# Market integration predicts human gut microbiome attributes across a gradient of economic development

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**ABSTRACT** Economic development is marked by dramatic increases in the incidence of microbiome-associated diseases, but the lifestyle changes that drive alterations in the human microbiome are not known. We surveyed numerous lifestyle factors associated with economic development and profiled fecal microbiomes of 213 participants from a contiguous, indigenous Ecuadorian population. Despite relatively modest differences in lifestyle across the population, greater economic development correlated with significantly lower within-host diversity, higher between-host dissimilarity, and a decrease in the relative abundance of the bacterium *Prevotella*. These microbiome shifts were most strongly associated with more modern housing, followed by reduced ownership of traditional subsistence lifestyle-associated items. Both factors are associated with decreased exposure to environmental microbes, indicating that decreased exposure may underlie the negative health outcomes associated with economic development such as allergy, autoimmunity, and inflammatory disorders.

**IMPORTANCE** Previous research has reported differences in the gut microbiome between populations residing in wealthy versus poorer countries, leading to the assertion that lifestyle changes associated with economic development promote changes in the gut microbiome that promote the proliferation of microbiome-associated diseases. However, a direct relationship between economic development and the gut microbiome has not previously been shown. We surveyed the gut microbiomes of a single indigenous population undergoing economic development and found significant associations between features of the gut microbiome and lifestyle changes associated with economic development. These findings suggest that even the earliest stages of economic development can drive changes in the gut microbiome, which may provide a warning sign for the development of microbiome-associated diseases.

**KEYWORDS:** microbiome, market integration, microbial ecology, biological anthropology.

## INTRODUCTION

It is increasingly evident that the gut microbiome—the collection of microbes found in the intestines of animals, including humans—plays a critical role in the development of

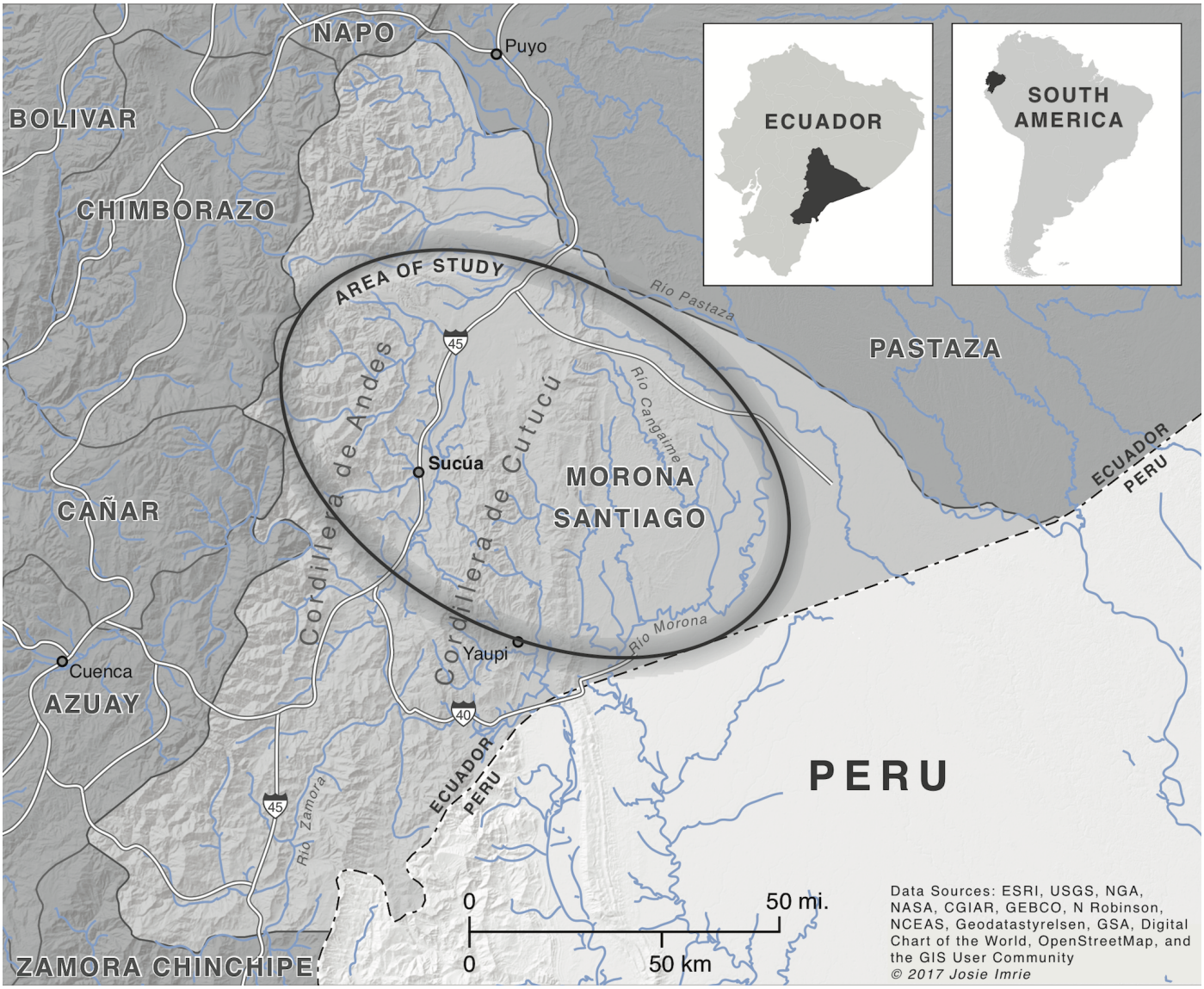


**FIG 1** **Map of Morona-Santiago Provence, Ecuador. The ellipse roughly corresponds to the area within which all five study villages reside. The two villages within the Upano Valley (west of the Cordillera de Cutucú and through which highway 45 runs), UV1 and UV2, have a travel time to the regional market center of Sucúa between 1 and 2 hours (including a 30-60 minute walk to the main road and a 30-60 minute car or bus ride). Travel times to Sucúa from three villages east of the Cordillera de Cutucú vary between 7 and 12 hours, based on time of departure, weather conditions, and river height. Estimates for typical travel times from each Cross-Cutucú village are as follows: 8.5-9.5 hr from CC1, 8-9 hr from CC2, and 10.5-11.5 hr from CC3.**

various diseases, including metabolic syndrome and immunoallergic disease (4, 30). Previous studies suggest that people from wealthier nations (e.g., those in western Europe and the United States) have gut microbiomes significantly different from people from nations undergoing economic development (e.g., Africa, South America, or the Pacific Islands) (5, 11, 20, 29, 37). This observation has led to the hypothesis that economic development results in substantial changes to the microbiome either through decreased exposure to environmental microbes (31) or loss of ancestral commensal microbes (2), resulting in the increased prevalence of major health problems associated with economic development, including cardiovascular disease, obesity, allergy, and autoimmune disorders (19, 23, 25, 36). However, these assertions derive from studies comparing the gut microbiomes of disparate populations (20, 29, 37), and thus confound the impact of economic development with many other important factors that influence microbiome composition and diversity, such as genotype, ethnicity, and geographic location (8, 26).

To test the role of economic development on intestinal microbiota diversity without such confounding factors, we conducted a survey of the fecal microbiome of a single indigenous population, the Shuar of southeastern Ecuador, and recorded household-level metrics of "market integration" (i.e. producing for and consuming from a market-based economy) to measure participants' level of economic development (9, 10, 17). The Shuar are experiencing rapid market integration, but share a recent common cultural and genetic history, having rapidly spread from a constrained geographic area in the last hundred years (Figure 1). The degree of market integration varies between individuals, households, and communities, but to a much lesser degree than between the populations studied in previous work. The impact of market integration on the health and well-being of the Shuar has been extensively studied (3, 14, 33). For example, as a whole the Shuar have favorable cardiovascular and metabolic health, and market integration is associated with both positive and negative health outcomes (14, 33). However, little is known regarding how market integration influences the Shuar's microbiomes.

For our study, samples were provided by participants living in five villages across a geographical region divided by the Cordillera de Cutucú mountain range. Two sample communities in the Upano Valley west of the Cordillera de Cutucú (UV1 and UV2) are approximately one hour by truck from the town of Sucúa, a local market center. Shuar in these communities tend to own more industrially produced items (e.g., televisions and portable propane stoves), and most reside in homes made from wood plank or recently introduced cinder block construction (14, 33). Three sample communities (CC1, CC2, CC3) in the region east of the Cordillera de Cutucú mountain range (referred to as "Cross-Cutucú") are much farther from market centers (1.5-3 hours by motor canoe to a road where they might sell produce, and an additional 5-8 hours by bus to Sucúa). Residents of these villages tend to own more subsistence-associated items (e.g., hunting or fishing equipment), more often live in traditional homes comprised of palmwood and thatch with dirt floors, and none live in cinderblock houses (14, 33). There is, however, substantial variation in market integration within each village, regardless of region (33). For example, some houses in the Upano Valley are still made using traditional materials, while more recently, houses in the Cross-Cutucú region have been built using wood planks. We therefore directly quantified the level of household market integration experienced by participants in this study, rather than simply using geographic location as a proxy measure of market integration, as previous studies have done (5, 11, 20, 29, 37). To do so, we used three style-of-life (SOL) metrics (see (8) and (9) for details). The first metric, SOL-House, is a composite

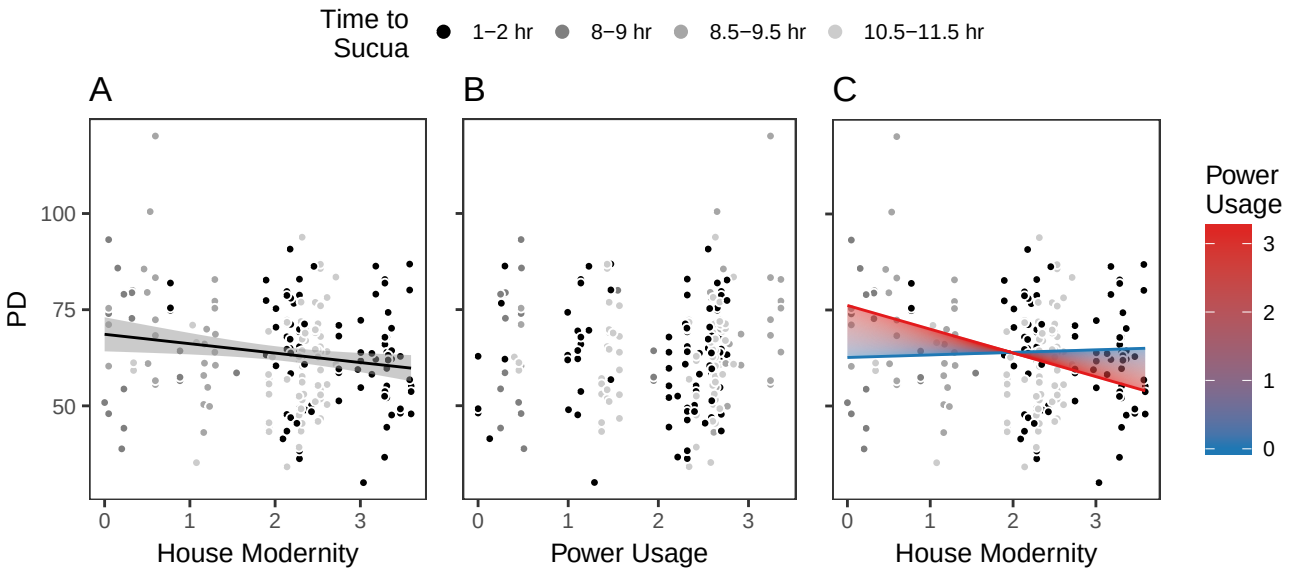


**FIG 2 Phylogenetic Diversity (PD) by significant market integration factors. (A) *House Modernity* (Factor 1), the black line is the best fit line from regressing PD by *House Modernity* ($R^2$ = 0.024, $p$ = 0.013); (B) *Power Usage* (Factor 3, $p$ = *n.s.*); and (C), the interaction between *House Modernity* and *Power Usage* ($R^2$ = 0.037, $p$ = 0.012). The blue line is the predicted relationship (using the full regression model) between PD and *House Modernity* when *Power Usage* is held to zero, the red line is the predicted relationship when *Power Usage* is set to its maximum, and the gradient between the two prediction lines represents predictions for each of 100 steps between the minimum and maximum values of *Power Usage*. (n = 213 for all panels).**

metric of five codes indicating type of housing construction and infrastructure. The second metric, SOL-Traditional, is the proportion of important items owned that reflect investment in a traditional foraging lifestyle. The third, SOL-Market, is the proportion of important items owned that reflect degree of investment in manufactured goods associated with the market economy. The codes and items for these metrics can be found in Table S1.

To reduce the number of variables in our analysis and to identify latent factors, we performed exploratory factor analysis including all individual items used in the SOL metrics. The factor analysis produced three factors, which we call (in order of variance explained): “House Modernity”, “Subsistence Items”, and “Power Usage” (the latter indicating the number of objects owned that require external electrical or petrochemical power such as radios, refrigerators, and gasoline engines). The results of the factor analysis and an explanation of the factor labels can be found in Table S2.

## RESULTS

Based on previous studies suggesting that market integration is inversely related to intra-individual microbiome diversity (α-diversity) (5, 11, 20, 37, 29), we predicted a negative correlation between the phylogenetic diversity (PD) of the gut microbiome and the factors associated with greater market integration: *House Modernity* and *Power Usage*. Similarly, we expected a positive correlation between PD and the *Subsistence Items* factor. As detailed in the methods, we performed model selection starting from a full model that included all three style-of-life factors, participant age, and the rank travel time from Sucúa and determined that the best fit model only included age, *House Modernity*, and *Power Usage*.

Because age followed the expected trends and did not interact with any other factors (Table S3), we omitted it from the rest of the analyses. Figure 2A shows the

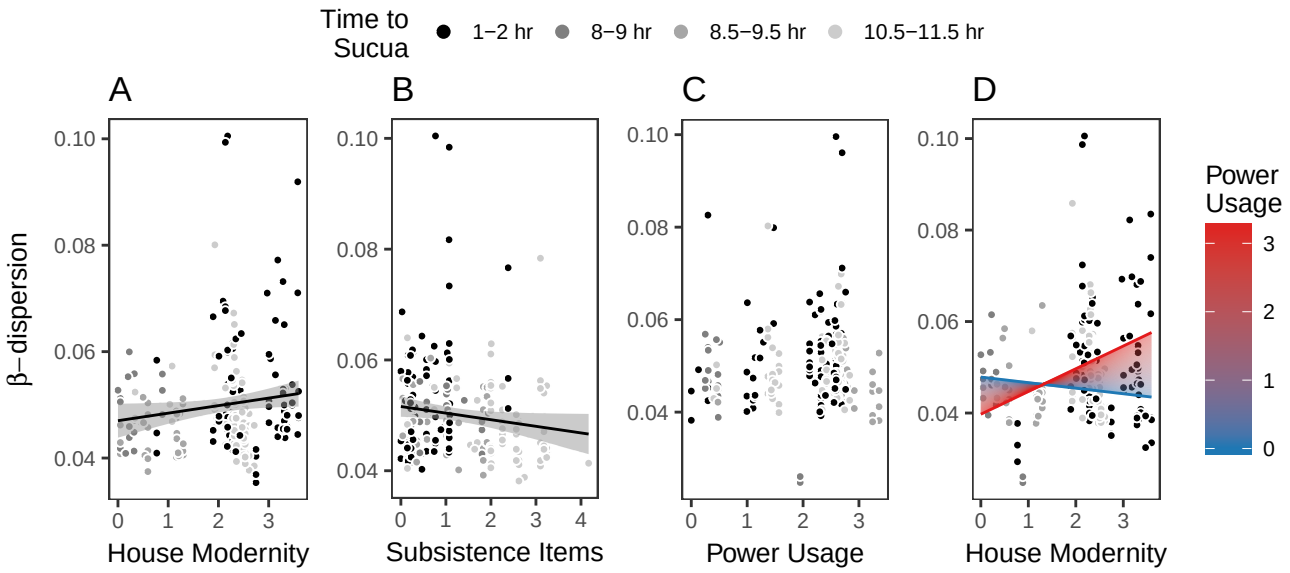


**FIG 3** **β-dispersion by each market integration factor. The term β-dispersion is often used when comparing the β-diversity of subjects within the same treatment or group. (A), *House Modernity*** ($n$ = 212, $R^2$ = 0.014, $p$ = 0.045)**, (B) *Subsistence Items*** ($n$ = 213, $R^2$ = 0.014, $p$ = 0.046)**, (C) *Power Usage*** ($n$ = 213, $p$ = *n.s.*)**, (D) the interaction between *House Modernity* and *Power Usage*** ($n$ = 209, $R^2$ = 0.034, $p$ = 0.018)**, β-dispersion was calculated as described in the methods. Black lines represent best fit regression lines for β-dispersion by each individual factor. The colored lines in panel D represent the predicted relationship between β-dispersion and *House Modernity* when *Power Usage* is held at zero up to its maximum observed value, divided into 100 steps.**

predicted significant negative relationship between PD and *House Modernity*. That is, participants with homes built from more modern materials have lower gut microbiome phylogenetic diversity than people with homes built from more traditional materials.

There was no significant relationship between PD and *Subsistence Items* or *Power Usage* (Figure 2B). However, there was a significant interaction between *Power Usage* and *House Modernity* such that as participants' *Power Usage* increases, the strength of relationship between PD and *House Modernity* increases (Figure 2C). Thus, *House Modernity* and *Power Usage* appear to be separate but related measures of market integration that are significantly associated with the diversity of the human gut microbiome.

Previous studies that compared disparate populations found that those in regions with higher market integration tend to have greater among-subject variation (β-diversity) than more traditionally living populations (20). It is hypothesized that this may be due to either lower levels of exposure to a common pool of environmental microbes or lower levels of microbial dispersal between individuals. We predicted that greater *House Modernity* and *Power Usage* would be associated with greater dissimilarity among participants' microbiomes, whereas higher *Subsistence Items* scores would be associated with greater homogeneity of participants' microbiomes. We calculated the mean weighted Unifrac (16) distance between the gut microbiomes of each subject and those of other subjects who experience similar levels of market integration (see Methods for details). These analyses confirmed our hypotheses: *House Modernity* was positively associated with among-subject variation (i.e., microbiomes were more dissimilar as *House Modernity* increased; Figure 3A), while *Subsistence Items* were negatively related to among-subject variation (i.e., microbiomes were more homogeneous as *Subsistence Items* increased; Figure 3B). Alone, *Power Usage* did not have a significant effect on among-subject variation (Figure 3C). However, as with within-host diversity, there was a significant interaction between *House Modernity* and *Power Usage* (Figure 3D),

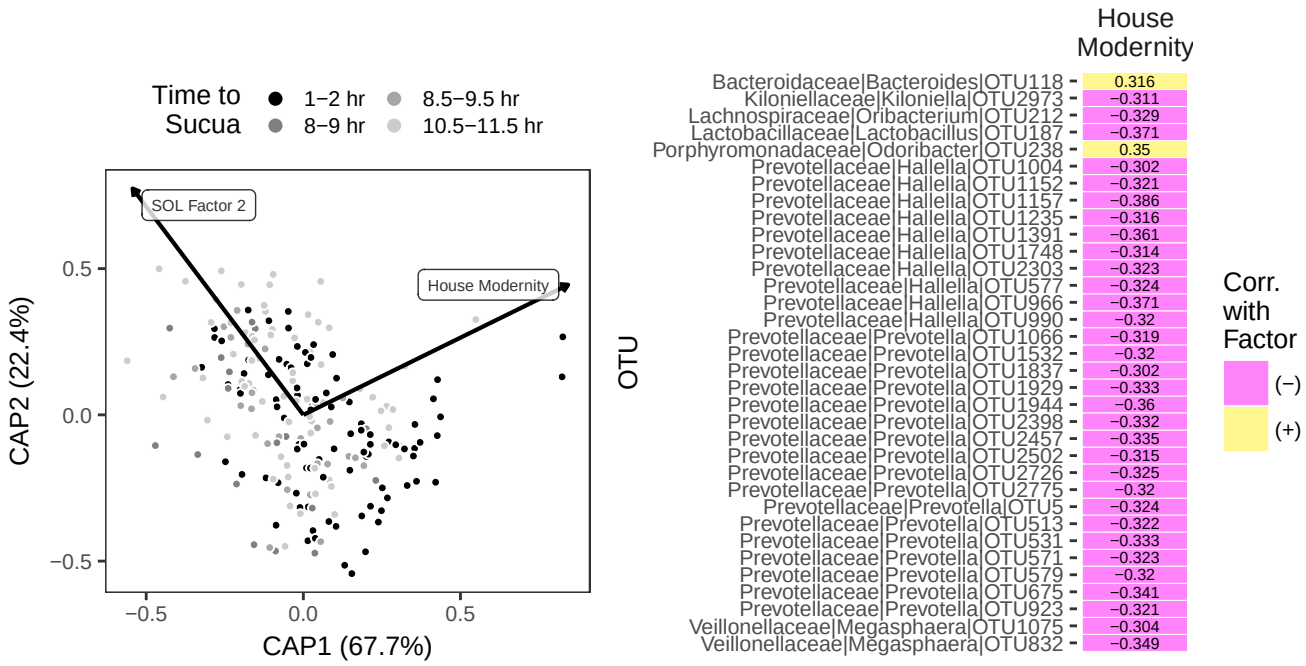


**FIG 4** **β-dispersion by each market integration factor. The term β-dispersion is often used when comparing the β-diversity of subjects within the same treatment or group. (A), *House Modernity* ($n$ = 212, $R^2$ = 0.014, $p$ = 0.045), (B) *Subsistence Items* ($n$ = 213, $R^2$ = 0.014, $p$ = 0.046), (C) *Power Usage* ($n$ = 213, $p$ = *n.s.*), (D) the interaction between *House Modernity* and *Power Usage* ($n$ = 209, $R^2$ = 0.034, $p$ = 0.018), β-dispersion was calculated as described in the methods. Black lines represent best fit regression lines for β-dispersion by each individual factor. The colored lines in panel D represent the predicted relationship between β-dispersion and *House Modernity* when *Power Usage* is held at zero up to its maximum observed value, divided into 100 steps.**

such that as *Power Usage* increases, the strength of the relationship between *House Modernity* and among-subject variation increases.

We analyzed the taxonomic composition of the gut microbiome of each subject via distance-based RDA (Figure 4A) and PERMANOVA (Table S4). These analyses reveal that the *House Modernity* and *Subsistence Items* factors are significantly associated with gut microbiome composition. Furthermore, these two factors are nearly orthogonal in the ordination space, implying that they have nearly independent relationships with microbiome composition. This result is to be expected if these style-of-life factors are measuring aspects of participants' lives that expose them to, isolate them from, or select for, non-overlapping consortia of environmental microbes. Thus, it further highlights the importance of providing specific measures of market integration, something rarely done in past studies of microbiome variation.

Finally, a multiple correlation test ($\alpha$ = 0.05, false discovery rate corrected) of the relationships among the abundances of all microbiome taxa and the three market integration factors revealed 32 operational taxonomic units (OTUs) that were negatively correlated, and two that were positively correlated, with *House Modernity* (Figure 4B). Of these 32 OTUs, 16 were assigned to the genus *Prevotella*, and another ten to the genus *Hallela*, a member of the Prevotellaceae family. Of the two OTUs positively correlated with *House Modernity*, one was assigned to *Bacteroides*. These results are consistent with previous studies. For example, Yatsunenko et al. (37) reported that 23 of 73 OTUs that were over-represented in Amerindian or Malawian versus U.S. adults were assigned to *Prevotella*, and De Filippo et al. (5) found that the intestinal microbiomes of participants from Burkina Faso harbored a much larger proportion of *Prevotella* than

that of participants from the United States. Additionally, Yatsunenko et al. (37) reported a negative relationship between the abundance of *Prevotella* and *Bacteroides* in adults, while De Filippo et al. (5) reported a greater proportion of *Bacteroides* in microbiomes from US individuals relative to microbiomes from Burkina Faso individuals.

## DISCUSSION

Our results suggest that even within a single ethnicity living in a constrained geographic region, the early stages of market integration affect the diversity and composition of the gut microbiome. In particular, the modernity of participants' homes consistently predicts gut microbiome attributes. The mechanism by which *House Modernity* affects the gut microbiome cannot be definitively determined from our study, but it could plausibly be due to the isolation from environmental microbes afforded by more modern housing. For example, related work with the Shuar showed reduced exposure to helminth soil parasites in more modern homes (28). Traditional housing consists of palm thatch structures with dirt floors, which allow more exposure to microbes from the "outside" (i.e. those associated with soil and plants) than does more modern housing (which consists of wood or cinder block structures with plank or concrete floors). The idea that more modern housing excludes environmental microbes is consistent with our previous work associating house modernity with reduced exposure to soil-transmitted parasites (28). The intensifying effect of *Power Usage* on the relationship between *House Modernity* and microbiome diversity metrics may be the result of numerous lifestyle changes that reduce a person's exposure to environmental microbes, such as remaining in their homes to use powered devices, employment in jobs (such as teaching) that are primarily indoors, or having access to a vehicle and a refrigerator increases the likelihood that food is bought commercially rather than foraged, fished, or hunted. Ownership of *Subsistence Items*, on the other hand, could be positively correlated with environmental microbe exposure associated with outdoor activities and non-domesticated animals, such as hunting. Alternatively, *Subsistence Items* and *House Modernity* (and its interaction with *Power Usage*) may together be a proxy for a suite of other lifestyle factors (e.g. dietary changes, healthcare practices, etc.) associated with economic development, which could be the actual drivers of the microbiome differences we observed.

Cardiovascular disease is now the leading cause of death in all but the lowest income nations (36). Obesity, already a major public health problem in wealthier nations, is rapidly increasing in the developing world (36). Allergy and autoimmune disorders continue to rise in the west (25). The increasing incidence of these and other microbiome-associated disorders currently experienced by populations in wealthy nations has been hypothesize to be driven by the loss of microbes essential to human health (the "Hygiene Hypothesis" (31) and the "Disappearing Microbiota Hypothesis" (2)). These hypotheses assert that recent lifestyle changes have either limited our exposure to or have driven extinct certain members of the microbiome in economically developed nations. The association between early market integration and gut microbiome composition and diversity observed in our study demonstrates that economic development can, indeed, alter the human microbiome, as predicted by these hypotheses. Furthermore, we show that these changes occur even in the early stages of market integration. This indicates that slower mechanisms, such as reduce trans-generational microbiome transmission, are unlikely to explain these effects. Our results are consistent with the assertion that reduced exposure to environmental microbes is a major driver of microbiome changes in economically developing countries, although further research is needed to definitively test this hypothesis. Finally, our results suggest

that the microbiome differences we observed may provide an early warning sign for microbiome-associated disorders in rapidly developing countries.

## MATERIALS AND METHODS

**Quantification of market integration and factor analysis** The three style-of-life (SOL) metrics were determined as described in previous work (14, 33). In short, researchers conducted structured interviews, administered mostly in Spanish (or through a bilingual translator for subjects who did not speak Spanish), to collect a range of demographic and lifestyle information. Ages of participants ranged from one to 100 years. Dietary data were collected in the form of a food frequency questionnaire, but as we did not directly quantify caloric amount and nutritional content of food consumed by each participant, these data were excluded from the analysis. Ethnographic observations and pilot testing over the course of a decade led to the selection of items in the House, Traditional, and Market style-of-life metrics. The final SOL-Traditional scale contained six items reflecting investment in a foraging lifestyle, while the SOL-Market scale included 12 items reflecting investment in a market economy. Individual scores were calculated as the fraction of list items owned (range 0–1). The SOL-House metric included five household measures as indices of household permanence, access to infrastructure, market participation, and pathogen risk. Individual scores for these metrics broken down by village can be found in Supplemental Figure 1. We conducted an exploratory factor analysis on the two item-based metrics (SOL-Traditional and SOL-Market), along with the five components of the SOL-House metric (type or presence of wall, floor, bathroom, water, and electricity in a participant's home) using the factanal function from the basic R stats package (27). Starting with fitting a single factor, we increased the number of fitted factors until either we reached the maximum allowed by the method (three for seven input variables) or until the p-value of the analysis was less than 0.05. This analysis resulted in three market integration factors that were similar to the style-of-life metrics except that the electricity type (from SOL-House) loaded most strongly on the third factor with SOL-Market. Biplots from the factor analysis can be found in Figure S1.

**Stool collection and DNA extraction** Three hundred stool samples were collected as described previously (3). Briefly, participants were given a pre-packed plastic bag containing an empty stool container and clean implements with which to collect the stool, and instructed on the collection technique. Participants returned the containers, and samples were preserved in RNAlater (ThermoFisher Scientific, Waltham, MA, USA) within an hour of sample collection. Preserved samples were stored in a portable freezer at −20 °C over the course of data collection, and then shipped to the lab on dry ice, where it was stored at −80 °C until analysis. DNA was extracted from the samples using the Blood and Stool kit (Qiagen, Hilden, Germany) in accordance with the kit protocol. No human data was gathered as part of this project, and the bacterial data gathered was purged of all sequences that aligned to the human genome (including mitochondrial genome) before archiving. Genetic material resulting from this research will never be used for human DNA research or commercial cell-line patenting.

**Ethics Statement** Informed verbal consent was obtained from adult participants. For participants under 15 years old (the local age of consent), parental verbal consent and child assent were obtained. Individuals were informed that they could choose not to participate, to participate only in individual portions of the study, or to participate in the full study. The study and consent procedures were approved by the Institutional Review Board (IRB) of the University of Oregon, and a central Shuar governing organization authorized research in member villages. The precise locations of the villages

were omitted from Figure 1 to protect the anonymity of the participants.

**Illumina library preparation and 16S rRNA gene sequence analysis** We characterized the intestinal microbial communities of fecal samples via Illumina (San Diego, CA, USA) sequencing of 16S rRNA gene amplicons. To prepare amplicons for Illumina sequencing, we used a single-step PCR method to add dual indices and adapter sequences to the V4 region of the bacterial 16S rRNA gene (no human sequences were specifically targeted) and generate paired-end 150 nucleotide reads on the Illumina HiSeq 2000 platform. Sequences can be accessed under the NCBI BioProject number PRJNA362944.

The 16S rRNA gene Illumina reads were processed using methods implemented by FLASH (18), the FASTX Toolkit (1), and the USEARCH pipeline (6). The processing pipeline can be found at http://www.github.com/kstagaman/Process_16S. Operational taxonomic units (OTUs) were defined using 97% sequence similarity. Any amplicons that matched the human genome were removed from the analysis with bowtie (13) prior to OTU clustering. Read assembly, quality control, and OTU table building were done on the University of Oregon ACISS cluster, and all subsequent data processing and diversity analyses were done in R (27).

**Intestinal microbiota diversity analyses** Samples were not included in the analysis if they had fewer than 20,000 total reads, or from individuals lacking complete SOL metric data. OTU abundances of the remaining 213 samples were variance-stabilized using phyloseq (21) and DESeq2 (15) as recommended (22). We measured phylogenetic diversity using Faith's PD (7), which takes into account taxon abundances as well as their phylogenetic relationship, as implemented in the picante package (12), and chose the best linear model using the anova function from the base R stats package (27). We used the distance function from the phyloseq package to calculate weighted Unifrac distances (16) between microbiomes. When comparing the β-diversity of subjects within the same treatment or group, the term β-dispersion is often used. We calculated β-dispersion as the mean weighted Unifrac community distance between each participant and other participants within 5% of the same factor score (thus comparing similarly market-integrated participants; analyses using between 2.5 and 10% of factor scores resulted in qualitatively similar results). Using the same distance matrix, we generated a distance-based redundancy analysis (db-RDA) ordination using the capscale function and measured individual factor R-squared values via PERMANOVA using the adonis function, both from the vegan package (24). Other distance metrics were used and produced qualitatively similar results. Diversity data visualization was done with the ggplot2 (35), ggfortify (32), and ggbiplot (34) packages.

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## SUPPLEMENTARY MATERIAL

**TABLE S1** Composite codes for the SOL-House metric and item lists for SOL-Traditional and SOL-Market metrics.

| Code Name | Code | Description |
|---|---|---|
| House Code | 0 | palmwood |
| House Code | 1 | mixed |
| House Code | 2 | milled lumber |
| House Code | 3 | cinder block |
| Floor Code | 0 | dirt |
| Floor Code | 1 | palmwood |
| Floor Code | 2 | milled lumber |
| Floor Code | 3 | concrete |
| Floor Code | 4 | tile |
| Bathroom Code | 0 | none |
| Bathroom Code | 1 | pit |
| Bathroom Code | 2 | indoor without water |
| Bathroom Code | 3 | outdoor with water |
| Bathroom Code | 4 | indoor with water |
| Water Code | 0 | river/stream |
| Water Code | 1 | well/outdoor pipe |
| Water Code | 2 | indoor pipe |
| Electricity Code | 0 | none |
| Electricity Code | 1 | lights only |
| Electricity Code | 2 | outlets |

| SOL-Traditional Item List |
|---|
| Fishing hook/line |
| Hunting dog |
| Blowgun |
| Firearm |
| Fishing net |
| Canoe |

| SOL-Market Item List |
|---|
| Radio |
| Propane stove |
| Mobile phone |
| Television |
| Chainsaw |
| Bicycle |
| Refrigerator |
| Computer |
| Outboard motor |
| Motorcycle |
| Car |
| Truck |

**TABLE S2** Results from factor analysis on the components of the SOL-House metric, SOL-Traditional, and SOL-Market. The first factor is most strongly composed of the wall type and the floor type of a subject's home, and to a lesser extent access to water and the type of latrine associated with the home. The more manufactured the materials used to build a subject's house (e.g., cinder block vs palmwood), the higher their Factor 1 score. Therefore, we named Factor 1 "House Modernity". The second factor is almost exclusively defined by the proportion of objects a subject owns from the SOL-Traditional list, thus we called it "Subsistence Items". The third factor's strongest loadings are the level of access to electricity in a subject's house and the proportion of objects a subject own from the SOL-Market list, which is mostly composed of items that use either electrical or petrochemical power. Factor 3 is therefore called "Power Usage".

| | Wall | Floor | Bathroom | Water | Electricity | SOL-Traditional | SOL-Market |
|---|---|---|---|---|---|---|---|
| Uniquenesses | 0.19 | 0.26 | 0.60 | 0.66 | 0.26 | 0.01 | 0.81 |

| | Factor1 | Factor2 | Factor3 |
|---|---|---|---|
| Wall Code | **0.89** | -0.092 | 0.068 |
| Floor Code | **0.8** | -0.29 | 0.16 |
| Bathroom Code | **0.55** | -0.28 | -0.15 |
| Water Code | **0.56** | 0.12 | -0.11 |
| Electricity Code | 0.21 | -0.14 | **0.82** |
| SOL-Traditional | -0.12 | **0.99** | -0.053 |
| SOL-Market | -0.14 | 0.049 | **0.41** |

| | Factor1 | Factor2 | Factor3 |
|---|---|---|---|
| SS Loadings | 2.12 | 1.18 | 0.91 |
| Proportion Var. | 0.30 | 0.17 | 0.13 |
| Cumulative Var. | 0.30 | 0.47 | 0.60 |

**TABLE S3** Significance of terms in the full model for predicting phylogenetic diversity (PD). Terms with p-values less than 0.05 are bolded.

| | D.f. | Sum Sq. | Mean Sq. | *F* value | Pr(> *F*) |
|---|---|---|---|---|---|
| Time to Sucúa (rank) | 1 | 4.72 | 4.72 | 0.03 | 0.867 |
| **Age** | 1 | 819.61 | 819.61 | 4.90 | **0.028** |
| **House Modernity** | 1 | 1158.04 | 1158.04 | 6.92 | **0.00918** |
| **Subsistence Items** | 1 | 808.48 | 808.48 | 4.83 | **0.0291** |
| Power Usage | 1 | 124.27 | 124.27 | 0.74 | 0.39 |
| Age:House Modernity | 1 | 39.28 | 39.28 | 0.23 | 0.628 |
| Age:Subsistence Items | 1 | 0.14 | 0.14 | 0.00 | 0.977 |
| House Modernity:Subsistence Items | 1 | 609.87 | 609.87 | 3.65 | 0.0576 |
| Age:Power Usage | 1 | 138.36 | 138.36 | 0.83 | 0.364 |
| **House Modernity:Power Usage** | 1 | 968.02 | 968.02 | 5.79 | **0.0171** |
| Subsistence Items:Power Usage | 1 | 265.41 | 265.41 | 1.59 | 0.209 |
| Age:House Modernity:Subsistence Items | 1 | 37.11 | 37.11 | 0.22 | 0.638 |
| Age:House Modernity:Power Usage | 1 | 113.75 | 113.75 | 0.68 | 0.411 |
| Age:Subsistence Items:Power Usage | 1 | 395.05 | 395.05 | 2.36 | 0.126 |
| House Modernity:Subsistence Items:Power Usage | 1 | 0.27 | 0.27 | 0.00 | 0.968 |
| Age:House Modernity:Subsistence Items:Power Usage | 1 | 380.01 | 380.01 | 2.27 | 0.133 |
| Residuals | 196 | 32780.68 | 167.25 | | |

**TABLE S4** Result of PERMANOVA analysis of contribution of style-of-life factors to microbiota composition. Terms with p-values less than 0.05 are bolded.

| | D.f. | Sum of Sqs. | Mean Sqs. | $F$ Model | $R^2$ | Pr($>F$) |
|---|---|---|---|---|---|---|
| **House Modernity** | 1 | 0.01 | 0.01 | 3.98 | 0.02 | **0.0092** |
| **Subsistence Items** | 1 | 0.00 | 0.00 | 2.54 | 0.01 | **0.0431** |
| Power Usage | 1 | 0.00 | 0.00 | 0.86 | 0.00 | 0.437 |
| Residuals | 209 | 0.30 | 0.00 | | 0.97 | |
| Total | 212 | 0.31 | | | 1.00 | |

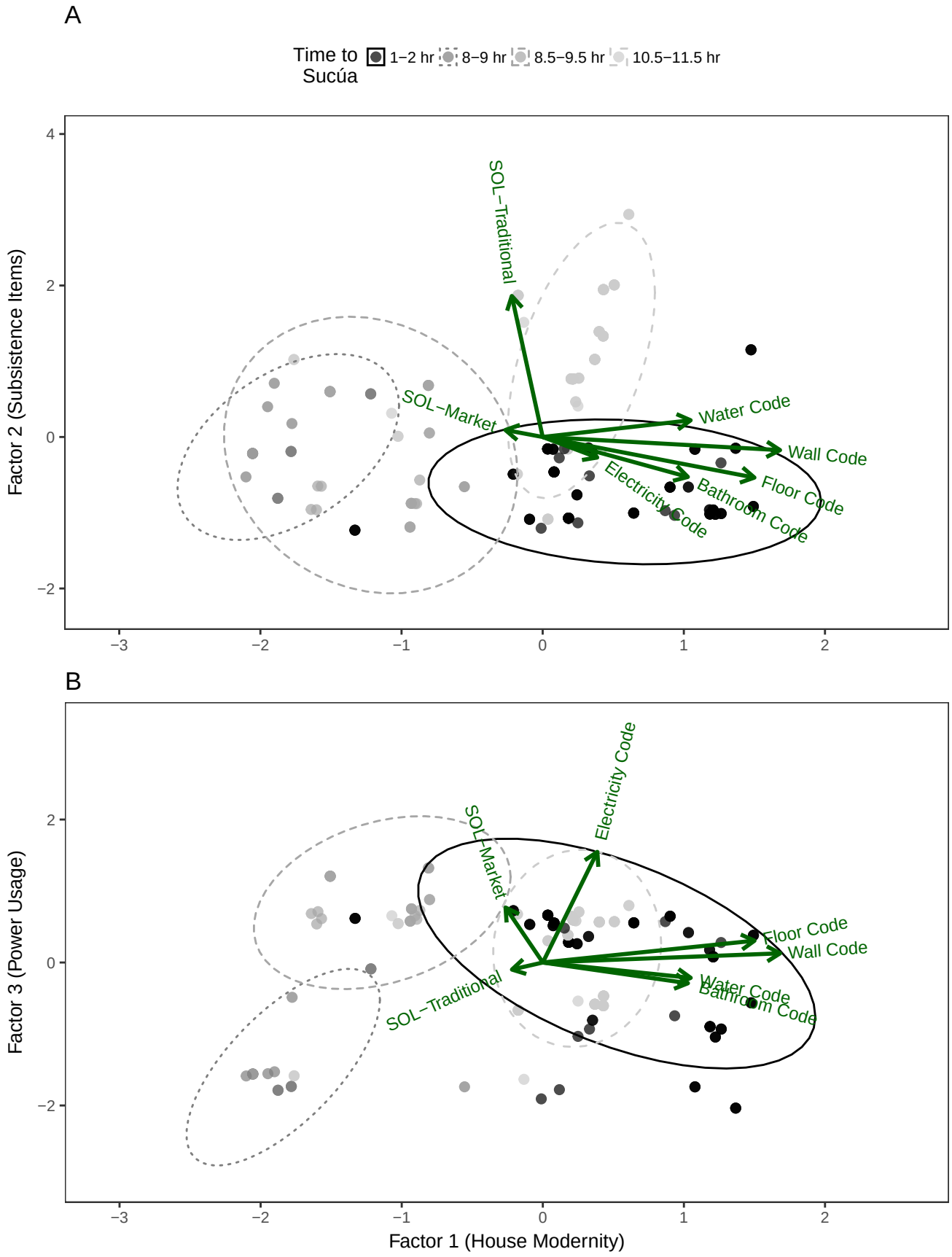


**FIG S1** **Biplots of item codes and style-of-life metrics with factor scores for each participant. The contribution of item codes and style-of-life metrics to each factor are represented by the direction and magnitude of its labelled green vector. Points represent scores for each participant (n = 213) for each factor and are colored by the average travel time from each village to Sucúa. Ellipses represent the standard error around the centroid for each estimated travel time. The top**