) PCoA of unweighted UniFrac diet-based distances reveals significant clustering by sample group (ANOSIM $R = 0.29$, $p = 0.001$). Dietary acculturation can be seen along PC1 with Thai-resident groups on the left and European controls on the right.

(C) Redundancy analysis (RDA) of the unweighted UniFrac microbiome-distances constrained by the first five principal coordinates of the PCoA of unweighted UniFrac food distances. The resulting RDA explains 16.8% of the total variation explained by PC1 and PC2 of the microbiome PCoA (Figure 2A). See also Figure S5.

with diversity in any of the other groups (Pearson correlation, HmongThai $p = 0.065$, Hmong2nd $p = 0.79$, control $p = 0.37$). Thus, we found evidence that increased duration of US residence is associated with decreased microbiome diversity, but further study is needed to separate the effects of age and duration of US residence.

Prevotella Displacement Continues for More Than One Decade

The longer immigrants spend living in the United States, the more their microbiome compositions diverge from their Thai counterparts and converge toward Euro-

pean Americans (Spearman correlation, $\rho = -0.41$, $p = 1.3 \times 10^{-12}$ and $\rho = 0.35$, $p = 1.2 \times 10^{-09}$, respectively; Figure 6A), with continued displacement of *Prevotella* with *Bacteroides* (Spearman's correlation, $\rho = 0.44$, $p = 8.76 \times 10^{-15}$; Figure 6B) over time. We confirmed that this significant association persisted after stratifying the first-generation immigrants by ethnicity, despite the shorter time frame of US residence in first-generation Karen (Spearman's correlation, Hmong $\rho = 0.47$, $p = 8.16 \times 10^{-19}$; Karen $\rho = 0.19$, $p = 0.023$; Figure 6B, inset). As in the case of diversity above, we note that age is highly correlated with years in the United States. However, we used the European American group as a control group to test for association of age with similarity to the HmongThai reference group and found no association ($p = 0.57$) (Figure S6A), and the B/P ratio in first-generation immigrants was significantly associated

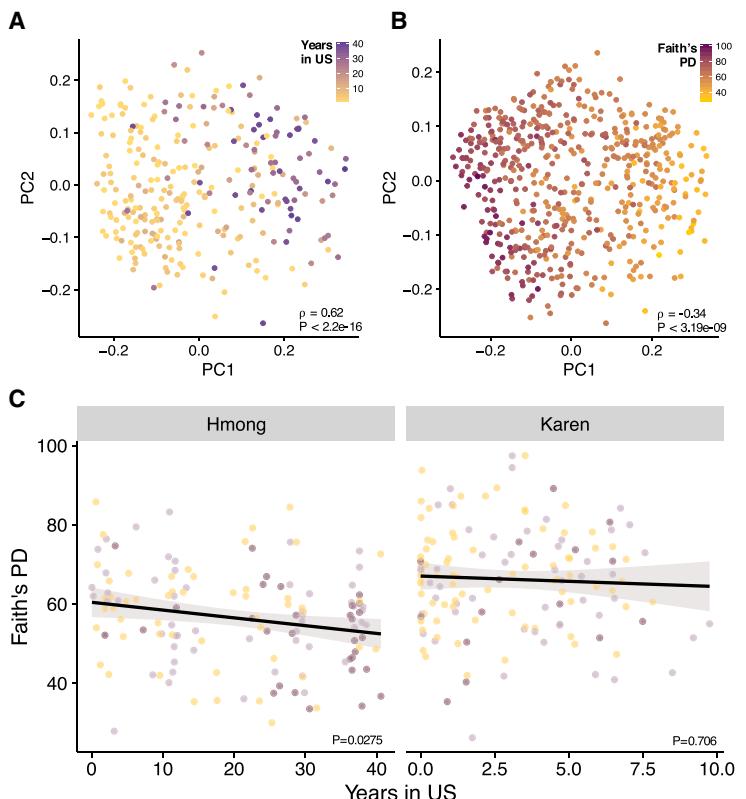


Figure 5. Gut Biodiversity Decreases with Time Spent in the United States

(A) Unweighted UniFrac PCoA of gut microbiomes of first-generation Hmong and Karen participants ($n = 281$), colored by years spent in the United States, which ranges from 1 day to 40.6 years. PC1 is strongly correlated with the amount of time spent in the United States ($\rho = 0.62$, $p < 2.2 \times 10^{-16}$).

(B) Unweighted UniFrac PCoA of gut microbiomes of cross-sectional participants ($n = 550$), colored by Faith's PD. PC1 is negatively correlated with phylogenetic richness ($\rho = -0.34$, $p < 3.19 \times 10^{-9}$).

(C) In first-generation Hmong, diversity significantly decreases over time in the United States (multiple regression: years in United States $\beta = -0.18$, $p = 0.0275$; BMI $\beta = -0.05$, $p = 0.81$), but a significant association was not observed in first-generation Karen (years in United States $\beta = -0.17$, $p = 0.71$; BMI $\beta = -0.27$, $p = 0.28$) (gray area represents 95% confidence interval).

with years in the United States regardless of age (multiple linear regression, years in United States $\beta = 0.096$, $p = 0.0094$; age $\beta = 0.039$, $p = 0.0065$) (Figure S6B). These findings show that changes to the dominant members of the gut microbiome begin during the first decade of US residence and continue for multiple decades.

Microbiome Westernization Begins within 9 Months of Immigration

To understand whether changes in the gut microbiome can be detected immediately after relocation to the United States, we examined the gut microbiomes of 19 newly arrived Karen over their first 6–9 months of US residence. Within this short time frame, all but one participant gained weight (paired t test, $p = 8.3 \times 10^{-5}$; Figure 7A), and protein consumption increased (paired t test, FDR-adjusted $q = 0.048$; Figure 7B), while the total variety of foods consumed decreased (paired t test, $p = 0.017$; Figure 7C), suggesting a period of acclimation to newly available foods. This is in contrast to the Hmong1st group, whose diet diversity tended to increase following US immigration (Figure S7B). Hmong participants' diets may be more acculturated due to the longer duration of residence of the Hmong community (mean time US residence = 20.2 years among study participants) when compared to the Karen community (mean time US residence = 1.9 years among study participants). Hmong1st diets tend to be more similar on average than the Karen1st diets to the European American diets (t test of mean tree-based diet distance to European American group, $p = 0.028$). We

again observed the displacement of *Prevotella* by *Bacteroides* (paired t test, $p = 0.0013$; Figure 7D) within this longitudinal cohort, in many cases involving a 10-fold increase in the *Bacteroides*-*Prevotella* ratio, indicating that microbiome Westernization begins immediately after arrival to the United States.

This longitudinal cohort also included six Karen participants from whom we collected samples in Thailand, prior to their relocation to the United States. Using deep shotgun metagenomics sequencing on 13 samples from these 6 participants, we found that *Prevotella* and *Bacteroides* strain profiles remained largely stable over 6 months but sometimes underwent substantial changes (subject highlighted in blue, Figure 7E). We observed in general that relocating to the United States induced a variety of short-term gut microbiome responses, including disruption to the gut microbiome immediately after arrival in two subjects (ID.273 and ID.304), expansion of opportunistic pathogens (ID.305), gut disruption several months after arrival (ID.275), and stability (ID.274, ID.308) (Figure 7F). Thus, we found that short-term responses to immigration of overall microbiome composition were variable across individuals, but the displacement of dominant native taxa with dominant US taxa begins within 6 to 9 months of US residence.

DISCUSSION

This article reports the first large-cohort study of the effects of migration from a non-Western country to a Western country on the human gut microbiome. In multi-ethnic, multi-generational cohorts of immigrants and refugees, we observed that gut microbiome diversity, function, and strain composition are strongly impacted by US immigration and that both short-term and long-term US residence and being born in the United

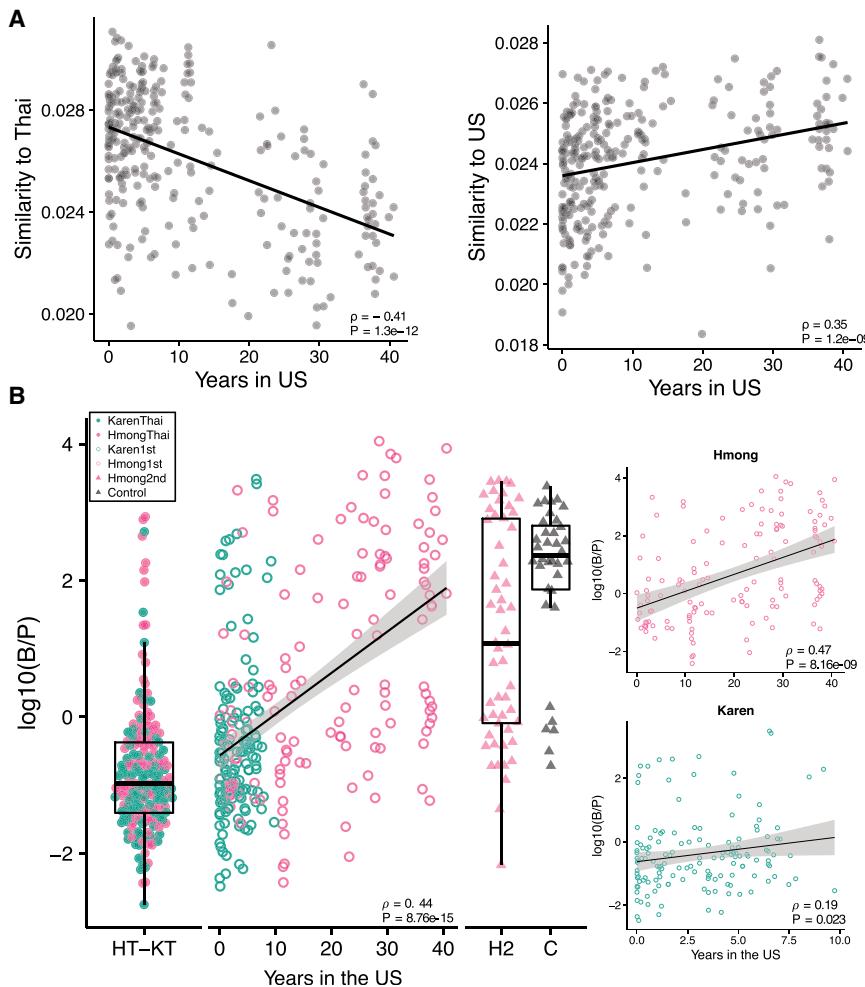


Figure 6. Prevotella Displacement Continues over Decades of US Residence

(A) Similarity (1/Aitchison's distance) of microbiomes relative to Thai-based groups (Spearman's correlation, $\rho = -0.41$, $P = 1.3 \times 10^{-12}$) and to controls (Spearman's correlation, $\rho = 0.35$, $P = 1.2 \times 10^{-9}$) (gray area represents 95% confidence interval). See also Figure S6A.

(B) Log ratio of *Bacteroides* to *Prevotella* of first-generation groups is significantly correlated to years spent in the United States (Spearman's correlation, $\rho = 0.44$, $P = 8.76 \times 10^{-15}$). Significantly correlated trends persist after stratification by ethnicity (Spearman's correlation, Hmong $\rho = 0.47$, $P = 8.16 \times 10^{-19}$; Karen $\rho = 0.19$, $P = 0.023$). HT, HmongThai; KT, KarenThai; H2, Hmong2nd; C, controls; 0–40 = years spent in the United States by Hmong1st and Karen1st (gray area represents 95% confidence interval). See also Figure S6B.

States shift an individual's microbiome along an axis toward a more Westernized state. Even a short period of residence in the United States was sufficient to induce pronounced increases, in some cases over 10-fold, in the ratio of *Bacteroides* to *Prevotella*. Metagenome assembly showed that the observed loss of *Prevotella* strains was associated with loss of carbohydrate-active enzymes dominant in the gut microbiota, including a near-complete loss of certain β -glucanases and other glycoside hydrolases that break down specific dietary fibers. Previous studies have demonstrated intergenerational effects of microbiome perturbations in animal models. These include loss of microbiota carbohydrate degradation function following removal of dietary fiber (Sonnenburg et al., 2016) and intergenerational loss of diversity following antibiotic perturbation (Schulfer et al., 2018). The data presented here extend these findings to humans by providing evidence that compounded intergenerational loss of taxonomic and functional diversity is occurring in US immigrant populations, supporting the model of disappearing human microbiota proposed by Blaser and Falkow (2009).

We also performed extensive analysis and modeling of differences in dietary intake, as diet is known to be a strong driver of

microbiome variation (Bokulich et al., 2016; David et al., 2014; Muegge et al., 2011). Although we observed clear patterns of dietary acculturation associated with US residence, dietary variation only partly explained microbiome variation across individuals. Interestingly, the diets of second-generation immigrants remained quite distinct from the controls, while their microbiomes did not. It is possible that different diets are driving the microbiome toward a similar state; an alternative explanation is that a limited set of metabolic capabilities in the microbiome are transmitted from one generation to the next, resulting in decreased overall functionality with each successive generation, consistent with the disappearing microbiota model.

This study has several limitations. Immigration-related microbiome changes are likely driven by a combination of diet and other factors associated with adjustment to life in the United States, and most of these factors were not examined in the context of this study. These include changes in exposure to stress, exercise, municipal drinking water, antibiotics, and treatment with antiparasitics. In addition, our study design did not allow us to test directly whether immigration causes the observed changes in the microbiome nor whether changes in microbiome are directly contributing to the high incidence of obesity in US immigrants.

Our findings demonstrate that US immigration is associated with profound perturbations to the gut microbiome, including loss of diversity, loss of native strains, loss of fiber degradation capability, and shifts from *Prevotella* dominance to *Bacteroides* dominance. These changes begin immediately upon arrival, continue over decades of US residence, and are compounded in obese individuals and in second-generation immigrants born in the United States. These results improve our fundamental understanding of how

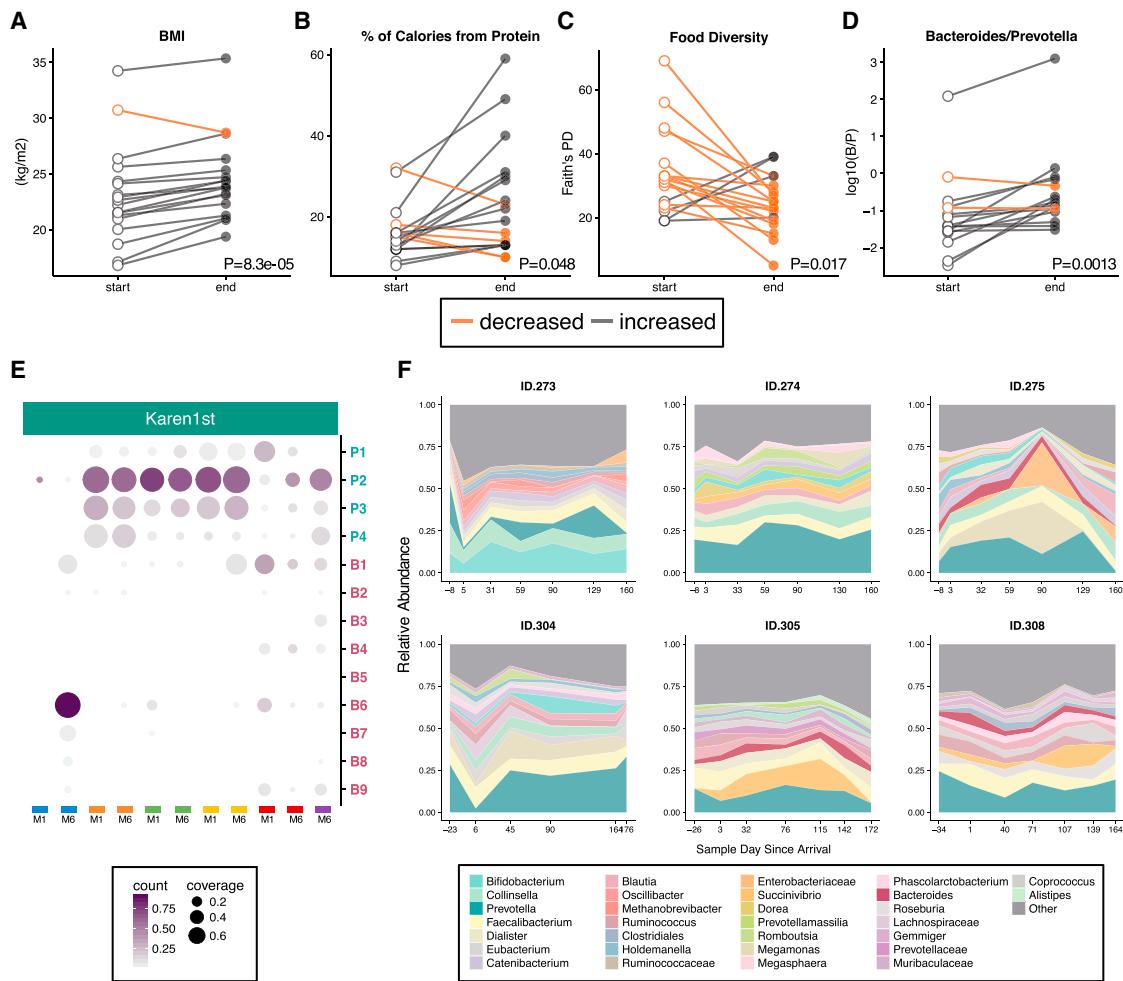


Figure 7. Longitudinal Microbiome Variation during Relocation to the United States

(A–D) (A) Comparison of per-participant changes between first and last months of the study in BMI (paired t test, $p = 8.3 \times 10^{-5}$), (B) protein consumption (paired t test, macronutrients adjusted for multiple comparisons using FDR < 0.05 , $p = 0.048$), (C) dietary diversity (Faith's PD) (paired t test, $p = 0.017$), and (D) *Bacteroides*-to-*Prevotella* ratios (paired t test, $p = 0.0013$).

(E) *Bacteroides* and *Prevotella* strain profiles are mostly stable after 6 months. Samples (columns) from the same participant are denoted by color, and M1 and M6 correspond to month 1 sample and month 6 sample, respectively. Selected strains are identical to Figure 3B (at least 50% coverage per sample across n = 55 samples; see Table S5).

(F) Taxonomic area charts of relative abundances of dominant genera (other taxa not shown) in six individuals who began the longitudinal study while in a refugee camp in Thailand and then continued after relocation to the United States. First available samples were collected 6 to 34 days before departure, and second samples were collected 1 to 6 days after arrival to the United States.

See also Figure S7.

migration affects the human microbiome and underscore the importance of considering the impact of the gut microbiome in future research into immigrant and refugee health.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and six tables and can be found with this article online at <https://doi.org/10.1016/j.cell.2018.10.029>.

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AUTHOR CONTRIBUTIONS

Conceptualization, P.V., K.A.C.-P., and D.K.; Methodology, P.V., K.A.C.-P., R.B., S.L.P., C.A., T.L.W., L.M.T., L.K.B., S.K.L., D.M., R.M., P.C.K., and D.K.; Software, G.A.A.-K., B.M.H., and A.D.K.; Formal Analysis, P.V., R.R.S.-C., A.J.J., and D.K.; Investigation, C.A., R.M., B.P., E.C.M., P.S., and M.X.; Data Curation, G.K.; Writing—Original Draft, P.V. and D.K.; Writing—Review and Editing, K.A.C.-P., S.L.P., C.A., L.K.B., S.K.L., E.A.T., E.C.M., D.M., P.C.K., R.R.S.-C., P.V., and D.K.; Visualization, P.V., R.R.S.-C., A.J.J., and D.K.; Supervision, D.K., K.A.C.P., and S.L.P.; Project Administration, P.V.; Funding Acquisition: P.V. and D.K.

DECLARATION OF INTERESTS

D.K. serves as CEO and holds equity in CoreBiome, a company involved in the commercialization of microbiome analysis. The University of Minnesota also has financial interests in CoreBiome under the terms of a license agreement with CoreBiome. These interests have been reviewed and managed by the University of Minnesota in accordance with its Conflict-of-Interest policies.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
16S rRNA gene and shotgun metagenomics DNA sequencing data	European Nucleotide Archive	Primary accession # PRJEB28687; RRID: SCR_006515
RefSeq version 87	O'Leary et al., 2016	ftp://ftp.ncbi.nlm.nih.gov/genomes/refseq/bacteria
Software and Algorithms		
Custom analysis scripts	This paper	https://github.com/knights-lab/IMP_analyses
BURST DNA aligner	Al-Ghalith and Knights, 2017	https://github.com/knights-lab/BURST
HUMAnN2 metabolic pathway annotation	Abubucker et al., 2012	http://huttenhower.sph.harvard.edu/humann

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dan Knights (dknights@umn.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study setting, population, and recruitment

Our inclusion criteria included individuals who were Hmong or Karen, female, at least 18 years old, and either were born and are currently living in Thailand, were born in Southeast Asia and moved to the U.S., or were born in the U.S. but whose parents were born in Southeast Asia. Our inclusion criteria for controls included European American females at least 18 years of age who were born in the U.S. and whose parents and grandparents were also born in the U.S. Our exclusion criteria consisted of use of any antibiotics in the previous 6 months, current use of probiotic supplements, known presence of gastrointestinal, cancer, immunodeficiency or autoimmune disorders, adults lacking capacity to consent, or pregnancy. Additionally, control subjects could not have traveled outside of the U.S. within the last 12 months. We recruited using multiple methods which included flyers, emails, social media, oral presentations, tabling, letters followed by phone calls to West Side Community Health Services (West Side) patients who met criteria, and by word of mouth. We recruited throughout the Minneapolis-St. Paul metro area at local community centers, faith-based organizations, adult education centers, health care centers, and health fairs. We recruited in Thailand at Khun Chang Khian (KCK), a rural Hmong village located one hour from Chiang Mai city, as well as from Mae La (ML) Camp, a Burmese refugee camp in Tak province located on the Myanmar-Thailand border (Figure S1). Interested subjects were then screened and interviewed privately or as a group, as preferred by the participants. Interviews and body measurements were conducted by trained Hmong and Karen community researchers and a graduate student researcher. This study was approved for human subject research by the University of Minnesota Institutional Review Board (1510S79446), and the Thailand-based portion of the study was additionally approved for human subject research by the Chiang Mai University Institutional Review Board (475/2015) and the Chiang Mai Public Health Office (0032.002/9930). Informed consent was obtained from all subjects.

Community-based Research methods

This project used a community-based participatory action research (CBPAR) approach, with a multidisciplinary team composed of academic researchers, Hmong and Karen community researchers, and staff from the Somali, Latino and Hmong Partnership for Health and Wellness (SoLaHmo). SoLaHmo is a multi-ethnic, community-driven CBPAR program of West Side Community Health Services, whose mission is to build upon the unique cultural strengths of ethnic communities to promote health and wellness through research, education and policy. All SoLaHmo members are trained in qualitative research processes using a previously developed training curriculum (Allen et al., 2011). In addition, all phases of our project were further guided by community advisory boards (CABs) composed of Hmong and Karen health professionals and community experts. The study design, recruitment methods and strategies, and dissemination of results were developed in partnership with both academic and community researchers, and through multiple discussions with the CABs. As noted in Results, we learned from the Hmong CAB and research team members that substantially more Hmong women than men were relocating to U.S. in recent years. Thus, to ensure feasibility of recruitment for this study we limited our population to women. In Thailand, we used a modified CPBAR approach in that Thai community researchers were members of the communities that we worked with, and were trained with qualitative research methods, recruitment, and sample and data collection, but were not directly involved with study design. We note that Hmong refugee camps have long been closed (Bureau of

[Population, Refugees and Migration, 2004](#)), hence Hmong in Khun Chang Khian are not refugees but serve as acceptable pre-immigration representatives available for US-based Hmong.

Cross-sectional specimen and data collection

For U.S. sample collection, research team members obtained informed consent and conducted interviews in the participants' preferred languages (English, Hmong, or Karen), and recorded participants' responses onto an English paper survey. Weights were measured using standard electronic scales, heights were measured against a wall using a pre-positioned measuring tape, and waist circumferences were measured with a tape measure at the uppermost lateral border of the iliac crest ([Center for Disease Control, 2007](#)). 24-hour dietary recalls were conducted using a multiple pass system ([Tippett et al., 1999](#)) with food models and measuring cups and spoons for portion size estimations. Participants were provided with a stool collection kit and instructions describing how to collect a stool sample. Stool samples were collected into preservative (see below) and were either returned to the research staff by mail or were stored at room temperature for up to 5 days before they were collected by the research team.

Procedures for consent, interviews, anthropometrics, and stool sampling in Thailand were as described above for the cross-sectional specimen and data collection. 24-hour dietary recalls and sample collections were conducted as described previously. Stool samples from KCK were transported on dry ice then placed in a -20C freezer for 2 days then transferred to a -80C freezer. Stool samples from ML were placed in a -20C freezer for up to 8 hours then transferred to a -80C freezer. All samples collected in Thailand were shipped overnight on dry ice from Thailand to the U.S., and stored in a -80C freezer in the U.S.

Research team members instructed participants in stool collection, using an instructional video, written visual instructions, and verbal reinforcement. Participants placed their stool sample onto a FecesCatcher (Tag Hemi VOF) and 1 g was collected using a sterile swab into a 1.5 mL cryogenic tube pre-filled with 900 ul of RNALater and mixed thoroughly. Larger samples (longitudinal first and last month samples) were collected using a Sarstedt 80.9924.014/CS500 tube and scoop without mixing or RNALater. Large samples collected in the U.S. were aliquoted into 1.5 mL tubes with and without 50% glycerol upon arrival and stored at -80C. Large samples collected in Thailand were stored at -80C until arrival to the U.S., at which point they were thawed over ice, aliquoted, and stored in the same manner.

Longitudinal specimen and data collection

Procedures for consent, interviews, anthropometrics, and stool sampling were as described above for the cross-sectional specimen and data collection. Once per month over six months, 24-hour dietary recalls were conducted as described previously. Month 1 and 6 samples were stored in a home freezer and picked up within 24 hours of stool collection. These samples were transported with an ice pack and immediately placed in a -80C freezer. Month 2-5 samples were stored in preservative (see below), mailed to the research team in prepaid mailers at room temperature, and placed in a -80C freezer upon receipt.

METHOD DETAILS

Dietary data processing

De-identified survey data was entered into an electronic spreadsheet. Foods and portions from 24-hour dietary recalls were entered into the USDA SuperTracker system ([Britten, 2013](#)). Foods that were not found in the USDA database were studied individually ([Speak et al., 1991](#)) for macronutrient content and entered in as custom foods. SuperTracker macronutrient and food grouping summaries, as well as foods and their respective portions were downloaded directly from the SuperTracker website or using custom Python ([van Rossum and Drake, 2011](#)) scripts. Foods and portions were mapped to the SuperTracker and USDA databases to obtain respective food and portion identification numbers; food and portion identification numbers were used in tree-based food analysis. Custom foods not in the USDA database were manually assigned appropriate existing or new food identification numbers by group consensus. Micronutrients were excluded from dietary analyses due to the high number of custom foods with limited information on micronutrients.

16S ribosomal RNA gene DNA sequencing

All fecal samples were submitted to the University of Minnesota Genomics Center (UMGC) for DNA extraction, amplification, and sequencing. 16S ribosomal rRNA gene sequences were extracted and amplified following the UMGC-developed protocol ([Gohl et al., 2016](#)).

Shotgun metagenomics DNA sequencing

Shotgun DNA sequencing was performed on the Illumina HiSeq platform. All fecal samples were submitted to the UMN Genomics Center for DNA extraction, amplification, and sequencing. Amplification, quantification, and normalization of extracted DNA was performed using the Illumina NeoPrep Library System. A HiSeq 2x125 cycle v4 kit was used to sequence samples.

QUANTIFICATION AND STATISTICAL ANALYSIS

16S sequencing analysis

We trimmed and processed all 16S marker-gene sequencing data for quality using SHI7 ([Al-Ghalith et al., 2018](#)) and picked *de novo* operational-taxonomic units (OTUs) as follows. We first filtered for reads with at least 100 exact duplicates as representative

sequences, and assigned taxonomy by alignment at 0% to the NCBI RefSeq 16 s reference database (O'Leary et al., 2016) using the BURST (Al-Ghalith and Knights, 2017) OTU-picking algorithm in CAPITALIST mode, which ensures optimal alignment of sequences and minimizes the set of aligned reference genomes. All original sequences were then re-aligned with BURST (Al-Ghalith and Knights, 2017) in CAPITALIST mode at 98% identity against this representative set, resulting in 93.54% of all available sequences aligned. Singleton OTUs and samples with depth less than 2,143 were removed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (Caporaso et al., 2010). Using QIIME, we measured within-sample biodiversity (alpha diversity) with rarefied OTU tables (at 2,143 sequences/sample) using whole-tree phylogenetic diversity (Faith, 1992) and a custom generated phylogeny constructed with the representative sequences using aKronyMer (Al-Ghalith and Knights, 2018). To quantify differences in composition between subjects, we calculated the phylogeny-based UniFrac distance (Lozupone et al., 2011) between all pairs of samples. To visualize between-subject differences (beta diversity) and to obtain principal components for subsequent statistical testing, we performed dimensionality reduction using principal coordinates analysis (Caporaso et al., 2010). Aitchison's distances were calculated by first imputing zeros from an abundance OTU table, then applying a centered log ratio transform using the *robCompositions* R package (Pawlowsky-Glahn and Buccianti, 2011). To enable tests for shifts in the relative abundances of *Bacteroides* and *Prevotella*, we collapsed the reference-based OTUs according to taxonomy at the genus level. P values, sample numbers, and names of statistical tests are provided in the main text and figure legends for Figures 2A, 2B, 3A, 3C, 4A, 4B, 5A–5C, 6A–6C, and 7A–7D.

Shotgun metagenomics analysis

Shotgun metagenomics sequences were identified at the species level via genomic alignment against a custom database created from aligning human samples from various public datasets against the comprehensive NCBI RefSeq database (Tatusova et al., 2013) release 87, and all matched bacterial species, as well as all species in matched representative genera, were included. Genome coverage estimates were calculated using the *bcov* utility from BURST (Al-Ghalith and Knights, 2017). Functional annotations were obtained using the HUMAnN2 (Abubucker et al., 2012) pipeline with UniRef50 (Suzek et al., 2015). Resulting functional pathways were mapped to and colored by the top-level categories of the MetaCyc (Caspi et al., 2008) ontology. CAZyme annotations were obtained using metaSPAdes (Nurk et al., 2017), filtered for scaffolds with minimum 1000 bp, then further processed with Prokka (Seemann, 2014), dbCAN (Yin et al., 2012) with E-value < 1e-5, and the CAZy database (Lombard et al., 2014). Taxonomic contributions of differentiated glycoside hydrolases were identified as follows: (1) scaffolds that contributed to GH17, GH64, GH87 were identified and respective DNA sequences were obtained and used as a reference database, (2) shotgun metagenomic reads were quality filtered as described previously, (3) quality reads were aligned against the scaffold reference database using BURST (Al-Ghalith and Knights, 2017) at 95% identity, (4) quality filtered reads from step 2 were aligned with BURST at 98% identity against the previously described custom database with taxonomy assigned from the NCBI database, (5) sequences that hit both the scaffolds reference and the custom NCBI-based reference were used to construct an OTU table.

Dietary data analysis

Food tree visualizations were generated with GraPhlAn (Asnicar et al., 2015). Dietary record and food item associations were generated using custom scripts, then visualized in Cytoscape (Shannon et al., 2003). Food-Microbiome Procrustes distance association P values are from the “vegan” implementation in function “protest” with 999 permutations (performed for each of the permuted data structures).

DATA AND SOFTWARE AVAILABILITY

Software

Software used to perform statistical testing and generate figures for this manuscript are available here: https://github.com/knights-lab/IMP_analyses.

Data Resources

The 16S rRNA gene and shotgun metagenomic sequencing data have been deposited in the European Nucleotide Archive under accession number PRJEB28687.

Supplemental Figures

Cell

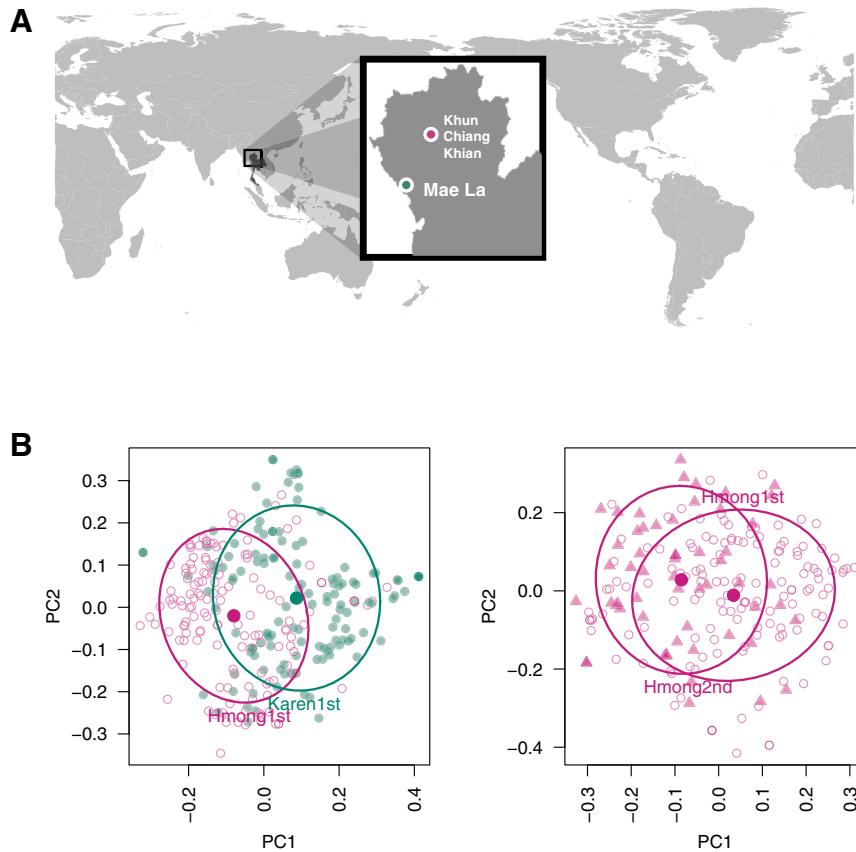


Figure S1. Geographical Locations of Recruitment Sites in Thailand and Validation of Food Tree for Separating Immigrant Groups, Related to Figure 1

(A) Khun Chang Khian in Chiang Mai province and Mae La camp in Tak Province.

(B) Principal coordinates analysis of tree-based unweighted UniFrac diet distances between Karen1st and Hmong1st (left) (Adonis F statistic = 43.85, $p < 0.001$) and between Hmong1st and Hmong2nd (right) (Adonis F statistic = 13.05, $p < 0.001$), showing the ability of the method to discriminate between these immigrant groups.

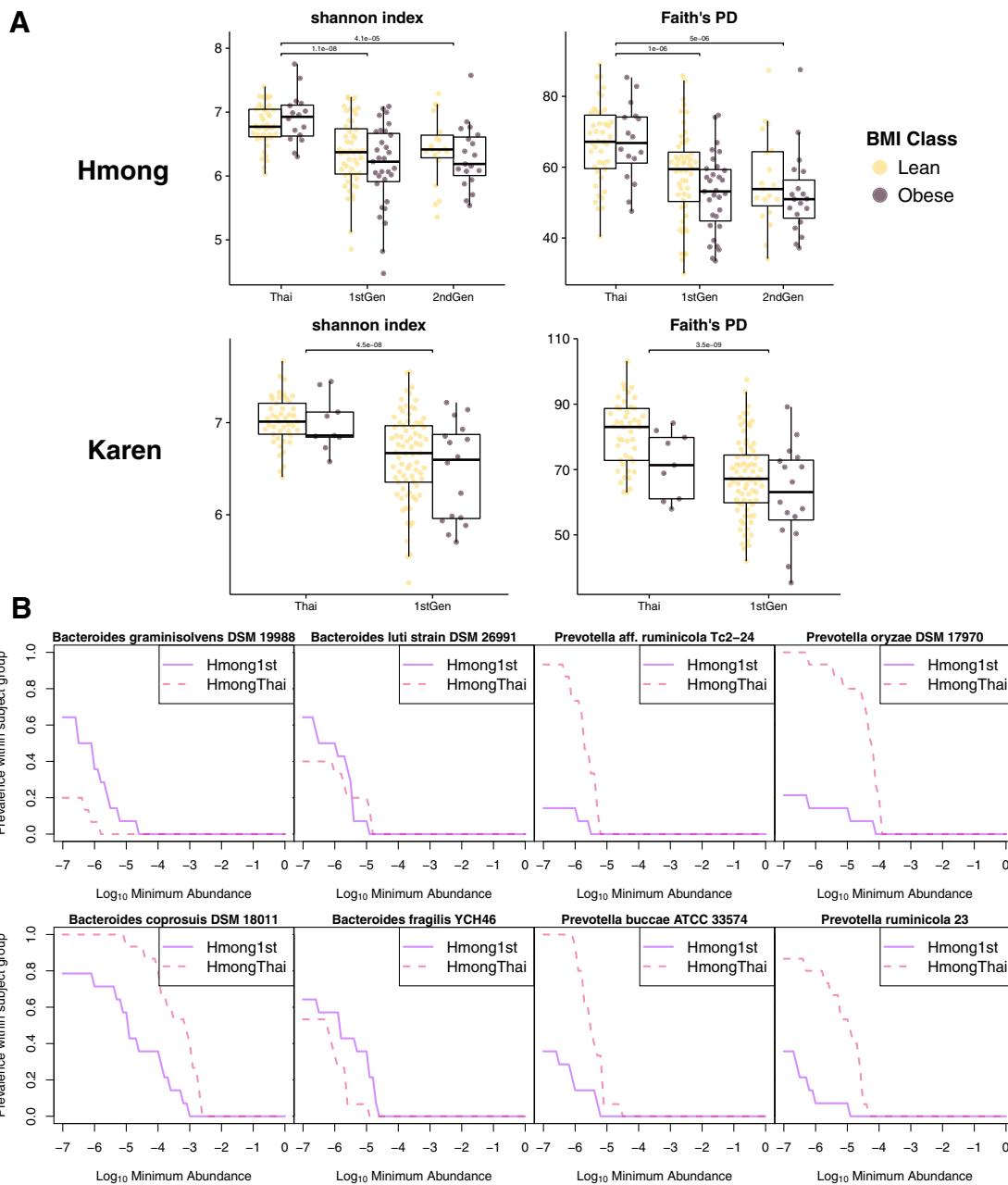


Figure S2. Alpha Diversity Boxplots of Obese and Lean Individuals Separated by Ethnicity and Prevalence-Abundance Analysis, Related to Figure 2

(A) Post hoc analysis with Tukey's HSD test across sample groups ($p < 0.01$).

(B) Prevalence-abundance curves comparing the four *Bacteroides* (top) and four *Prevotella* (bottom) strains with the largest change in overall prevalence between Hmong1st and HmongThai.

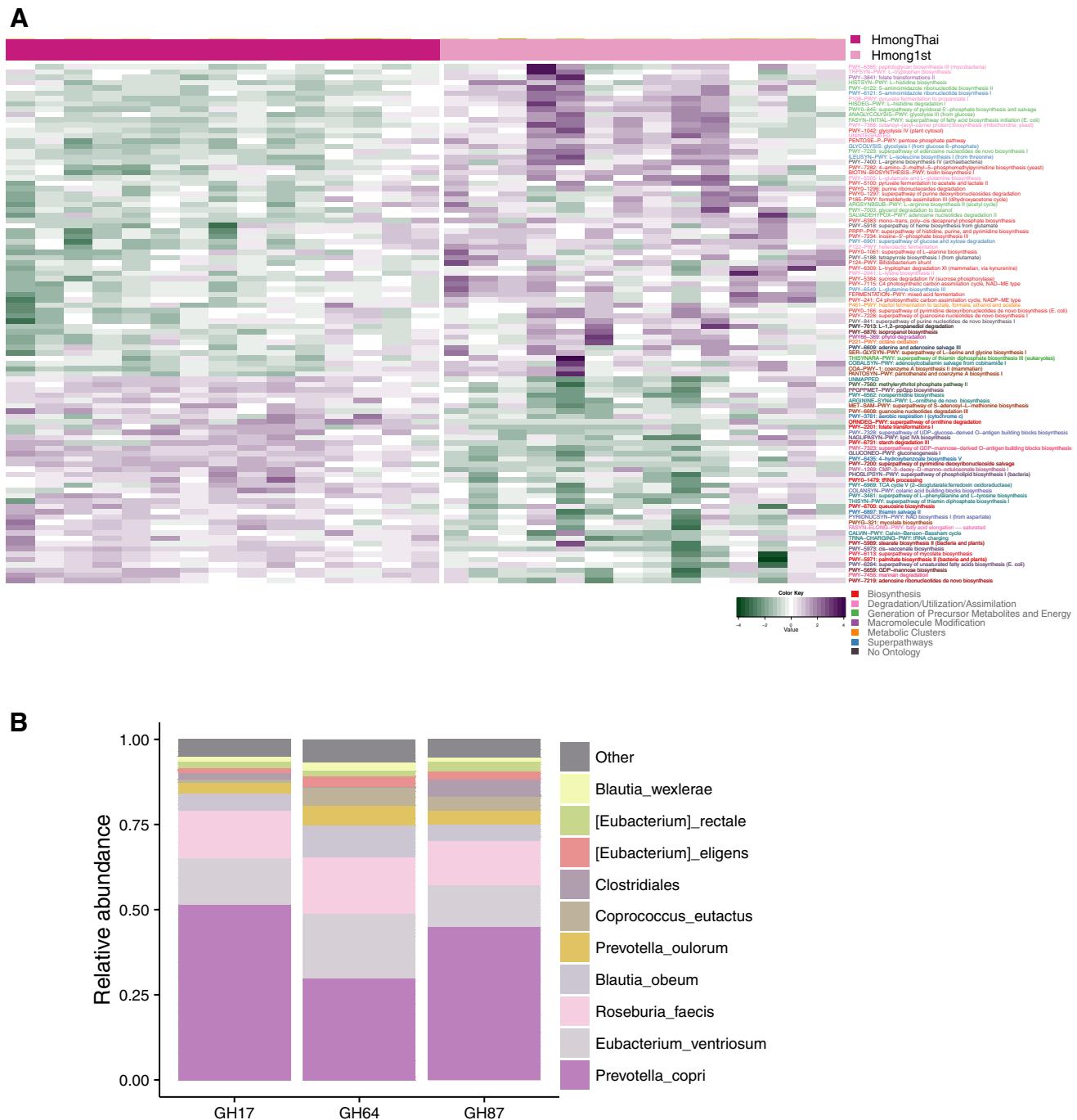
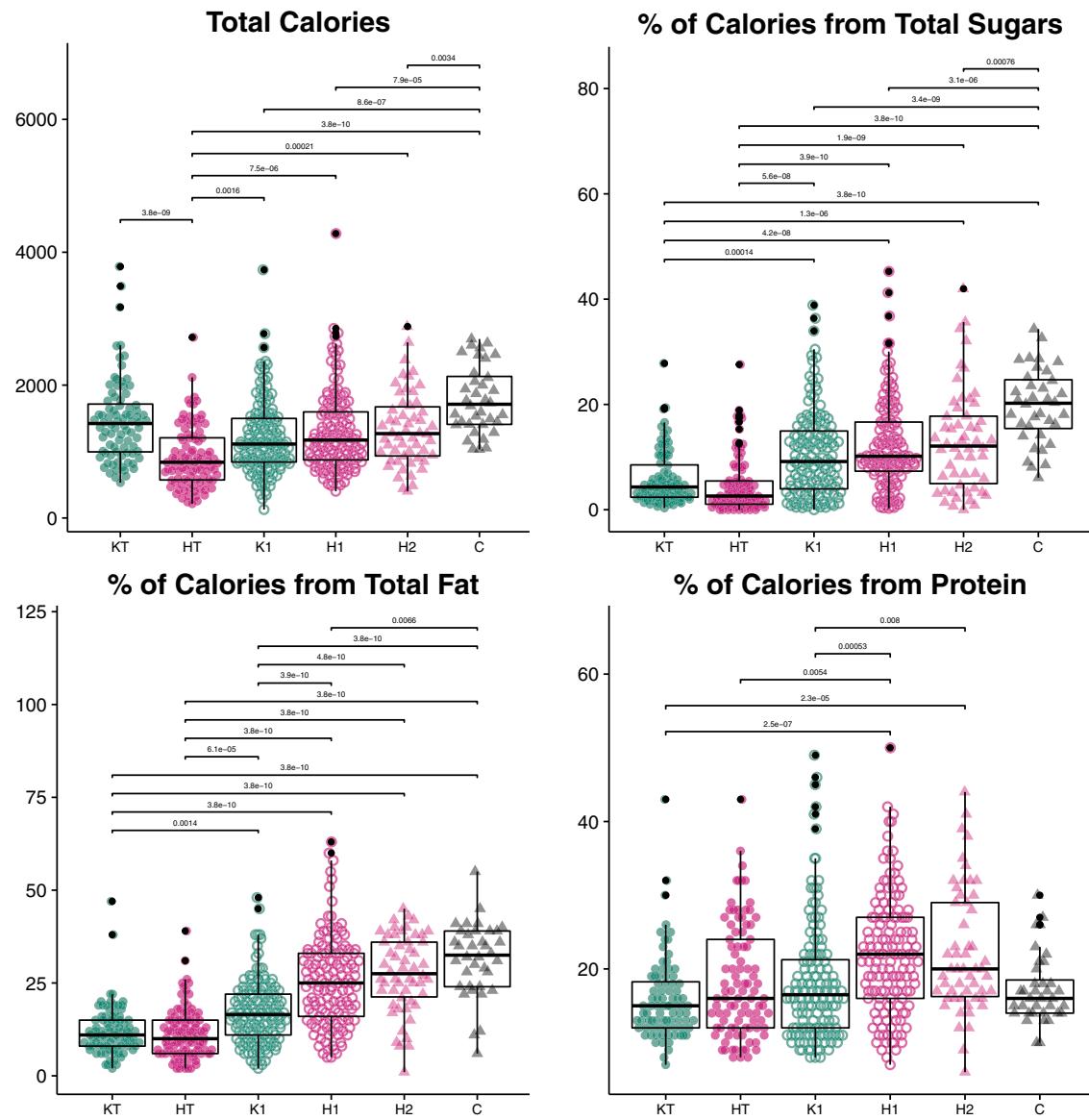


Figure S3. Functional Annotations and Glycoside Hydrolase Taxonomic Contributions, Related to Figure 3

(A) Differentiated relative abundances of functional pathways between HmongThai and Hmong1st (asin-sqrt transformed abundances, ANOVA, FDR-corrected $q < 0.10$).

(B) Taxonomic contributions of scaffolds contributing to beta-glucan-targeting glycoside hydrolases.

**Figure S4. Macronutrient Pairwise Comparisons, Related to Figure 4**Pairwise comparisons with Tukeys' HSD, significant p values < 0.01 are shown.

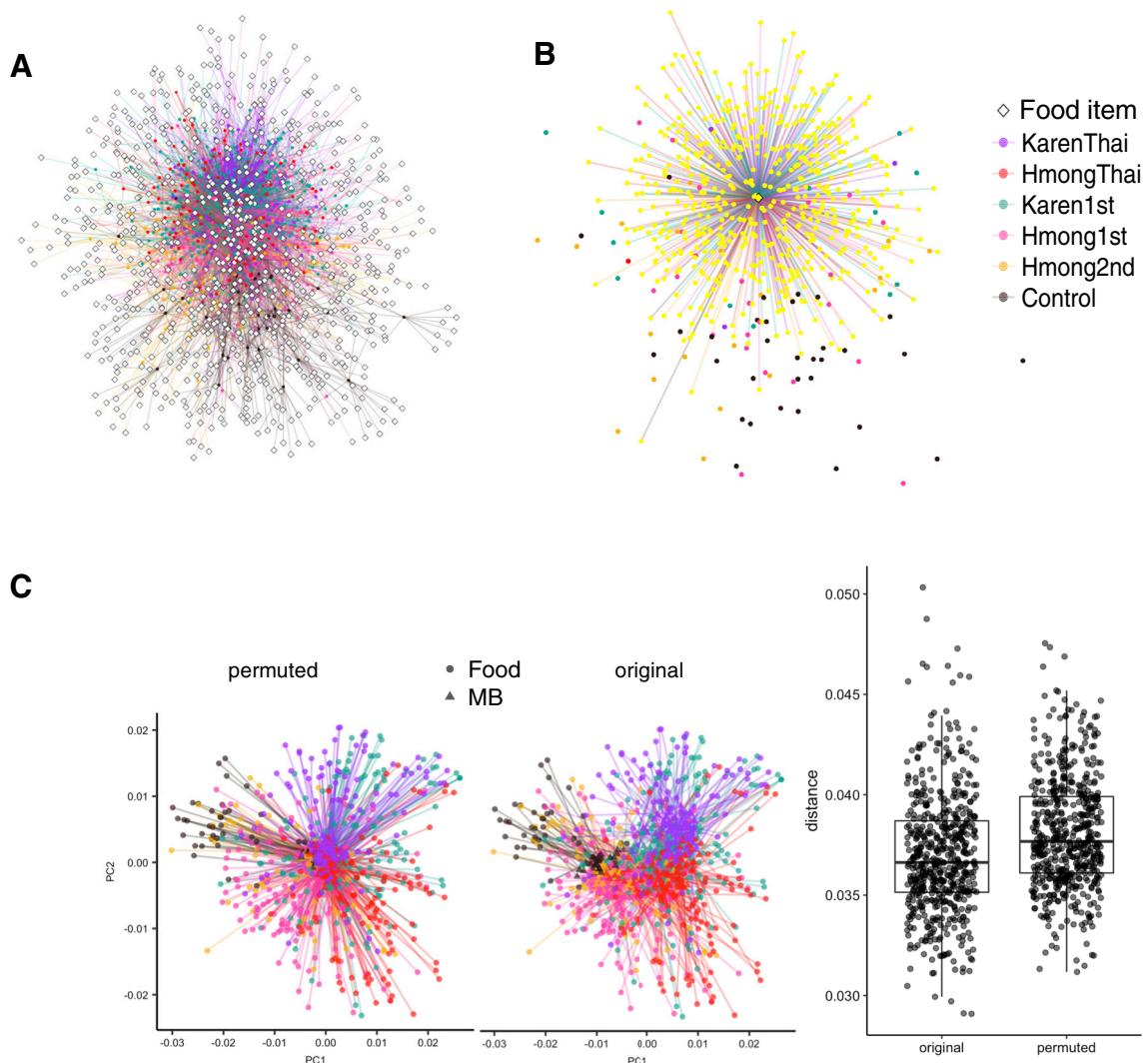


Figure S5. Bipartite Network of Participant Dietary Records and Food Items, Related to Figure 4

(A) Edges and participants are colored by sample group, and food items are shown as white-filled diamonds.

(B) We highlight the high prevalence of rice consumption. Participants who consumed rice are denoted as yellow nodes and yellow edges connected to the centroid (rice), otherwise participants were colored by sample group.

(C) Procrustes permutation shows significant relatedness between individuals' food and microbiome profiles. Procrustes PCoA for a representative permutation (median of 9) and the original data (left), and a boxplot of distances between each individuals' food and microbiome data in the original and permuted data after Procrustes rotation (distances are smaller in original data, Mann Whitney U test $p = 1e-10$).

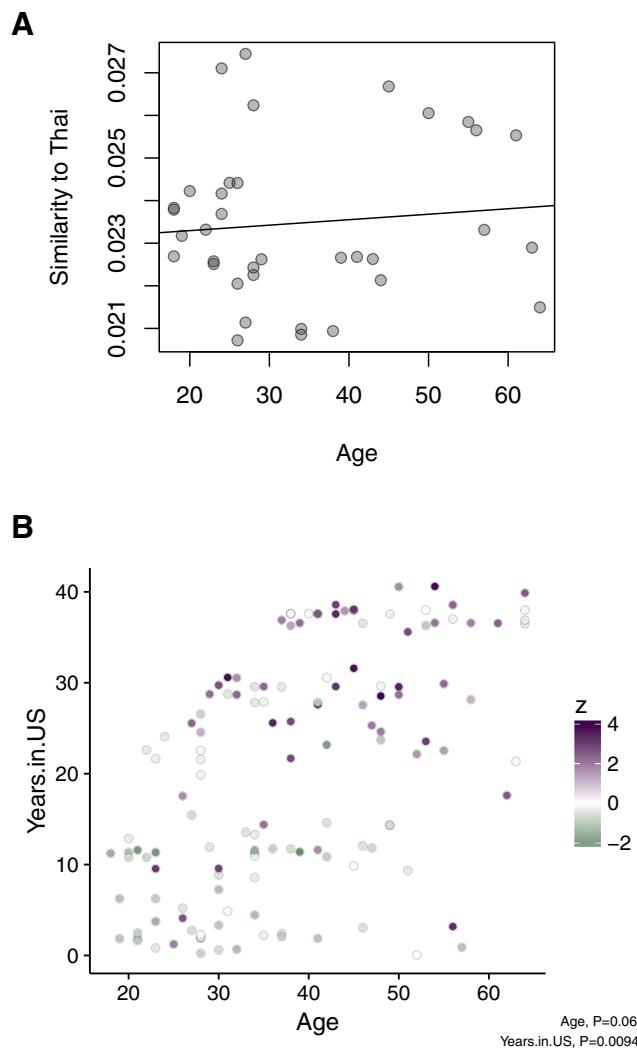


Figure S6. Bipartite Network of Participant Dietary Records and Food Items, Related to Figure 6

(A) Scatterplot of overall microbiome similarity (1 / Aitchison distance) of European Americans to the HmongThai reference group against Age with best-fit line (linear regression Age $p = 0.57$).

(B) Scatterplot of years in U.S. against Age in Hmong1st, colored by *Bacteroides-Prevotella* ratio. Years in U.S. was significantly associated with the B-P ratio in a multiple linear regression ($p = 0.0094$) while Age was not ($p = 0.065$).

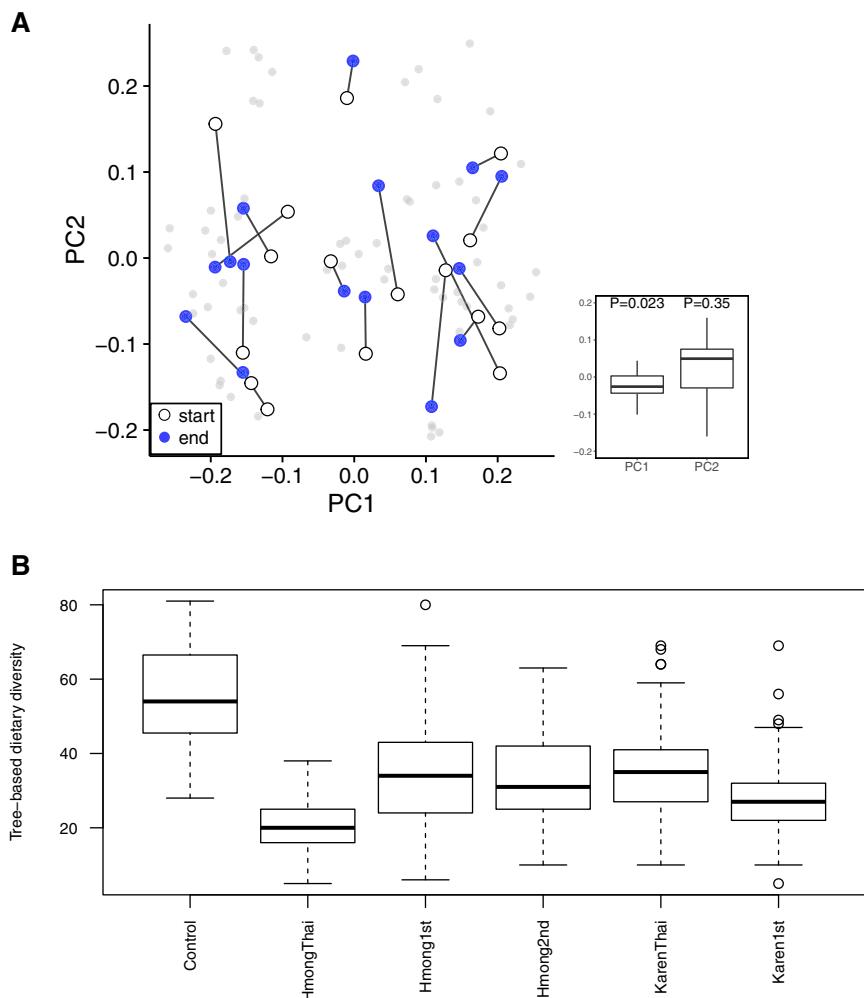


Figure S7. PCoA of Unweighted UniFrac Distances of Longitudinal Samples, Related to Figure 7

(A) First and last month samples are highlighted and connected by participant, with all intermediate monthly samples in gray. Inset shows the within-individual changes along PC1 and PC2 from first to last months (one sample t test, PC1 $p = 0.023$, PC2 $p = 0.35$).

(B) Boxplot of tree-based dietary diversity by study group.