HHS Public Access

Author manuscript

Nature. Author manuscript; available in PMC 2016 July 14.

Published in final edited form as:

Nature. 2016 January 14; 529(7585): 212–215. doi:10.1038/nature16504.

Diet-induced extinction in the gut microbiota compounds over generations

Erica D. Sonnenburg[1,*], **Samuel A. Smits**[1,*], **Mikhail Tikhonov**[2], **Steven K. Higginbottom**[1], **Ned S. Wingreen**[3], and **Justin L. Sonnenburg**[1,†]

[1]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA

[2]Department of Mathematics, Harvard University, Cambridge, MA 02138, USA

[3]Department of Molecular Biology, Princeton University, Princeton, NJ 08540

Abstract

The gut is home to trillions of microbes that play a fundamental role in many aspects of human biology including immune function and metabolism [1,2]. The reduced diversity of the Western microbiota compared to populations living traditional lifestyles presents the question of which factors have driven microbiota change during modernization. Microbiota accessible carbohydrates (MACs) found in dietary fiber, play a key role in shaping this microbial ecosystem, and are strikingly reduced in the Western diet relative to more traditional diets [3]. Here we show that changes in the microbiota of mice consuming a low-MAC diet and harboring a human microbiota are largely reversible within a single generation, however over multiple generations a low-MAC diet results in a progressive loss of diversity, which is not recoverable upon the reintroduction of dietary MACs. To restore the microbiota to its original state requires the administration of missing taxa in combination with dietary MAC consumption. Our data illustrate that taxa driven to low abundance when dietary MACs are scarce are inefficiently transferred to the next generation and are at increased risk of becoming extinct within an isolated population. As more diseases are linked to the Western microbiota and the microbiota is targeted therapeutically, microbiota reprogramming may need to involve strategies that incorporate dietary MACs as well as taxa not currently present in the Western gut.

†Correspondence and requests for materials should be addressed to J.L.S., jsonnenburg@stanford.edu.
*These authors contributed equally

Author Contributions. E.D.S. and J.L.S. conceived and designed the project. E.D.S., J.L.S., and S.K.H. designed and supervised the experiments. E.D.S. and S.K.H. performed the experiments. E.D.S., S.A.S., and M.T. analyzed the experimental data. N.S.W. designed and supervised data analysis. E.D.S. and S.A.S. wrote the manuscript. All authors discussed the results and commented on the manuscript.

The 16S sequence data has been deposited in the Sequence Read Archive (SRA) under the project ID: PRJNA303185 (http://www.ncbi.nlm.nih.gov/sra).

The authors declare no competing financial interests.

The gut microbiota of hunter-gatherers and populations consuming a rural agrarian diet is distinct and harbors greater diversity than the microbiota of Westerners [4–9] (Extended Data Fig. 1). One possible explanation for the greater microbiota diversity seen in hunter-gatherers and agrarians is the large quantity of dietary fiber they consume relative to Westerners. Microbiota-accessible carbohydrates (MACs), which are abundant in dietary fiber, serve as the primary source of carbon and energy for the distal gut microbiota [3,4,6,10–12]. Therefore, we wished to determine whether a diet low in MACs could drive loss of taxa within the gut microbiota.

Humanized mice (4 weeks old, n=10) were fed a diet rich in fiber derived from a variety of plants (high-MAC) for six weeks and randomly divided into two groups (Extended Data Fig. 2). One group was switched to a low-MAC diet for seven weeks, after which they were returned to the high-MAC diet for four weeks (Figure 1A; Extended Data Table 1). The control group was maintained on the high-MAC diet throughout the experiment. At the start of the experiment the microbiota composition from both groups of mice was indistinguishable (Student's t-test p=0.2; UniFrac distance; no significant difference in OTU frequency observed between groups, Mann-Whitney *U* test). The diet-switching mice, while consuming the low-MAC diet, had an altered composition relative to controls (Student's t-test $p=10^{-25}$; UniFrac distance). Weeks after return to the high-MAC diet, the microbiota of the diet-switching mice remained distinct from controls (Student's t-test $p=3\times10^{-8}$; UniFrac distance at 15 weeks) (Figure 1B). To determine whether taxa had been lost over the course of the diet perturbation, we focused on a subset of OTUs that met stringent measures of prevalence and abundance and could be confidently monitored over the course of the experiment ("high-confidence" OTUs, see Methods). We identified 208 high-confidence OTUs in the diet-switching group and 213 high-confidence OTUs in the control group (Extended Data Table 2). When mice were switched from the high-MAC diet to the low-MAC diet, we observed that 60% of taxa (124 out of 208) decreased in abundance at least two-fold compared with only 11% of the control group (25 out of 213). When these mice were returned to a high-MAC diet, 33% (71 out of 208) were two-fold less abundant. The control group did not change significantly (10% were two-fold less abundant; 22 out of 213) (Figure 1C; Table S1 and S2). These data reveal two divergent qualities of the microbiota. First, 58 of the 208 high-confidence OTUs that exhibit diet-induced decline in abundance recovered (were no longer at least two-fold less abundant) with the reintroduction of MACs illustrating microbiota resilience over short time scales (Table S3). However, secondly, the low-MAC diet perturbation induced "scars" on the microbiota.

We hypothesized that diet-induced microbiota diversity loss would be magnified over generations. Mice from the previous experiment consuming the low-MAC diet or the high-MAC diet were used to generate a litter of pups. Pups were weaned onto the respective diets of their parents. This breeding strategy was repeated for four generations. For each generation, low-MAC diet parents were switched to the high-MAC diet after their pups were weaned, to see if taxa that became undetectable while MACs were scarce would bloom in the presence of MACs (Figure 2A, Extended Data Fig. 3). At five weeks old, mice propagated in the low-MAC diet condition (born to low-MAC diet parents and consuming a low-MAC diet) had a lower diversity microbiota than high-MAC fed controls (Student's t-test, Shannon Index $p=3\times10^{-6}$, $p=8\times10^{-5}$, $p=8\times10^{-6}$; generations two, three, and four,

respectively) (Figure 2B, top). Even after mice were switched to the high-MAC diet for several weeks, their microbiota diversity did not recover to control levels (Student's t-test $p=2\times10^{-6}$, $p=1\times10^{-8}$, $p=1\times10^{-4}$, generations two, three, and four, respectively at week 15) (Figure 2B, bottom). With each generation, the microbiota composition of the diet-switching group showed increasing departure from that of controls (Figure 2C). Weaning the diet-switching lineage directly onto the high-MAC diet did not correct the diversity loss relative to controls (Student's t-test, Shannon Index $p=3\times10^{-6}$) and there was no difference in composition between this group and the generation four diet-switching group (Student's t-test p=0.9; UniFrac distance; week 13, generation four and week 5, generation five) (Extended Data Fig. 4).

Plotting the relative abundance of the high confidence OTUs over time revealed a pattern of taxa loss over generations in the diet-switching group (Figure 2D). Specifically, in the diet-switching group generations one, two, and three exhibited a progressive loss in high-confidence OTUs while consuming the low-MAC diet (72%; 150 of 208 lost by generation four, week 15) (Table S3). In each generation, switching to the high-MAC diet allowed for the recovery of a small number of taxa (grey vs. yellow highlighted rows within each generation in Figure 2D), but most did not return (141 of 208 were undetectable by generation four, week 15) (Table S3). Most of the lost taxa (112 of the 141) were from the Bacteroidales order with an additional loss of 26 taxa from the Clostridiales, making the Clostridiales the most numerous high confidence taxon present in the fourth generation (Extended Data Fig. 5a,b).

To determine whether the carbohydrate degrading capacity of the microbiota had been altered over the four generations, we compared imputed glycoside hydrolases (GH) between the first and fourth generations of both the diet-switching and control groups after validating this method (Table S4)[13,14]. Although representation of glycoside hydrolase families is not a perfect correlate of specificity for polysaccharide degradation (e.g., due to combinatorial activity or polyspecificity within a family) loss of representation within GH families provides one measure of changes in glycan degrading capacity. Twenty-two GH families showed a loss in abundance in the diet-switching group between the initial time point in generation one and four weeks after the switch to the high-MAC diet in generation four (Bonferroni-corrected Student's t-test, $p<0.05$ plus at least a twofold change) (Extended Data Fig. 5c) (Table S5). No differences in GH families were observed in the control group. An overall loss in GH diversity occurred between generation four diet-switching mice on the high-MAC diet relative to generation one mice (Shannon Index of GH subfamilies, p=0.0002; Table S6, Extended Data Table 3). No difference in GH subfamilies was observed in the control group. These data demonstrate that, in addition to the loss of high-confidence OTUs, the diet-switching group sustained a widespread and marked loss in glycoside hydrolase repertoire over the four generations. Future experiments will be needed to reveal the functional consequences of these observations in terms of fibre degrading capacity.

We next wanted to determine whether low abundance OTUs that bloom when MACs are reintroduced are more likely to be lost due to inefficient inter-generational transmission. Low abundance taxa (average abundance < 25 reads) in a given generation were less efficiently transferred to the next generation (average abundance > 10 reads to be considered

present, four weeks after high-MAC diet) compared to high abundance taxa (average abundance > 25 reads) (hypergeometric distribution p=0.002, $p=4\times10^{-15}$, p=0.01, inheritance between generations one and two, two and three, and three and four; respectively) (Table S7). The overall diversity and composition did not change between the third and fourth generation (Figure 2B, 2C), however, we wondered whether additional loss could be obscured due to lack of resolution of OTUs. Therefore, we identified 280 high-confidence sub-OTUs in the control group and 261 high-confidence sub-OTUs in the diet-switching group using a cluster-free filtering approach (Table S8)[15]. A similar decline in the number of sub-OTUs with each generation was observed (114 of 261 sub-OTUs were undetectable by generation four, week 15) (Extended Data Fig. 6 and Table S8). Most of the lost taxa (77 of the 114) were from the Bacteroidales order with an additional loss of 32 taxa from the Clostridiales. Between generation three and four, we detected loss of 22 sub-OTUs, compared to four using the lower resolution high-confidence OTUs (Table S8).

Since high dietary MACs were insufficient to restore microbiota composition or diversity to control levels, we tested whether reintroduction of lost bacteria was required. Fourth generation, diet-switching mice were gavaged with fecal contents (fecal microbiota transplant group; FMT) from fourth generation high-MAC diet controls. Since the low-MAC diet does not support full microbiota diversity (Figure 2D), the fourth generation FMT recipients were fed a high-MAC diet for two weeks (Figure 3A). Within ten days, microbiota composition and diversity of the FMT group was indistinguishable from fourth generation high-MAC diet controls (Student's t-test p=0.4 UniFrac distance; Student's t-test p=0.4 Shannon Index) (Figure 3B, 3C) and 110 taxa were restored (average abundance ≥1 sequencing read; no taxa restored in no FMT controls) (Figure 3D, Table S9). Restored taxa were predominantly from the Bacteroidales (99 taxa), which experienced the greatest loss in high-confidence OTUs (Extended Data Fig. 7a). Similar results were observed using the high-confidence sub-OTUs (Extended Data Fig. 7b, Table S10).

These data demonstrate a diet-induced ratcheting effect in which certain taxa decrease in abundance upon reduced MACs and are not effectively transferred to the next generation. Notably, the majority of the lost taxa are Bacteroidales, an order that is proficient in consumption of dietary fiber [16]. Introduction of dietary MACs are insufficient to regain "lost" taxa in the absence of their deliberate re-introduction.

Over our history, humans have experienced major dietary changes from gathered to farmed foods during the Agricultural Revolution, and more recently to the mass consumption of processed foods in the industrialized world. Each dietary shift was likely accompanied by a concomitant adjustment in the microbiota. Here we have used a model in which mice have been colonized with a human microbiota from a Westerner to determine the effect of fiber deprivation over four generations on the gut microbiota. This model does not allow us to address microbiota changes that may have occurred as humans shifted from a hunter-gatherer lifestyle to one from a modern industrialized country. Our data support a model in which consuming a modern diet low in fiber contributes to the loss of taxa over generations and may be responsible for the lower diversity microbiota observed in the industrialized world compared to present-day hunter-gatherers and rural agrarians. The data we present also hint that further deterioration of the Western microbiota is possible.

The gut microbiota regulates numerous facets of human biology suggesting that our human genome has been shaped by interactions with these microbes over our co-evolutionary history. However, the microbiota can change on a timescale that is much faster than the host allowing for the possibility that the microbiota, if pressed by severe selective forces, could undergo change so rapidly that it cannot be accommodated by our human biology. While the roles of different types of microbiota diversity in host health remain to be defined, it is possible that rewilding the modern microbiota with extinct species may be necessary to restore evolutionarily important functionality to our gut.

Methods

Meta-analysis of human populations

16S rRNA datasets from Hadza (n=27), Malawian and Venezuelan (n=213) and American (n=315) [4,5] were trimmed to match FLX chemistry as previously described (MG-RAST Projects 111, 528, 7058) [12]. OTUs were picked on the Greengenes 13.8 database with a 97% similarity threshold using UCLUST [17]. Alpha-diversity and beta-diversity measures were calculated using unweighted UniFrac [18] on rarified OTU tables. PCoA coordinates were computed using QIIME 1.8 [19]. Fecal 16S rRNA data was not included from other studies of traditional populations because they 1.) did not use the V4 region of the 16S rRNA for amplification and were thus not comparable (Papua New Guinea[9]), 2.) have not made the data publicly available (Yanomami[7]), or 3.) the control Western data was not comparable to other studies (Matses[8]).

Mice

All mouse experiments were performed in accordance with A-PLAC, the Stanford IACUC on mixed gender germ-free Swiss Webster mice that were humanized by oral gavage of human fecal sample obtained from a healthy anonymous donor (American male living in the San Francisco Bay Area, CA, age 42, omnivorous diet) as previously described [20]. Humanized mice faithfully reconstituted the diversity and phylogenetic make-up of the donor (Pearson's r=0.96; Extended Data Fig. 2). Mice were randomly assigned to two groups and were fed either a high MAC diet (LabDiet 5010) or a low MAC diet (Harlan TD. 86489). The high-MAC diet is a plant polysaccharide-rich diet in which the microbiota accessible carbohydrates come from a diverse source of plants including: corn, soybean, wheat, oats, alfalfa, and beet. The reported neutral detergent fiber content of the high-MAC diet is 15% by weight. The low-MAC diet is defined diet in which carbohydrates are from sucrose (31% by weight), corn starch (31% by weight), and cellulose (5% by weight). The accessibility of cellulose to gut microbiota is known to be extremely low and we have been unable to isolate bacteria from the microbiota that utilize this substrate[20]. Mice from the fecal transplant experiment were from the fourth generation of mice from the diet-switching group. Fecal transplants were carried out by gavage using freshly collected fecal samples from fourth generation control mice consuming a high MAC diet using a procedure identical to the original humanization [21]. Fecal samples were collected throughout all mouse experiments and stored at −80°C.

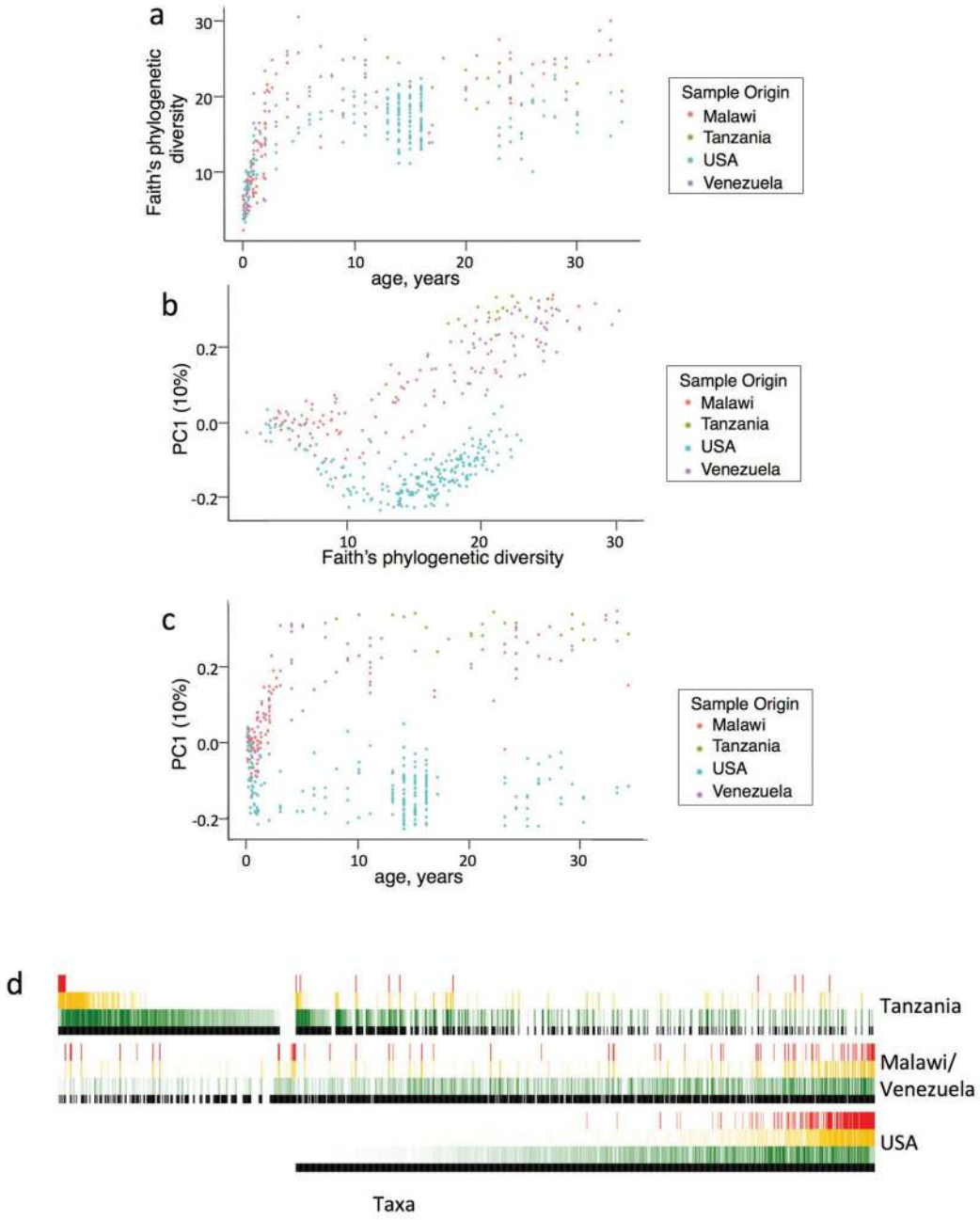
16S rRNA amplicon sequencing and analysis

16S rRNA amplicons were generated for the v4 region from fecal pellets collected and the 16,878,145 Illumina generated sequencing reads were analyzed using Qiime 1.8 as described previously [19]. Sequencing data underwent quality filtering as described previously and data was rarefied to the lowest number of reads observed in a single sample (28,596 reads) [22]. OTUs were identified by open-reference picking using the UCLUST algorithm and taxonomy was assigned using the Greengenes 13.8 database. Plots of UniFrac distances are from unweighted analyses and UniFrac distance values are reported as within group versus between groups. Microbiota diversity was measured by Shannon Index, which takes into account both overall richness and evenness. High confidence OTUs were identified using the following criteria: present in at least three mice at the start of the experiment (four weeks after humanization) and had a collective abundance of greater than 25 reads. High-confidence sub-OTUs were selected by first filtering out duplicate sequences whose distribution across samples is highly correlated. These duplicates correspond to the same bacterial populations, either as uncommonly frequent errors or as multiple 16S copies within the same bacterium. Second, for each experimental group all sequences whose initial raw abundance was at least 5 reads in at least 3 out of 5 mice in that isolator were selected as high-confidence sub-OTUs. Sub-OTU level analysis was performed as described previously [15].

Glycoside Hydrolase Profiles

This method was validated using samples for which 16S rRNA profiles and metagenomic data were available (imputed GH profiles explained 84% of the annotated metagenomic data using a simple linear fit without any model corrections; $p=10^{-28}$)[5]. Glycoside hydrolase (GH) imputations were performed by annotating 2,746 reference genomes with GH families using validated hidden Markov models many of which were derived from Conserved Domain Database models that are capable of identifying multiple domains within a putative enzyme with increased sensitivity[23,24]. The GHs from taxonomically-assigned communities were then calculated by applying a weighted average of the lowest taxonomic level with representative genomes. To account for the fact that many GH families have wide ranging functions while each GH subfamily may possess distinct function, we further clustered the annotated GHs into subfamilies as previously described[23,25,26]. Fold-changes of ymes database (CAZy) in 2013. Nucleic Acids Res. 42, 490ration four mice to the the mean generation one GH profiles with significance testing performed across treatment and control groups.

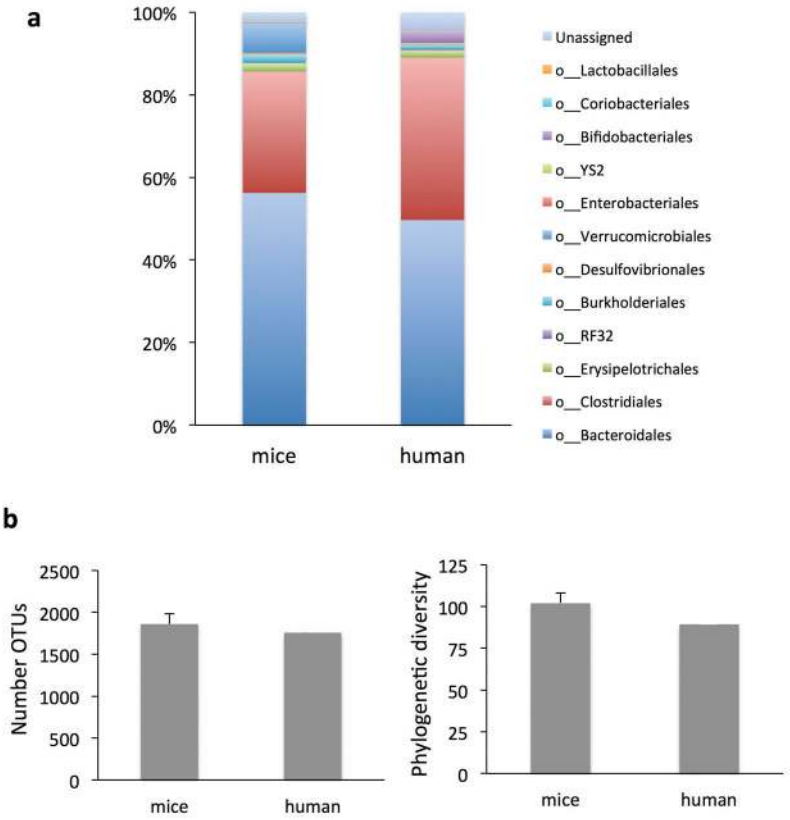
Extended Data



Extended Data Figure 1. Collating data from studies of the microbiota of hunter-gatherers in Tanzania, agrarians from Malawi and Venezuela, and Westerners from the United States reveals that Western populations have depleted alpha diversity from birth through childbearing years and are missing bacterial taxa present in the traditional groups

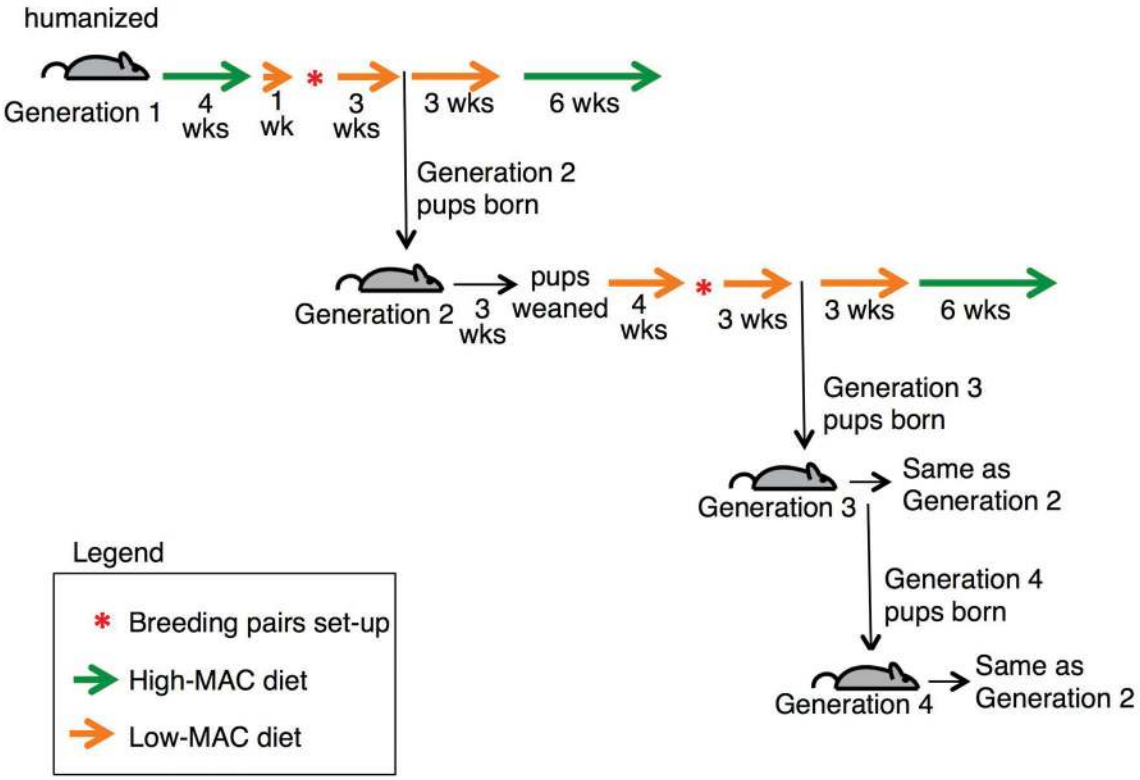
a, Scatterplot of fecal microbiota of individuals plotted by phylogenetic diversity against age of the Hadza hunter-gatherers from Tanzania (n=16, green), agrarians from Malawi (n=81, red) and Venezuela (n=78, purple) and Americans (n=213, blue) **b**, Individuals plotted by unweighted UniFrac PC1 versus phylogenetic diversity. **c**, Individuals plotted by unweighted

UniFrac PC1 versus age. **d**, Line plot of unique OTUs from fecal microbiota across populations (Americans, n=315; Malawi and Venezuela, n=213; Tanzania, n=27). OTUs (x-axis; black, present; white, absent) are considered present if represented by ≥0.001% of reads within each population. OTUs were sorted along the x-axis by their relative abundance in the U.S. and Tanzanian populations and further subdivided by their distributions within a population into tracks (red > 0.05%, yellow ≤0.05%, and green ≤0.01%, relative abundance). The line's opacity is the proportion of that population that meets the criteria for that respective track.



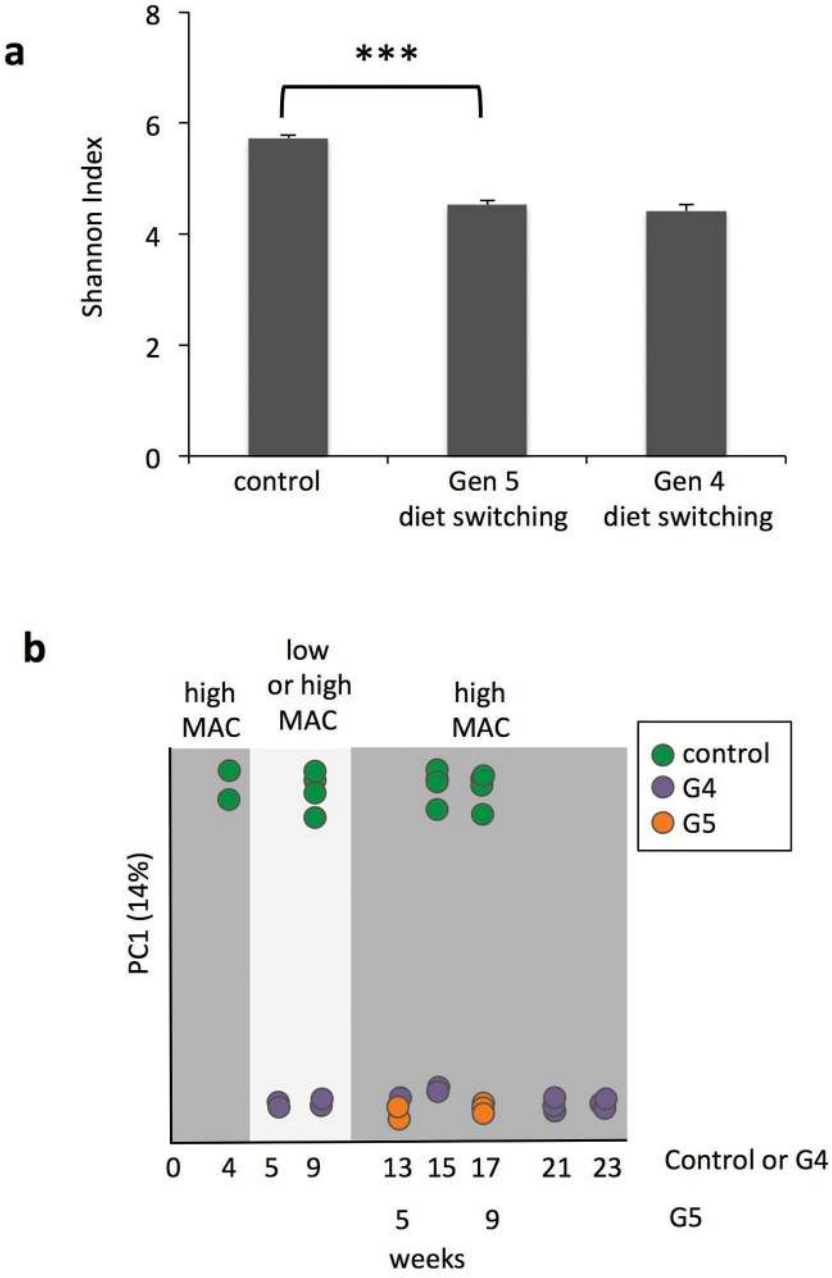
Extended Data Figure 2. Comparison of human donor and humanized mice

a, Taxa summary plot of the relative abundance of taxa from humanized mice feces (mice) (n=10) and human donor feces (human) (n=1). **b**, Alpha-diversity of the fecal microbiota from humanized mice (mice) and human donor (human) expressed as number of OTUs (top panel) and phylogenetic diversity (bottom panel). Error bars are shown as s.e.m.



Extended Data Figure 3. Detailed schematic of multigeneration experiment

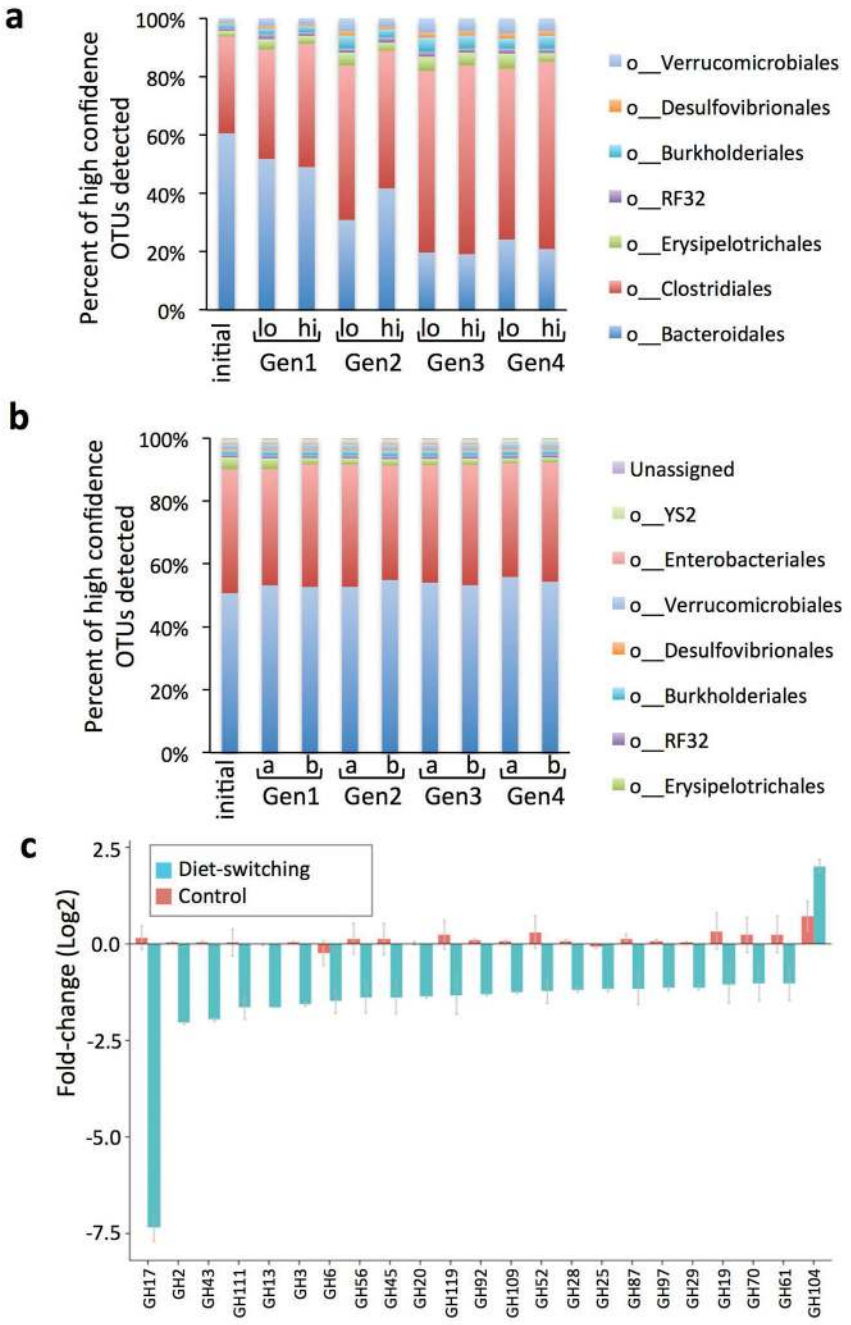
Generation 1. Humanized mice were fed a high-MAC diet for four weeks then switched to a low-MAC diet. One week after diet switch, the mice were bred to generate a litter of pups. After three additional weeks on the low-MAC diet, generation two pups were born and remained in the cage with their mother for three weeks (generation 1 still consuming the low-MAC diet). After pups were weaned, generation 1 mice were returned to the high-MAC diet for six weeks. Generation 2. Pups were weaned from their mother at three weeks old onto a low-MAC diet, which they consumed for 10 weeks. Breeding pairs for generation 2 mice were set-up at 7 weeks old. After three additional weeks on the low-MAC diet, generation 3 pups were born and remained in the cage with their mother for three weeks (generation 2 still consuming the low-MAC diet). After pups were weaned, generation 2 mice were returned to the high-MAC diet for six weeks. Generations 3 and 4 followed the same protocol as generation 2 described above.



Extended Data Figure 4. Microbiota diversity is not regained upon direct weaning the diet-switching group onto the high-MAC diet

a, Alpha-diversity as measured by Shannon index of fecal microbiota from generation 5 mice from the high-MAC diet control (control) (n=6), generation 5, diet-switching group that was weaned directly onto the high-MAC diet (Gen 5 diet switching) (n=6), and generation 4 mice from the diet switching group after weaning and maintenance on the low-MAC diet for 13 weeks and returned to the high-MAC diet for four weeks (Gen 4 diet switching) (n=5). Error bars are shown as s.e.m. and P values are from a two-tailed Student's t-test **b**, Principal coordinate analysis of unweighted UniFrac distance for 16S rRNA amplicon profiles from fecal samples collected from first generation control mice on a high-

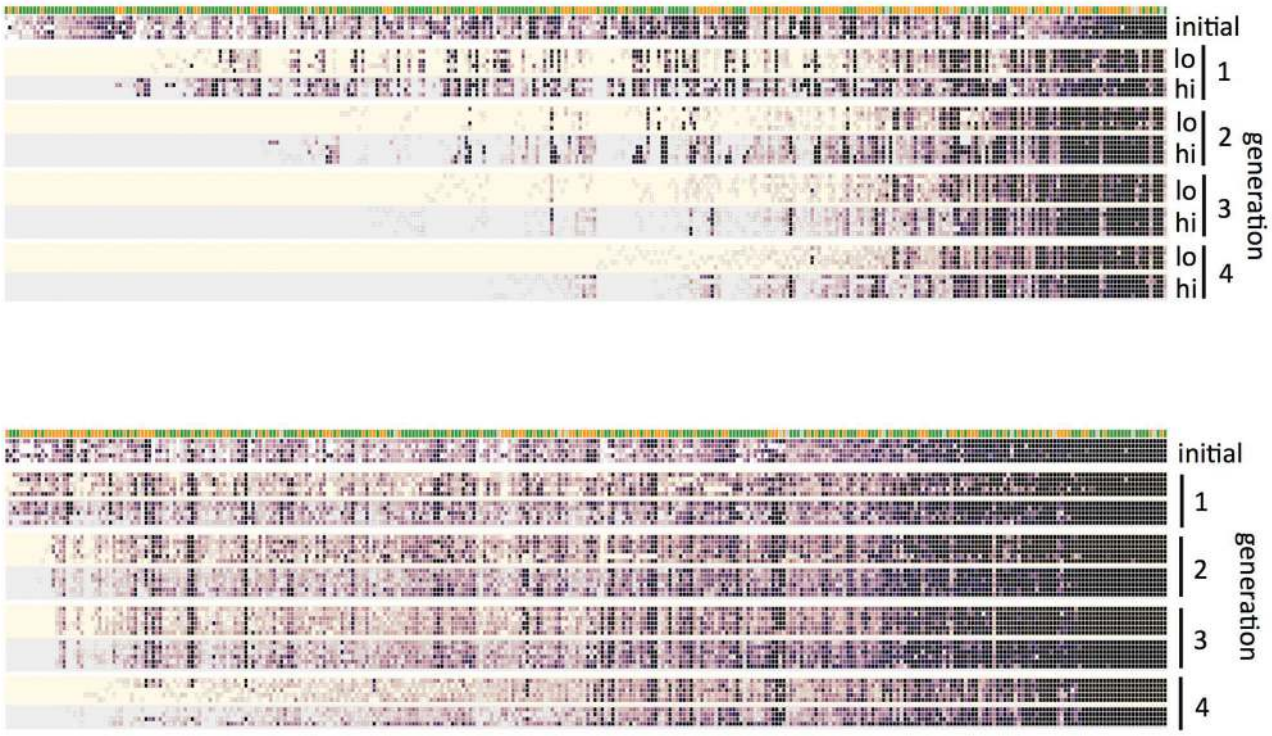
MAC diet (green), fourth generation, diet-switching mice (purple), and fifth generation mice from the diet-switching lineage weaned directly onto the high-MAC diet (orange). Control is plotted as weeks post-humanization and generation 4 and 5 are plotted as age.



Extended Data Figure 5. Fraction of high-confidence OTUs from the Clostridiales order increases and from the Bacteroidales order decreases over multiple generations in the low-MAC consuming mice

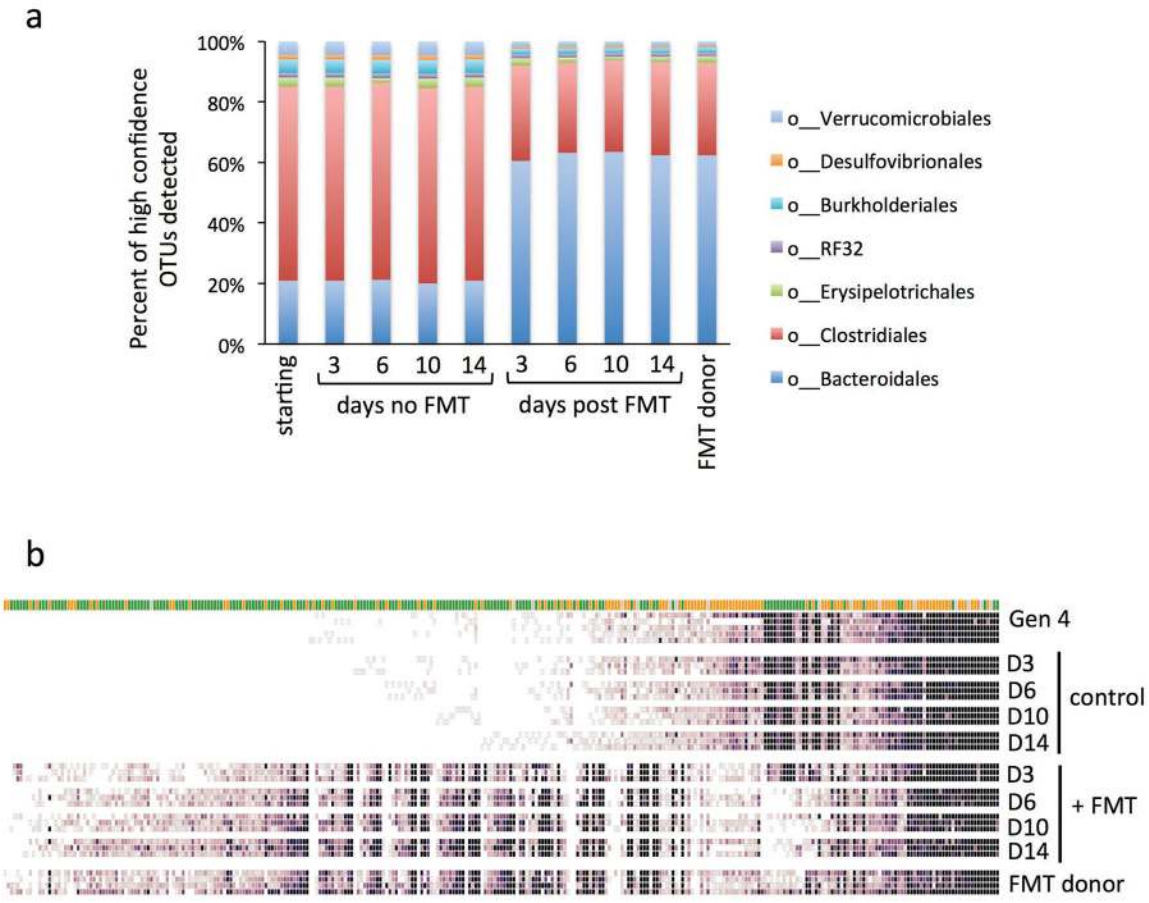
a, Percent of high-confidence OTUs, grouped by order, detected in mice feces over four generations in the diet-switching lineage on the low-MAC diet (low) and high-MAC diet (hi)

(n=5 for Gen 1; n=6 for Gen 2-4). **b**, Percent of high-confidence OTUs, grouped by order, detected in mice feces over four generations in the control high-MAC diet lineage at the equivalent time points to the high-MAC diet (a) and low-MAC diet (b) of the diet-switching group (n=5 for Gen 1; n=6 for Gen 2–4). **c**, Imputed gycloside hydrolase family members (GH) that show significant differences (at least 2-fold change and $p<0.05$, Bonferroni-corrected t test) between generation 4 diet-switching mice after four weeks on the high MAC diet (teal) (n=5) and the starting generation one mice (salmon) (n=10). Error bars depict s.e.m. No GH families showed significant changes in the control group.



Extended Data Figure 6. Inefficient inter-generational transfer of taxa driven to low abundance by low dietary MACs

Heat map of abundance of high-confidence sub-OTUs (number of sequencing reads, columns) from feces of the diet switching group (top panel) and control group (bottom panel). Each row represents an individual mouse fecal microbiota from four weeks post-humanization (initial), while consuming the low-MAC diet (week 9, lo, shaded yellow), and four weeks after switching to the high-MAC diet (week 15, hi, shaded grey). Corresponding time points from controls are also shaded. Top row shows the taxanomic assignment for the OTUs plotted, Bacteroidetes are green, Firmicutes are orange, and others are grey.



Extended Data Figure 7. Reintroduction of lost taxa and a high-MAC diet restores microbiota diversity and composition with Clostridiales order decreasing and Bacteroidales order increasing in low-MAC consuming mice that receive a fecal transplant

a, Plot of percent representation of high-confidence OTUs from generation 4 mice feces in the diet-switching group at day 0 before the FMT (starting) (n=6) and then 3–14 days no FMT control (n=3) or post FMT (n=3). FMT donor is plotted on the right. **b**, Heat map of abundance of high-confidence sub-OTUs (number of sequencing reads, columns) from the feces of the diet switching group at day 0 (Gen 4), day 3 to day 14 that did not receive an FMT (control) (n=3 for each day), day 3 to day 14 that received an FMT (+ FMT), and the FMT donor. Each row represents an individual mouse fecal microbiota. Top row shows the taxonomic assignment for the OTUs plotted, Bacteroidetes are green, Firmicutes are orange, and others are grey.

Extended Data Table 1

Nutritional Information of Mouse Diets

Diet Name	Supplier/Product Name	Protein (% by weight)	Carbohydrates (% by weight)	Fat (% by weight)	Fiber (% by weight)	Neutral Detergent Fiber (% by weight)
High-MAC	LabDiet 5010	25	50	5	5	15

Diet Name	Supplier/Product Name	Protein (% by weight)	Carbohydrates (% by weight)	Fat (% by weight)	Fiber (% by weight)	Neutral Detergent Fiber (% by weight)
Low-MAC	Harlan TD.86489	18	62	5	5	5 *

* Exclusively from added cellulose

Extended Data Table 2

High-confidence OTUs at Experiment Start

Taxonomy	Control Isolator (# OTUs)	Experimental Isolator (# OTUs)
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__[Odoribacteraceae]: g__Butyricimonas: s__	0	1
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Bacteroidaceae: g__Bacteroides: s__	49	48
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Bacteroidaceae: g__Bacteroides: s__fragilis	2	2
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Bacteroidaceae: g__Bacteroides: s__ovatus	2	2
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Bacteroidaceae: g__Bacteroides: s__uniformis	5	6
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Porphyromonadaceae: g__Parabacteroides: s__	3	5
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Porphyromonadaceae: g__Parabacteroides: s__distasonis	2	1
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Porphyromonadaceae: g__Parabacteroides: s__gordonii	1	2
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Rikenellaceae: g__: s__	1	1
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__S24-7: g__: s__	43	58
p__Cyanobacteria: c__4C0d-2: o__YS2: f__: g__: s__	2	0
p__Firmicutes: c__Clostridia: o__Clostridials: f__: g__: s__	3	3
p__Firmicutes: c__Clostridia: o__Clostridials: f__Lachnospiraceae: g__: s__	22	17
p__Firmicutes: c__Clostridia: o__Clostridials: f__Lachnospiraceae: g__[Ruminococcus]: s__	3	3
p__Firmicutes: c__Clostridia: o__Clostridials: f__Lachnospiraceae: g__[Ruminococcus]: s__gnavus	6	0
p__Firmicutes: c__Clostridia: o__Clostridials: f__Lachnospiraceae: g__Blautia: s__	20	17
p__Firmicutes: c__Clostridia: o__Clostridials: f__Lachnospiraceae: g__Blautia: s__producta	5	3
p__Firmicutes: c__Clostridia: o__Clostridials: f__Lachnospiraceae: g__Coprococcus: s__	6	4
p__Firmicutes: c__Clostridia: o__Clostridials: f__Lachnospiraceae: g__Dorea: s__	4	3
p__Firmicutes: c__Clostridia: o__Clostridials: f__Ruminococcaceae: g__: s__	6	9
p__Firmicutes: c__Clostridia: o__Clostridials: f__Ruminococcaceae: g__Faecalibacterium: s__prausnitzii	0	4

Taxonomy	Control Isolator (# OTUs)	Experimental Isolator (# OTUs)
p__Firmicutes: c__Clostridia: o__Clostridials: f__Ruminococcaceae: g__Oscillospira: s__	4	3
p__Firmicutes: c__Clostridia: o__Clostridials: f__Ruminococcaceae: g__Ruminococcus: s__	5	3
p__Firmicutes: c__Erysipelotrichi: o__Erysipelotrichales: f__Erysipelotrichaceae: g__: s__	4	1
p__Firmicutes: c__Erysipelotrichi: o__Erysipelotrichales: f__Erysipelotrichaceae: g__[Eubacterium]: s__dolichum	2	2
p__Firmicutes: c__Erysipelotrichi: o__Erysipelotrichales: f__Erysipelotrichaceae: g__Coprobacillus: s__	1	1
p__Firmicutes: c__Erysipelotrichi: o__Erysipelotrichales: f__Erysipelotrichaceae: g__Holdemania: s__	1	0
p__Proteobacteria: c__Alphaproteobacteria: o__RF32: f__: g__: s__	2	2
p__Proteobacteria: c__Betaproteobacteria: o__Burkholderiales: f__Alcaligenaceae: g__Sutterella: s__	3	3
p__Proteobacteria: c__Deltaproteobacteria: o__Desulfovibrionales: f__Desulfovibrionaceae: g__Bilophila: s__	1	1
p__Proteobacteria: c__Gammaproteobacteria: o__Enterobacteriales: f__Enterobacteriaceae: g__: s__	1	0
p__Verrucomicrobia: c__Verrucomicrobiae: o__Verrucomicrobiales: f__Verrucomicrobiaceae: g__Akkermansia: s__muciniphila	3	3
Unassigned	1	0

Extended Data Table 3

Shannon Index of Glycoside Hydrolase Subfamilies

Comparisons	Gen1 Mouse1	Gen1 Mouse2	Gen1 Mouse3	Gen1 Mouse4	Gen1 Mouse5	Gen4 Mouse1	Gen4 Mouse2	Gen4 Mouse3	Gen4 Mouse4	Gen4 Mouse5	p value	signifi
Diet-switching	9.39	9.42	9.34	9.35	9.40	9.23	9.21	9.12	9.26	9.17	2.2E-04	
Control	9.41	9.39	9.45	9.48	9.45	9.42	9.34	9.42	9.41	9.41	0.13	

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Michelle St. Onge for technical assistance. This work was funded by a grant from National Institutes of Health NIDDK (R01-DK085025 to J.L.S.). Justin L. Sonnenburg, Ph.D., holds an Investigators in the Pathogenesis of Infectious Disease Award from the Burroughs Wellcome Fund.

References

1. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science (New York, NY). 2012; 336:1268–1273.10.1126/science.1223490
2. Karlsson F, Tremaroli V, Nielsen J, Backhed F. Assessing the human gut microbiota in metabolic diseases. Diabetes. 2013; 62:3341–3349.10.2337/db13-0844 [PubMed: 24065795]

3. Sonnenburg ED, Sonnenburg JL. Starving our Microbial Self: The Deleterious Consequences of a Diet Deficient in Microbiota-Accessible Carbohydrates. Cell metabolism. 201410.1016/j.cmet.2014.07.003
4. Schnorr SL, et al. Gut microbiome of the Hadza hunter-gatherers. Nature communications. 2014; 5:3654.10.1038/ncomms4654
5. Yatsunenko T, et al. Human gut microbiome viewed across age and geography. Nature. 2012; 486:222–227.10.1038/nature11053 [PubMed: 22699611]
6. De Filippo C, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:14691–14696.10.1073/pnas.1005963107 [PubMed: 20679230]
7. Clemente JC, et al. The microbiome of uncontacted Amerindians. Science Advances. 2015; 1:e1500183. [PubMed: 26229982]
8. Obregon-Tito AJ, et al. Subsistence strategies in traditional societies distinguish gut microbiomes. 2015; 6:6505.10.1038/ncomms7505
9. Martinez I, et al. The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. Cell reports. 2015; 11:527–538.10.1016/j.celrep.2015.03.049 [PubMed: 25892234]
10. McGill CR, Fulgoni VL 3rd, Devareddy L. Ten-year trends in fiber and whole grain intakes and food sources for the United States population: National Health and Nutrition Examination Survey 2001–2010. Nutrients. 2015; 7:1119–1130.10.3390/nu7021119 [PubMed: 25671414]
11. King DE, Mainous AG 3rd, Lambourne CA. Trends in dietary fiber intake in the United States, 1999–2008. Journal of the Academy of Nutrition and Dietetics. 2012; 112:642–648.10.1016/j.jand.2012.01.019 [PubMed: 22709768]
12. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012; 489:220–230.10.1038/nature11550 [PubMed: 22972295]
13. Kashyap PC, et al. Genetically dictated change in host mucus carbohydrate landscape exerts a diet-dependent effect on the gut microbiota. Proc Natl Acad Sci U S A. 2013; 110:17059–64. [PubMed: 24062455]
14. Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 2014; 42:490–495.
15. Tikhonov M, Leach RW, Wingreen NS. Interpreting 16S metagenomic data without clustering to achieve sub-OTU resolution. The ISME journal. 2015; 9:68–80.10.1038/ismej.2014.117 [PubMed: 25012900]
16. El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. Nature reviews Microbiology. 2013; 11:497–504.10.1038/nrmicro3050
17. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics (Oxford, England). 2010; 26:2460–2461.10.1093/bioinformatics/btq461
18. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and environmental microbiology. 2005; 71:8228–8235.10.1128/aem.71.12.8228-8235.2005 [PubMed: 16332807]
19. Caporaso JG, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods. 2010; 7:335–336.10.1038/nmeth.f.303 [PubMed: 20383131]
20. Kashyap PC, et al. Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. Gastroenterology. 2013; 144:967–977.10.1053/j.gastro.2013.01.047 [PubMed: 23380084]
21. Turnbaugh PJ, et al. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Science translational medicine. 2009; 1:6ra14.10.1126/scitranslmed.3000322
22. Bokulich NA, et al. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nature methods. 2013; 10:57–59.10.1038/nmeth.2276 [PubMed: 23202435]
23. Yin Y, et al. dbCAN: a web resource for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. 2012; 40:W445–51. [PubMed: 22645317]

24. Marchler-Bauer A, et al. CDD: Specific functional annotation with the Conserved Domain Database. Nucleic Acids Res. 2009; 37:205–210.

25. Aspeborg H, Coutinho PM, Wang Y, Brumer H, Henrissat B. Evolution, substrate specificity and subfamily classification of glycoside hydrolase family 5 (GH5). BMC Evol Biol. 2012; 12:186. [PubMed: 22992189]

26. Stam MR, Danchin EGJ, Rancurel C, Coutinho PM, Henrissat B. Dividing the large glycoside hydrolase family 13 into subfamilies: towards improved functional annotations of alpha-amylase-related proteins. Protein Eng Des Sel. 2006; 19:555–62. [PubMed: 17085431]

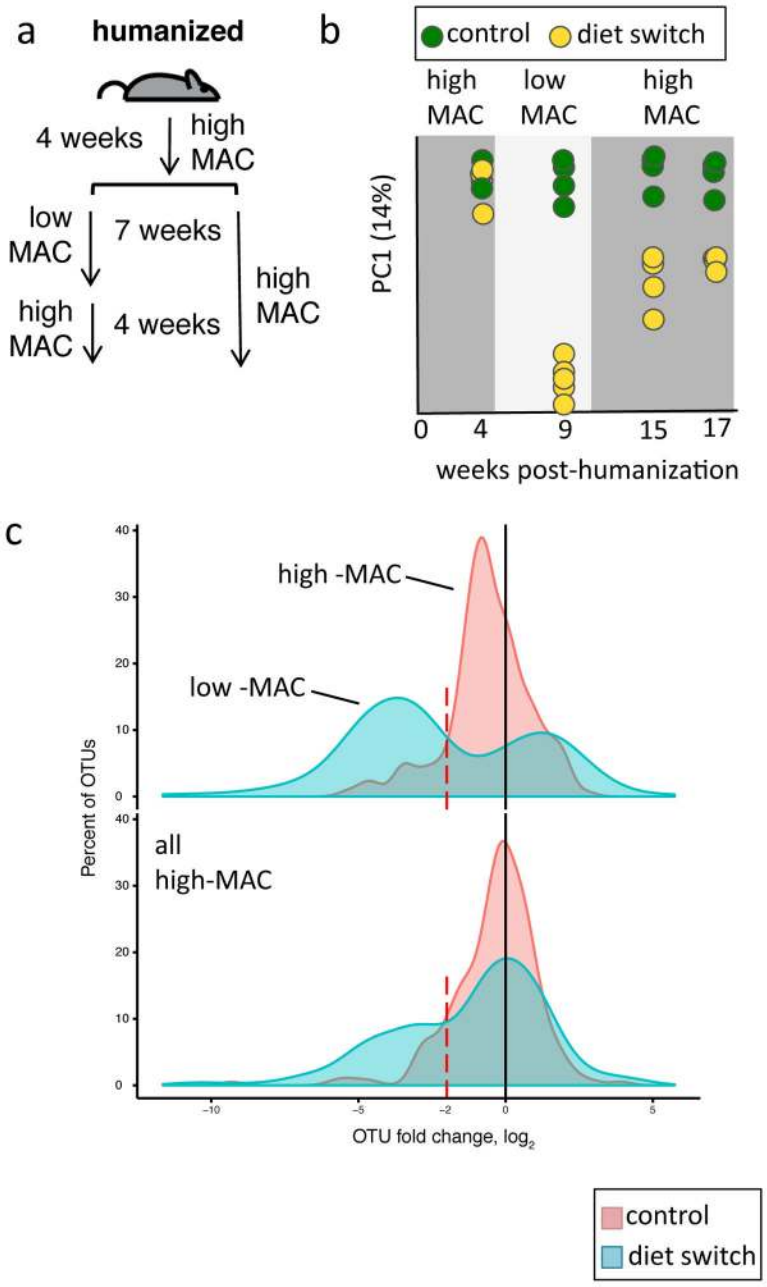


Figure 1. Taxa reduction observed in low-MAC diet is largely reversible in a single generation

a, Schematic of mouse experiment. Humanized mice (n=10) were maintained on a high-MAC diet for four weeks after which half of the mice were switched to a low-MAC diet for 7 weeks. These mice were then switched back to the high-MAC diet for >4 weeks. **b**, Principle coordinate analysis of the UniFrac distance for 16S rRNA amplicon profiles from fecal samples collected from the diet switching mice (yellow, n=5) and control high-MAC diet mice (green, n=5). **c**, Distribution of OTUs fold changes for diet switching (blue, n=5) or control (red, n=5) groups comparing baseline (4 weeks post-humanization) versus week 9 (5 weeks post-low MAC diet for "diet switch" group; top panel) and baseline versus week 15 (4 weeks after return to high-MAC diet for "diet switch" group, bottom panel).

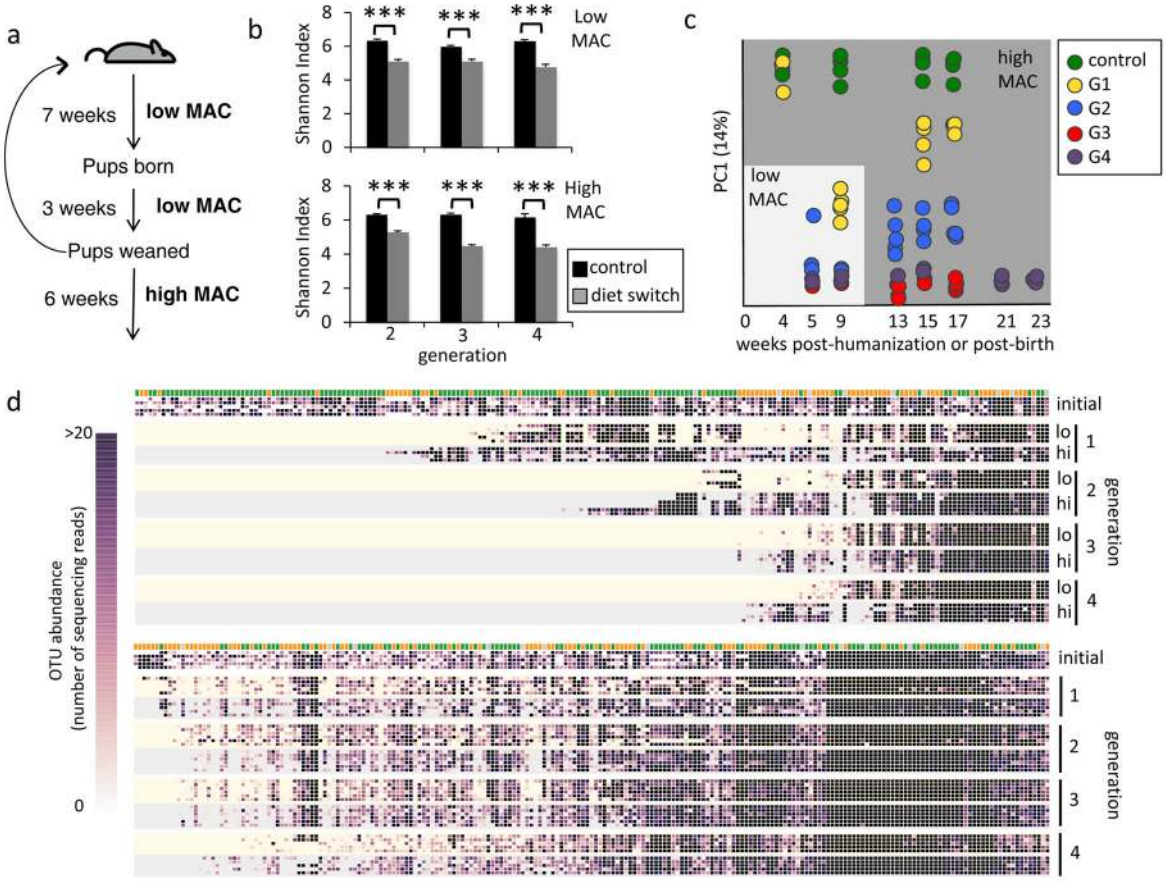


Figure 2. Inefficient inter-generational transfer of taxa driven to low abundance by low dietary MACs

a, Schematic of multigeneration mouse experiment. Second (n=6), third (n=6), and fourth generation mice (n=6) were weaned onto a low-MAC diet. After mice generated a litter of pups that were weaned, low-MAC diet mice were switched to the high-MAC diet for 4 weeks. A parallel group of control mice were maintained on the high-MAC diet throughout (generation 2, n=6; generation 3, n=6; generation 4, n=5). **b**, Microbiota diversity as measured by Shannon index observed in the microbiota of mice at five weeks old (top panel, n=6 for each group) or four weeks after shift to high-MAC diet (bottom panel, n=6 for each group) from three generations of diet switching mice (grey) or control high-MAC diet mice (black). Error bars are shown as s.e.m and P values are from two-tailed Student's t-test. **c**, Principal coordinate analysis of UniFrac distance for 16S rRNA amplicon profiles from fecal samples collected from first generation mice from the control group consuming a high-MAC diet (green, n=5) or the diet switching group from generation 1 (yellow, n=5), 2 (blue, n=6), 3 (red, n=6), and 4 (purple, n=6). **d**, Heat map of abundance of high-confidence OTUs (number of sequencing reads, columns) from the diet switching group (top panel) and controls (bottom panel); taxonomic assignment is indicated at the top of each column (Bacteroidetes, green; Firmicutes, orange; other, grey). Each row represents an individual mouse microbiota from four weeks post-humanization (initial), while consuming the low-MAC diet (week 9, lo, shaded yellow), and four weeks after switching to the high-MAC diet (week 15, hi, shaded grey). Corresponding time points from controls are similarly shaded.

N=5, 6, 6, and 6 for the diet-switching group and n=5, 6, 6, and 5 for the control group for generations one through four respectively.

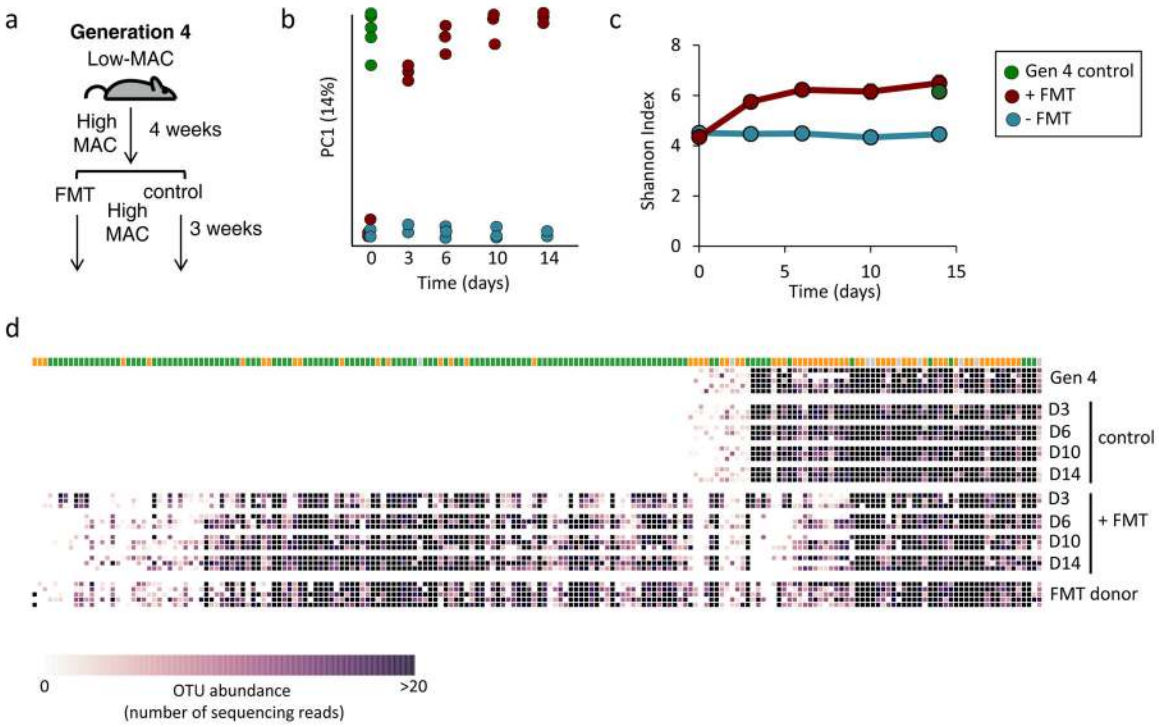


Figure 3. Reintroduction of lost taxa and a high-MAC diet restores microbiota diversity and composition

a, Schematic of fecal transplant mouse experiment. **b**, Principal coordinate analysis of UniFrac distance for 16S rRNA amplicon profiles from fecal samples collected from fourth generation control mice on a high-MAC diet (green, n=6), fourth generation, diet-switching mice that received a fecal transplant (red, n=3), or did not (blue, n=3). **c**, Microbiota diversity as measured by Shannon index observed in the microbiota of mice that received a fecal transplant (red, n=3) or did not (blue, n=3). A green circle denotes the number of OTUs observed in fourth generation control mice consuming a high-MAC diet (n=6). Error bars are shown as s.e.m. **d**, Heat map of abundance of high-confidence OTUs (number of sequencing reads) from fourth generation diet-switching mice (n=3) three to 14 days after FMT (fecal microbiota transplant) and no FMT controls (n=3); taxonomic assignment is indicated at the top of each column (Bacteroidetes, green; Firmicutes, orange; other, grey). FMT donor (fourth generation control mice, n=5) and fourth generation diet-switching mice (n=5) four weeks after consuming high-MAC diet are also shown.