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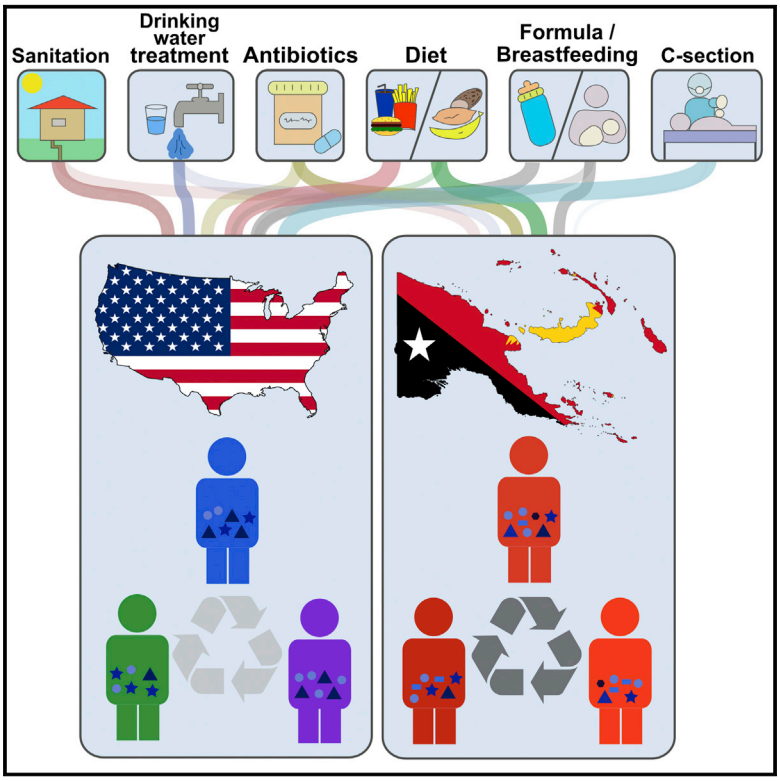
The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes

Graphical Abstract



Highlights

- The fecal microbiota in PNG is more diverse but less individualized than in the US
- Most bacterial species are shared among PNG and the US, but abundance profiles differ
- Impact of lifestyle on ecological assembly processes might explain these patterns
- Westernization may decrease bacterial dispersal rates, altering microbiota structure

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In Brief

The gut microbiome differs substantially between westernized and non-industrialized societies. Martínez et al. characterize the fecal microbiome structure, diversity patterns, and assembly processes in rural Papua New Guineans and United States residents and propose a model by which reduced bacterial dispersal due to modern lifestyle practices causes microbiome alterations associated with westernization.

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The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes

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SUMMARY

Although recent research revealed an impact of westernization on diversity and composition of the human gut microbiota, the exact consequences on metacommunity characteristics are insufficiently understood, and the underlying ecological mechanisms have not been elucidated. Here, we have compared the fecal microbiota of adults from two non-industrialized regions in Papua New Guinea (PNG) with that of United States (US) residents. Papua New Guineans harbor communities with greater bacterial diversity, lower inter-individual variation, vastly different abundance profiles, and bacterial lineages undetectable in US residents. A quantification of the ecological processes that govern community assembly identified bacterial dispersal as the dominant process that shapes the microbiome in PNG but not in the US. These findings suggest that the microbiome alterations detected in industrialized societies might arise from modern lifestyle factors limiting bacterial dispersal, which has implications for human health and the development of strategies aimed to redress the impact of westernization.

INTRODUCTION

The human gastrointestinal tract is colonized by an abundant and diverse microbial consortium (the gut microbiota) that impacts host physiology and health. Recent research in animal models has demonstrated an essential contribution of the gut microbiota in non-communicable diseases that have higher prevalence in westernized societies (western diseases), such as inflammatory bowel disease, autoimmune diseases (i.e., multiple sclerosis, type 1 diabetes, and rheumatoid arthritis), obesity-associated metabolic aberrancies, allergies, and colon cancer (Berer et al., 2011; Devkota et al., 2012; Koeth et al., 2013; Noval Rivas et al., 2013; Ochoa-Repáraz et al., 2010; Trompette et al., 2014; Wen et al., 2008). Epidemiological data further support that lifestyle practices (caesarian sections, antibiotic use, and formula feeding of infants) that affect the assembly of the microbiota are associated with an increased risk of disease (Conradi et al., 2013; Marra et al., 2009; Risnes et al., 2011; Tenconi et al., 2007). These observations have led scientists to hypothesize that aberrant (dysbiotic) microbiomes (Noverr and Huffnagle, 2005) and/or the loss of specific symbionts (Blaser and Falkow, 2009) predispose westerners to non-communicable diseases. On the other hand, non-industrialized societies are burdened with a high incidence of infectious diseases, including life-threatening diarrhea (Pop et al., 2014). The importance of the gut microbiome for non-communicable diseases in westernized societies and the prevalence of infectious diseases in non-industrialized communities warrant studies that compare the microbiome in both settings.

To determine how lifestyle, and especially westernization, resonates in the structure of the human gut microbiome, scientists have begun to systematically compare the fecal microbiota of humans from non-industrialized societies to those with a westernized lifestyle (De Filippo et al., 2010; Schnorr et al., 2014; Yatsunenko et al., 2012). Studies to date have compared the gut microbiome of Europeans and Americans to that of children in Burkina Faso (De Filippo et al., 2010), children and adults in Malawi and Amazonian Amerindians (Yatsunenko et al., 2012), and adult Hadza hunter-gatherers in Tanzania (Schnorr et al., 2014). Collectively, these studies have revealed higher fecal bacteria α diversity (within individuals) and lower β diversity (between individuals) in non-industrialized societies. These diversity patterns were accompanied by major compositional differences, likely reflecting distinct dietary habits, such as higher proportions of fiber-utilizing bacteria and lower abundances of bacterial lineages associated with intake of animal-derived products. Although these studies have begun to unravel the biogeographic

variations of the human microbiome and the impact of westernization, the exact repercussions are still insufficiently understood, and we lack a conceptual understanding on how modern lifestyle alters the gut microbiota.

An understanding of the factors that drive distinct microbiome configurations across human populations will require the application of ecological theory. The collection of microbes associated with a human population can be conceived as a co-evolved metacommunity, in which individuals represent local, island-like habitats occupied by spatially separated microbial assemblies linked through the transmission and dispersal of symbionts (Costello et al., 2012; Dethlefsen et al., 2007; Leach, 2013; Mihaljevic, 2012). An application of Vellend's general synthesis in community ecology (Vellend, 2010) toward microbial ecosystems postulates that diversity at local scales is shaped by a combination of only four processes: selection, drift, diversification, and dispersal (Costello et al., 2012; Nemergut et al., 2013). As in other ecological communities, it is likely that these processes operate in combination to govern the assembly of the gut microbiota (Walter and Ley, 2011). The temporal characteristics of the adult human fecal microbiota, as well as theoretical model calculations, indicate that neutral processes are unlikely to contribute significantly to gut microbiota assembly (Jeraldo et al., 2012; Martínez et al., 2013b). Still, the relative contribution of the ecological processes and the impact of geography, environment, and lifestyle remain largely undetermined. Clearly, an ecologic perspective based on theory can provide a framework by which to interpret microbiome configurations in different human populations and infer how environment and lifestyle impact these patterns. Such an approach can be combined with quantitative analyses developed by community ecologists to characterize the simultaneous influence of ecological processes in shaping communities (Stegen et al., 2012, 2013).

The goal of this study was to apply such an ecological framework and compare the fecal microbiome of individuals from Papua New Guinea living a traditional lifestyle with that of United States (US) residents. Papua New Guinea (PNG) is one of the most diverse countries in the world, with 823 languages spoken according to the 2000 PNG census (about one-quarter of the world's languages; UNESCO, 2002; Frawley, 2003) and a similar number of ethnic groups. PNG remains one of the least urbanized countries in the world, and for several PNG populations, contact with outside communities remains limited. The majority of Papua New Guineans live a traditional, subsistence agriculture-based lifestyle. PNG has poor general health and socioeconomic predictors: infant and maternal mortality rates are high and the life expectancy is low. Like other tropical developing countries, prevalence of infectious diseases is high. Leading causes of death in PNG include pneumonia, malaria, tuberculosis, neonatal sepsis, diarrhea, and meningitis (Riley, 2009). On the other hand, non-communicable diseases such as multiple sclerosis, rheumatic arthritis, lupus erythematosus, and type 1 diabetes have been rarely described in PNG (Currie et al., 1989; Fisher, 1988; Hulcombe et al., 1999; Karvonen et al., 2000; Kemiki et al., 2001; Ogle et al., 2001; Saweri et al., 1993; Scrimgeour et al., 1987). The fecal microbiota of different regions within PNG has recently been compared by targeted qPCR analysis, revealing some of the patterns (e.g., high *Prevotella*/*Bacteroides* ratio) found in other non-westernized populations (Greenhill et al., 2015). However, a systematic comparison of the PNG microbiome to that of a westernized population has not been performed.

In this study, we used 16S rRNA-tag Illumina sequencing to compare the fecal microbiota of Papua New Guineans residing in two rural communities to individuals living in the US (Figure 1A). Emphasis was given to standardize methods of fecal collection, DNA extraction, and gut microbiota characterization with the goal of comparing community composition and diversity measures and determining unique and shared lineages. To gain insight into the ecological processes that shape the microbiome in the different geographic locations, we quantified the relative influences of community assembly processes using an analytical framework specifically conceived for bacterial metacommunities (Stegen et al., 2013).

RESULTS

Synopsis of Lifestyle in PNG and US Participants

Study participants included individuals from two traditional societies of PNG: the Asaro and the Sausi. The Asaro live in the highlands (Figure 1A), approximately a 30-min drive from the provincial capital of Goroka, and are one of the larger ethnic groups in PNG with approximately 50,000 people. The Sausi live in the lowlands in the Ramu Valley, Madang province, and consist of approximately 1,000 people. The two study sites are 45 km apart and are connected by pedestrian tracks (2 or 3 days walk in steep terrain) and by road (235 km; 4–6 hr traveling time). Contact between the villages does occur but is infrequent.

Both populations live in traditional settings (Figure 1B). No sewage, wastewater, or drinking water treatment facilities exist. Drinking water is derived primarily from rivers, streams, or rainwater and is mainly consumed without boiling or any other treatments. Both communities rely on subsistence agriculture for their food supply, with households having their own gardens (Figure 1C). Persistent under-nutrition is rare in PNG, as carbohydrate sources are generally available. Dietary information collected though surveys showed a large overlap in the diet of the Asaro and Sausi participants (Table S1). The staple foods are sweet potato, taro, and plantain, which are traditionally cooked in open fires (Figures 1D and 1E). Meat-derived protein (principally pork and fish) is consumed less frequently (typically twice weekly; Table S1). Antibiotic use is high in PNG (Duke, 2000) due to the high burden of infectious diseases, poorly regulated administration, and the lack of diagnostic capacity, which leads to empirical treatments. More information on PNG study participants is included in the Supplemental Information.

Twenty participants at each location in PNG (40 in total) were included in the study. The Asaro (Figure 1F) participants had a mean age of 35 years (17–50 years), and two-thirds of them were females. The Sausi individuals had a mean age of 32 years (23–50 years), 80% of which were females.

Fecal samples from 22 subjects (10 males and 12 females; 27 years old on average), part of an independent study conducted at the University of Nebraska (M.X.M.-G., I.M., A.M.E., R.W. Hutkins, and J.W., unpublished data), were used as western controls. All individuals in this cohort were currently residing at

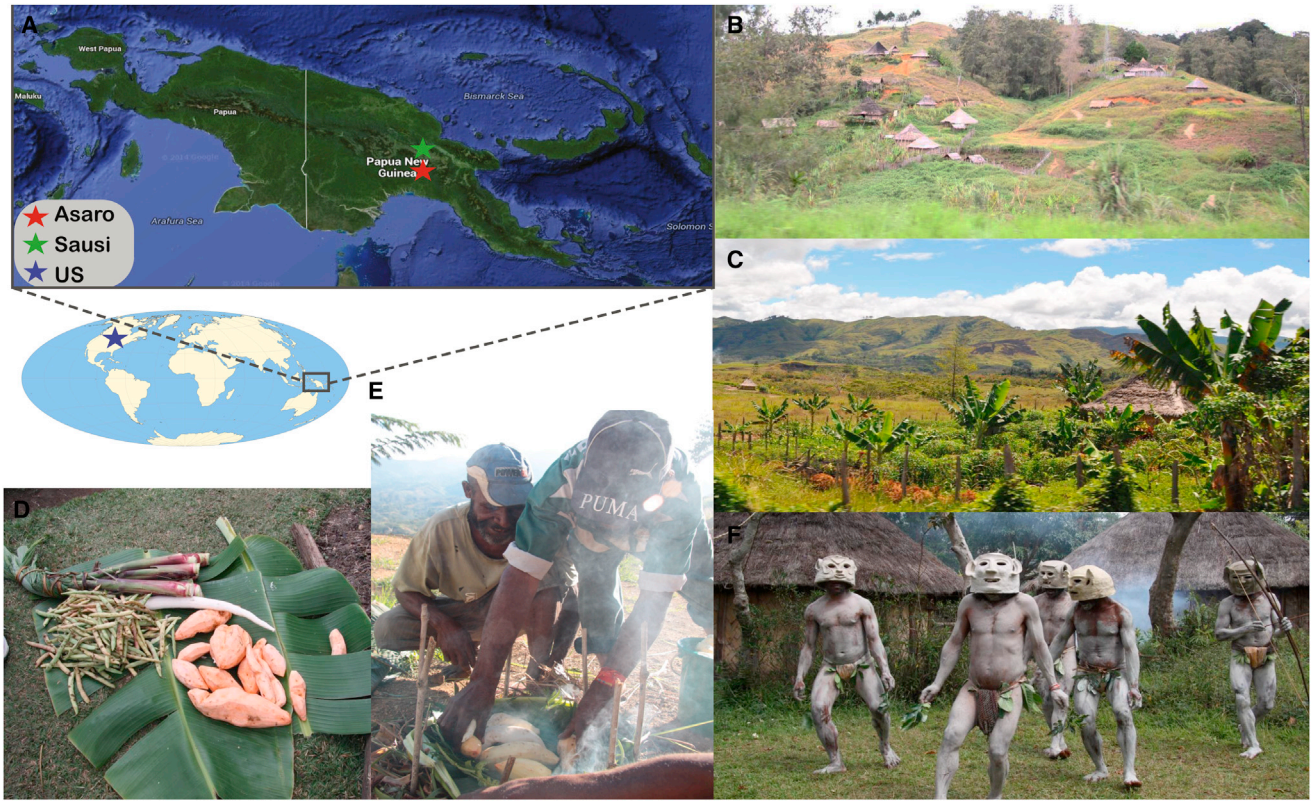

Figure 1. Geography and Traditional Lifestyle Features of PNG

(A) Geographic locations of Asaro and Sausi study sites.

(B) Traditional huts in Eastern Highlands province.

(C) Garden of an individual household in Eastern Highlands province.

(D and E) Staple foods such as taro and sweet potatoes, as well as plantain, banana, and leafy greens among others, which are traditionally cooked in open fire on plantain leaves.

(F) Asaro people are well known for their traditional mudmen attire.

World map downloaded from http://www.freeworldmaps.net; island of New Guinea downloaded from Google Earth; photographs (C)–(G) by A.R.G. See also Table S1.

Lincoln, Nebraska, an urban area. These individuals were born in Colombia (five), Costa Rica (three), Guatemala (two), United States (two), Ghana (one), China (two), Honduras (one), Thailand (two), Nepal (two), Mexico (one), Brazil (one), and Nicaragua (one). The non-US-born participants had been residing in the US for 1.4 ± 0.8 years by the time of sampling. All were college graduates with reliable sources of income. Participants had standard westernized omnivorous diets.

The PNG Fecal Microbiota Has Higher Biodiversity and Lower Inter-individual Variation

The fecal microbiota was characterized by sequencing of 16S rRNA gene tags (V5-V6 region) with Illumina MiSeq technology. Sequencing resulted in 16,072 ± 2,250 quality-controlled and chimera-checked reads per sample. OTU clustering (98% cutoff) yielded a total of 1,520 OTUs for the entire data set, 1,251 OTUs associated with the PNG data set, and 931 with the US samples, indicating higher γ diversity (Hunter, 2002) in PNG.

Rarefied α diversity metrics showed that the fecal microbiome of the PNG cohort had higher biodiversity (Shannon index; $p = 0.01$) and a significantly higher average number of bacterial OTUs ($p = 0.02$; 224 ± 30 in PNG versus 197 ± 50 in US; Figure 2A). No difference in α diversity was observed between samples of the Asaro and Sausi communities (observed OTUs $p = 0.81$; Shannon diversity index $p = 0.75$; Figure 2B). In terms of β diversity based on Bray-Curtis dissimilarity index, fecal bacteria community profiles across Papua New Guineans was more homogeneous than that of US individuals (lower β diversity; $p < 0.001$; Figure 2C), whereas no differences were determined between the Asaro and Sausi fecal communities ($p = 0.96$; Figure 2D). The different geographic origins of the US residents did not contribute to higher β diversity, as microbiome dissimilarities among individuals of the same country of origin did not differ from those of individuals born in different countries ($p = 0.90$; Figure S1A). NMDS ordination plots based on the Bray-Curtis distances (Figure 2E) showed separate clustering of US and PNG samples (Figure 2E), whereas within the US cohort, samples of the same country of origin did not cluster together (Figures S1B and S1C). In addition, higher dispersion of US samples was observed (Figure 2E), confirming their higher β diversity.

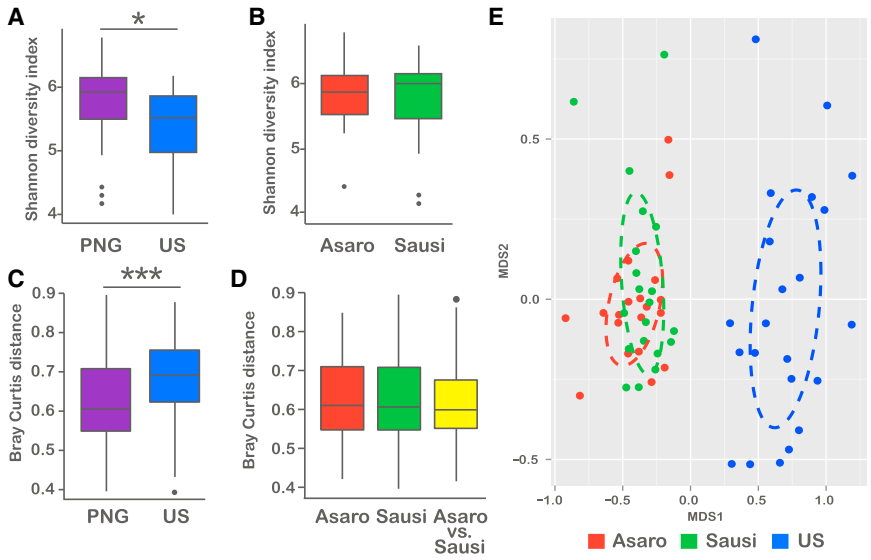

Figure 2. Bacterial Biodiversity of the Fecal Samples of PNG and US Participants

Diversity of the fecal microbiome was evaluated using OTUs defined by 98% sequence similarity cutoff.

(A and B) Comparison of rarefied Shannon diversity in the fecal microbiota of PNG and US individuals (A) and between the Asaro and Sausi individuals (B).

(C and D) β diversity of the bacterial communities computed with Bray-Curtis diversity indices within the PNG and US fecal samples (C) and within/between the Asaro and Sausi participants (D).

(E) NMDS ordination plot of fecal bacterial communities based on the Bray-Curtis distance metric.

(A–D) Mean ± SD; (A–C) Student's t test; (D) ANOVA; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. See also Figure S1.

Abundance Profiles

Ordination analyses revealed distinct clustering of PNG and US fecal communities but no segregation of Asaro and Sausi communities (Figure 2E). In order to determine the bacterial groups that segregated PNG and US gut bacterial communities, microbiomes were compared at different taxonomic scales. No differences were detected at the phylum level (Table S2); however, substantial differences were detected at lower taxonomic levels (Figure 3A; Tables S2 and S3). Altogether, 25 families, 45 genera, and 230 OTUs differed in abundance between the PNG and US cohorts (Figure 3A; Tables S2 and S3). In contrast, we did not detect any taxa with differential abundances between Asaro and Sausi samples.

Within the Bacteroidetes phylum, *Prevotella* abundance was significantly higher in PNG, whereas the proportions of *Alistipes*, *Bacteroides*, *Parabacteroides*, *Odoribacter*, and *Barnesiella* were all significantly higher in the US (Figure 3A). In the Actinobacteria phylum, there was a significantly lower abundance of the genus *Bifidobacterium* in PNG individuals. The proportions of Coriobacteriaceae were similar between PNG and US participants, but several genera within this family showed distinct abundance profiles; significantly higher abundance of *Slackia* and *Propionibacterium* were determined in Papua New Guineans, whereas *Eggerthella* and *Gordonibacter* were higher in the US cohort. Within the Firmicutes, a significantly higher abundance of *Streptococcus* was detected in PNG samples, which constituted 21% ± 11% of the total sequences per participant (Figure 3A; Table S2). Moreover, a substantial phylogenetic diversity (68 OTUs) was observed within this genus in Papua New Guineans, but not US subjects (Figure S2). Other differences in the makeup of the Firmicutes were significantly higher proportions of *Staphylococcus*, *Eubacterium*, Erysipelotrichaceae Incertae sedis, *Clostridium* senso stricto, *Sarcina*, *Enterococcus*, and *Lactobacillus* and significantly lower abundance of *Faecalibacterium*; *Blautia*; *Clostridium* XIVb, XIVa, and IV; *Ruminococcus*; Lachnospiraceae Incertae sedis; *Gemella*; *Turicibacter*; and *Phascolarctobacterium* in PNG. Regarding the Proteobacteria, PNG individuals had significantly higher abundance of the family Enterobacteriaceae and Helicobacteriaceae and the genera *Helicobacter* and *Pseudomonas*, whereas the family Comamonadaceae and the genera *Bilophila*, *Aquabacterium*, and *Acidovorax* were significantly enriched in US volunteers.

The Most-Abundant Phylotypes Were Shared by PNG and US Individuals

Previous analyses of the fecal microbiota of US and European individuals have revealed 50–80 core bacterial species that are shared by >50% of subjects (Qin et al., 2010; Schloissnig et al., 2013; Tap et al., 2009) and collectively constitute the vast majority (99%) of the bacterial population within individuals (Schloissnig et al., 2013). However, whether a human core fecal microbiota exists on a global scale or distinct cores characterize geographically separated human populations has not been evaluated. We therefore determined the number of OTUs that were jointly detected across cohorts and the average abundance that these OTUs represented in each cohort as a function of the fraction of subjects.

Of the 1,520 OTUs detected in this data set, 664 were detected in both PNG and the US. Notably, these shared OTUs represented the majority of the individual fecal microbiota in both cohorts (Figure 3B), comprising an average of 97% ± 2% and 87% ± 5% of the sequences in US and PNG individuals, respectively. To determine whether a high proportion of the microbiome was also shared between other westernized and non-industrialized microbiomes, we analyzed published data sets of the Hadza hunter gatherers, Amerindians, and Malawians and their respective controls (Schnorr et al., 2014; Yatsunenko et al., 2012). This analysis confirmed that shared OTUs dominate the microbiome in both western and non-industrialized settings (Figure S3; Table S4). Interestingly, in all data sets, the OTUs concurrently detected across cohorts constituted a higher proportion of the microbiome of westernized cohorts (Figures 3B and S3), which is in agreement

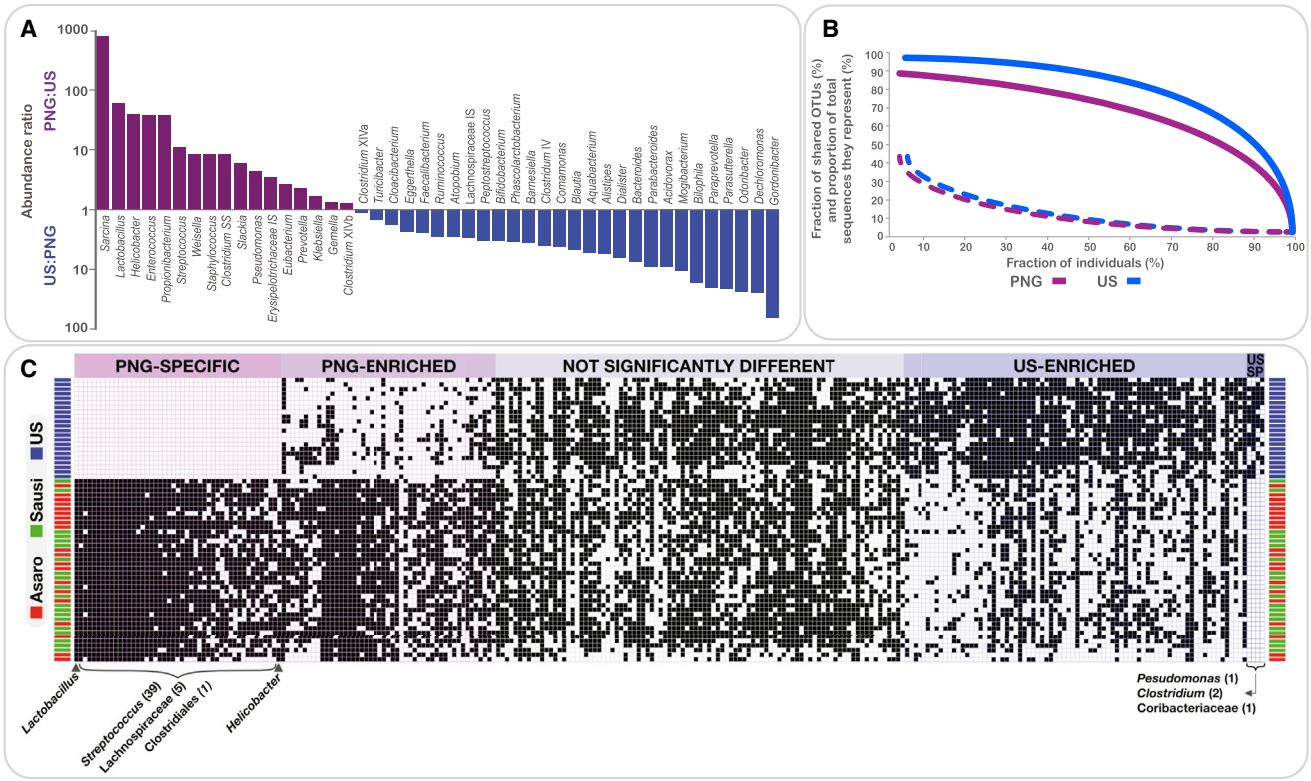

Figure 3. Compositional Comparison of the Microbiome of PNG and US Individuals

(A) Barplots indicate the ratio of the average relative abundance of significantly different genera across the US and PNG populations.

(B) PNG and US microbiomes were analyzed to determine OTUs that were shared between the cohorts. The average fraction of shared OTUs (dashed lines) and the average proportion of total sequences that they represent (non-dashed lines) is plotted as a function of the fraction of individuals within that cohort.

(C) Heatmap indicating presence/absence patterns of shared and cohort-specific OTUs in PNG and US fecal samples. OTUs that belonged to the core microbiome were identified for the US and the PNG data set (present in ≥50% of individuals in each cohort). Samples (rows) in the heatmap were clustered in R using Ward hierarchical clustering algorithm based on binomial distances (calculated with vegan package).

See also Figures S2–S4 and Tables S2, S3, S4, and S5.

with the reduced α diversity (Figure 2A). The number of common OTUs across cohorts and the average relative proportion that they represent decreased as a function of the number of individuals included in the analysis (Figure 3B), reflecting the individualized nature of the human gut microbiome. In our PNG/US comparison, out of the 664 jointly detected OTUs, only 14 were detected in all US individuals and ten in all PNG individuals.

We next identified core members detected in 50% of individuals of each cohort (PNG and US). This analysis revealed a core of 186 OTUs in PNG, whereas US individuals shared a core of 169 OTUs. Core members accounted for an average of 78% ± 9% and 86% ± 12% of the total sequences in the individuals' microbiomes, respectively (Table S4). Two hundred and twenty-two of these OTUs, encompassing more than 40 distinct genera, were detected in both data sets (Figures 3C and S4; Table S4). Eighty-five OTUs were core members in both cohorts (detected in ≥50% of PNG and ≥50% US residents; Table S4), comprising an average of 49% ± 12% and 61% ± 14% of the sequences among individuals in the PNG and US cohorts (Table S4).

Identification of Core Members Exclusive to PNG or US Cohorts

Forty-seven of the 186 core OTUs in PNG were completely undetectable in the US samples (Figure 3C). Together, these OTUs comprised an average of 6.6% of the sequences obtained from Papua New Guineans. Thirty-nine OTUs belong to the genus *Streptococcus* (related to the species *S. lutetiensis/infantarius* and *equinus*), one as *Lactobacillus* (related to *L. reuteri*), one as *Helicobacter* (related to *H. macacae*), five belonged to the Lachnospiraceae family, and one to the Clostridiales with no close matches to described species (Figure 3C; Table S3). The OTU related to *L. reuteri*, and several OTUs related to *S. lutetiensis/infantarius*, and *S. equinus* were detected in all 40 PNG individuals. Only four core OTUs of the US population were not detected in PNG (Figure 3C). These OTUs represented the genus *Pseudomonas* (related to *P. aeruginosa*), two clostridia (related to *C. leptum* and *C. spiroforme*), and one member of the Coriobacteriaceae family (Figure 3C; Table S3).

To investigate whether the core OTUs exclusively detected in PNG could represent specific lineages of the non-westernized fecal microbiota, we examined whether related lineages could

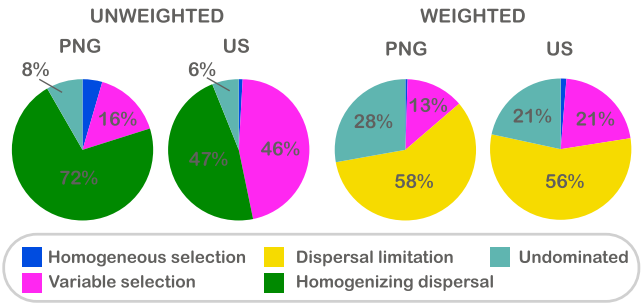

Figure 4. Summary of the Contribution of the Ecological Processes that Determine Gut Community Assembly in PNG and US Microbiomes

Pie charts illustrate the fraction that selection, dispersal, and undominated processes contribute to the community assembly when considering only presence/absence OTU patterns (unweighted) and their abundance (weighted) observed in the data set. See also Figure S5.

be detected in the Hadza hunter-gatherers (Schnorr et al., 2014), Malawi and Amerindian (Yatsunenko et al., 2012), and children from Burkina Faso (De Filippo et al., 2010; see details in the Supplemental Information). This analysis revealed that, although *Helicobacter* and *Lactobacillus* lineages were also detected in other non-industrialized microbiomes (and absent in the western controls), they consist of species other than the ones exclusively associated with the PNG cohort (Table S5).

Differences in the Relative Importance of Fundamental Assembly Processes Might Contribute to the Observed Diversity Patterns in the Gut Microbiomes in PNG and the US

In an effort to gain insight into the mechanisms that drive the distinct ecological patterns and community features, we employed a specifically developed analytical framework for the elucidation of assembly processes in bacterial ecosystems (Chase et al., 2011; Stegen et al., 2013). Using a combination of null models, we estimated the relative influences of homogeneous selection, variable selection, dispersal limitation, and homogenizing dispersal. Homogeneous selection results when a consistent selective environment among local scales causes community composition to be similar, whereas variable selection (*sensu*; Vellend 2010) results when differences in selective environments among local scales case differences in community composition. Homogenizing dispersal (*sensu*; Stegen et al., 2013) results when microbial dispersal causes community composition to be similar among local scales, whereas dispersal limitation (*sensu*; Stegen et al., 2013) results when limited exchange of microbes causes divergence in community composition. The model further estimates the fraction in which neither selection nor dispersal is the primary cause of between-community compositional differences (referred to as undominated).

Process estimates were generated based on OTU presence/absence patterns (unweighted) or OTU relative abundances (weighted). In terms of OTU presence/absence, PNG microbiomes had a larger influence of homogenizing dispersal, whereas in the US, variable selection was higher when compared to PNG (Figure 4). With respect to relative abundances, there was a strong signal of dispersal limitation for both groups, but the US microbiome was again more impacted by variable selection. Whereas this inference holds for both weighted and unweighted microbiome characterization, there was a distinction in the type of dispersal; homogenizing dispersal was most influential over the presence/absence of taxa whereas dispersal limitation influenced relative abundances (Figure 4). Dispersal rates in PNG are thus high enough to influence which taxa are present, potentially increasing the occurrence of rare species, but too low to determine the relative abundances of those taxa.

Our findings indicate that the relative contribution of the ecological processes that structure the gut microbiome (dispersal and variable selection) differs between PNG and the US. However, the question remains whether this is caused mainly through increased dispersal rates in PNG (linked to lifestyle) or higher variable selection in the US cohort (linked to its increased cultural, dietary, and genetic heterogeneity). The latter may be especially pronounced in our study, as the US participants were of various geographic and ethnic origins. To gain additional insight into the factors that influence the relative contribution of community assembly processes in westernized and non-industrialized microbiomes, we performed the same null-modeling approach on the Hadza/Italy data set (Schnorr et al., 2014). In this data set, geography, culture, diet, and genetics of the western cohort are likely to be more homogenous, as all participants are Italian. Although the estimates of the community assembly processes differed in magnitude to the ones observed for the PNG/US data set, similar findings were obtained regarding the dominant processes in each cohort; significantly higher homogenizing dispersal characterized the Hadza community, whereas there was a stronger signal of variable selection in Italian individuals (Figure S5).

DISCUSSION

Here, we compared the fecal microbiota of rural Papua New Guineans to that of US residents. Several of our findings are in agreement with previous comparisons of westernized and non-westernized fecal microbiomes, suggesting a general impact of westernization on the gut microbiome. We argue that metacommunity theory can offer a framework to explain the observed biogeographic patterns and provides the first evidence suggesting that the distinct ecological configurations in non-industrialized and westernized microbiomes are caused by differences in the relative influence of assembly processes.

The PNG fecal microbiota had significantly higher OTU richness compared to the US, both at a population (γ diversity) and individual (α diversity) level, whereas variation among individuals (β diversity) was lower. Analogous findings have been shown in other non-westernized populations in Africa and South America (De Filippo et al., 2010; Schnorr et al., 2014; Yatsunenko et al., 2012). Several factors could contribute to these findings. A higher intake of plant-derived carbohydrates and dietary fiber in non-westernized societies could result in higher α diversity (Sonnenburg and Sonnenburg, 2014). Accordingly, the addition of whole grains to a standard US diet can increase the diversity of the fecal microbiota (Martínez et al., 2013a). Although the use of antibiotics is often regarded a cause of reduced

biodiversity in westernized microbiomes (Schnorr et al., 2014), this is an unlikely explanation for our findings, as antibiotic use is common in PNG. A major yet unexplored contributor to the observed diversity patterns could be that differences in lifestyle impact ecological processes that shape the gut microbiota.

When interpreted in the light of metacommunity theory, the diversity patterns in PNG and the US, as well as in other comparisons of westernized and non-industrialized microbiomes (De Filippo et al., 2010; Schnorr et al., 2014; Yatsunenko et al., 2012), are concordant with differences in rates of microbial dispersal (which relates to horizontal transmission). Dispersal (the movement of organisms across space; Vellend, 2010) constitutes a major process through which diversity accumulates in local microbial communities (Cadotte, 2006; Costello et al., 2012), thereby increasing α diversity (Chase and Myers, 2011). Further, dispersal reinforces homogenization of local communities, thus decreasing β diversity (Cadotte, 2006). Therefore, the observed ecological patterns might be driven by the substantial differences in sanitation, water treatment, and other hygienic practices that were specifically conceived in westernized societies to reduce human exposure to feces and decrease the incidence and spreading of infectious diseases. These practices have likely affected not only the transmission of pathogens but also gut symbionts. The results from our null-modeling analysis now provide empirical evidence for an elevated relative importance of dispersal in the assembly of the non-industrialized microbiomes. This occurred despite the PNG data set covering two geographically separated locations, which one would expect to reduce the influence of homogenizing dispersal. Therefore, both the diversity patterns (low α diversity and high β diversity) and the quantitative analysis of assembly processes suggest that westernized lifestyle might alter the gut microbiome by reducing dispersal of symbionts.

However, dissimilar community configurations among westernized and non-industrialized microbiomes might also arise due to differences in heterogeneity of the studied populations. Westernized societies are characterized by greater variability in host genetics, cultural backgrounds, and dietary habits when compared with non-westernized populations, and in our study, the US cohort consisted of individuals from various geographic and ethnic origins. This heterogeneity likely imposes variable selective pressures that could contribute to the observed increased β diversity. In accordance, our null-model analysis revealed higher variable selection in the US residents. However, four considerations question the role of variable selection as the main driver of the profound dissimilarities between westernized and non-industrialized microbiomes. First, microbiome comparisons between different industrialized countries (Japan, Italy, Spain, Denmark, France, and the US) have revealed no major differences across nations (Arumugam et al., 2011), suggesting that genetic and cultural factors exert minor effects. Second, in our study, β diversity was not different among US residents of the same country of origin and those born in different countries. Third, despite profound geographic, genetic, and cultural differences, the microbiomes of Malawians and Venezuelan-Amerindians cluster together (and separate from the US controls; Yatsunenko et al., 2012). Finally, the Italian cohort analyzed by Schnorr and coworkers, although composed of individuals of the same cultural background, still showed higher β diversity when compared to Hadza hunter-gatherers (Schnorr et al., 2014). These considerations support the dominant role of modern lifestyle in causing the observed gut microbiome patterns despite the confounding influence of host genetics, culture, and dietary habits. However, it is likely that various factors act together to influence the ecological processes that structure the microbiome. An intriguing hypothesis is that dispersal limitation (induced by westernized lifestyle) and high variable selection (induced by broader genetic and cultural heterogeneity) converge to generate the observed alterations in western microbiomes. Dispersal limitation alone reduces homogenization among the metacommunity (increasing β diversity), whereas inter-individual differences in selective environments do not only increase β diversity but also limit successful colonization of microbes following dispersal, thereby decreasing α diversity. Lineages unable to adapt to heterogeneous selective pressures may disappear from the population, and dispersal rates across individuals might be too low to "rescue" species from extinction (Costello et al., 2012). Although this hypothesis is consistent with the data, the elucidation of the exact factors that drive differences among westernized and non-industrialized societies will require studies that control for confounding factors.

Abundance profiles of bacterial taxa greatly differed between PNG and US samples. Several of these differences have been reported in previous comparisons of westernized and non-westernized societies, indicating that some of these changes are driven by lifestyle. Overall, westernization consistently increased proportions of *Faecalibacterium*, *Ruminococcus*, *Bifidobacterium*, *Bacteroides*, *Blautia*, *Bilophila*, and *Alistipes*, whereas *Prevotella* is generally increased in non-industrialized societies (De Filippo et al., 2010; Schnorr et al., 2014; Yatsunenko et al., 2012). The altered *Prevotella*/*Bacteroides* ratio could be caused by diet, as intake of lipids, cholesterol, amino acids, and dairy have been linked to the enrichment of *Bacteroides* (Wu et al., 2011), whereas *Prevotella* is favored by sugars (Wu et al., 2011) and diets rich in complex carbohydrates (De Filippo et al., 2010; Schnorr et al., 2014). Increased abundance of *Alistipes* and *Bilophila* has been also linked to animal-based diets (David et al., 2014; Wu et al., 2011). In addition, lower fecal proportions of bifidobacteria have been observed in vegans (David et al., 2014), and the lower abundance of this genus in non-westernized societies might be attributed to the absence of dairy products from their diet (Schnorr et al., 2014). Overall, non-westernized microbiomes resemble those of vegetarians and vegans (David et al., 2014; Wu et al., 2011). A notable finding in this study was the elevated proportion of streptococci in PNG (see the Supplemental Information for additional details).

Thus far, the focus of studies comparing the gut microbiome of humans living in westernized versus non-industrialized societies has centered upon differences in the abundance of taxa (De Filippo et al., 2010; Schnorr et al., 2014; Yatsunenko et al., 2012). We have now included an additional perspective by comparing microbiomes in terms of membership. Despite the substantial differences in community structure, shared OTUs in PNG and the US constituted a majority of the total sequences and we obtained similar findings for other westernized and non-westernized populations. Collectively, these findings are consistent

with the strong influence of homogenizing dispersal inferred through our null-modeling analyses and indicate that, despite large geographic distances and cultural differences, the human gut microbiome is dominated by globally distributed species (although sub-species- and strain-level differences were not assessed in our study). International travel has been suggested as a possible mechanism for the global transmission of gut microbes (Dethlefsen et al., 2007). However, given the limited contact of some populations (i.e., Venezuelan Amazonia and Tanzania Hadza land) with westerners, the findings support the view of gut bacteria as real symbionts that have remained stably host associated even after human populations became separated (Falush et al., 2003). Despite the high conservation of membership, plasticity of the gut microbiome in response to environmental conditions and lifestyle is apparent, as shown by the substantial differences in abundance profiles and the strong signal for variable selection in our null-modeling analyses.

Although bacterial membership was to a large degree conserved between PNG and the US, each cohort had exclusively associated core OTUs. Interestingly, there were a larger number of exclusive OTUs associated to the PNG cohort, congruent with the higher bacterial diversity (Figure 2) and the lower proportion of shared OTUs within the fecal microbiota (Figures 3C and S4). An OTU related to *Lactobacillus reuteri* was detected in all PNG subjects. This species used to be regularly isolated from human fecal samples in studies conducted around 1960 in western societies; however, it has been detected only rarely in more-recent studies (Walter et al., 2011). Therefore, our findings support the hypothesis that westernization led to a loss of certain bacterial lineages (Blaser and Falkow, 2009). A *Helicobacter-macacae*-like OTU was also exclusively detected in PNG samples as a core member. Deliberate eradication of *Helicobacter pylori* has led to a significant reduction of this species in westernized countries (Blaser and Falkow, 2009), and other species of this genus might also have been affected. However, PNG-specific core OTUs were not detected in data sets from other non-westernized samples and we therefore cannot conclude that they represent members of an ancestral microbiome. Methodological differences across studies might hinder the detection of PNG-specific species-level OTUs in other data sets, and further studies using standardized techniques are needed. Overall, our comparisons suggest that the *Helicobacter* genus is less prevalent in individuals living in westernized societies, as this genus was exclusively detected in the non-industrialized data sets analyzed. The dissimilarities between the westernized and non-westernized microbiota might have important health implications (further details in the Supplemental Information).

Overall Conclusions

The findings obtained in this study provide novel insight into the ecology and biogeography of the human gut microbiome, and their interpretation in the light of metacommunity theory provides a possible explanation by which human lifestyle, and specifically westernization, impacts microbiome configurations. Humans harbor a gut microbiome whose dominant members are largely globally distributed, supporting the concept of humans as holobionts that, independent of geographic location, acquire for the most part the same bacterial symbionts. However, community structure and abundance profiles vary significantly among geographically separated human populations, suggesting that environmental selection at local scales (especially diet) has a major impact on the gut microbiome. This plasticity might constitute a mechanism by which the human holobiont can rapidly adapt to environmental changes that require metabolic capabilities beyond what is encoded by the human genome. In addition, westernization has been consistently associated with lower α diversity and higher β diversity. We propose a model in which microbial dispersal (which can relate to both horizontal transmission of symbionts and environmental exposure; de Vrieze, 2014) exerts a prominent role in structuring the gut microbiome in non-industrialized societies, whereas microbiome alterations associated with westernization are caused through dispersal limitation in combination with high inter-individual differences in selective environments (Figure 5). Theory predicts that a combination of low dispersal with distinct selective environments will reduce rates of successful colonization, which might, together with antibiotics and insufficient dietary support through low fiber intake (Sonnenburg and Sonnenburg, 2014), lead to the extinction of bacterial lineages. The importance of dispersal for microbiome assembly and maintaining diversity has substantial implications for human health in non-industrialized and westernized populations alike. High microbial dispersal is at the core of the epidemic levels of infectious diseases in low-income communities such as PNG, whereas lifestyle practices that decrease dispersal in westernized societies might preclude the acquisition of microbes or microbial consortia that protect from non-communicable diseases. An important implication of our findings would be the need to develop strategies by which to reduce pathogen transmission while supporting symbiont dispersal. Clearly, the characterization of the gut microbiota in non-westernized populations provides information that might aid in the development of strategies to reintroduce bacterial lineages that have been eradicated in westernized human populations. Studies such as ours are timely, as human populations that live a non-westernized lifestyle are in decline.

EXPERIMENTAL PROCEDURES

Study Participants

Ethics approval was granted by the Papua New Guinea Institute of Medical Research Institutional Review Board (no. 1030) and the Papua New Guinea Medical Research Advisory Committee (MRAC no. 11.05). The two PNG study sites, which differed from those studied previously (Greenhill et al., 2015), were selected on account of (1) having a large proportion of the population that live a traditional, subsistence-agriculture-based lifestyle, (2) being geographically distinct from one another, and (3) being accessible by road for the Papua New Guinea Institute of Medical Research (PNGIMR)-based research team. At both study sites, an experienced study nurse conducted a community information session to inform leaders and the general community of the study. Thereafter, volunteers were sought and selected based on convenience. Written informed consent was obtained from each participant. Twenty participants at each location in PNG (40 in total) were included in the study, and socio-demographic and dietary questionnaires were answered by all participants of the Sausi village and 8 of the 20 Asaro village participants. The socio-demographic characterization of participants included age, gender, marital status, highest level of education reached, and occupation. Food questionnaires included a description of the meals consumed in the previous 24 hr plus sources of carbohydrate, protein, and other nutrients (fruits, leafy greens, nuts, and beans) as well as the weekly frequency consumption of protein.

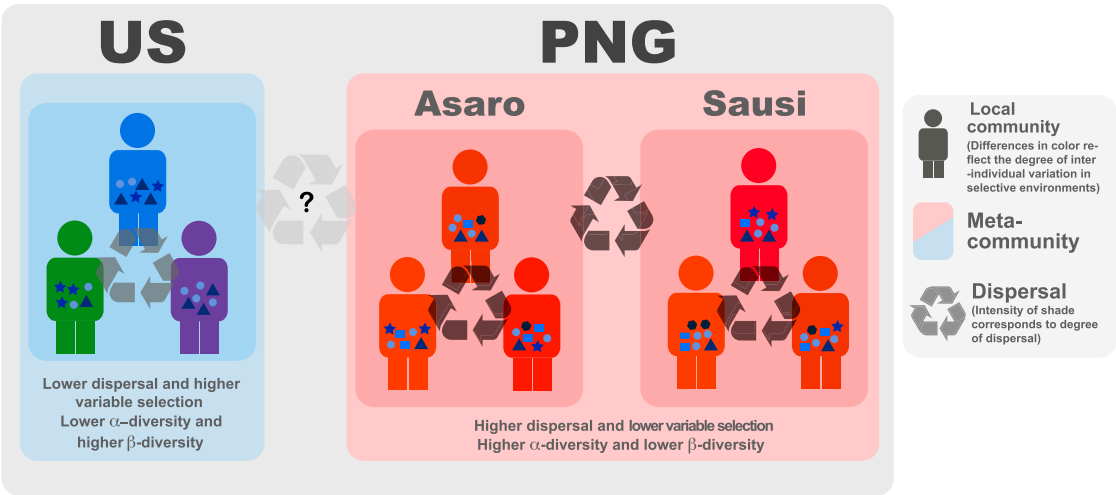

Figure 5. Model of the Ecological Processes that Drive Diversity Patterns of the Fecal Microbiota in PNG and the US

The human gut microbiome can be viewed as a metacommunity, with human individuals representing local communities connected through dispersal and transmission of microbes. Lower dispersal in the US might lead to a decrease of α diversity. The increased β diversity in westernized microbiomes results from the combination of lower homogenizing dispersal and higher variable selection. Relative proportions of bacterial groups differ vastly between the US and PNG, indicating that structure of local assemblages is shaped by selection, likely caused by dietary differences (although host genetics cannot be excluded). Facets of westernized diet, especially low levels of complex, plant-derived carbohydrates, might also contribute to the loss of bacterial lineages that rely on dietary substrates for growth.

The western controls were recruited as part of an independent study conducted at the University of Nebraska. Exclusion criteria of the Nebraska participants included being less than 19 years of age, being underweight or obese (having a BMI < 18.5 kg/m^2 or ≥30 kg/m^2), antibiotic usage within 3 months prior to sample collection, have an acute or chronic existing illness, prior gastrointestinal surgery (except for appendectomy or hernia repair), recent unexplained bleeding, pregnant or lactating, participation in another experimental trial 30 days prior to sample collection, vaccinated within 6 months prior to sample collection, or undergoing treatment with steroids or an immunosuppressant. Individual meetings were conducted with the potential participants to explain the study protocol, and written consent was obtained for every participant in the US cohort. Ethics approval for the US cohort was granted by the Institutional Review Board of the University of Nebraska (IRB protocol no. 20120612289COLLB).

Sample Collection and Processing

PNG participants were provided with sterile specimen jars for fecal collection. Participants were informed of the need for fresh samples; thus samples were given to the study nurse within 1 hr of defecation. Samples were placed in an insulated container with ice bricks to keep the sample at approximately 4°C–8°C during transit to the laboratory, which was up to 10 hr after sample collection. Immediately upon receipt in the laboratory, samples were diluted 1:10 in PBS and stored at −80°C. Samples were then shipped in liquid nitrogen to the University of Nebraska for analysis. Fecal samples from US participants were processed as close as possible to the fecal samples collected from the US individuals to reduce bias in comparisons.

DNA Extraction from Feces

DNA extraction from all fecal slurries were conducted by the same person using a standardized approach that combined enzymatic and mechanical lysis of cells as described previously (Martínez et al., 2009), with minor modifications. Briefly, 1 ml of fecal homogenate was transferred to sterile bead beating tubes containing 300 mg of zirconium beads (Biospec Products). Cells were recovered by centrifugation (8,000 × *g* for 5 min at room temperature) and washed three times in ice-cold PBS. Next, 750 μl of the QIAGEN lysis buffer for Gram-positive bacteria was added to the cell pellet (20 mM Tris [pH 8.0], 2 mM EDTA, 1.2% v/v Triton X-100, and 20 mg/ml lysozyme). This lysis buffer was used to ensure DNA recovery from Gram-positive cells (e.g., bifidobacteria), which are often underrepresented in the analyses of bacterial communities. The inclusion of this step led to a lower representation of genera and species within Bacteroidetes (Table S2), although all species within this phylum that were previously detected using a less-harsh cell lysis (Martínez et al., 2013b) were still detectable (see Table S3). Solutions were homogenized and incubated at 37°C for 20 min. Eighty-five microliters of 10% SDS solution and 30 μl proteinase K (20 mg/ml) were added, and samples were incubated for 30 min at 60°C. Five hundred microliters of phenol-chloroform-isoamyl alcohol (25:24:1) was added, and the samples were homogenized in a MiniBeadbeater-8 (BioSpec Products) at maximum speed for 2 min. Next, the samples were placed on ice and centrifuged at 10,000 *g* for 5 min. The aqueous layer was extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1) and twice with chloroform-isoamyl alcohol. DNA was recovered by standard ethanol precipitation and dissolved in 100 μl of Tris-HCl buffer (10 mM; pH 8.0).

16S rRNA Gene Illumina Sequencing

PCR (targeting V5-V6 region of the 16S rRNA gene with primers 784F [5′-RGGATTAGATACCC-3′] and 1064R [5′-CGACRRCCATGCANCACCT-3′]), and amplicon sequencing was performed at the University of Minnesota Genomics Center as described previously (Krumbeck et al., 2015). All PNG and US samples were included in the same sequencing run.

Microbial Community Analysis

Quality control, merging of pair ends, OTU clustering, and taxonomic assignation was done as described (Krumbeck et al., 2015). Samples exceeding 20,000 high-quality reads were subsampled to this number using Mothur v.1.31.1 (Schloss et al., 2009) to minimize potential biases due to sequencing depth across samples. After quality control and chimera removal, samples contained an average of 16,072 ± 2,255 sequences.

Comparison to Other Non-industrialized Microbiomes

16S rRNA-sequencing data from studies on the Hadza hunter-gatherers (Schnorr et al., 2014), Malawian and Venezuelan-Amerindians (Yatsunenko et al., 2012), and children from Burkina Faso (De Filippo et al., 2010) and westernized controls were downloaded from the MG-RAST (project ID 7058), MG-RAST (under "qiime:850" accession numbers), and the European Nucleotide Archive (project ERP000133), respectively. The sequences from Hadza hunter-gatherers (Schnorr et al., 2014) of 230–238 bases (V4 region

of 16S rRNA gene) were analyzed analogous to sequences from this study using the UPARSE pipeline. Samples of individuals from the study by Yatsunenko et al. (2012) of 23 Amerindian, 21 Malawian, and 21 US subjects that matched the age group of the PNG individuals were used in the analysis (ages 20–55 years). The sequences were first quality filtered with the fastq_quality_filter script of the FASTX_toolkit (parameters used: -q 30 -p 90), length filtered (sequences with 90–105 bases were kept), and subsampled to 100,000 sequences per file. Thereafter, sequences were analyzed in an analogous manner to our reads in UPARSE. The Burkina Faso data set was first filtered by length of the reads (250–400 bp) and then subjected to the UPARSE pipeline.

Calculation of Ecological Processes Estimates

For each data set (PNG/US and Hadza/Italy; Schnorr et al., 2014), OTU representative sequences were aligned in QIIME (Caporaso et al., 2010) with default parameters and distance matrices were constructed. Next, the procedures described by Stegen et al. (2013) were followed to calculate the ecological processes estimates. Briefly, β-mean-nearest taxon distances (β-MNTD)—the mean distance between each taxon and its nearest neighbor—was computed. Null-model expectations of this parameter were calculated by random shuffling of OTUs and their abundances across phylogenetic tips. Microbial communities were compared pairwise, and β-nearest taxon indices (β-NTI)—the difference between the calculated β-MNTD and the null-model estimate—were determined. β-NTI values were quantified by either accounting for (weighted) or not accounting for (unweighted) taxa relative abundances. Values of β-NTI $>+2$ or <-2 represent community turnover governed by variable or homogeneous selection, respectively. The fractions of all β-NTI values that were $>+2$ or <-2 were used as estimates of variable and homogeneous selection, respectively; these processes were estimated separately using weighted or unweighted β-NTI. Pairwise comparisons with $|\beta\text{-NTI}| < 2$ were further subjected to either Bray-Curtis-based Raup-Crick (as in Stegen et al., 2013) or presence/absence Raup-Crick (as in Chase et al., 2011, and Stegen et al., 2013). Both metrics were used in combination with β-NTI to estimate the contribution of homogenizing dispersal and dispersal limitation as in Stegen et al. (2013); the fraction of pairwise comparisons with $|\beta\text{-NTI}| < 2$ and Raup-Crick < -0.95 estimated the homogenizing dispersal influence; the fraction of pairwise comparisons with $|\beta\text{-NTI}| < 2$ and Raup-Crick $> +0.95$ estimated the dispersal limitation influence; the fraction of pairwise comparisons with $|\beta\text{-NTI}| < 2$ and $|RC_{bray}| < 0.95$ represented the component of compositional turnover undominated by a single process (note that this "undominated" component was referred to as "drift" in Stegen et al., 2013). We note that null-model deviations measure how different observed data are from an expectation; our approach does not fit a model to data—a distinct advantage relative to alternative approaches—such that it does not generate confidence intervals or a goodness-of-fit measurement.

Statistical Analysis

Rarefied α diversity indices (observed species; Shannon) were calculated in QIIME (Caporaso et al., 2010; with 1,000 min number of sequences, 6,000 max number of sequences to cover the minimum number of sequences in all samples, and steps of 500 sequences). β diversity indices (Bray-Curtis) were calculated in QIIME. Ordination plots for β diversity metrics were generated by non-parametric multidimensional scaling (NMDS) ordination in R (R Core Team, 2014), based on Bray-Curtis distance calculated with the VEGAN package (Oksanen et al., 2013), with scaled and centered results. PCoA plot was generated based on the binary Jaccard distance. Significant differences between the PNG and US cohorts in taxonomic and diversity data were compared with Student's t tests or Wilcoxon signed-rank test in R, depending on whether the variable was normally distributed or not. For taxonomic data, FDR correction of the p values was conducted in R.

ACCESSION NUMBERS

Quality-controlled sequences used for analysis were deposited in the MG-RAST database and are available under accession numbers 4576511.3–4576572.3.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.03.049.

AUTHOR CONTRIBUTIONS

I.M., A.R.G., and J.W. designed the project. A.R.G. and P.M.S. got the authorization and ethical approval for collection of fecal samples in PNG. A.R.G. collected PNG samples, and M.X.M.-G. collected US residents' samples. M.X.M.-G. performed DNA extractions on all samples. I.M. coordinated sequencing and processed sequencing data. A.M.E. and I.M. performed statistical analyses and data visualization. J.C.S. performed the quantification of ecological processes. I.M. and J.W. wrote the paper. All authors (I.M., J.C.S., M.X.M.-G., A.M.E., P.M.S., A.R.G., and J.W.) interpreted data, discussed the results, and revised and edited the draft.

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REFERENCES

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes, G.R., Tap, J., Bruls, T., Batto, J.M., et al.; MetaHIT Consortium (2011). Enterotypes of the human gut microbiome. Nature *473*, 174–180.

Berer, K., Mues, M., Koutrolos, M., Rasbi, Z.A., Boziki, M., Johner, C., Wekerle, H., and Krishnamoorthy, G. (2011). Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature *479*, 538–541.

Blaser, M.J., and Falkow, S. (2009). What are the consequences of the disappearing human microbiota? Nat. Rev. Microbiol. *7*, 887–894.

Cadotte, M.W. (2006). Dispersal and species diversity: a meta-analysis. Am. Nat. *167*, 913–924.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. Nat. Methods *7*, 335–336.

Chase, J.M., and Myers, J.A. (2011). Disentangling the importance of ecological niches from stochastic processes across scales. Philos. Trans. R. Soc. Lond. B Biol. Sci. *366*, 2351–2363.

Chase, J.M., Kraft, N.J.B., Smith, K.G., Vellend, M., and Inouye, B.D. (2011). Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere *2*, art24.

Conradi, S., Malzahn, U., Paul, F., Quill, S., Harms, L., Then Bergh, F., Ditzenbach, A., Georgi, T., Heuschmann, P., and Rosche, B. (2013). Breastfeeding is associated with lower risk for multiple sclerosis. Mult. Scler. *19*, 553–558.

Costello, E.K., Stagaman, K., Dethlefsen, L., Bohannan, B.J.M., and Relman, D.A. (2012). The application of ecological theory toward an understanding of the human microbiome. Science *336*, 1255–1262.

Currie, B., Kevau, I., and Naraqi, S. (1989). Acute myocardial infarction in a young Papuan woman with systemic lupus erythematosus. P. N. G. Med. J. *32*, 181–184.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. Nature *505*, 559–563.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G., and Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc. Natl. Acad. Sci. USA *107*, 14691–14696.

de Vrieze, J. (2014). Gut instinct. Science *343*, 241–243.

Dethlefsen, L., McFall-Ngai, M., and Relman, D.A. (2007). An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature *449*, 811–818.

Devkota, S., Wang, Y., Musch, M.W., Leone, V., Fehlner-Peach, H., Nadimpalli, A., Antonopoulos, D.A., Jabri, B., and Chang, E.B. (2012). Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. Nature *487*, 104–108.

Duke, T. (2000). Antibiotic-resistant bacterial sepsis in Papua New Guinea. P. N. G. Med. J. *43*, 82–90.

Falush, D., Wirth, T., Linz, B., Pritchard, J.K., Stephens, M., Kidd, M., Blaser, M.J., Graham, D.Y., Vacher, S., Perez-Perez, G.I., et al. (2003). Traces of human migrations in Helicobacter pylori populations. Science *299*, 1582–1585.

Fisher, B.W. (1988). Systemic lupus erythematosis in a Papua New Guinean highlander. P. N. G. Med. J. *31*, 57–59.

Frawley, W. (2003). International Encyclopedia of Linguistics, Second Edition (Oxford University Press).

Greenhill, A.R., Tsuji, H., Ogata, K., Natsuhara, K., Morita, A., Soli, K., Larkins, J.A., Tadokoro, K., Odani, S., Baba, J., et al. (2015). Characterization of the gut microbiota of Papua New Guineans using reverse transcription quantitative PCR. PLoS ONE *10*, e0117427.

Hulcombe, J.E., Bradfield, J.M., Tannenberg, A.E., and Pender, M.P. (1999). Baló's concentric sclerosis in a woman from Papua New Guinea. J. Clin. Neurosci. *6*, 46–48.

Hunter, M.J. (2002). Fundamentals of Conservation Biology (Massachusetts: Blackwell Science).

Jeraldo, P., Sipos, M., Chia, N., Brulc, J.M., Dhillon, A.S., Konkel, M.E., Larson, C.L., Nelson, K.E., Qu, A., Schook, L.B., et al. (2012). Quantification of the relative roles of niche and neutral processes in structuring gastrointestinal microbiomes. Proc. Natl. Acad. Sci. USA *109*, 9692–9698.

Karvonen, M., Viik-Kajander, M., Moltchanova, E., Libman, I., LaPorte, R., and Tuomilehto, J. (2000). Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. Diabetes Care *23*, 1516–1526.

Kemiki, A.D., Jimmy, S., and Rongap, A. (2001). Systemic lupus erythematosus in children: a case report and review. P. N. G. Med. J. *44*, 57–62.

Koeth, R.A., Wang, Z., Levison, B.S., Buffa, J.A., Org, E., Sheehy, B.T., Britt, E.B., Fu, X., Wu, Y., Li, L., et al. (2013). Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat. Med. *19*, 576–585.

Krumbeck, J.A., Maldonado-Gomez, M.X., Martínez, I., Frese, S.A., Burkey, T.E., Rasineni, K., Ramer-Tait, A.E., Harris, E.N., Hutkins, R.W., and Walter, J. (2015). In vivo selection to identify bacterial strains with enhanced ecological performance in synbiotic applications. Appl. Environ. Microbiol. *81*, 2455–2465.

Leach, J. (2013). Gut microbiota: Please pass the microbes. Nature *504*, 33.

Marra, F., Marra, C.A., Richardson, K., Lynd, L.D., Kozyrskyj, A., Patrick, D.M., Bowie, W.R., and Fitzgerald, J.M. (2009). Antibiotic use in children is associated with increased risk of asthma. Pediatrics *123*, 1003–1010.

Martínez, I., Wallace, G., Zhang, C., Legge, R., Benson, A.K., Carr, T.P., Moriyama, E.N., and Walter, J. (2009). Diet-induced metabolic improvements in a hamster model of hypercholesterolemia are strongly linked to alterations of the gut microbiota. Appl. Environ. Microbiol. *75*, 4175–4184.

Martínez, I., Lattimer, J.M., Hubach, K.L., Case, J.A., Yang, J., Weber, C.G., Louk, J.A., Rose, D.J., Kyureghian, G., Peterson, D.A., et al. (2013a). Gut microbiome composition is linked to whole grain-induced immunological improvements. ISME J. *7*, 269–280.

Martínez, I., Muller, C.E., and Walter, J. (2013b). Long-term temporal analysis of the human fecal microbiota revealed a stable core of dominant bacterial species. PLoS ONE *8*, e69621.

Mihaljevic, J.R. (2012). Linking metacommunity theory and symbiont evolutionary ecology. Trends Ecol. Evol. *27*, 323–329.

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman, J.E., Darcy, J.L., Lynch, R.C., Wickey, P., and Ferrenberg, S. (2013). Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. *77*, 342–356.

Noval Rivas, M., Burton, O.T., Wise, P., Zhang, Y.Q., Hobson, S.A., Garcia Lloret, M., Chehoud, C., Kuczynski, J., DeSantis, T., Warrington, J., et al. (2013). A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. J. Allergy Clin. Immunol. *131*, 201–212.

Noverr, M.C., and Huffnagle, G.B. (2005). The 'microflora hypothesis' of allergic diseases. Clin. Exp. Allergy *35*, 1511–1520.

Ochoa-Repáraz, J., Mielcarz, D.W., Haque-Begum, S., and Kasper, L.H. (2010). Induction of a regulatory B cell population in experimental allergic encephalomyelitis by alteration of the gut commensal microflora. Gut Microbes *1*, 103–108.

Ogle, G.D., Lesley, J., Sine, P., and McMaster, P. (2001). Type 1 diabetes mellitus in children in Papua New Guinea. P. N. G. Med. J. *44*, 96–100.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., and Wagner, H. (2013). vegan: Community Ecology Package. R package version 2.0-10.

Pop, M., Walker, A.W., Paulson, J., Lindsay, B., Antonio, M., Hossain, M.A., Oundo, J., Tamboura, B., Mai, V., Astrovskaya, I., et al. (2014). Diarrhea in young children from low-income countries leads to large-scale alterations in intestinal microbiota composition. Genome Biol. *15*, R76.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., et al.; MetaHIT Consortium (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature *464*, 59–65.

R Core Team (2014). R: A Language and Environment for Statistical Computing (Vienna, Austria: R Found. Stat. Comput).

Riley, I. (2009). Demography and the epidemiology of disease in Papua New Guinea. P. N. G. Med. J. *52*, 83–95.

Risnes, K.R., Belanger, K., Murk, W., and Bracken, M.B. (2011). Antibiotic exposure by 6 months and asthma and allergy at 6 years: Findings in a cohort of 1,401 US children. Am. J. Epidemiol. *173*, 310–318.

Saweri, A., Dakulala, P., Tuck, R.R., and Naraqi, S. (1993). Multiple sclerosis: report of a case in a Papua New Guinean. P. N. G. Med. J. *36*, 53–55.

Schloissnig, S., Arumugam, M., Sunagawa, S., Mitreva, M., Tap, J., Zhu, A., Waller, A., Mende, D.R., Kultima, J.R., Martin, J., et al. (2013). Genomic variation landscape of the human gut microbiome. Nature *493*, 45–50.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. *75*, 7537–7541.

Schnorr, S.L., Candela, M., Rampelli, S., Centanni, M., Consolandi, C., Basaglia, G., Turroni, S., Biagi, E., Peano, C., Severgnini, M., et al. (2014). Gut microbiome of the Hadza hunter-gatherers. Nat. Commun. *5*, 3654.

Scrimgeour, E.M., Matz, L.R., and Aaskov, J.G. (1987). A study of arthritis in Papua New Guinea. Aust. N. Z. J. Med. *17*, 51–54.

Sonnenburg, E.D., and Sonnenburg, J.L. (2014). Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. Cell Metab. *20*, 779–786.

Stegen, J.C., Lin, X., Konopka, A.E., and Fredrickson, J.K. (2012). Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J. *6*, 1653–1664.

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., and Konopka, A. (2013). Quantifying community assembly processes and identifying features that impose them. ISME J. *7*, 2069–2079.

Tap, J., Mondot, S., Levenez, F., Pelletier, E., Caron, C., Furet, J.P., Ugarte, E., Muñoz-Tamayo, R., Paslier, D.L.E., Nalin, R., et al. (2009). Towards the human intestinal microbiota phylogenetic core. Environ. Microbiol. *11*, 2574–2584.

Tenconi, M.T., Devoti, G., Comelli, M., Pinon, M., Capocchiano, A., Calcaterra, V., and Pretti, G.; Pavia T1DM Registry Group (2007). Major childhood infectious diseases and other determinants associated with type 1 diabetes: a case-control study. Acta Diabetol. *44*, 14–19.

Trompette, A., Gollwitzer, E.S., Yadava, K., Sichelstiel, A.K., Sprenger, N., Ngom-Bru, C., Blanchard, C., Junt, T., Nicod, L.P., Harris, N.L., and Marsland, B.J. (2014). Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat. Med. *20*, 159–166.

UNESCO (2002). Papua New Guinea's vernacular language preschool programme. UNESCO Policy Brief on Early Childhood. October 2002. http://unesdoc.unesco.org/images/0013/001373/137383e.pdf.

Vellend, M. (2010). Conceptual synthesis in community ecology. Q. Rev. Biol. *85*, 183–206.

Walter, J., and Ley, R. (2011). The human gut microbiome: ecology and recent evolutionary changes. Annu. Rev. Microbiol. *65*, 411–429.

Walter, J., Britton, R.A., and Roos, S. (2011). Host-microbial symbiosis in the vertebrate gastrointestinal tract and the Lactobacillus reuteri paradigm. Proc. Natl. Acad. Sci. USA *108* (*1*), 4645–4652.

Wen, L., Ley, R.E., Volchkov, P.Y., Stranges, P.B., Avanesyan, L., Stonebraker, A.C., Hu, C., Wong, F.S., Szot, G.L., Bluestone, J.A., et al. (2008). Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature *455*, 1109–1113.

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., Knight, R., et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. Science *334*, 105–108.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., et al. (2012). Human gut microbiome viewed across age and geography. Nature *486*, 222–227.