**Dynamics is a missing link for understanding microbiota-dependent heterogeneity in dietary responses**

**Abstract.** The endpoint responses of prebiotic fiber intervention are highly individualized and their associations with pretreatment gut microbiota have been previously reported. The missing link between baseline microbiota and endpoint heterogeneity is dynamics, yet its temporal pattern and role in transforming variations from start to end remain poorly understood. Here we administered inulin and resistant starch from maize to adult mice that carry distinct microbiota and monitored the dynamics of microbiome profiles and short-chain fatty acids (SCFAs) over four weeks. We found that inulin is more capable of stimulating production of SCFAs than resistant starch. In the majority of mice, inulin induced biphasic changes in SCFAs concentration and microbiota biodiversity, both of which were quickly perturbed in short-term but only partially recovered even under sustainable inulin supply. We further identified a consortium of five inulin responders that collectively shape short-term microbiota dynamics through an activation-then-competition mechanism. The observed dynamical responses depend on baseline microbiota composition and the baseline-dependency was found in 10%-20% bacterial species and the majority of SCFAs. In accordance with previous human studies, the strong baseline effects pose a great challenge to extrapolate prediction of SCFA concentration from gut microbiota to unobserved baseline composition. Our study reveals time- and baseline-dependence of dietary fiber responses as major challenges for attainable and robust personalized nutritional therapies.

Keywords: mouse, dietary fiber intervention, gut microbiome, time-series, dynamics, short-chain fatty acids, ecological network, biphasic responses, inulin, resistant starch

**Introduction**

# Dietary fiber intervention is often used to promote SCFA production

Biodiversity and inter-individual variability are two hallmarks of healthy human gut microbiome, which harbors hundreds of trillions of tiny microbes (bacteria, fungi, virus, etc.) that co-evolve with us in the gastrointestinal tract. Prebiotic fibers (e.g., inulin and resistant starches) are often used as an intervention on gut microbiota to promote production of short-chain fatty acids (SCFAs). SCFAs, especially butyrate, have broad impacts on human host physiology, immune system and intestinal homeostasis by playing versatile roles as histone deacetylases inhibitors, ligands of G-protein-coupled-receptors, and energy sources for colonocytes. Impaired intestinal SCFAs production has also been significantly associated with several metabolic diseases (e.g., type 2 diabetes mellitus, obesity and inflammatory bowel disorders) [2], chronic Graft-versus-host-disease, and efficacy of immunotherapy for solid tumors [3]. Despite a long history, the efficacy of using prebiotics to promote SCFAs has not been clearly validated. Several independent clinical trials of healthy adults have collectively revealed that the ability of prebiotic fibers to alter SCFAs profiles varies between fibers, SCFAs and human subjects.

# The interactions between dietary fiber, gut bacteria and SCFA metabolism is partially known

Supplementation of dietary fiber to selectively stimulate and enrich SCFAs producers is not as straightforward as it sounds: gut microbiome is an ecosystem and prebiotic interventions may lead to system-wide microbiota and metabolic shifts. Therefore, predicting whether SCFAs levels increase or decrease requires a system understanding of the complex interplay between dietary fiber, gut bacteria and SCFA metabolism, which is only partially known. Depending on the availability of fermentable fibers in the gut, gut bacteria choose between those fibers and other energetically less favorable sources (e.g., amino acids) and regulate the fatty acid composition accordingly, where fiber consumption produces SCFAs as the major end products. The microbial conversion from dietary fibers to SCFAs involve a number of metabolic cascading events operated by specialized members of the gut microbiota. Complex polysaccharide fibers are first hydrolyzed by primary fiber-degrading bacteria. These primary degraders can either produce SCFAs themselves or fuel the fermentation pathways of downstream SCFAs-producing bacteria by releasing a wide range of accessible carbohydrates (e.g., mono- or oligosaccharides) [10, 11]. For example, Eubacterium hallii can convert lactate produced by Bifidobacterium adolescents from breaking down starch or fructo-oligosaccharides to butyrate. Environmental cues other than dietary fiber can further modulate the metabolic processes. A mildly acidic pH (5.5) was reported to favor butyrate production while high propionate concentrations were observed upon just one unit pH change (6.5).

# Dietary intervention are heterogenous and how dynamics can bridge the gap for better understanding the heterogeneity

Previous studies have indicated that the effects of dietary fiber on gut microbiota and SCFAs can be unpredictable and individualized [14]. For example, Baxter et al. shows that inulin was able to promote butyrate production in only 63% healthy young adults. Understanding which particular recipients can benefit from the treatment is critical to ensure that appropriate prebiotics can be offered in a customized and optimal solution. There is growing evidence that baseline gut microbiota may be an important contributor to the observed heterogeneity, as the presence and abundance of primary fiber degraders and SCFAs producers prior to treatment may trigger different ecological and metabolic processes that ultimately lead to personalized outcomes [17]. While this association is promising, the statistical evidence was obtained mainly by comparing baseline and endpoint measurements in cross-sectional studies. The dynamic processes that bridge the two research timepoints remain unclear: Do prebiotic fibers induce generalized temporal reponses and how much of the variation in response dynamics is due to variation in baseline microbiota? Clearly, baseline differences do not always result in individualized responses; it is therefore important to identify the (latent) responding variables which can predict the individuality based on their initial differences. The key responding-bacteria in the baseline community and their ecological interactions may be inferred from time series data with assistances from ecological dynamics models. Generally, understanding the temporal changes in gut microbiome composition and metabolism, as well as identifying responders that empowers prediction of individualized responses, can aid in clinical trial design and move us closer to personalized medicine.

# Our approach to meet the challenges

Compared to antibiotics which can severely disrupt gut microbiota and lead to expansion and domination of facultative anaerobes (e.g., Vancomycin-resistant Enterococcus) in the gut, diet as a modifiable factor is much milder and its effects may be statistically masked by confounding variables in clinical trials. Studying dietary effects on gut microbiota using mouse models, as a complimentary alternative to clinical trials, has better controls over confounding variations between individuals driven by environmental and other host differences. For this purpose, we purchased age- and gender-matched isogenic C57BL/6J mice that harbor distinct gut microbiota composition from four commercial vendors (independent breeder sources), mimicking the efforts of stratifying human subjects into groups based on their gut microbiota to tackle the individuality issue in personalized nutrition. By collecting stool specimens over an experimental period of 4 weeks, we monitored temporal shifts in absolute abundances of fecal bacteria (16S rRNA gene sequencing and quantitative PCR), gene abundances (metagenomics sequencing), SCFAs concentration (targeted metabolome) as well as physiological changes of treated mice (e.g., body weight) following intervention of two high fermentable fibers (inulin and resistant starch from maize) and a low fermentable cellulose control (Fig 1A). Both inulin and resistant starch can escape hydrolysis by human enzymes in the upper gut and known to be degraded by gut bacteria in the cecum and colon [18][19]. Motivated by a central hypothesis that heterogenous dynamics of gut microbiota and SCFAs are primed by pretreatment microbial community and community ecology (Fig. 1B), we developed several computational approaches to address different aspects (quantifications, causes and consequences) of the diet-induced, baseline-microbiota-dependent heterogeneity.

**Results**

**Baseline characteristics of the murine gut microbiome**. All mice in our study were genetically identical, co-housed in the same cages, and fed with the same cellulose-based diet 7 days prior to dietary fiber intervention. While difficult to stratify human microbiomes, these mice can be naturally divided by vendor sources into groups with distinct microbiota composition (Fig. 1C, D). Beta-diversity (between-sample distance), calculated by Aitchison distance at the lowest classified taxonomy level, shows that the baseline compositions of our mice cluster by vendor (Adonis, P<0.001) and are characterized by distinct bacterial taxa (Fig. 1C). For example, Shanghai mice lack several commensal mucosal-monosaccharide-utilizers such as Muribaculaceae and Rikenellaceae. The profound inter-vendor difference (Adonis, P<0.001) was also observed at the family level (Fig. 1D). Due to the high between-vendor variation, mice from the same vendor can be effectively treated as independent biological replicates for each baseline microbiota composition.

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**Figure 1. Experimental design and computational framework for this study.** **A**. Schematics of the experiments performed on a mouse model. Gray dots indicate the days on which longitudinal fecal samples, microbiome and metabolomics data were collected. SCFA: short-chain fatty acid. **B**. An ecology-centric view of baseline-dependent dynamical fiber responses. We used mathematical modeling to infer ecological network and key fiber responders that determine baseline dependence of both microbiome and SCFA dynamics. **C**. Beta diversity of baseline microbiota composition shown in PCoA (principal coordinate analysis) plot. Gray dots represent baseline samples of isogenic mice purchased from four different vendors (Beijing, Guangdong, Hunan, Shanghai) and gray arrows represent dominant bacterial taxa in these samples. The sample whose dot projects furthest in the direction of a species has the highest relative abundance of that species. R2 and P-value were obtained from Adonis analysis, which tests for baseline differences across the four vendors. Taxonomic labels w/ “Un.” group bacteria that are unclassified or uncultured at lower taxonomic ranks. **D**. The baseline gut microbiota composition at family level. Bars in each subplot represent different mice purchased from the same vendor.

**Biphasic dynamics of SCFAs and gut biodiversity**. Dietary intake of inulin and resistant starch increases mice body weight gradually over time, but the amount of body weight gain is generally insignificant compared to mice treated with the cellulose control (Fig. S1A). Although there were no obvious temporal patterns in the fiber intake rate (Fig. S1B, C) and fecal weight (Fig. S1D), the fecal weight was dramatically lower in inulin- and resistant starch-fed mice, suggesting increased colonic transit time and decreased defecation frequency. Accordingly, the intestinal absorption of both fermentable fibers by gut microbiota promoted fecal SCFA concentration (Fig. 2A, S2A). Consistent with previous human studies, inulin stimulated higher production of total and three major individual SCFAs (acetate, propionate, and butyrate) than resistant starch regardless of baseline microbiota (Fig. S2B). Still, the quantitative differences in the stimulating effects of inulin and resistant starch are SCFA- and baseline-dependent.

Except for Shanghai mice whose propionate production was notably delayed and compromised, the inulin-induced changes in fecal SCFA concentration follow a universal biphasic dynamics: their levels peaked in short-term before gradually decreasing until steady states. Depending on the baseline microbiota, the mean endpoint level of total SCFA at day 31 is 60%-65% of its peak but 2.0-3.5 times as high as its baseline level at day 0. The long-term decrease in fecal SCFA concentration was not a result of reduced diet intake as the intake rate remains largely unchanged over time (Fig. S1B, C). Since fecal SCFAs are metabolites produced by colonic bacterial fermentation of dietary fibers, we observed similar biphasic, but undershoot, dynamics in the gut biodiversity, which drops initially before gradual increase (Fig. 2B). Concordantly, we observed rapid but non-monotonic complex changes in the relative abundance of several dominant bacterial taxa such as Bacteroides and unclassified Muribaculaceae (Fig. 2C). Similar to SCFA dynamics, the trends of biodiversity are qualitatively consistent but quantitatively different across the four vendors. We further showed that the undershoot dynamics of biodiversity primarily resulted from changes in evenness (Fig. S3A), not richness (Fig. S3B), suggesting that the decreased biodiversity was likely caused by an expansion of resident bacteria, rather than invaders. Supporting these findings, metagenomic sequencing revealed that the initial (day 0), short (day 5)- and long (day 31)-term microbiomes have distinct gene family profiles and functions (Fig. 2D). Our dynamics data confirms previous studies on the role of dietary fibers to quickly alter gut microbiota diversity and composition on the timescale of a day independent of the baseline microbiota.

On contrary to inulin, no consistent temporal patterns of SCFAs and gut microbiota biodiversity were found in the resistant starch intervention (Fig. 2A,B). The alterations in gut microbiota composition were also milder, despite the effects are more dramatic than cellulose (Fig. S2C).

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**Figure S1. Effects of inulin or resistant starch on (A) body weight, (B) daily food intake, (C) daily energy intake, and (D) 48-hr fecal sample weight of mice receiving diet supplementation used in this study.** Each symbol represents the mean body weight in panel A or a single data point in panels B-D. The body weight data were analyzed by ordinary one-way ANOVA (Analysis of variance) with Turkey post hoc test between inulin or resistant starch and cellulose group. \*, P < 0.05.

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**Figure S2. Dynamics (A) and mean levels (B) of fecal short-chain fatty acids (SCFAs) concentration following dietary fiber intervention.** In panel A, dots/lines represent mean concentrations across mice within the same vendor and shading areas represent standard error of the mean. In panel B, each colored dot means the time-averaged level of SCFAs (calculated by area under the concentration curve divided by the observation time) in a mouse and gray lines are the best linear regression fit.

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**Figure S3. Dynamics of evenness (A), number of observed ASVs (B), and cellulose-group microbiota composition (C) following dietary fiber intervention.** The height of lines (panels A, B) or stacked bands (panel C) represent mean values across mice within the same vendor and shading areas (panels A, B) represent standard error of the mean. In panel C, taxonomic labels w/ “Un.” group bacteria that are unclassified or uncultured at lower taxonomic ranks.

**Stabilization of gut microbiota composition under sustained stimulation.** In addition to biphasic responses, we also found strong tendency of gut microbiota composition to stabilize under sustained stimulation of inulin (Fig. 2E) and, to lesser degree, resistant starch (Fig. S4A). Regardless of the baseline microbiota, the steady-state compositions are distinct from their baselines for both fibers and thus represent new equilibria sustained by dietary fiber intake. Specifically, the gut microbiota in Guangdong mice was highly resilient to inulin intervention and almost returned to its baseline composition at day 31. In addition, inulin, as well as resistant starch and even cellulose, reduced baseline microbiota differences by ~35% at day 31 (Fig. S4B), suggesting that long-term dietary fiber intervention promotes adaptative convergence of baseline microbiota towards similar composition. The adaptation is also evident in the dynamics of the dominant bacterial taxa. For example, the relative abundance of unclassified Muribaculaceae in Shanghai mice was initially much lower at day 0 but finally reached similar levels as in the mice from the other three vendors at day 31.

To quantify the rate of stabilization, we fit a harmonic oscillator model (Eq. 1-2) from physics to time series of biodiversity and calculated damping ratio—a dimensionless measure of whether and how dynamical systems approach new steady states upon perturbations—from the best-fit parameters (see Methods, Table S1). The model fits the data reasonably well with mean R2 of 73% and 61% for inulin and resistant starch respectively. The damping ratios are all equal or greater than 1, meaning that their responses were critically or overdamped and no oscillations were involved in the adaptive responses. The oscillation-free property is critical for ecosystems to maintain dynamic stability and integrity during adaptation. Although the damping ratio varies among individual mice and treatment, the dynamical responses of 42% of our mice were critical damping which leads to the fastest non-oscillatory convergence towards stationary equilibria. Interestingly, the critical damping behavior was also found in human gut microbiome recovery after transient antibiotic exposure.

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**Figure 2.** **Dynamical responses of short-chain fatty acids (SCFAs) metabolism and murine gut microbiome to dietary fiber intervention. A.** Fecal concentration of three major SCFAs. **B**. Biodiversity of gut microbiota. **C**. Genus-level composition of gut microbiota. Taxonomic labels w/ “Un.” group bacteria that are unclassified or uncultured at lower taxonomic ranks. **D**. Clustering of gene family profiles at day 0 (baseline), 5 (short-term) and 31 (long-term) following inulin intervention in PCoA (principal coordinate analysis) plots. R2 and P-value were obtained from Adonis analysis. For each cluster, smaller dots represent individual mice. The bigger dot represents the cluster center, and an eclipse was drawn around the average to show the 95% confidence interval. **E.** Time trajectories of gut microbiota composition responses to inulin in PCoA coordinates. The heights of stacked bands (panels A, C), lines (panel B) and dots (panel E) represent the mean values across mice within the same vendor. Shading areas (panel B) and error bars (panel E) represent standard error of the mean.

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**Figure S4.** **Time trajectories of gut microbiota responses in PCoA (principal coordinate analysis) coordinates (A) and temporal changes in the distance of microbiota composition between vendors (B).** Panel A shows responses to resistant starch and panel B shows responses to inulin, resistant starch and cellulose. In panel A, dots represent the mean PCoA coordinate score across mice within the same vendor and error bars represent standard error of the mean. In panel B, dots/lines are the mean pairwise Aitchison distance between samples from different vendors and shading areas represent standard error of the mean.

**Inferring dietary fiber responders.** A number of culturable bacteria could metabolize inulin and resistant starch *in vitro* but an *in vivo* understanding of the bacterial fiber degraders and the ecology correlated with their fermentation has not been established yet. Since genomic approaches rely on strain-level identifiability and cultivability, we switched to a data-driven approach that aims to identify responders that can directly or indirectly metabolize dietary fibers. If such responders exist, we would expect increased abundance of their populations with a concomitant higher level of enzymes capable of fiber degradation following treatment. Indeed, the relative abundance of genes encoding inulinase significantly increased from day 0 to day 5 and 31 (Fig. 3A), suggesting that inulinase may be a trait that has been selected for by inulin.

To infer dietary fiber responders from the complex microbiota, we employed the generalized Lotka–Volterra (gLV) equation (Fig. 3B, Eq. 3) to model the observed dynamical responses. The gLV model summarizes the underlying ecology by three terms that additively determine bacterial growth rates: the basal growth rates (), the influences from other bacteria (), and the impacts of environment (). In this study, represents the growth responses of bacteria to dietary fiber. To estimate the uncertainties associated with these parameters, we further formulated the inference problem in a Bayesian framework (see Methods) and responders include any bacteria whose 95% credible interval of its posterior distribution of is completely to the right of 0. Since gLV is a differential-equation model, it is naturally more amenable to analyzing longitudinal data compared to cross-sectional statistical tests based on pre-to-post changes. Indeed, bacteria whose relative abundances were significantly altered by inulin vary depending on the day of sample collection (Fig. S5).

Since gLV models the absolute abundance of bacterial taxa, we measured the fecal bacterial density of each sample by quantitative PCR (Fig. 3C) and then multiplied the density by their relative abundance to calculate absolute abundance. Except for the Shanghai group (despite high inulinase abundance), inulin nourished gut microbes by rapidly (in a day) boosting their density to more than 70% of maximum load. Considering ecological forces driving microbiome dynamics are largely host-independent, the time-series data from all mice were simultaneously fed into the gLV model after grouping bacteria according to their lowest classified taxonomic ranks. We identified five inulin responders at different classification levels (Fig. 3D): (from highest to lowest signal strength) Bacteroides acidifaciens (species), unclassified Muribaculaceae (family), unclassified Faecalibaculum (genus), unclassified Parasutterella (genus), and unclassified Bacteroides (genus). Since gLV incorporates bacterial interactions as confounding variables, these responders are most likely to be primary inulin degraders. In fact, genetic or *in vitro* evidences have been found, except for unclassified Parasutterella, to support the their role in degrading inulin (Table S2). For example, members from Bacteroides and Muribaculaceae harbor gene clusters known as polysaccharide utilization loci (PULs), which contain *susC/susD* homologous gene pair that facilitates sensing and import of inulin [23, 24]. Putative inulin PULs were also detected in the metagenome-assembled genomes of Bacteroides acidifaciens and Muribaculaceae (Table S3). Other than competitions, we also found that Bacteroides acidifaciens positively influences growth of Akkermancia muciniphila—a mucin-degrading bacteria whose abundance often increases after prebiotic treatment [21]. Considering Akkermancia muciniphila does not grow well *in vitro* by supplementing inulin, the indirect effect may explain why it was not inferred as a responder but its relative abundance significantly increased in both short- and long-term (Fig. S5B,C).

For resistant starch, three bacterial taxa were inferred as responders: (from highest to lowest signal strength) unclassified Faecalibaculum (genus), unclassised Muribaculaceae (family), and unclassified Desulfovibrionaceae (family). Some indirect evidences have been shown for the first two responders in utilizing resistant starch (Table S2).

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**Figure S5.** **Significantly altered bacterial taxa in relative abundance between inulin and cellulose intervention.** Relative abundance changes were calculated between day 0 and day 1 (A), day 0 and day 5 (B), day 0 and day 31 (C). P-values were obtained from Wilcoxon rank-sum test after multiple test correction via false discovery rate (FDR) estimation. \*, FDR < 0.05; \*\*, FDR < 0.01; \*\*\*, FDR < 0.001.

**Additional dataset confirms our major findings**. To corroborate our findings on the inulin-induced dynamical responses as well as the identity of inulin responders, we reanalyzed the raw data from a recent longitudinal study with similar experimantal setup, which also profiled the gut microbiota compositions of mice based on 16S rRNA sequencing after inulin intervention. Their data independently showed undershoot dynamics of biodiversity and its tendency to converge after 14 days (Fig. S6). By applying our inference approach to the relative abundance of their gut microbiota data, Bacteroides acidifaciens was also inferred as a responder. The mutual agreement suggests that the characteristic responses of murine gut microbiota to inulin intervention may be robust and shared among mice with different microbiota compositions.

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**Figure S6. Reanalysis of data from Chijiiwa et al., where** **a similar mouse experiment was performed to measure gut microbiota dynamics following inulin treatment.** **A**. Biodiversity. **B**. Time trajectory of gut microbiota in PCoA (principal coordinate analysis) plot. Each dot represents the mean principal coordinate score across all mice and the corresponding error bar represents the standard error of the mean.

**Inulin responders as a functional guild shape microbiota dynamics.** Compared to taxonomic groups, ecological guilds (groups of bacteria that perform similar ecological functions) are more fundamental units for microbiome data analysis. In line with the guild-based concept, the inferred responders, though taxonomically heterogenous, define a core group that participates in dietary fiber degradation. By partitioning microbiota dynamics between responders and other bacteria (mainly Akkermansia municipila and Bacteroides unifomis), we showed that the total relative (Fig. S7A) and absolute (Fig. 3E) abundances of the five inulin responders dominate the changes of the entire microbiota in short-term (Guangdong, Hunan), long-term (Shanghai), or even the entire period of observation (Beijing). Interestingly, the total biomass of other bacteria in Hunan mice has a delayed response and the dynamics of the two guilds co-oscillated after the delay. It is likely the delayed bacterial succession [12] may result from niche opening and occupation by cross-feeders that utilize simple sugars released from the inferred responders.

Substantial heterogeneity was observed within the guild of inulin responders (Fig. 3F, S7B,C). Bacteroides acidifaciens and unclassified Facelibaculum showed transient dynamics with quick rise and drop in their relative and absolute abundances, which correspond to and may explain the undershoot dynamics of biodiversity (Fig. 2B). By contrast, the relative and absolute abundances of unclassified Muribaculaceae increased continuously and remained high throughout the entire period of study. Except for Shanghai mice, the total bacterial density reached local maximum rapidly within 1-3 days (Fig. 3C), suggesting that these responders may compete for inulin after the gut microbiota has reached its temporary maximum load. The within-guild competition was supported by the gLV-based inference of bacterial interactions (Fig. 3G), where unclassified Muribaculaceae negatively impact growth of Bacteroides acidifaciens and unclassified Facaelibaculum (highlighted in red). This activation-then-competition mechanism found *in vivo* was also observed in a synthetic community of two auxotrophic yeast strains under high amino acid supplementation.

Compared to inulin, the guild of resistant starch responders plays much less dominant role in shaping the overall response of gut microbiota (Fig. S8A). In Hunan and Shanghai mice, the total density of other bacteria, despite not inferred as responders, increased sharply to higher levels than that of the responders. One possibility of this seemingly counterintuitive result might be an uneven distribution of a large number of resistant starch-degrading bacteria in the baseline community across vendors, such that the responders showing mild effect in specific vendors did not pass the significance test. Nonetheless, we found similar trends, as observed in the inulin-group mice, for the dynamics of unclassified Muribaculaceae and unclassified Faecalibaculum (Fig. S8B): the absolute abundance of the former bacteria showed gradual but continued increase, while that of the latter bacteria showed a quick rise-and-drop pattern. GLV-based inference further suggests that the observed dynamics may be driven by mutual inhibition between the two taxa (Fig. S8C).

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**Figure 3. Inulin** **responders** **and associated ecological interactions. A.** Increased expression of inulinase genes following inulin treatment. Each dotted line represents an individual mouse. \*: P < 0.05; \*\*: P < 0.01; \*\*\*: P < 0.001. **B**. Generalized Lotka-Volterra model combined with Bayesian statistics to infer inulin responders and bacterial interactions. **C**. Total bacterial density measured by quantitative PCR. **D.** Posterior distribution of five bacterial taxa with significant growth responses (inulin responders). **E**,**F**. The absolute abundance of total (E) and individual (F) inulin responders shown in panel D. **G.** Core ecological interaction network consisting of significant interactions (95% credible interval of an interaction coefficient does not contain 0). Self-interactions are not shown. Inulin responders (bold font) and key inhibitions (red arrows) are highlighted. Point and blunt arrows represent positive and negative interactions respectively. The arrow thickness is proportional to the posterior mean of the corresponding interaction coefficient. For panels D, F, G, taxonomic labels w/ “Un.” group bacteria that are unclassified or uncultured at lower taxonomic ranks**.**

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**Figure S7. Comparison of temporal changes in relative abundance (A) between inulin responders and other bacteria and (B,C) between different inulin responders.** The dynamical responses were organized by responder in panel B or by vendor in panel C.

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**Figure S8. Inferring bacterial responders to resistant starch intervention and associated ecological interaction network.** The same figure legend applies as in the main text Fig. 3E-G (the same order).

**Dynamical responses of gut microbiota and SCFAs are baseline-dependent.** We have shown above that the Shanghai mice were initially inert to inulin and resistant starch stimulation (Fig. 3C). The lack of short-term response can be explained by the low abundance of Bacteroides acidifaciens (as inulin responder) and unclassified Muribaculaceae (as responder of both fibers) in their baseline microbiota (Fig. S3F, S7B, S8B). To formally quantify the baseline effects, we transformed all time series from both intervention and control group into a 2-dimensional space and then compared the coarse-grained representation among different vendors after adjusting the responses from the comparator arm (see Methods). Compared to previous approaches that measure intervention effects by inspection of cross-sectional changes in the intervention group alone, the control group data was purposely introduced to avoid the caveat that the pre-to-post changes may be entirely attributed to random variations within each individual. To determine whether an observational variable (bacteria or SCFAs in our study) exihibits a baseline-dependent response, we obtained two P-values that separately test for the responsiveness (differential responses between the intervention and control group) and baseline dependence (differential responses between the four vendors), and the variable has a baseline-dependent response if both P-values are smaller than 0.05.

We first confirmed that the dynamics of total bacterial load depends on baseline microbiota composition for both interventions of inulin (Fig. 4A) and resistant starch (Fig. S9A). By constrast, no significant difference was found between vendors by comparing the bacterial load change between day 0 and day 31 (Table S4), indicating the importance of incorporating whole time series for individualized response analysis. Additionally, we identified 20% and 11% bacterial taxa that exhibit baseline-dependent responses for the inulin (Fig. 4B, Table S5) and resistant starch (Fig. S9B, Table S6) intervention respectively. As a comparison, calculations based on pre-to-post changes (see Methods) vastly overestimated the percentages (about 50% for both fibers), further reinforcing the necessity of taking control group into accounts to avoid false discoveries. Finally, baseline effects were also detected for the dynamics of all three major SCFAs (except for acetate in the inulin intervention) following intervention of both fibers (Fig. 4C, S9C). For the inulin-induced butyrate and propionate dynamics, the simple statistical tests made variable conclusions regarding the significance of inter-vendor differences, depending on the endpoint day chosen for comparisons (Table S4). Interestingly, the dynamics of total SCFAs are baseline independent for both fibers, suggesting that baseline microbiota may only modulate the proportions of different SCFAs but not the total concentration.

As a byproduct, our approach also facilitates visualization of baseline dependence for any observational variable: the averaged response difference of the variable between intervention and control group is represented by an arrow and colored differently by vendors. Therefore, any differences in the magnitude and direction of these vendor-specific arrows indicate baseline effects. For example, an enrichment of Akkermansia muciniphila was observed in Hunan and Shanghai mice (from day 5 and day 19 respectively), but not in Beijing and Guangdong mice, following inulin intervention (Fig. 2C). The baseline effect was also reflected in our simplified plot: the green arrow (Hunan) is longest and points to northeast direction; the red arrow (Shanghai) is the second longest and closely aligns with the y axis; the orange (Guangdong) and blue (Beijing) arrows are much shorter and negligible.

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**Figure 4. Quantification and visualization of baseline-dependent responses of (A) total bacterial load, (B) individual bacterial taxa, and (C) short-chain fatty acids (SCFAs) to inulin intervention.** The scatter plot in the center of panel B distinguishes four different scenarios of baseline-dependent responses depending on the P-values for significance test of responsiveness (Pr) and baseline dependence (Pb). The non-responsive bacterial taxa are marked as gray dots, while the responsive ones are colored by the ratio of their averaged absolute abundances (abs. abun.) between inulin and cellulose group. We mapped the inulin-stimulated dynamical response of any variable relative to its corresponding response in the cellulose group onto a reduced two-dimensional (2D) space spanned by two factors extracted from sequential non-negative matrix factorization (see Methods for details). In the 2D plot, each symbol represents a mouse (dots: cellulose group, crosses: inulin group) and all mice data from the same vendor under the same dietary fiber treatment was used to fit an eclipse (ellipse’s radius was determined by 2 standard deviations). For each vendor, an arrow was drawn from the eclipse center of the vendor under cellulose treatment (standardized to the origin) to that under the inulin treatment. Numbers in parentheses on x- and y-axis labels indicate factor loadings. Taxonomic labels w/ “Un.” group bacteria that are unclassified or uncultured at lower taxonomic ranks.

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**Figure S9. Quantification and visualization of baseline-dependent responses of (A) total bacterial load, (B) individual bacterial taxa, and (C) short-chain fatty acids (SCFAs) to resistant starch intervention.** The same figure legend applies as in the main text Fig. 4A-C (the same order).

**Baseline dependence impedes prediction of SCFAs from microbiota composition.** Predicating metabolomes from microbiota has been of long-standing interest but there have been controversies regarding whether metabolome, especially SCFAs, is predictable. We evaluated the performance of machine learning models to predict the fecal SCFA concentrations using absolute abundance of bacterial taxa as predictors. All mice were split into training and test sets using two data-split approaches (Fig. 5A). The “interpolation” strategy generated balanced distribution of baseline microbiota composition between the training and test sets by randomly selecting a single mouse from each vendor as test data and leaving the other mice of the same vendor for training. By contrast, the “extrapolation” strategy produced highly unbalanced microbiota distribution by randomly selecting all mice from a vendor as test data and leaving the other vendors for training. Using a Random Forest (RF) regression model and data from inulin intervention, the training accuracy in terms of R2 is at least 66% regardless of SCFAs and data-split strategy. In the scenario of “interpolation”, the prediction accuracy of the three SCFAs varies from 10% to 45%, where butyrate is the most predictable SCFA probably due to its conserved pathways for *de novo* biosynthesis. However, though not surprisingly, the R2 value for model prediction is less than 0 for all SCFAs in the “extrapolation” scenario, meaning that our model is highly overfit and generalizes worse than any linear model. We further showed that the poor performance of predicting extrapolated dataset cannot be significantly improved by using alternative models (Fig. S10A) or predictors (Fig. S10B). In both data-split scenarios, the RF predictions are even more inaccurate using data from resistant starch intervention (Fig. S11A).

We hypothesized that the poor performance of RF model stemmed from the strong dependence of SCFAs concentrations on baseline gut microbiota composition. By counting the presence and absence of each bacterial taxa in the baseline samples (Fig. 5C, S11B), we found that about 85% bacteria were only present in a subset of mice and >70% was not present in all vendors regardless of the intervention. Some of these bacteria that are missing in at least one vendor have been inferred as SCFAs producers (Fig. S12A). Formally, we quantified the similarity of gut microbiota composition between the training and test datasets in the “interpolation” and “extrapolation” scenarios used a RF classifier (see Methods). For both dietary fibers, we found that the testing set was almost fully distinguishable from the training set when the entire data was split by the “extrapolation” approach, while the two sets are very much alike when split by the “interpolation” strategy (Fig. 5D, S11C). The problem of the changed distribution between training and test dataset is formally known as covariate shift and the distribution gap can be sometimes counteracted by assigning weights to the training sets (see Methods). Unfortunately, the baseline gut microbiota between vendors seems to be too different and adding weights does not improve the prediction accuracy (Fig. S10C).

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**Figure 5.** **Prediction of short-chain fatty acids concentrations from gut microbiota using machine learning models. A**. Two data-split strategies for testing model performance. Mice in the test sets were randomly selected on a one-per-vendor basis for “interpolation” and exclusively selected from a single vendor for “extrapolation”. Data before intervention (i.e., day 0) was not included. **B**.Performances of Random Forest regression models on the training and test datasets. **C**. Presence (threshold: 0.001%) and prevalence of bacterial taxa in baseline microbiota across mice and vendors. In the bottom panel, the prevalence score of a taxon across mice (solid line) was defined as the fraction of mice that contains this taxon in their baseline microbiota and that across vendors (dashed line) was defined as the fraction of vendors whose mice all contain this taxon. **D**. Receiver operating characteristic (ROC) curve analysis of the similarity between training and test datasets. A Random Forest classifier trained to discriminate the two datasets outputs area under the ROC curve (auc) as a dissimilarity metric.

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**Figure S10. Poor performance of Random Forest (RF) regression model in predicting short-chain fatty acids (SCFAs) concentration (see Fig. 5B of the main text for the results) cannot be rescued by using (A) alternative predictors, (B) alternative regression models, and (C,D) weighting of training samples. A**. Prediction accuracy of a RF model trained on different taxonomic- (ASV, Species, Genus, Family) or functional- (Gene, Pathway, Phenotype) predictors. For each taxonomic level, unclassified or uncultured taxa at this level were grouped by the lowest classified rank above this level. The abundances of genes, pathways and phenotypes were predicted using PICRUSt2. **B**. Prediction accuracy of the MelonnPan algorithm trained on the same predictors as used in panel A. **C**. Weights assigned to the training data. The gut microbiota composition of all samples was shown in a reduced two-dimensional UMAP (Uniform Manifold Approximation and Projection) space. The bigger the weights, the larger circle sizes. See Methods in the main text for details of weight calculation. **D**. Prediction accuracy of an RF model built from weighted training data. The absolute abundance of bacterial taxa (grouped by the lowest classified taxonomic level) was used as predictors.

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**Figure S11. Prediction of short-chain fatty acid (SCFA) concentration from gut microbiota using data from resistant starch-treated mice**. The same figure legend applies as in the main text Fig. 5B-D (the same order).

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**Figure S12. Inferred short-chain fatty acid (SCFA) producers in inulin-treated mice vary depending on the inference approaches (Random forest regression vs. Repeated correlation analysis). A.** Random forest (RF) regression. For each SCFA, we showed top 10 bacterial taxa with highest Gini importance score in model training using all data. The absolute abundances of bacterial taxa were standardized and filtered (threshold 10-5) by LASSO (least absolute shrinkage and selection operator) regression before passing to RF model. Several key hyperparameters in LASSO and RF were optimized using grid search cross-validation with R2 as the score metric. The vendor-level prevalence scores were obtained from Fig. 5C in the main text. **B**. Repeated correlation analysis. Longitudinal data and correlation trend lines are color-coded on a per-mouse basis. Repeated measures correlation coefficients (*r*rm) and FDR-corrected P-values are indicated in the plot.

**Discussions/Conclusions**

# Discuss how our study shows that dynamics is important for understanding individualized responses

In summary, our results indicated paramount importance of dynamics for understanding inter-individual differences in dietary responses. The significance of dynamics is two folds. First, inter-individual variability is a function of time and dynamic data provide temporal trends allowing for more accurate quantification of individualized responses. Compared to pre-to-post changes, temporal trends improve our ability to distinguish responses that have different shapes but the same endpoint values. For example, Shanghai mice showed delayed responses to inulin and their biomass levels at day 31 do not differ significantly from the other three vendors (Table S4). As a proof, our new approach that incorporated the whole time series correctly identified the inter-vendor difference (Fig. 4A). Second, dynamics bridge the gap between baseline state and endpoint state to reveal how input variability evolves and transforms into output variability. For instance, the inter-vendor variability of bacterial load was successfully linked to the baseline abundance of two inulin responders—Bacteroides acidifaciens and unclassified Muribaculaceae. Without time series data, it would be difficult to precisely pinpoint the sources of heterogeneity as the inference of inulin responders based on pre-to-post changes would possibly result in both false-positives (e.g., bacterial density increases just before study endpoint due to indirect effects) and false-negatives (e.g., bacterial density increases initially but drops to baseline levels before study endpoint). Taken together, characterizing dynamic responses to dietary fiber intervention across individuals, with integrated longitudinal analysis of 16S rRNA sequencing, metagenomics and metabolomics, is an important priority for microbiome research to further understanding of diet-induced responses. Such studies thus have great potential to improve human health and treat gut microbiome-associated disease via microbiome engineering.

# Discuss the relevance of biphasic SCFAs dynamics to the literature

Diet-induced changes in SCFAs are often transient and vanish shortly after cessation of dietary intervention [25-28]. Our experiments add to the literature by further showing that SCFA concentrations cannot be maintained at its peak and drop by 35%-40% even under continuous inulin intake until 4 weeks [29]. The transient responses under sustained dietary fiber intake were also observed in colorectal cancer patients [30]. Despite the drop, our data demonstrates that a continuous intervention that lasts for 31 days is sufficient to elevate and stabilize the SCFAs concentration, but it is not clear yet whether the elevated level persists after the intervention discontinues. We envision two possibilities that may explain the after-peak decrease of SCFA concentration. First, some bacteria are known to consume SCFAs and a net consumption of SCFAs may occur when SCFAs consumers dominate over producers. The other possibility is increased absorption of SCFAs by host cells, leaving less produced SCFAs excreted to feces. In healthy individuals, the percentage of bacteria-derived SCFAs that appear in feces is only 5%. Considering the majority of SCFAs are consumed by the host, it is important to note that fecal SCFA concentration may not reflect their concentration in the intestine.

# Discuss how our inference approach helps tackle the replicability issue

Using public datasets, we demonstrate that our approach for inferring dietary fiber responders helps tackle replicability issue in microbiome science. The structure of gLV allows for modeling bacterial interactions as covariates and accounting for confounding variables reduces the risk of producing biased estimates of indirect effects. Controlling for confounding variables is highly important for replicability between results of similar microbiome studies, as the underlying mechanisms driving microbiota dynamics is, to a great extent, shared and should play consistent roles. In fact, any scientific field that heavily relies on complex statistical analysis of large datasets may encounter challenges in replicability. Remarkably, our inference approach applied to different mouse or human gut microbiome datasets produced highly compatible results regarding inulin responders. In the mouse gut, both our data and data from Chijiiwa et al. agree that the strongest inulin responder is Bacteroides acidifaciens, which was also reported by a third study that treated mice with inulin (data not reanalzyed). On the other hand, reanalysis of four human datasets that all administered inulin revealed that unclassified Bifidobacterium (in three studies) and Anaerostipes (in all four studies) exhibit significant responses to inulin, despite the huge differences in the pretreatment microbiota composition among human subjects in these studies (Fig. S13).

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**Figure S13. Inference of inulin responders in human gut microbiome. A.** Principal coordinate analysis (PCoA) of baseline human gut microbiota (Bray-Curtis distance matrix of 16S or shallow shotgun metagenomics) in four cohorts of literature studies with inulin intervention. Inulin responders inferred from the four literature studies are showed to the right of the PCoA plot. We used the same generalized Lotka-Volterra model and Bayesian inference framework as we used for analyzing our mouse data (see Methods in the main text for details). Cross (x) marks an exception that the inferred responder can be classified to the species level (Anaerostipes hadrus). Taxonomic labels w/ “Un.” group bacteria that are unclassified or uncultured at lower taxonomic ranks. **B**. Dynamics of relative abundance (rel. abun.) of unclassified Bifidobacterium and unclassified Anaerostipes in our dataset. Lines represent mean concentrations across mice within the same vendor and shading areas represent standard error of the mean.

# Can we predict microbiota responses from baseline abundance of responders?

By combining gLV model and Bayesian regression, we identified multiple inulin and resistant starch responders from the complex microbial community of the murine intestine, which we believe play critical roles in bridging the variability in baseline microbiota to the variability in microbiota responses. There then comes a question: Can we predict microbiota responses simply from the relative abundance of these responders in the baseline community? To answer it, we first tested the statistical association between baseline relative abundance of each individual responder and time-averaged bacterial density (area under the curve divided by the observation time), where the latter was used as a proxy for overall microbiota response. Not all inulin responders are equally predictive and positive correlations were only found in Bacteroides acidifaciens and unclassified Muribaculace (Fig. S14A). We found that the total relative abundance of the two responders can explain 56% of the variability (Pearson correlation) in microbiota response. Similarly, the total relative abundance of unclassified Muribaculaceae and Desulfovibrionaceae in the baseline community explains 74% of heterogeneity in microbiota response induced by resistant starch (Fig. S14B). Although not perfect, our study provides guidance on how to develop predictive metrics of microbiota response under dietary fiber intervention.

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**Figure S14. Prediction of time-averaged gut microbiota response (area under the curve of total bacterial density divided by the observation time) from relative abundance of dietary fiber responders in the baseline community.** **A**. Inulin intervention. **B**. Resistant starch intervention. The combination of responders highlighted in red has the highest Pearson correlation coefficient. In both scatter plots, gray lines represent the best fitting line.

# Discuss the major reason for the failure of predicting SCFAs from microbiota composition

Considerable debates have been raised over the feasibility of predicting metabolite profiles solely based on microbiome sequencing data. For example, the MelonnPan algorithm based on linear regression accurately predicted relative abundances of >50% metabolites from metagenomes in patients with inflammatory bowel disease and healthy controls. Oppositely, it was also reported that only 14% of the observed variation in the SCFAs concentration can be explained by RF regression models trained on 16S rRNA or metagenomic gene sequences in colorectal cancer patients and healthy controls. While the possibility of using different regression models and patient cohorts between these controveral studies cannot be excluded, our study points out that the similarity of data distribution between training and test sets, which is strongly affected by the degree of inter-individual variability of gut microbiota, may contribute to the disagreement. Using mouse models, we showed that the predictability was completely lost when extrapolating models to predict SCFAs from gut microbiota compositions that were unseen during training (Fig. 5). In studies with humans, inconsistent model performances may be partially attributed to the neglection of stratified train-test split, leading to an undesired consequence that training and test set data are not independent and identically distributed (i.i.d.). Even though data split is stratified based on microbiome characteristics, regression models may still perform poorly in cases when inter-individual variability is too large to fulfill the i.i.d. assumption, the violation of which would cause covariate shift and machine learning to fail. To improve model predictability, large-scale human cohorts are therefore needed to cover the substantial inter-individual variation of human gut microbiome.

# Complex microbiome-metabolome relationship brings additional challenge for predicting SCFAs

Other than covariant shifts, the complex relationship between SCFAs and gut microbiota composition poses an additional challenge for predictive model development. By regressing SCFAs concentration on absolute abundance of microbes, we implicitly assume that SCFAs concentration remains unchanged if gut microbiota has a steady composition. However, this assumption does not always hold and here we provided two pieces of evidence proving that it can be violated. First, Shanghai mice showed delayed changes in total biomass (Fig. 3C) following inulin intervention, but their acetate and butyrate concentrations were neither delayed nor compromised (Fig. 2A). Second, SCFAs were highly produced by gut microbiota that maintains relatively stable composition between day 0 and day 1, regardless of vendors (Fig. S15A). It is likely the mouse gut resembles an *in vitro* culturing system at the beginning of intervention when the microbiome-metabolome relationship follows a rate model, i.e., gut microbiota composition determines the change in SCFAs concentration. This issue is more severe for densely sampled longitudinal data than cross-sectional data as the possibility of sampling very similar microbiota composition at two timepoints far from each other is quite low. Taken together, the decoupling of changes in SCFAs from gut microbiota responses suggests that SCFAs production may be additionally regulated at transcriptional (gene expression) and metabolically (enzyme activity) levels. Our evidence for potential molecular-level regulations justifies the emerging essentiality of integrating transcriptomics and metabolomics data in dietary response analysis.

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**Figure S15**. The quantitative microbiome-metabolome relationship varies in a complex, time-dependent manner. **A**. Dynamics of gut microbiota composition (x-axis) and total SCFA concentration (y-axis) plotted on the same graph. We used the first principal coordinate score from PCoA (principal coordinate analysis) ordination to represent changes in gut microbiota composition (relative abundance) along the direction of maximum variance. Note that SCFAs were substantially produced between day 0 and 1 while gut microbiota composition only changes slightly. Points represent the mean PCoA coordinate score across mice within each vendor and error bars represent the standard error of the mean. **B**. Correlation of baseline unclassified (Un.) Parabacteroides absolute abundance with initial propionate production rates on day 0 (upper panel) and rates in later days (lower panel). Gray line: linear regression.

# Discuss the challenge of finding SCFAs producers

The challenges of predicting SCFAs from gut microbiota composition, as discussed above, brings similar difficulties in robust inference of SCFAs producers. Using three different approaches, we showed that the inferred top producers do not agree with each other. Taking propionate producers as an example. Using the RF model developed in Fig. 5, we ranked propionate producers based on their Gini importance scores and the top three bacterial taxa are Bacteroides acidifaciens, unclassified Alloprevottella and Akkermansia muciniphila (Fig. S12A). The second approach is repeated correlation and we identified Parabacteroides distasonis as the sole significant producer (Fig. S12B), which ranked the 5th in the RF model. The third approach also trained a RF model but different variables and only subset of data were used. As discussed above, SCFAs production may follow a rate model within the first day (Fig. S15A) and we threrefore regressed the first-day changes in propionate concentration on absolute abundances of bacterial taxa. Based on Gini importance, the top three propionate producers are unclassified Parabacteroides, an unknown species from mouse gut metagenome, and Parabacteroides merdae. As shown in Fig. S15B, the absolute abundance of unclassified Parabacteroides showed a linear association (Pearson r: 0.71) with first-day propioinate change, while this association fades away with data after day 1 (Pearson r: 0.21). Despite the results of these three approaches have little overlap, the genus Parabacateroides as known propionate producers seems to regulate the proprionate level in our dataset.

# Limitations in translating knowledge from mice experiments to human

We note two major limitations that may hinder translating the insights provided from our mouse experiments to humans. First, mice have much less inter-individual variability than humans. Since individual mice in our study can be stratified into four distinct microbiota compositions based on vendor sources, the major conclusions we draw were essentially based comparative analysis of dietary responses among the four microbial communities each with several replicates. Whether and how well the four community types correspond to the enterotypes—distinct microbiome configurations—in humans are worth further study. To soften this limitation, we have made efforts to augment the microbiome diversity by reanalyzing public dataset from a similar study. Second, the murine gut microbiota has distinct compositions from humans [39]. Although Bacteroidetes and firmicutes are two major phyla shared between mice and human, 85% genera found in the mouse gut are not detectable in the human gut. For example, the family Muribaculaceae—a major inulin responder inferred by this study—was specific to the mouse gut. Moreover, the differences in microbiota composition between mice and human diversify the gut environment at functional levels, resulting in differential dietary responses of the same microbes between the two ecosystems. For instance, the two human inulin responders—Bifidobacterium and Anaerostipes—were present in the mice gut but their relative abundances remain low and unresponsive to inulin intervention throughout the entire period of observation (Fig. S13B). This example shows that bacteria responding to dietary fiber in one of the two mammalian systems may not consistently respond in the other, suggesting that community ecology may play a selective role in the activation of fiber degraders depending on the surrounding environment.

**Methods**

**Animal experiment.** Specific-pathogen-free (SPF) female C57BL/6J mice for different gut microbial composition were obtained at 6 weeks of age from Beijing (A Charles River Company, Beijing, China), Hunan (Hunan Slac Jingda Laboratory Animal Company, Ltd., Changsha, China), Guangdong (Guangdong Medical Laboratory Animal Center, Foshan, China)), Shanghai (SLAC Laboratory Animal Co., Ltd., Shanghai, China). Mice were maintained on a 12-h light/dark cycle and allowed ad libitum access to food and water throughout the experiment. After acclimatizing to the diet and housing environment for 1 week, mice from each vendor were randomly separated into three groups: cellulose group, resistant starch group, and inulin group (n = 5). Composition of all diets including the source of dietary fibers cellulose, resistant starch, and inulin are provided in supplementary table 1 (Table S7). Fecal pellets from each mouse were freshly collected over multiple time points: day 0 (before diet change), day 1, 3, 5, 8, 13, 19, 25, and 31 (Figure 1A). Fecal samples were snap-frozen in liquid nitrogen and stored at −80 °C until further processing. At every cage change (moving the mice to a new clean cage with fresh bedding twice in one week), body weight was individually measured, and food intake and fecal output of each cage mice during the past three days per cage were measured. This study was performed in accordance with the recommendations of the National Care and Use of Animals Guidelines (China) and approved by the Institutional Animal Care and Use Committee (IACUC) of the Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences.

**GC-MS analysis of fecal SCFA concentration.** The SCFAs were analyzed according to the previous studies with modifications [42]. For the sample extraction, 0.05 g of frozen feces were mixed with 300 µL of pure water containing caproic acid-6,6,6-d3 (CDN Isotopes, Quebec, Canada) as internal standard (IS, final concentration 20 µg/mL). After adding 1.0 mm diameter zirconia/silica beads (BioSpec, Bartlesville, OK), feces were homogenized for 20 s under 6500 rpm for three times, then incubated at 4 °C with shaking for 30 min, followed by centrifugation for 30 min at 13,000×g. Following extraction with anhydrous diethyl ether, the SCFA extract accurately transferred into a glass insert in a GC vial and capped tightly after added 5 µl of N, O-bis(trimethyl-silyl)-trifluoroacetamide and vortexed for 5 s. The mixture was kept in the GC vial and incubated at room temperature (22 °C) overnight (or over 8 h) before loading to GC/MS. The analysis of acetic, propionic and butyric acids was performed by Agilent 8890/7000D triple quadrupole GC/MS equipped with a capillary HP-5 ms capillary column (30 m × 0.25 mm × 0.25 µm film thickness) (Agilent Technologies). The analytes were quantified in the selected ion monitoring (SIM) mode using the target ion and confirmed by confirmative ions. The concentration was determined with reference to the peak side of IS.

**DNA extraction and quantification of bacterial load.** For extraction of DNA, fecal material from pellets was extracted using the QIAmp PowerFecal DNA kit (Qiagen, #12830–50) following standard manufacturer procedures. DNA samples were resuspended in Buffer C6 and quantitated using the Qubit fluorometer (ThermoFisher Scientific). To quantitatively assess bacterial load, total bacteria cell counts were determined using qPCR as described recently [43].

**Amplicon and metagenomic sequencing.** 16S rRNA gene sequencing was performed as previously described with modifications [44]. Library preparation was done using a two-step PCR method. During the first step of PCR, primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 were used to target and amplify the v3-4 region, as well as to add second-step priming sites. Dual index codes were added to each sample at the second PCR step. The PCR products were purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) and quality controlled with TapeStation (Agilent Technologies, Santa Clara, CA, USA). The final DNA concentrations of the purified products were measured with a Qubit 2.0 fluorometer (Thermo Fisher Scientific). The purified products were pooled in equal molar concentrations, and denatured following the Illumina protocol. All sequencing was done in a single run, which was performed with a 250-cycle SP kit on the NovaSeq 6000 following the NovaSeq XP workflow (Illumina, USA). Blank controls (no sample added, processed routinely, n = 4) were included in the extraction process to control for contamination throughout processing.

Metagenomic sequencing was performed using fecal samples from the inulin diet group at day 0, 5 and 31. Extracted DNA sample was purified using silica-based columns. Metagenomics sequencing libraries were prepared with at least 2 μg of total DNA using the Nextera XT DNA sample Prep Kit (Illumina, San Diego, USA) with an equimolar pool of libraries achieved independently based on Qubit 2.0 fluorometer results combined with SYBR Green quantification (Thermo Fisher Scientific, Massachusetts, USA). The indexed libraries were sequenced with a 150-cycle S4 kit on the NovaSeq 6000 following the NovaSeq XP workflow (Illumina, USA).

**Bioinformatic and statistical analysis**. The 16S rRNA sequencing reads were analyzed by QIIME 2-2020.2 software [45]. Demultiplexed paired-end reads were trimmed to remove primers and low-quality bases with q2-cutadapt plugin. The trimmed sequences were denoised and joined with q2-dada2 plugin. Potential reagent contaminants were identified using decontam package based on either the frequency of the ASV in the blank control or the negative correlation with DNA concentration [46]. The generated feature table was filtered to remove ASVs present in only a single sample and remaining ASVs were used to construct a rooted phylogenetic tree via q2-phylogeny. Rarefaction curve analysis of the data obtained was used to estimate the completeness of microbial communities sampling and performed using the iNEXT R package [47]. Subsequently, in order to avoid sample-to-sample bias due to variable sequencing depth (different number of reads per sample), samples were rarefied to 38,980 sequences per sample. Rarefaction analysis showed that great majority of the bacteria species diversity and richness that could be sampled was captured by our sequencing depth (Fig. S18), indicated sufficient sequencing depth for majority of the analyzed samples. Estimated alpha diversity metrics, beta diversity metrics (Aitchison distance [20]) and Principle Coordinate Analysis (PCoA) using q2-diversity. Group significance between alpha and beta diversity indexes was calculated with QIIME2 plugins using the Kruskal–Wallis test and permutational multivariate analysis of variance (PERMANOVA), respectively. To assign taxonomy to the amplicon sequence variants (ASVs), the q2-feature-classifier basing on the classify-sklearn naïve Bayes taxonomy classifier was used with the SILVA (v.138) as reference database.

For metagenome analysis, raw sequencing reads were subjected to quality filtering and barcode trimming using KneadData (v0.5.4) by employing trimmomatic settings of 4-base wide sliding window, with average quality per base >20 and minimum length 90 bp. Reads mapping to the mouse genome were also removed. Kraken2 was run against genome taxonomy database (GTDB\_r89\_54k) with default parameters [47]. Following classification by Kraken2, Bracken was used to re-estimate bacterial abundances at taxonomic levels from species to phylum using a read length parameter of 150. Next, the filtered sequences were assembled into contigs using metaSPAdes with default settings [49]. The gene abundance was analyzed and calculated as previously described with modifications [50]. Putative genes were then predicted on contigs longer than 200 base pairs using Prodigal under metagenome mode (-p meta) [51]. A non-redundant gene catalogue was constructed with CD-HIT using the parameters “-c 0.95 –aS 0.9” [52]. The abundance of each predicted gene was evaluated by mapping reads back with KMA algorithm and then normalized with the following equation: RPM = 1M × (mapped reads/gene length)/(sum of mapped reads/gene length) [53]. For all the predicted genes, CAZymes were annotated using hmmsearch against the dbCAN2 database V9 (e value <1 × 10−10; coverage >0.3) [54]. The domain with the highest coverage was selected for sequences overlapping multiple CAZyme domains. For all samples, short genomic assemblies (<2,000 bp) that could have biased the subsequent analysis were first excluded. Genomes were then binned using VAMB [55]. The binning results were refined based on the bin quality assessment (completeness >75, and contamination <15) of different binners from CheckM [56]. Taxonomic classification of each bin was determined by GTDB-tk [57], and subjected to prediction of polysaccharide utilization loci (PULz) using pipeline PULpy [58].

**Statistical analysis.** R packages *qiime2R*, *ANCOM*, *phyloseq*, *DESeq2*, *RandomForests*, *vegan*, *cluster*, *ggpubr*, *ggtern*, *rstatix*, and *ggplot2* were used for normalization, analyses, and visualization. For normally distributed continuous variables, the mean values were examined using an unpaired Student’s t test or One-way ANOVA with Tukey’s post hoc test. The level of significance was set at *P* < 0.05. Differential CAZY genes and bacterial CAGs between time points were identified using Wilcoxon matched-pair signed-rank tests (two-tailed) followed by FDR corrections. Beta diversity, which illustrates differences in taxonomic diversity between different vendors were examined using recently developed tools that are robust for investigating compositional data: DEICODE [20].

**Harmonic oscillation model.** The dynamic behavior of a damped harmonic oscillator is modeled by the following second-order differential equation

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| --- | --- |
|  | Eq. (1) |

where is the displacement from its equilibrium position in the absence of external force , is the frictional force constant and is the spring constant. Given the initial position (fixed to data) and initial velocity (free parameter), the model can be solved analytically

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| --- | --- |
|  | Eq. (2) |
|  |  |

where and . Parameters () were optimized using modified Powell algorithm with constraints . The best-fit values of and were then used to calculate the damping ratio .

**Ecological inference of dietary fiber responses.** Bayesian regression techniques were used to parameterize the generalized Lotka-Volterra (gLV) model, as similarly used in Morjaria et al. The generalized Lotka-Volterra (gLV) model describes how absolute abundance of bacterial species change over time

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| --- | --- | --- |
|  |  | Eq. (3) |

where is the number of bacterial taxa, is the absolute abundance of taxon () obtained by multiplying its relative abundance by the qPCR value, is the basal growth rate, represents the influence of taxon () on the growth of taxon , is the susceptibility coefficient that represents growth response to any given fiber, is a binary variable that indicates whether the fiber is administed at time . For each mice (), Eq. (1) can be transformed into a matrix form that incorporates all discrete time points of measurements (, )

|  |  |  |
| --- | --- | --- |
|  |  | Eq. (4) |

where and . The log-derivatives of on the left-hand side of Eq. (2) were estimated from a cubic spline interpolation. Using a simplified notation for Eq. (2), i.e., , we can incorpates data from all mice into a single regression model

|  |  |
| --- | --- |
|  | Eq. (5) |

The linear regression as described in Eq. (3) (for brevity ) can be further transformed into a Bayesian regression where and are normal distribution and standard deviation respectively. We used uninformative priors for all gLV parameters and Stan program to produce posterior distributions for each parameter after “no U-turn” sampling of 10,000 samples from at least 3 independent Markov chain Monte Carlo traces. Since Stan is computationally expensive, we limited the inferences of dietary fiber responders to the top 20 bacterial species with the highest absolute abundances.

**Significance test of baseline-dependent responses.** Sequential non-negative matrix factorization was applied to transform all high-dimensional time series data from both intervention (inulin and resistant starch) and control group into two-dimensional space. We chose two factors because (1) reconstructed time series from the two latent factors preserve the quantitative trends of the untransformed time series sufficiently well (Fig. S17) and (2) two-dimensional data can be easily visualized. Suppose the reduced representation for the intervention group and control group are vectors and respectively, where () refers to the index of vendor and ( and ) refers to the index of mouse. For each vendor , both vectors were then standardized by subtracting the mean vector of the vendor in the control group, i.e., and . The significance test of the responsiveness (i.e., whether time series in the intervention group differs from that in the control group regardless of vendor) and baseline dependence (i.e., whether time series in the intervention group varies among vendors ) were performed separately using Permutational Multivariate Analaysis of Variance (PERMANOVA) with Minkowski distance as the distance metric. The Benjamini-Hochberg procedure was used for controlling the false discovery rate in multiple test correction. The same PERMANOVA and muitiple test correction methods were used to test the baseline dependence based on pre-to-post changes (different of absolute abundance between day 0 and day 31).

**Random forest (RF) model.** Model development was run in a pipeline by combining normalization for data transformation, LASSO (least absolute shrinkage and selection operator) for feature selection, and RF regression for data fitting and prediction. The tolerance used in LASSO is 1e-5 and features whose coefficients below this threshold were discarded and not used to build RF regression model. Regarding model training, five hyperparameters were tuned using 5-fold cross validation and R2 as the scoring metric: constant that multiplies the L1 term in LASSO (1e-4, 1e-3, 1e-2, 1e-1, 1), the number of features to consider when looking for the best split in RF (square root, log2, 16%, 32%, 64%, 100% of all features), the maximum depth of the tree in RF (2, 4, 8, 16), the minium number of samples required to split an internal node in RF (2, 4, 8, 16), and the minimum number of samples required to be at a leaf node (1, 2, 4). We fixed the number of trees in RF model to 2,000.

ROC (Receiver Operating Characteristic) curves in Fig. 5D were obtained for RF classifiers that compute the probability of samples in the full datasets (both training and test sets) predicted as being taken from the training distribution. Specificially, we first concatenated the training and test sets and assign labels 1 and 0 respectively. The new dataset was stratified into 20 folds and each time, a RF classifier was trainined on 19 folds and the used to predict the probability of the remaining fold being sampled from the training set. Suppose the probabilities of sample taken from the training and test set are and respectively. The weight assigned to sample was given by , which makes intuitive sense: the higher the numerator and the lower the denominator, the closer the sample gets to the high-density regions of the test data.

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**Figure S16. Rarefaction analysis of 16S rRNA gene clone libraries in terms of species richness, Shannon diversity, and Simpson diversity.** Rarefaction curves were generated using the iNEXT package. Solid lines represent the observed alpha diversity with the number of reads sampled, and dashed lines represent the extrapolation of the solid lines until 25% more reads. To avoid sample-to-sample bias due to variable sequencing depth (different number of reads per sample), all samples were rarefied to 38,980 sequences (black dashed line) per sample before downstream analysis.

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**Figure S17. Reconstructed time series (lines) of (A) five inulin responders and (B) short-chain fatty acids from two-dimensional space, which was constructed by using sequential Non-negative matrix factorization.** Dots represent observations and both lines and dots are color-coded on a per-mouse basis.

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