Quantifying the daily harvest of fermentation products from the human gut microbiota

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Abstract

Fermentation products released by the gut microbiota provide energy and important regulatory functions to the host. Yet, little quantitative information is available on the metabolite exchange between the microbiota and the human host, and thus the effective doses of fermentation products. Here, we introduce an integrative framework combining experimental characterization of major gut bacteria with a quantitative analysis of human digestive physiology to put numbers on this exchange and its dependence on diet and microbiota composition. From the complex carbohydrates fueling microbiota growth, we find most carbon ends up in fermentation products which are largely utilized by the host. This harvest of mixed fermentation products varies strongly with diet, from between 100-700 mmol/day within the US population to up to 1300 for the Hadza people of Tanzania. Accordingly, fermentation products cover between 2% and 12% of the daily energy demand of human hosts, substantially less than the 21±4% estimated for laboratory mice. In contrast, microbiota composition has little impact on the total daily harvest but determines the harvest of specific fermentation products. Butyrate, known for promoting epithelial health, shows the biggest variation. Our framework thus identifies and quantifies major factors driving the metabolic interactions and exchange of information between microbiota and host, crucial to mechanistically dissect the role of the microbiota in health and disease.

Introduction

The exchange of fermentation products (mostly acetate, propionate, and butyrate) is a major avenue for the gut microbiota to exert its effects on the host [1,2]. These molecules are excreted by anaerobically growing microbes in the large intestine which feed mostly on complex carbohydrates from plant-based foods, such as dietary fiber and resistant starches. Microbial fermentation products are absorbed by the colonic epithelium and perform a variety of important functions in the host. For example, they serve as an energy source [3,4], influence immune cell regulation and recruitment [5–7], and have a role in satiety signaling [8,9] and the gut-brain axis more generally [1,10]. By changing the pH in the gut lumen, they also allow gut microbes to shape their local environment with strong effects on microbiota composition [11,12].

Fermentation product concentrations have been measured in feces and within the human digestive tract [13,14]. However, these concentrations are the result of a highly dynamic interplay between microbial fermentation product release and uptake by the host which varies strongly with changes in the host's food intake and digestion activity throughout the day [15,16]. Therefore, concentration measurements in gut content are only snapshots and provide little insight into

the overall flux of fermentation products that microbes produce and that the human body takes up.

Here, we integrate our own experimental measurements of bacterial fermentation with a quantitative analysis of human digestive physiology to estimate the daily amount of fermentation products the gut microbiota provides to the human host. With this framework, we analyze the variation of the daily fermentation product harvest and its contribution to the host's energy demand, depending on diet and microbiome composition.

Results

Quantifying fermentative metabolism of major gut bacteria

To maintain their redox balance and generate energy in the anoxic environment of the large intestine, gut bacteria utilize different fermentation pathways which mostly produce the acids of acetate, propionate, butyrate, lactate, formate, and succinate (**Figure 1A, SI Text 1.1**). To quantify the production of these fermentation products and the consumption of carbohydrates, we grew selected gut bacteria in pure cultures and tracked changes in metabolite concentrations and bacterial biomass over time. Metabolite concentrations changed linearly with biomass during steady-state growth, allowing us to determine the per biomass uptake and excretion rates with high precision (**Figures 1B and S1, Methods**).

We picked sixteen gut microbiota species (**Figure 1C and Table S1**) based on their relative abundances in reported metagenomic data [17,18]. While hundreds of species are typically detectable in a healthy human gut microbiome, abundances are highly uneven and the sixteen species we chose commonly account for close to 60% of total bacterial biomass in the human gut (**Figure S2**). When using these species as representatives of their respective families (**SI Text 1.1**), coverage increases even to around 90% of biomass (**Figures 1C, S2**). For growth in rich but carbon-limited medium (YCA), uptake of glucose or maltose varied in a relatively narrow range (**Figure 1D; SI Text 2**), in line with the idea that different bacterial strains need similar amounts of carbohydrates to sustain growth (**SI Text 1.1**). Types of secreted fermentation products, however, varied substantially between different bacterial species, reflecting their metabolic pathways (**Figure 1E**). For example, *Bacteroides* strains excrete succinate and Lachnospiraceae produce butyrate (**SI Text 1.1**). Considering the fate of carbohydrate-derived carbon, we find that most ends up in fermentation products (**Figure 1F**), illustrating the high demand for carbohydrates required for fermentative growth. We further repeated these experiments in other media of different complexities (BHI, ϵ, and γ media) (**Figure S3**). Per-biomass fermentation product excretion is very similar across media despite strong variations in growth rates (**Figure 1G, SI dataset**), in line with the theoretical ATP requirements for biomass synthesis which change little in different growth media. This indicates that bacteria face the same fundamental metabolic constraints across conditions, making the excretion numbers we obtain robust estimates that hold beyond the axenic cultures we have characterized, and are thus applicable in more complex environmental and ecological settings (**SI Text 1.2**). Per-biomass carbohydrate uptake is comparable in epsilon and YCA media, which both contain amino acids and other complex nutrients, but substantially higher in minimal medium (γ), reflecting the increased carbohydrate demand for biomass synthesis in minimal media (**Figure 1G**). In the analysis following in the main text, we use uptake and excretion rates measured in YCA medium.

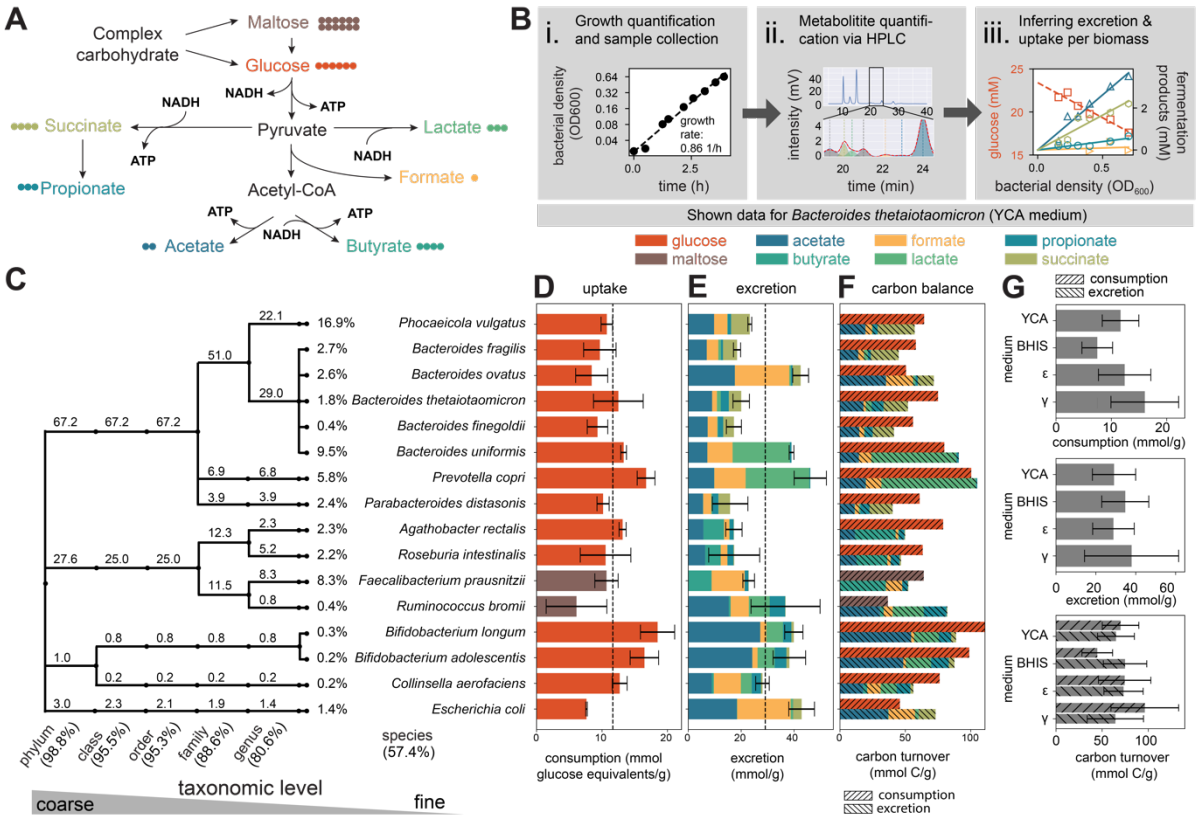

Figure 1. Growth, nutrient uptake, and fermentation product excretion of major gut strains. (A) Major fermentation pathways that promote energy synthesis (ATP) and ensure redox balance during anaerobic growth. Colored circles indicate the number of carbon atoms in the corresponding molecules (details on metabolism with full stoichiometry in **SI Text 1**). **(B)** Experimental setup to determine nutrient uptake and fermentation product release. Samples taken at multiple time points throughout steady exponential growth (i) were analyzed for metabolite concentration using liquid chromatography (ii). With this data, we calculated linear concentration trends with bacterial density (iii) to quantify per biomass uptake and excretion. Further details in **Figure S1**. **(C)** Taxonomic classification of the 16 gut microbiota species characterized. Numbers indicate the typical fraction of bacterial biomass these species and their taxonomic groups represent in healthy individuals (samples from [17,18]). **(D,E)** Per biomass glucose/maltose uptake and fermentation product excretion for growth in YCA medium. Error bars denote SD of three biological replicates. Dashed lines indicate averages of all measured species. **(F)** Carbon content in taken-up carbohydrates and excreted fermentation products. Mismatched carbon balances for *E. coli* and *R. bromii* likely reflect utilization of other media components, such as amino acids, as carbon sources. **(G)** Species averaged uptake and excretion rates in different media (YCA, BHIS, ϵ, γ). Error bars denote SD of species-to-species variation.

Point estimates of the daily fermentation product harvest

Integrating our experimental dataset and a quantitative analysis of diet and digestion, we next estimated the daily amount of fermentation products released by the gut microbiota in two different ways. To do so, we consider averages from measurements in 1970's Britain since dietary and digestion data needed for our estimation is available for this cohort in unparalleled quality. First, we estimated the fermentation product release based on fecal weight, following the logic presented in [19] (**Figure 2A and SI Text 3.1**). In the British reference scenario, humans excrete around 30g of fecal dry weight every day [20] (**Figure 2A(i), Figure S4**) of which around 16g is bacterial biomass [21] (**Figure 2A(ii)**). To maintain a stable microbiota in the gut, this loss of bacterial biomass must be compensated by bacterial growth. Using the measured amount of fermentation products that bacteria release to support growth (on average 29 mmol per g

dry biomass in YCA medium, **Figure 1E,** dashed line), we get a daily production of fermentation products of approximately 16g/day x 29mmol/g = 464mmol/day (**Figure 2A(iii)**). Second, we estimated the fermentation product release based on the amount of carbohydrates in the diet (**Figure 2B and SI Text 3.2**). From the amount of fiber, sugar, and other carbohydrates in the diet and their digestibility, we estimated the amount of carbohydrates reaching the large intestine (**SI Text 4**). For the British reference scenario, 36g (corresponding to 198mmol equivalents of glucose) of these microbiota-available carbohydrates reach the large intestine [22] (**Figure 2C**, with exact values depending on the starch and fiber content in the diet (**Figure S5**, **SI Text 4**). Using the measured amount of carbohydrate consumption per biomass (12mmol/g, **Figure 1D,** dashed line) approximately 198mmol / 12mmol/g = 17g bacterial biomass is produced per day (**Figure 2B(ii)**), leading to a daily fermentation product release of 17g * 29mmol/g = 493mmol (**Figure 2B(iii)**, in good agreement with the estimation via fecal weight. When estimating fermentation product release based on the experimentally measured uptake and excretion for other media, we arrive at similar results (**Figure S6**), highlighting the consistency of the estimation independent of growth conditions.

Given the high carbohydrate demand of anaerobic growth (**Figure 1F**), most of the carbon from microbiota-available carbohydrates will end up in fermentation products (>90%, **Figure 2D**). In contrast, following measurements of fermentation products in feces [23], less than 2% of released fermentation products exit the host via feces (**Figures 2D and S6B,C**, **SI Text 5**). As the consumption of fermentation products via cross-feeding is likely limited (**SI Text 5**), the daily amount of fermentation products released by bacteria represents a good upper bound for the daily amount absorbed by the gut epithelium and thus the daily fermentation product harvest by the human host.

Variation in total fermentation product harvest with changing microbiota composition

The presented point estimates for the daily fermentation product harvest rely on average metabolite consumption and production rates for 16 highly abundant gut microbiota members. However, gut microbiota composition varies[24]. For example, the relative abundance of primary fermenters from the families Bacteroidaceae and Lachnospiraceae varies considerably between healthy individuals (**Figure 3A**, upper panel). To assess the effect of these changes in microbiota composition on fermentation product harvest, we weighted measured consumption and production rates of different species with their relative abundance, using them as metabolic representatives of their respective families (**Figure S7**, **SI Text 6**). Taking microbiota-available carbohydrates corresponding to the British reference diet, the relative amount of different fermentation products released varies substantially between individuals (**Figures 3A** lower panel, and **B**). However, both the daily production of bacterial biomass (**Figure 3C**) and the total daily release of fermentation products (**Figure 3D**) remain within a narrow range. These differences in variation are quantified by the coefficients of variation (**Figure 3E**), with butyrate showing the highest value. Changes in fermentation product release with host age, health, and lifestyle, obtained from a collection of published metagenomic data [25], can be explored in the **Interactive Figure 1** (https://cremerlab.github.io/fermentation_products/study-explorer) accompanying this paper.

In summary, this analysis shows that, given a specific diet, differences in microbiota composition lead to differences in the types of fermentation products released, while the total daily release of fermentation products remains within a narrow range.

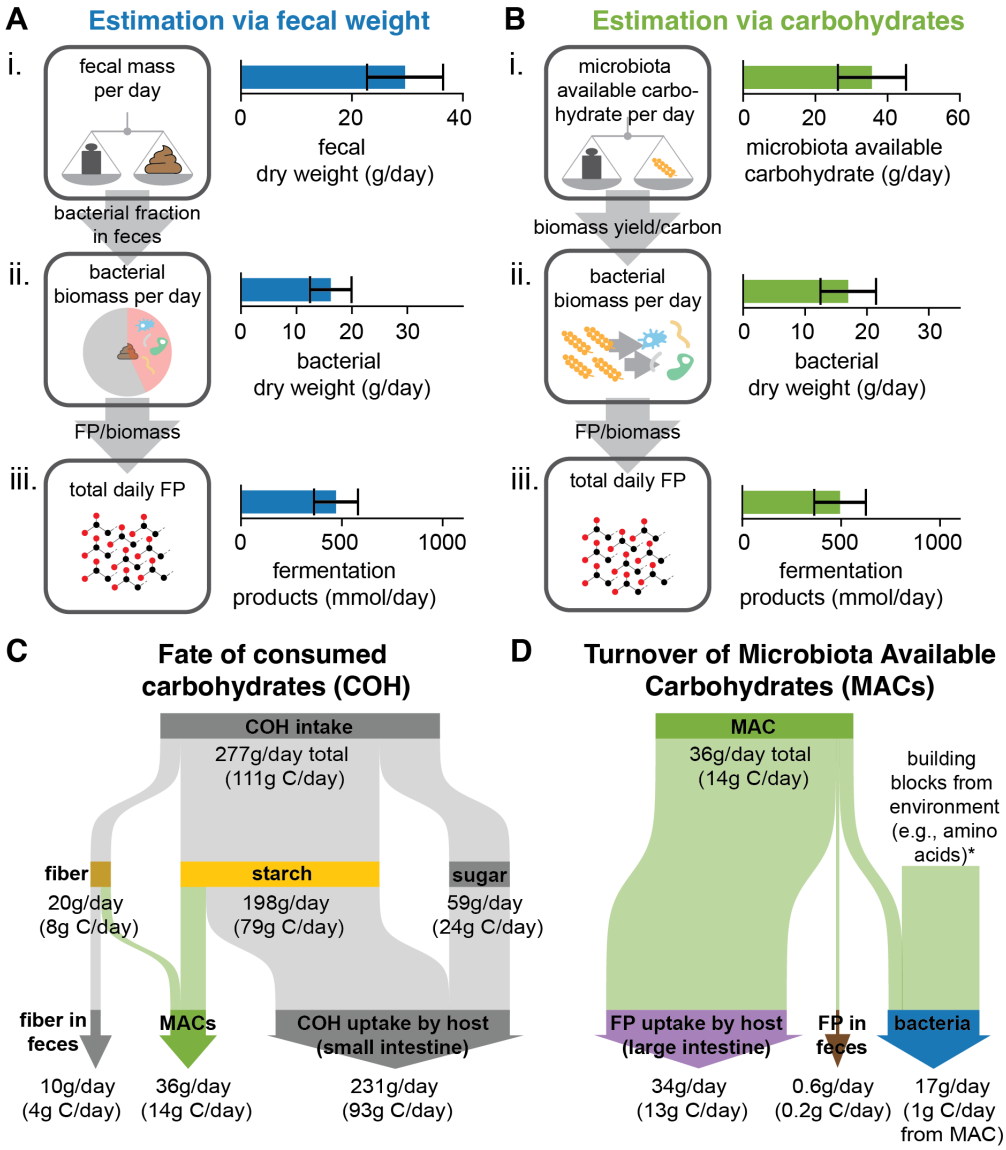

Figure 2. Daily fermentation product harvest for British reference scenario. (A) Fecal-weight-based point estimate of the total daily fermentation product release. Starting point is the measurement of daily fecal weight [20] (i). Given the fraction of bacteria in fecal weight [21], bacterial biomass in feces follows (ii) which, given the measured fermentation product excretion, sets the total daily fermentation product release (iii). **(B)** Carbohydrate-based point estimate of the total daily fermentation product release. Starting point is the estimation of microbiota available carbohydrates (i). With experimentally determined uptake and excretion rates (**Figure 1**), the bacterial biomass that gut bacteria synthesize (ii) and the resulting fermentation product harvest when bacteria grow on these carbohydrates (iii) follows. **(C)** Flow diagram showing the daily turnover of consumed carbohydrates along the upper digestive tract with only a fraction being available for the microbiota (details of this mapping described in **SI Text 4**). **(D)** Flow diagram showing resulting carbohydrate and carbon flow along the large intestine, with most of the carbon ending up in fermentation products that are absorbed by the host. Calculations are based on species-averaged uptake and excretion rates (**Table S2**) obtained from our growth characterization of major gut species in YCA medium commonly used to emulate intestinal conditions (**Figure 1**). g C/day of total carbohydrates, fiber, starch, sugar, and microbiota-available carbohydrates were calculated by assuming a fraction of carbon per total weight equal to glucose (0.4); the fraction of carbon in bacterial biomass was taken to be 0.39, based on data in [26]. Plots for other media shown in **Figure S6**. Error bars in A and C denote SD based on the variation in daily fecal weight and microbiota-available carbohydrates, respectively. Detailed calculations are described in **SI Text 3**.

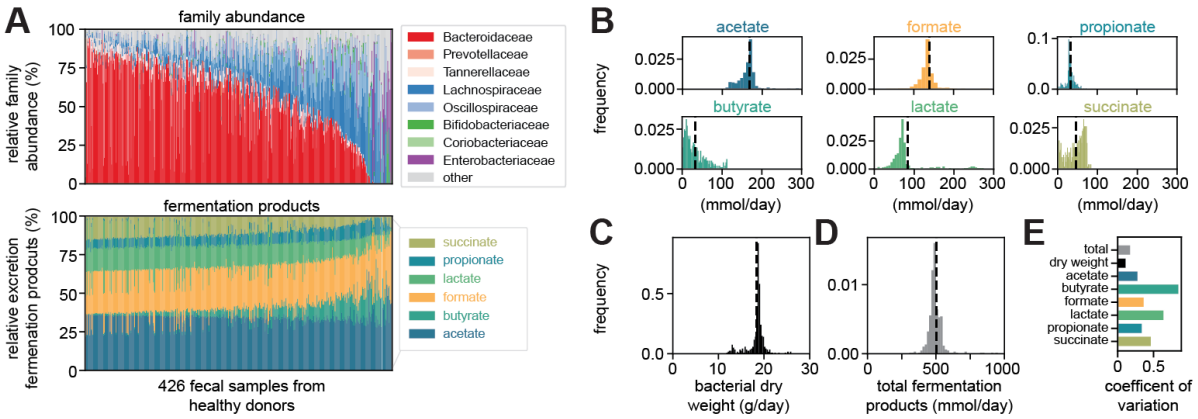

Figure 3. The effect of microbiota composition on daily fermentation product harvest. (A) Variation of gut microbiota composition on the family level (top) and corresponding molar fraction of different fermentation products (bottom) across fecal samples from healthy individuals [17,18]. **(B,C,D)** Distribution of the daily release of single fermentation products (**B**), total bacterial biomass (**C**), and total amount of fermentation products (**D**) for the same set of samples. **(E)** Coefficients of variation quantify these variations, with the variation in total fermentation product release being much smaller than variations in the production of specific fermentation products. Estimations assume carbohydrate consumption as for the 1970's British reference scenario (**Figure 2B,C**). Details of calculations introduced in **SI Text 6**. The variation with carbohydrate consumption and for further datasets on microbiota composition can be explored in **Interactive Figure 1**.

Variation in total fermentation product harvest with changing diet

To quantify the variation in daily fermentation product harvest with diet, we next analyzed data on diet composition and digestion from three different cohorts, covering much of the global variation in human diets.

First, we estimated the variation in the US population using nutrition data from the 2017/2018 National Health and Nutrition Examination Survey (NHANES) study [27] (**Figure S8A-F**). Using the same mapping as above (**Figure 2B, SI Text 4**), we inferred daily intake of microbiota-available carbohydrates in this cohort (**Figure S9A-C**) and estimated the fermentation product harvest based on these values (**Figure 4A**), with constant uptake and excretion characteristics given by the averages of the experimentally characterized species (**Figure 1DE**, dashed lines). The fermentation product harvest is broadly distributed around a mean of 340mmol/day, with most individuals staying substantially below the estimate for the British reference scenario (**Figure 4A**, purple dashed line), reflecting how modern western diets lead to a lower fermentation product harvest from the gut microbiota [28–30].

Second, we analyzed nutrition data available for the Hadza, an indigenous hunter-gatherer group in Tanzania. Their diet, including the amount of carbohydrates consumed, differs substantially from a modern western diet, has strong seasonal variation, and often includes fiber-rich tubers [31,32] (**Figure S8G, H**). Accordingly, the estimated amount of microbiota-available carbohydrates (**Figure S9D-F**) and the resulting daily fermentation product harvest show strong seasonal variation (**Figure 4B, SI Text 4**). Harvests are much higher than those typically observed for people consuming western diets and can reach up to approximately 1300mmol/day.

Third, we estimated the global variation in fermentation product harvest based on fecal weight data representing different geographical regions and lifestyles around 1970 [33]. Daily excretion of fecal wet weight varies strongly, with maximum values about five times higher than in the

British cohort (**Figure S10A**). Correcting for changes in water content with fecal mass [14,21,34–38] and assuming a constant bacterial fraction per fecal dry weight (**Figure S10**), we estimated the corresponding variation in fermentation product harvest (**Figure 4C**). As differences in fecal weight are largely due to differences in diet [34,38,39], this broad variation with a maximum value of 1500mmol/day again emphasizes the importance of diet for the fermentation product harvest. In summary, we find that diet, and not microbiota composition, is the dominant factor in setting this number. Particularly, we estimate how diets low in microbiota-available carbohydrates, as consumed in the US and increasingly around the globe, exhibit much lower fermentation product yields than fiber-rich diets with potentially detrimental health outcomes [28–30].

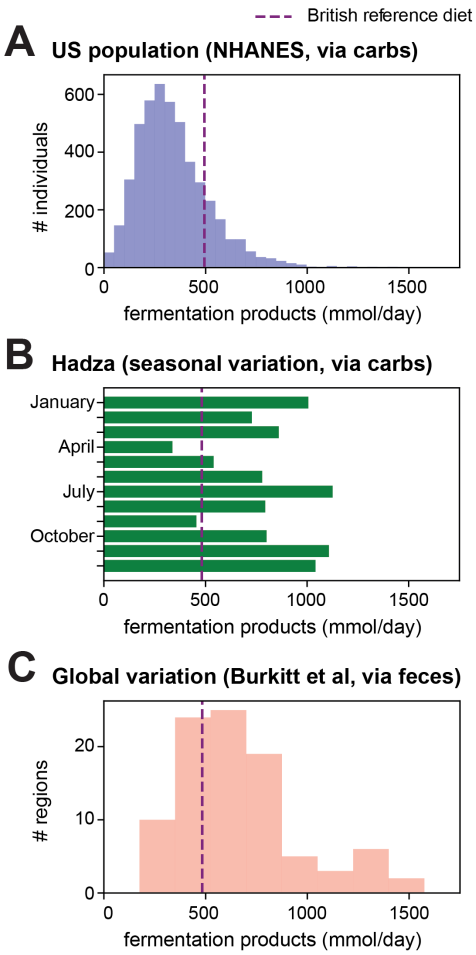

Figure 4. Variation of the daily fermentation product harvest with diet and digestion. (A) Inter-individual variation in total daily fermentation product release for the US population. Carbohydrate-based estimate using reported variation in diet for the NHANES 2017/2018 cohort [27]. **(B)** Month-to-month variation of total daily fermentation product release for the Hadza. Carbohydrate-based estimate using reported seasonal variation in diet [31]. **(C)** Variation in fermentation product release based on fecal-based estimate and global variation in fecal weight reported in Burkitt et al [33]. These numbers represent an upper bound estimation (**Figure S10** for detailed discussion). Estimates in this figure are based on average per-biomass uptake and excretion rates for the 16 strains characterized in **Figure 1**.

Gut microbiota derived fermentation products as energy source for the host

We next compared the human fermentation product harvest with that of the mouse, the most common experimental model in gut microbiota research. Hoces and co-workers have recently

compared the energy extraction from food in germ-free and conventionally colonized mice [16]. Energy extraction is lower by 8.1kJ/day in germ-free mice (**Figure 5A,B**), likely due to a lack of microbiota-available-carbohydrate-derived fermentation products that serve as energy sources for the host (**SI Text 8.1**). When estimating the harvest of fermentation products (via fecal weight, analogous to **Figure 2A**) and their energy content *(i.e.,* combustion enthalpy, see **SI Text 7**) for these mice, we find that fermentation products can explain this difference in energy extraction (**Figure 5C**, **SI Text 8.2**), indicating that most fermentation products are utilized as an energy source by the host. In the same way, we calculated the energy content of the fermentation product harvest in humans. Depending on diet and digestion (**Figure 4**), these values vary between 0.1MJ/day and 1.2 MJ/day. To put these numbers into perspective and allow a comparison with values in mice, we normalized them by the daily energy expenditure of the respective host (approximately 10MJ/day for humans [40] and 38kJ/day for mice [16], **SI Text 7**). For the British reference scenario, the gut microbiota contributes 4.4% of the host's daily energy expenditure (**Figure 6,** purple dashed line). We find broad variation around this number, with values varying from 1.8% (5th percentile of the NHANES cohort, **Figure 6**, blue) to 12.1% in the extreme cases of non-western diets (**Figure 6**, red; 95th percentile from Burkitt data). In contrast, for mice we arrive at 21.4% or more of the total energy demand of 38kJ/day [16] (**Figure 6**, grey, and **SI Text 8**). Notably, these mice were fed autoclaved laboratory chow, and mice consuming non-autoclaved food or a more natural diet are likely to consume more microbiota-available carbohydrates, resulting in an even higher microbiota derived energy supply [41]. Therefore, our analysis reveals and quantifies a substantial difference in microbiota-supplied energy between humans and mice.

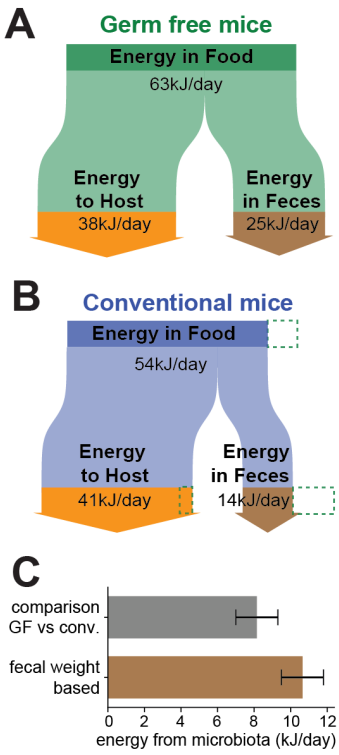

Figure 5. Daily metabolic energy contribution of the gut microbiota in mice. (A, B) Flow charts illustrate the flow of the energy consumed as food in germ-free (**A**) and conventionally colonized mice (**B**). Around 60% or 75% of the consumed energy is extracted in germ-free and conventionally colonized mice, respectively. The rest is excreted in feces. Accordingly, the energy consumed in food and energy in feces is lower in conventional mice, while the energy retained in the host is slightly higher. Green dashed lines in B indicate these differences. **(C)** Daily host energy provided by the gut

microbiota when comparing the energy retention in germ-free and conventional mice (upper bar, **SI Text 8** for calculation) or when estimating fermentation product release using fecal mass in mice (lower bar, analogous to **Figure 2A**). Error bars denote SD based on the variation of consumed food and fecal weight of different mice (upper estimation). Based on data reported in [16].

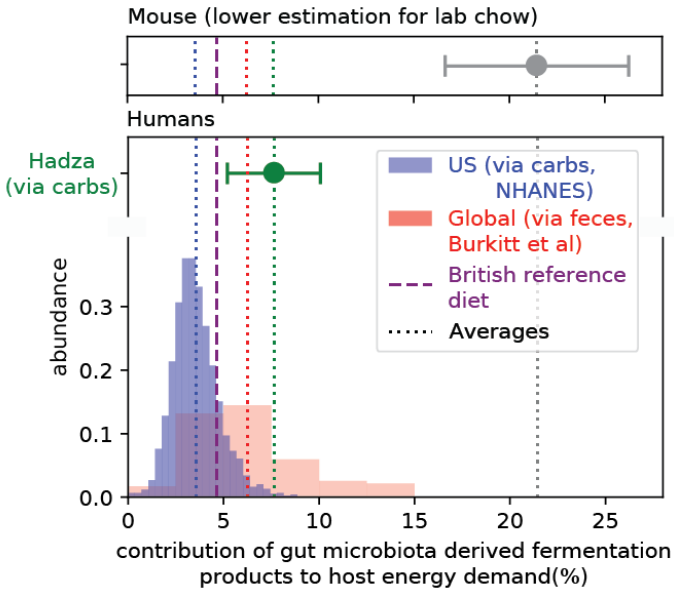

Figure 6. Microbiota contribution to energy supply. Variation of fermentation product harvest leads to strong variation in the fraction of daily energy demand supplied by the gut microbiota. Non-western diets show substantially higher values (green, red), with an upper bound estimation of 15%, when compared to a cohort of US residents (blue). Laboratory mice feeding on autoclaved lab chow (grey) show a substantially higher percentage of daily energy covered by the microbiota. We expect values in mice to be even higher when they consume non-autoclaved food reflecting their natural diet. Error bars indicate variation (SD) based on monthly variation in food intake (Hadza data, green, based on data in [31]), and on error propagation using data originally published in [16] (mouse data, gray, see **SI Text 8.2**), respectively,

Discussion

Recent studies have emphasized the importance of quantitative physiological considerations in microbiome research [11,42,43]. By integrating data on nutrient intake and fecal mass with experimental measurements of bacterial growth and fermentation rates, we quantified the daily turnover of bacterial biomass and the fermentation product harvest in the human intestine.

Notably, complementary analyses led to similar outcomes, confirming the validity of our integrative framework. First, the fermentation product harvest is highly comparable when deriving it from consumed carbohydrates or released feces, both for the point estimate of the British reference scenario and when analyzing its variation (**Figures 2D,H and 4**). Second, a theoretical consideration based on the ATP turnover required to generate bacterial biomass predicts a similar total fermentation product harvest for the British reference scenario [19] (**SI Text 3.3**). Third, estimating the fermentation product harvests via fecal weight matches an analysis of energy extraction by the microbiota in mice (**Figure 5C**).

When utilizing this framework to analyze fermentation product harvest, several findings stand out: the bulk of carbon in microbiota available carbohydrates, >90%, ends up in fermentation products, most of which are taken up by the host (**Figure 2D**). Quantitatively, fermentation

products therefore dominate the metabolite exchange between the microbiota and the host, affecting host signaling, behavior, and energy homeostasis. They are thus a factor which should always be included in studies of host-microbiota interactions. Differences in the total fermentation product harvest is primarily determined by variation in diet, with higher fermentation product harvests resulting from the consumption of less processed foods rich in complex carbohydrates. In contrast, microbiota composition has less impact on the total daily fermentation product harvest. However, microbiota composition strongly impacts the harvest of specific fermentation products, with butyrate and lactate showing the strongest dependence on composition (**Figure 3E**). Given the different ways specific fermentation products affect host processes [44], this finding may underlie many of the commonly reported links between health status and microbiota composition, even without explicitly considering the dominant effect of diet on total fermentation product harvest. However, as dietary changes also cause changes in microbiota composition [45], diet is likely the key determinant for both, the abundance of specific fermentation products and the total fermentation product harvest. Thus, our results highlight that the axis between diet, microbiota composition and fermentation product harvest should routinely be considered when investigating host-microbiota interactions, their role in host health, and the etiology and progression of different microbiota-associated diseases. Critically, we also demonstrate that fermentation products measured in feces represent only a small fraction of the total production, which should rather be estimated using our integrative framework based on diet and microbiome composition.

Our analysis also reveals an important systemic difference between humans and mice. Specifically, the fermentation product harvest per body weight is much higher in mice than in humans (approximately 393 mmol/kg/day for the mouse vs. 7 mmol/kg/day for humans, **SI Text 8.3**). This difference is also reflected in the contribution of microbiota-derived fermentation products to the daily energy demand, which is much higher in mice compared to humans (1.8-12.1% in humans vs 21.4 % in laboratory mice, **Figure 6**). Together with previously reported differences in microbiome composition and anatomy of the digestive tract [46–50], this difference in fermentation product harvest needs to be accounted for when utilizing mouse studies to explore possible systemic effects of the microbiota on the human host. For example, while specific microbiota perturbations have strong physiological and behavioral effects in mice, comparable perturbations in humans might have a smaller impact [51], simply because mice rely more on fermentation product-derived energy than humans. On the other hand, local characteristics like the absorption of fermentation products per epithelial surface area might be more comparable (171 and 233mmol/m2/day in mice and humans, respectively; **SI Text 8.3**), suggesting that local interactions between the human microbiota and the intestinal mucosa can be emulated more realistically in mouse studies.

More generally, this study emphasizes the strong potential of quantitative analyses that incorporate microbial metabolism, diet, host physiology, and microbiota turnover to put numbers on the metabolic exchange between microbiota and host. By providing information on specific doses, this goes well beyond the identification of microbiota-derived molecules and their possible interactions with the host. Besides the quantification of major fermentation products and their harvest in this study, many other interaction paths would benefit from such an integrative analysis. This includes the nitrogen cycling between the host and the gut microbiota [52], the role of cross-feeding in the exchange of gases such as H2 [53] and CO2, and the microbial digestion of proteins which exposes the host to branched-chained fatty acids and toxic waste products like hydrogen sulfide. In the context of the dynamic, flowing environment of the intestine [54], developing such frameworks is critical to move the field away from

misleading assumptions based on point measurements of metabolite concentrations towards an integrated model of host-microbiome functions driving health and disease.

Methods

Bacterial strains and culturing conditions. All characterized strains were obtained from established strain repositories and are listed in **Table S1**. Details for all media components and laboratory supplies are listed in **Table S5**. Bacteria were grown in the media indicated in the respective figures. BHIS medium is Brain Heart Infusion supplemented with 10mg/L hemin (prepared as in [55]) and 1g/L L-cysteine. YCA medium is a modified version of YCFA [56], containing 1% casitone 0.25% yeast extract, 0.1% meat extract, 4g/L $NaHCO_3$, 1g/L cysteine, 0.45g/L K_2HPO_4, 0.45g/L KH_2PO_4, 0.9g/L NaCl, 0.09g/l $MgSO_4$•7 H_2O, 0.12 g/L $CaCl_2$•$2H_2O$, 10mg/L hemin, 0.02mg/L cobalamin, 0.06mg/L p-aminobenzoic acid, 2mL/L BME vitamins, 10mM sodium acetate, 20mM NH_4Cl, and 20mM glucose or 10mM maltose, respectively. Gamma medium is a fully defined medium, containing 72mM K_2HPO_4, 28mM KH_2PO_4, 50mM NaCl, 0.5mM $CaCl_2$, 0.4mM $MgCl_2$, 0.05mM $MnCl_2$, 0.05mM $CoCl_2$, 0.004mM $FeSO_4$, 5mM Cysteine, 20mM $NaHCO_3$, 20mM NH_4Cl, 1.2mg/mL Hemin, 1mg/mL menadione, 2mg/mL folinic acid, 2mg/mL cobalamine, 10mM acetate, 0.2% BME vitamins, and 20mM glucose. Epsilon medium has the same composition as gamma medium, but also contains 1% Tryptone. All bacteria were cultured in glass tubes in anaerobic work benches, in temperature controlled dry baths at 37°C, shaking with 450rpm. Cultures were inoculated from glycerol stocks stored at -80°C, which were transferred to the anaerobic work benches before opening.

Growth experiments and sampling for metabolite analysis. For growth experiments, glycerol stocks of bacterial cells were taken from a -80°C freezer to an anaerobic workbench. Cells were then transferred from stocks to sterile glass tubes with YCA or BHIS media (seeding culture). After substantial growth, as indicated by an optical density at 600nm (OD_{600}) over 0.1, cultures were diluted into new culture tubes with YCA (pre-culture). Before cells reached saturation, $OD_{600} < 1$, cultures were then diluted again into fresh media to $OD_{600} \approx 0.02$ to start the experimental cultures. Growth was then tracked by regular measurements of OD_{600} (around 7 measurements between OD_{600} 0.05 and 0.5). OD_{600} measurements were performed in Quartz semi-micro-cuvettes in a benchtop spectrophotometer or by directly determining optical density of the culture tubes. We also collected four to six samples of 200µl culture volume for the subsequent quantification of sugar and fermentation products concentrations. These samples were transferred to sterile 0.2µm filter centrifuge tubes, and centrifuged at 11'000g for 2min to remove cells. Samples were then kept at 4°C until further analysis. For all samples, optical densities were typically kept between $OD_{600} \approx 0.04$ to 0.5 to ensure linearity between absorbance and bacterial biomass. These measurements were done in YCA, BHIS, epsilon, and gamma media, typically for three biological replicates each. Depending on the medium used, not all strains were able to grow and we report only results for strains which showed substantial growth in the respective media. Media compositions are provided above.

Chromatography Method to Determine Nutrient Uptake and Fermentation Product Secretion. Metabolite concentrations in filtered samples from culture supernatants were analyzed using isocratic HPLC with refractive index detection (RID), as described in [11]. In short, 20µl of sample was injected using an autosampler cooled to 10°C, separated over an

ion exchange column (Phenomenex Rezex RoA organic acid H+ (8%), LC column 300 x 7.8mm) in a column oven at 40°C, at a flow rate of 0.4ml/min, with 2.5mM H2SO4 in water as mobile phase. Chromatography data from the RID detector was recorded for 40min, exported as plain text files, and analyzed using custom Python scripts which has subsequently been released as a standalone software package [57]. An example of the analysis is discussed in **Figure S1** and the analysis scripts are available on the GitHub repository of this study. In short, a background correction based on the algorithm described in [58] was applied and areas under all the peaks representing the metabolites of interest were extracted. These areas were compared to a standard curve of known metabolite concentrations, and values for uptake or excretion per biomass were then calculated for every growth experiment.

Estimate daily fermentation product release: To calculate the daily release of fermentation bacteria in the large intestine we started with estimations on the bacterial biomass released daily in feces $M_{fec,bac}$, or the daily amount of complex carbohydrates which reach the large intestine and are digestible by bacteria, M_{carb}. These values either follow from fecal mass measurements more direct characterizations of luminal content, or from a mapping estimating the link between consumed carbohydrates and those available for the microbiota to utilize. We then calculated the daily release as $FP_{tot} = \epsilon_{tot} \cdot M_{bact}$ or $FP_{tot} = \epsilon_{tot} \cdot Y_{carb} \cdot M_{carb}$, were we took the average of the excretion and yield values measured for all strains. Full details of this approach are provided in **SI Text 3**. To account for variations in microbiome composition, we further weighted the values of different strains based on their metabolically determined relative abundance in different samples covering different studies from the curated metagenomic dataset [25]. Full details of this approach are described in **SI Text 6** and results for different studies are shown in the **Interactive Figure 1**.

Calculation of variation with diet. To obtain variations with diet, we analyzed different datasets on dietary composition and fecal weight as introduced. From dietary data we estimated the variation in microbiota available carbohydrates which we then used to calculate the variation in the daily release of fermentation products using the via-carbohydrate estimation (**Figures 2BC and S8, SI Text 3**). From fecal wet weight data we estimated the variation of daily bacterial biomass released in feces, accounting for changes in fecal water uptake (**Figure S10**). With this data we then calculated the daily fermentation product release using the via-feces estimation (**Figure 2A and SI Text 3**).

Code and Data Availability

All Python code, raw chromatograms and processed data are available on the paper's GitHub repository (https://doi.org/10.5281/zenodo.10445504) accessible via the website https://github.com/cremerlab/fermentation_products. Interactive figures are available at https://cremerlab.github.io/fermentation_products/study-explorer.

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