**Microbial ecology regulates baseline-dependent dietary dynamics of gut microbiota and metabolism**

**Running title: Baseline microbiota modulates prebiotic-mediated ecological dynamics of gut microbiota and SCFA metabolism**

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**Abstract**

Fiber intake indirectly promotes human health via gut microbiome and associated metabolism but the effects are highly personalized. While many factors are possibly at play, the well-documented individuality has been mostly associated with baseline microbiota before dietary intervention. However, little is known about the fiber-induced ecological dynamics of gut community and its baseline dependence, thus precluding in-depth understanding of the end-point response heterogeneity. Here we supplied inulin to isogenic mice that carry four distinct baseline microbiota for four weeks and longitudinally profiled microbiome and short-chain fatty acids (SCFAs) in absolute abundance. Despite the microbiota and SCFAs share consensus two-phase responses—short-term rapid stimulation and long-term partial recovery, we confirmed that the dynamical shifts in the total bacterial load, butyrate and propionate concentrations are indeed baseline-dependent. By combining statistical inference and ecological modeling, we revealed that a subcommunity of seven bacterial responders of inulin, including five putative degraders, almost fully determine the dynamics of microbiota and their relative profiles were largely controlled by baseline abundance and interspecies competition. Despite a challenge in accurate prediction of SCFAs from gut microbiota, we provided statistical evidences that the previously found correlation between bacterial load and propionate concentration may be mediated by these keystone bacteria. Finally, we demonstrated that some major findings from inulin are reproducible and generalizable to resistant starch intervention. Our study emphasizes the importance of ecological network analysis in mechanistic understanding of individualized responses to dietary fiber.

**Keywords:** gut microbiome, dietary fiber, ecological dynamics, short-chain fatty acids, baseline-dependent dependence, generalized Lotka-Volterra

**Introduction**

Fermentable dietary fibers such as inulin and resistant starch are edible carbohydrate polymers that can escape colonic digestions in the small intestine but are fermented by gut microbiota in the large intestine. Human gut microbiome harbors hundreds of trillions of microbes and their associated metabolism that co-evolve with us. The major anaerobic fermentation products of gut bacteria in the large intestine are short-chain fatty acids (SCFAs). SCFAs, mainly acetate, propionate and butyrate, have broad impacts on human host physiology (e.g., stimulate glucagon-like peptide-1 secretion), immune system (e.g., regulate expression of pro-inflammatory cytokines) and intestinal homeostasis (e.g., serve as energy sources for colonocytes) [1]. Converely, impaired SCFAs production has been associated with gut microbiota dysbiosis and a wide range of dieseases [2][3][4]. To increase intestinal levels of SCFAs and eventually improve overall digestive health, some fiber molecules have been considered as “prebiotics” and made commercially available based on the premise that they can selectively enrich beneficial SCFA-producing bacteria. To test this hypothesis, diets supplemented with prebiotics have been administered as a therapeutic strategy to modulate and restore intestinal gut microbiota and SCFA levels in clinical trials.

To date, it has been well established that prebiotics can rapidly alter gut microbiota composition, including both relative abundance of individual bacteria and their total load. However, their efficacy in stimulating SCFAs remains unclear yet. Several clinical trials of healthy adults have collectively revealed that the ability of prebiotic fibers to induce SCFAs production varies among individuals [9-12]. For example, Baxter *et al.* shows that resistant starch was able to promote butyrate production in only 63% participants [10]. Although the individualized response can arise from a combination of factors such as genetics and diet history, there is growing evidence that baseline gut microbiota is a critical factor for explaning the observed heterogeneity at multiple levels [12, 13]. The reason for the inter-individual variation in fiber consumption can be explained by the presence and absence of key fiber-degrading bacteria. One famous example is the degradation of resistant starch by *Ruminococcus brommi* and the impaired ability of healthy human donors’ fecal samples to ferment resistant starch can be *in vitro* restored by their co-incubation with *R. brommi*. The baseline dependence of microbiome and metabolome variations was much less understood, although a few studies have attributed this individuality to the lack of specific keystone species (e.g., SCFAs producers) in the composition of baseline microbiota or the differences in their principle components. The variations in gut multi-omics can further propogate to human host, resulting in heterogenous clinical outcomes such as body mass index and glucose tolerance.

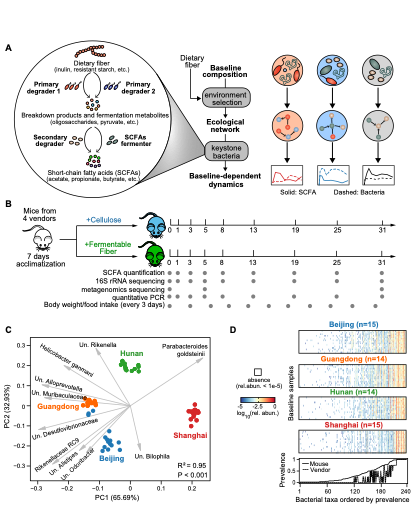
As externally provided substrates, intake of dietary fibers perturbs ecological niche of substrate utilization in the gut and thus selects for specific shifts away from the baseline composition. We propose that the compositional shift in time can be abstracted by the dynamics of a baseline-specific ecological network selected by the infleunces of dietary fibers on bacterial fitness and interactions (Fig. 1A). Several robust characteristics of ecological responses of gut microbiota have been found so far. First, consumption of fibers selectively enriches a fairly limited number of bacteria which rapidly expand and dominate the gut microbiota after substantial induction. In healthy people, *Bifidobacterium* species are one of few bacterial taxa whose abundance consistently increases following intake of diverse fibers. Second, interspecies competitions shape the relative profiles of these key bacteria. By administering multiple fibers to the murine gut, Patnode *et al.* identified competitive inhibition as the ecological mechanism for consistent dominance of *Bacteroides cellulosilyticus* over *Bacteroids vulgatus* even though both species contain fiber-processing polysaccharide utilization loci (PULs). Third, production of SCFAs, especially butyrate, involves cross-feeding cooperations among specialized gut bacteria (Fig. 1A). By hydrolyzing complex polysaccharide fibers, primary degraders release into the gut partially breakdown products (e.g., mono- and oligo-saccharides) and fermentation metabolites (e.g., pyruvate), which can respectively benefit the secondary fiber degraders and SCFAs producers [5, 6, 7, 8]. Despite these invaluable insights, our knowledge about the fiber-induced ecological dyanmics and its dependence on baseline microbiota remains limited.

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n this study, we used age- and gender-matched isogenic mice that harbor distinct baseline gut microbiota composition to study its ecological dynamics in response to dietary interventions and the inter-individual variation within these dynamical trajectories [19]. Murine models, as a complimentary alternative to clinical trials on humans, has better controls over confounding variations between individuals driven by genetic, environmental and other host differences. We monitored temporal shifts in the absolute abundance of gut bacteria and genes (quantitative PCR, 16S rRNA amplicon sequencing, metagenomics sequencing), SCFAs concentration (targeted metabolome) as well as physiological changes following the intervention of two fermentable fibers (inulin, resistant starch from maize) and cellulose (control group) (Fig. 1B). Both inulin and resistant starch are degraded by gut bacteria in the cecum and colon [20, 21], and thus have been harnessed to stimulate the production of SCFAs [10, 22]. Motivated by the hypothesis that individualized dynamics of gut microbiota and SCFAs are primed by the baseline microbial community (Fig. 1A), we developed two computational approaches that employed time series data to analyze the heterogeneity in dietary responses and study its relationships with baseline composition. As the major findings, we showed that both bacterial load and propionate concentration exihibit baseline-dependent responses which can be linked to the baseline abundance of a few responsive and potentially fiber-degrading bacteria. The dynamics of these keystone responders constitute the major shifts in the gut microbiota composition due to their ability of fiber degradation and competitative advantage over other community members, while their relative profiles are controlled by fiber-induced interspecific competitions. Our study indicated that ecological inference of keystone fiber responders from longitudinal data is critical for understanding the differential responses of gut microbiota to dietary fiber perturbations.

**Results**

**Heterogenous baseline gut microbiota composition across different vendors**. Age- and gender-matched isogenic mice were purchased from four commercial vendors (Beijing, Guangdong, Hunan, Shanghai), i.e. independent breeder sources. All mice were fed with cellulose-based diet 7 days prior to dietary fiber intervention. Consistent with previous studies [23, 24], these mice can be naturally divided by vendor sources into groups with distinct microbiota composition. Beta-diversity (between-sample distance) calculated by Aitchison distance shows that the baseline compositions of our mice cluster by vendor (Adonis, *P* < 0.001) and are characterized by distinct bacterial taxa (Fig. 1C, Fig. S1). For example, Shanghai mice have low relative abundances of several commensal polysaccharide-degrading bacteria such as *Muribaculaceae* and *Rikenellaceae* [25, 26]. The profound inter-vendor differences are also noticeable at the level of presence and absence of bacteria: ~65% taxa were entirely absent in at least one vendor and only ~10% bacterial taxa were present in all mice (and thus all vendors) (Fig. 1D). Due to the high between-vendor variation, mice from the same vendor can be effectively considered as independent biological replicates for each baseline microbiota composition.

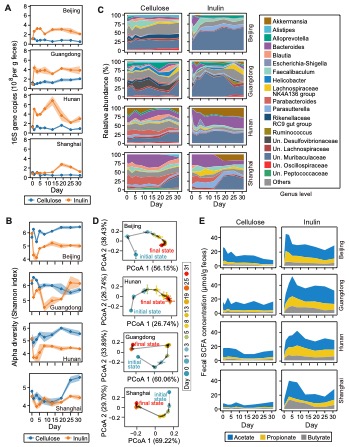


**Figure 1. High-resolution temporal profiling of murine gut microbiota and metabolome to study their individualized responses during dietary fiber intervention.** **A**. An ecological perspective of baseline-dependent dynamical responses of gut microbiota and SCFAs. Administration of dietary fibers alters ecological substrate niche in the gut and selects for an unique ecological network for each baseline microbiota type. Within the network, a few gut bacteria playing key metabolic roles as primary/secondary degraders and SCFAs fermenters drives heterogenous responses of bacteria and SCFAs via diverse ecological interactions (e.g., resource compeitition and cross-feeding). **B**. Experimental design. All mice from the four vendors were continuously fed with either dietary fiber (inulin or resistant starch)- or cellulose-supplemented diets for four weeks. Gray dots indicate the days on which data were collected from fecal samples. **C**. Baseline microbiota composition shown in robust PCoA (principal coordinate analysis) biplot. Isogenic age- and gender-matced mice were purchased from four different vendors (Beijing, Guangdong, Hunan, Shanghai). Gray arrows represent the dominant bacterial taxa in these samples. Adonis analysis was performed to test for differences in baseline gut microbiota composition across the four vendors (P<0.001). Un.: unclassified/uncultured. **D**. Top four panels: pwhite blocks indicate absence and abundance (colored blocks) the samples. Bottom panel: tall ) or ).

**Two-phase dynamics of gut microbiome and SCFA metabolism**. Dietary intake of inulin 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. S2A). Although there were no obvious temporal patterns in the inulin intake (Fig. S2B, C) and fecal weight (Fig. S2D), the fecal weight was dramatically lower in inulin-fed mice, suggesting that intestinal absorption of inulin may cause increased colonic transit time and decreased defecation frequency. Except for Shanghai mice, the inulin absorption nourished gut microbes by rapidly boosting their density to more than 70% of maximum load within 24 hours (Fig. 2A). More interestingly, inulin induced a qualitatively consistent two-phase dynamics of microbiota (Fig. 2B)rapidly in the short-terma in the long-term (i.e., undershoot)CTinitial loss of is due tothe an ecological mechanism caused bygut invasionNotably, the long-term recovery is only partial for Beijing and Hunan mice with much lower gut microbiota diversity at day 31 compared to day 0. Collectively, our dynamics data confirms previous studies on the role of dietary fibers to quickly alter gut microbiota diversity and composition on the time scale of a day [29, 30].

In addition to phase-dependent responses, we also found strong tendency of gut microbiota composition to stabilize under sustained stimulation of inulin (Fig. 2D). Regardless of the baseline microbiota, the steady-state compositions are distinct from their baselines and thus represent new equilibria sustained by inulin intake. Specifically, the gut microbiota in Guangdong mice was highly resilient to inulin intervention and almost returned to its baseline composition 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% (Table S1). 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, the dynamical responses of 61% 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 [31].

By metagenomic sequencing, we observed temporal changes in the functional capacity of gut microbiome, supporting the time-dependent shifts in microbial composition. Specificially, the initial (day 0), short (day 5)- and long (day 31)-term microbiomes have distinct gene family profiles (Fig. S4A) and the s/fructanases for inulin hydrolysisS4B. Consistent with the shift in gene-level functions, we observed a strong stimulating effects of inulin on the metabolism of three major SCFAs and valerate (Fig. 2E, S5). The mean peak-to-baseline concentration ratios of total SCFAs are 3.3, 3.9, 4.5 and 4.2 for Beijing, Guangdong, Hunan and Shanghai mice respectively. Since SCFAs are metabolites produced by colonic bacterial fermentation of inulin, we expect a similar phase-dependent dynamics of fecal SCFAs concentration. Indeed, both total and the three major SCFAs show two temproal phases: their levels peaked in short-term before gradually decreasing until steady states (i.e., overshoot), with an exception of Shanghai mice whose propionate production was notably delayed and compromised. Despite reduced SCFAs in the second phase, the mean concentrations of total SCFAs at day 31 still remain 60%-65% of its peak levels and 2.0-3.5 fold of its baseline levels. We confirmed that the long-term decline in SCFAs was not a result of reduced diet intake as the intake rate remains largely unchanged over time (Fig. S2B).



**Figure 2.** **Inulin-induced temporal shifts in murine gut microbiome and short-chain fatty acids (SCFAs) metabolism. A.** Bload. **B.** Alpha diversity of gut microbiota composition. **C.** Relative abundance of bacterial genera shown in stacked band plot. **D.** Adaptive shifts in gut microbiota composition represented by robust PCoA (principal coordinate analysis) plot. Initial and final states represent the microbiota compositions at day 0 and day 31 respectively. **E.** Fecal concentration of three major SCFAs. Beijing, Guangdong, Hunan, Shanghai are four different mice vendors. Lines (panels A,B), dots (panels A,B,D), and stacked bands (panels C, E) represent the mean values over mouse replicates from the same vendor. Shading areas (panels A,B) and error bars (panel D) represent standard error of the mean. Un.: unclassified/uncultured.

**bacterial load and two major SCFAs** had delayed increase in bacterial load2A and produced low levels of propionate (Fig. 2E) in response to inulinThe distinct behavior of Shanghai mice indicated that the responses of total biomass and SCFAs may depend on the vendor and its associated baseline microbiota. test if they (or any other quantity) exihibit baseline-dependent responsesdeveloped a novel framework that separately tests for the significance of two orthogonal concepts—“baseline dependence” and “responsiveness”—based on the longitudinal data from intervention and control group (see Methods). As shown in a schematic diagram (Fig. 3A), both groups of time series were projected onto a 2-dimensional space by sequential non-negative matrix factorization for capturing representative temporal trends (Fig. S6) and easy visual analytics. Using the coarse-grained data representation, we then obtained two *P*-values by comparing the differential responses between the intervention and control group (“responsiveness”, *Pr*) as well as those between the four vendors in the intervention group (“baseline dependence”, *Pb*). We determined a quantity has a significant baseline-dependent response if both *P*-valus are smaller than 0.05. Using this approach, we confirmed that the temporal responses of bacterial load (Fig. 3B) and propionate concentration (Fig. 3C) are indeed baseline-dependent. We further applied it to time series of acetate and butyrate concentrations: only butyrate shows significant baseline dependence (Fig. 3C).

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**3Quantifying significance of temporal shifts induced by inulinA.**A schematic diagram of our statistical framework to test for the significance of baseline-dependent response. The framework involves two steps: (1) projecting all time series from both intervention and control groups onto the same 2-dimensional space and (2) performing two separate statistical tests using the projected data to quantify the significance of “responsiveness” (*Pr*) and “baseline-dependence” (*Pb*). Abbreviations: Significant (Sig.); dependence (dep.). **B**,**C**. Reduced 2-dimensional representation of the inulin-induced responses in bacterial load (B) and three major SCFAs (C). In all panels, each symbol represents a mouse (dots: cellulose group, crosses: inulin group) and all mice data from the same vendor under the same intervention (inulin or cellulose) was used to fit an eclipse (ellipse’s radius was determined by 2 standard deviations). Beijing, Guangdong, Hunan, Shanghai are four different mice vendors. For each vendor, an arrow was drawn from the eclipse center of the vendor under cellulose intervention (standardized to the origin) to that under the inulin intervention.

**Ecological inference of inulin degraders.** The computational assay we described in the proceeding section provides a statistical way of defining “dietary fiber responders”—*P*-value for the test of “responsiveness” is smaller than 0.05 after multiple test correction. We found a total of 37 bacterial taxa with significant dynamical responses (Table S2) and these responders include bacterial species well known for inulin degradation, such as species of the genus *Bacteroides* and *Bifidobacterium*. However, the ability to ferment fibers, which is testable by *in vitro* growth experiment, is only a sufficient (but not necessary) condition for the *in vivo* selectivity of fiber-induced bacterial expansion, which also requires fitness advantage over other community members for substrate utilization. Since the inference of inulin degraders needs to take bacterial interactions into accounts, we developed a data-driven approach based on the generalized Lotka–Volterra (gLV) equation to identify putative degraders that initialize the utilization of inulin (Fig. 4A, see Methods). The gLV model implicitly assumes that fiber degradation and subsequent utilization boost bacterial growth (the amount of increment is parameterized by ) after controlling for the confounding growth benefits indirectly acquired from other bacteria. To estimate the uncertainty associated with model parameters, we further formulated the gLV-based inference problem in a rigorous Bayesian framework which outputs posterior distributions, rather than point estimates, of these parameters. In this probabilistic framework, any bacteria with a significant positive is considered a candidate of inulin degrader.

The gLV-based ecological model identified five taxonomically heterogenous candidates for inulin degradation (Fig. 4B) , all of which have been previously statistically inferred as the responders. Ranked from highest to lowest confidence, these putative degraders are *Bacteroides acidifaciens* (species), *unclassified Muribaculaceae* (family), *unclassified Faecalibaculum* (genus), *unclassified Parasutterella* (genus), and *unclassified Bacteroides* (genus). Genetic or *in vitro* evidences have been found, except for *unclassified Parasutterella*, to support their functional roles in inulin degradation (Table S3). For example, members from *Bacteroides* and *Muribaculaceae* contain PULs with a *susC/susD* homologous gene pair that facilitates sensing and import of inulin [35, 36]. Putative inulin PULs were also detected in the metagenome-assembled genomes of *B. acidifaciens* and *un. Muribaculaceae* (Table S4). *Akkermancia muciniphila* and *Bacteroides uniformis*—two bacterial species whose safter day 57, S8—were inferred as responders but not degraders (Fig. 4B). Literature search revealed that *B. uniformis*, but not *A. muciniphila* [38], can grow well on inulin *in vitro*. Other than inulin-induced growth, our gLV inference also predicted intense competitions among the five degraders as well as a significant positive interaction from *B. acidifaciens* to *A. muciniphila* (Fig. 4C).

**Inulin responders drive the baseline-dependent dynamics of gut microbiota.** Compared to taxonomic groups, ecological groups (eco-group) made by bacteria that perform similar ecological functions are more fundamental units for microbiome data analysis [39, 40]. In line with the notion, we divided the entire gut microbiota into three eco-groups: (1) the five putative inulin degraders; (2) the 37 inulin responders excluding the degraders (referred as generic responders below); (3) the non-responders. The group-level dynamics in Fig. 4D shows that the inulin degraders as a guild dominated the response of total bioimass in short-term (Guangdong, Hunan), long-term (Shanghai), or even the entire period of study (Beijing). The short-term rise in the absolute abundance of only a few bacteria corresponds to the initial drop of the gut microbiota biodiversity soon after the intervention (Fig. 2B). More interestingly, both Hunan and Shanghai mice showed individualized responses of several inulin responders and the reason of individuality is linked to their baseline levels (Fig. 4D). For example, the abundances of *A. municiphila* and *B. uniformis* increased mostly in Hunan mice (Fig. S8) which also contain highest abundance of the two species in their baseline samples (dark yellow box frames in Fig. 4E). On the other hand, the extremely low baseline abundances of *B. acidifaciens* and *un. Muribaculaceae* in Shanghai mice (violet box frames in Fig. 4E) may explain their own sluggish responses (Fig. S8). Spearman correlation confirms that the averaged absolute abundances of *B. uniformis* (P<0.001), *B. acidifaciens* (P=0.002), and *un. Muribaculaceae* (P=0.051), but not *A. municiphia* (P=0.766), are (marginally) significantly correlated with their baseline levels.

Although inulin stimulated growth of all five inulin degraders, their relative profiles did not remain constant and distinct temporal trends have been found (Fig. 4F, S8). *B. acidifaciens* and *un.* *Facelibaculum* showed transient dynamics with quick rise and drop in their absolute abundances, while the abundance of *un*. *Muribaculaceae* increased steadily and remained high throughout the entire period of study. Compared to the three degraders, *un. Parasutterella* and *un. Bacteroides* have relatively low abundances and showed complex patterns. Since the total biomass reached local maximum rapidly in less than 3 days (Fig. 2A, except for Shanghai mice), these degraders may compete for inulin soon after the gut microbiota has reached its temporary carrying capacity. The competition for inulin among the degraders was supported by the gLV-based inference of pairwise interactions, where *un.* *Muribaculaceae* negatively impacts growth of *B. acidifaciens* and *un.* *Facaelibaculum* (Fig. 4C).

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**Figure 4. Inulin** **responders shape gut microbiota dynamics in a baseline-depednent manner. A.** Generalized Lotka-Volterra (gLV) model combined with Bayesian statistics to infer inulin degraders and pairwise interactions. 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 dietary fiber (). A primary degrader is determined when 95% credible interval of the posterior distribution of is completely to the right of 0. **B**. Posterior distribution of for five primary degraders (violet) and two generic responders (dark yellow). Generic responders are those bacteria showing statistical significance of inulin-induced response (i.e., responders) but not inferred as primary degraders. Bacterial taxa are ranked according to their posterior mean of . **C**. Core ecological interaction network composed of six bacterial taxa shown in the panel B. Point and blunt arrows represent positive and negative interactions respectively. The arrow thickness is proportional to the posterior mean of the corresponding interaction coefficient. **D**. Ecological group dynamics of primary inulin degraders, generic responders (presented with two subgroups) and non-responders. **E**. Mean absolute baseline abundance of the seven bacterial taxa shown in the panel B. **F**. Temporal changes in the absolute abundance of the top three inulin degraders. In panels D, F, lines and dots represent the mean values across mice from the same vendor (Beijing, Guangdong, Hunan, Shanghai are four different mice vendors) and shading areas represent the standard error of the mean. Un: unclassified/uncultured.

**Inulin responders mediate the baseline-dependent propionate production and its correlation with bacterial load.** We have shown above that the dynamics of propionate and butyrate are baseline-dependent and varied substantially across vendors. Particularly, Shanghai mice produced low levels of propionate, which motivated us to explore whether the compromised propionate production is linked to the low bacterial load of their gut microbiota. Positive correlations between the bacterial load and the three major SCFAs have been reported in a previous study that administered resistant starch to obese males. However, a strong positive correlation was only found for propionate (P<0.001), but not acetate (P=0.047) or butyrate (P=0.190), using our data (Fig. 5A, right panel). Since the response of bacterial load to inulin mainly involves the changes in the abundance of 7 inulin responders (5 inulin degraders plus *A. municiphila* and *B. uniformis*), it is likely that the baseline levels of some of these responders are hidden factors that play simultaneous roles as inulin responders and propionate producers, thus leading to a robust correlation between the two. Supporting this hypothesis, the baseline abundances of four inulin responders—*B. acidifaciens*, *un. Muribaculaceae*, *A. municiphila*, *B. uniformis*—are all significantly associated with the averaged bacterial load (Fig. 5A, top left) and propionate concentration (Fig. 5A, bottom left). According to the literature, all four bacteria, except for *B. acidifaciens*, are capable of producing propionate *in vitro* and/or *in vivo* (Table S3).

The correlations of propionate concentration with bacterial load and inulin responders indicate that the SCFAs concentration may be predictable from gut microbiota composition. We evaluated the performance of machine learning models to predict the fecal SCFA concentrations using absolute abundance of all bacterial taxa as predictors (Fig. 5B). All mice were split into training and test sets using two data-split approaches (Fig. 5B). The “interpolation” strategy generated balanced distribution of baseline microbiota composition between the training and test sets (Fig. S9A) 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 (Fig. S9B) by randomly selecting all mice from a vendor as test data and leaving the other vendors for training. Despite it fits the training data generally well (R2 ≥ 0.66 regardless of SCFAs and data-split strategy), a Random Forest (RF) regression model generalizes poorly to the test data: the R2 scores varied among SCFAs in a range between 0.1 and 0.45 when test data were intrapolated but dropped below 0 for the “extrapolation” scenario (meaning that these RF models generalized worse than training data average). low predictability of the substantially or adding weights to training samples (Fig. 10C).

**A screenshot of a video game

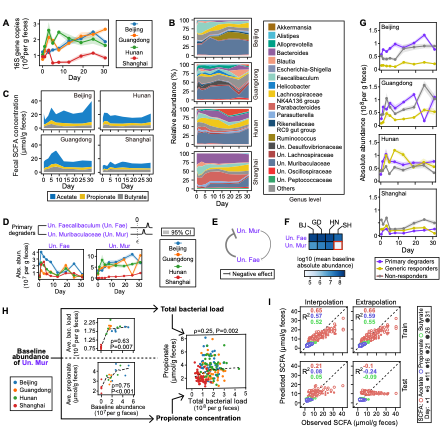
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**Figure 5.** **Short-chain fatty acids (SCFAs) are statistically linked to gut microbiota composition but with low predictability.** **A.** Correlation between bacterial load and propionate concentration (right big panel). We proposed that the correlation is mediated by some inulin responders which causally and simultaenoulsy affect both observations. Eight small panels to the left: correlations of baseline abundance of four inulin responders with the mean bacterial load (top row) or propionate concentration (bottome row) averaged across the interveion period. Beijing, Guangdong, Hunan, Shanghai are four different mice vendors. Dashed line: Lowess regression. Spearman correlation coefficient (ρ) and adjusted P-value are indicated in each plot. **B**. Prediction of SCFAs concentrations from gut microbiota composition using machine learning models. Two data-split strategies for testing model performance were designed: 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. **C**.Performances of Random Forest regression models on the training and test datasets.

**Our major findings are reproducible and generalizable.** degraderphase-dependent gut microbiota 11 Although quantitative microbiota compositions are unavailable, weapplied gLV-basedir profilesand *.* again s a putative inulin degrader. Treproducibility suggests that the rapid enrichement of *B. acidifaciens* is a hallmark of inulin-induced response of murine gut microbiota.

To further test how well our findings can be generalized to other dietary fibers, we administered resistant starch to mice from the same four vendors following the same experimental procedure. Compared to inulin, resistant starch stimulated much less and milder changes in the bacterial load (Fig. 6A), gut microbiota composition (Fig. 6B), and SCFAs production (Fig. 6C). Despite the weak signals, 27 bacterial taxa were determined as responders (Table S2), among which *un. Faecalibaculum* and *un. Muribaculaceae*—two putative inulin degraders—were also inferred as degraders of resistant starch (Fig. 6D). Genetic evidences from the literature supports their functional role (Table S3). Moreover, the dynamical patterns of both degraders are also preserved between inulin and resistant starch interventions (Fig. 6D): the absolute abundance of *un. Muribaculaceae* increased rapidly and soon reached a plateau (except for Shanghai mice), while that of *un. Faecalibaculum* quickly declined after an initial burst. GLV-based inference suggests that the observed dynamics may be driven by mutual inhibitions (Fig. 6E).

Similar to inulin intervention, bacterial load (Fig. S12A) and all three major SCFAs (Fig. S12B) exihibited baseline-dependent responses to resistant starch intervention. Particularly, the low bacterial load in Shanghai mice (Fig. 6A) can be linked to the low abundance of *un. Muribaculaceae* in their baseline communities (Fig. 6F, highlighted in red box frame). Consistent with inulin-induced changes, there is substantial growth of generic responders in Hunan mice (Fig. 6G), although the dominant bacterial taxa in this eco-group are no longer *A. muciniphila* and *B. uniformis*. Furthermore, we show weaker but still significant (P=0.002) positive association between bacterial load and propionate concentaiton (Fig. 6H, right panel) and both are significantly associated with the baseline abundance of *un. Muribaculaceae* (Fig. 6H, left panel). Finally, Random Forest models revealed that gut microbiota compostion is generally not predictive of SCFAs concentration (Fig. 6I). Collectively, our major findings related to fiber degraders, including their identity, dynamical trends, as well as relationships with bacterial load and propionate concentration, are qualitatively consistant between inulin and resistant starch interventions, suggesting that the gut ecosystem may respond to fiber-based perturbation by following universal microecological rules.

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**Figure 6. Longitudinal measurement and analysis of resistant starch-induced shifts in gut microbiota and short-chain fatty acids (SCFAs). A-C.** Dynamical responses of bacterial load (A), gut microbiota composition (B), and SCFAs concentration (C) following resistant starch intervention. **D**.Dynamics of two putative resistant starch degraders. represents the growth impact of resistant starch and its posterior distributions are shown for each degrader. CI: credible interval. **E**. Ecological interactions between the two degraders. The arrow thickness is proportional to the posterior mean of the corresponding interaction coefficient. **F**. Mean baseline abundances of the two degraders. **G**. Ecological group dynamics of primary resistant starch degraders, generic responders (resistant starch responders excluding the two degraders), and non-responders. **H**. Correlations among baseline abundance of *un. Muribaculaceae*, bacterial load and propionate concentration. The averaged responses were calculated as the ratio of the area under the curve to the length of observation. Dashed lines: Lowess regression. **I**. Prediction of SCFAs concentration from gut microbiota compsotion using a random forest regression model. “Interpolation” and “extrapolation” are two strategies of splitting all data into the training and test sets (see Fig. 5B for details). In all panels, Beijing (BJ), Guangdong (GD), Hunan (HN), Shanghai (SH) are four mice vendors. Lines (panels A,D,G) or height of stacked bands represent mean values across mice from the same vendor and shading areas (panels A,D,G) represent the standard error of the mean. Un.: unclassified/uncultured.

**Discussion**

# Emphasize the importance of ecological network analysis

Our study emphasizes that ecological network analysis is paramount to understanding the dietary responses of gut microbiota and metabolism. By integrating gLV model with Bayesian regression, we inferred a competitive network of fiber degraders as keystone bacteria that mediate the response of gut microbiota to inulin and resistant starch intervention. GLV-based ecological inference from gut microbiome time series data has yielded mechanistic insights into the stability of probiotic community under dietary perturbation, colonization resistance of pathogenic *Clostridioides difficile*, and community assembly dynamics within preterm infant gut. Besides evidences supporting the fiber-degrading function of these putative degraders, our findings correspond to and advance previous literature at multiple systems level understanding of the effects of dietrary changes. First, the small number of fiber degraders (five for inulin and two for resistant starch) suggested that fiber-induced bacterial shifts are very selective and occur to a restricted number of taxa. Second, the absolute abudance of many fiber-degrading bacteria such as taxa related to the genus *Bifidobacterium*, failed to expand in the mouse gut on both fibers (Fig. S13), indicating that fiber-induced bacterial enrichement cannot be simply predicted from their *in vitro* growth. Third, our results revelaed that fiber-induced dynamics are largely driven by competitions and *un. Muribaculaceae* outcompetes other degraders in consuming both fibers. Since the family *Muribaculaceae* was specific to the mouse gut [74], it might have been adapted to the murine gut with higher fitness in utilizing fiber. Fourth, we offered new insights into the fiber-induced shift in propionate production by linking its inter-individual variability with baseline abundance of *un. Muribaculaceae*. We propose that bacteria from this family can also produce propionate, thus correlating bacterial load and propionate concentration in the gut during dietary fiber intake.

# Discuss the relevance of two-phase SCFAs dynamics to the literature

Diet-induced changes in SCFAs are often transient and vanish shortly after cessation of dietary intervention [52-55]. 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 four weeks. The transient responses under sustained dietary fiber intake were also observed in colorectal cancer patients [56]. 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. The *in vivo* SCFAs dynamics is jointly determined by multiple metabolic processes, where the two major ones are microbial production and host absorption. In healthy individuals, 90%-95% SCFAs produced in the colonic lumen are absorbed by the gut mucosa [58]. While many studies used fecal SCFAs concentrations as proxy of their luminal levels, neither of both represents the rate of production or absorption so the declined phase of SCFAs in our study may be explained by reduced production rate, increased absorption rate or both. Due to the difficulty of measuring fluxes *in vivo*, mathematical models that take both processes into accounts show great premise in the estimation of their flux rates from SCFAs concentrations.

# Discuss the advantages of our approach for quantifying baseline-dependent response

Quantifying the association of an observable quantity with baseline microbiota composition is a critical step in individualized response analysis of dietary fiber intervention. In previous studies, the statistical test of the association was mostly performed between the pre-to-post changes in the quantity during intervention and the relative abundance of all or selected taxa in the baseline samples. There are two main potential caveats to this conventional approach. First, the significance of association may vary depending on the study endpoint used to calculate pre-to-post changes. In our experiments, the changes in propionate concentration from their baseline levels differ significantly among the four vendors at day 5 but not at day 31 (Fig. S14). Second, due to the lack of control group data to assess the intervention effects, pre-to-post changes that are supposedly to capture fiber-indcued effects may be entirely attributable to random variations within each individual [43]. By constrast, our new approach avoids these two caveats by incorporating longtitudinal and comparator arm data. Additionally, the use of dimensionality reduction in our approach further benefits visualization of inter-vendor variations in gut microbiota composition (Fig. 3B,C). For mice from the same vendor, the mean response difference between the intervention and control group is represented by an arrow in a vendor-specific color. Therefore, any differences in the magnitude and direction of these vendor-specific arrows indicate baseline effects.

# Discuss the major reasons 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 [44-46]. For example, the MelonnPan algorithm based on linear regression accurately predicted relative abundances of >50% metabolites [45], while RF regression can only explain 14% of the observed variation in the SCFAs concentration [46]. While the possibility of using different regression models and patient cohorts between these controversal studies cannot be excluded, our study points out that the dissimilarity of gut microbiota composition between training and test sets, which is formally known as covariate shift [48], may contribute to the disagreement. Due to strong inter-individual variations of human gut microbiome, it seems to be difficult to sufficiently balance train and test subsets especially when the sample size is small, calling for the need to examine the presence of distribution shift before model traning. Other than covariant shifts, the complex relationship between SCFAs and gut microbiota also challenges predictive model development. By regressing SCFAs concentration on bacterial abundances, we implicitly assume that SCFAs level remains unchanged if gut microbiota has a steady bacterial load and composition. However, this assumption can be violated. For example, Shanghai mice showed delayed changes in bacterial load to inulin (Fig. 2A) but the acetate and butyrate concentrations in these mice were neither delayed nor compromised (Fig. 2E). Additionally, SCFAs were highly produced within the first 24 hours, while gut microbiota maintains relatively stable composition regardless of vendors (Fig. S15). 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 [65], i.e., gut microbiota composition determines the change in SCFAs concentration. This is consistent with previous human studies showing that short‐term diet interventions could rapidly and significantly alter gut microbitome gene expression without changing the community structure [54, 66, 67]. Our evidence for potential molecular-level regulations justifies the emerging essentiality of integrating transcriptomics and metabolomics data in dietary response analysis.

# Perspectives on how our findings can facilitate microbiome engineering

Characterizing dynamics of gut microbiota and their inter-individual variability with multi-omics data is an important priority for microbiome research to further understanding of diet-induced responses [51]. Such studies thus hold great premise to improve human health and treat gut microbiome-associated disease via microbiome engineering. Despite the murine gut microbiota has distinct compositions from humans [72], our gLV-based inference assay and the derived ecological insights can be potentially translated to facilitate human-based research. A key question in microbiome engineering with prebiotics is whether and to what extent a beneficial bacterial population can be selectively induced by a prebiotic compound. Microbiome engineering, as with other engineering disciplines, requires computational tools to aid the design process. Prediction of bacterial growth in human gut is nontrivial: We and other researches have repeatedly shown that bacteria able to consume a fiber supplement *in vitro* may not be selectively enriched *in vivo*, suggesting that dietary response of an organism depends on its ecological traits, i.e., the organism’s ability to compete, occupy and defend for the newly opened niche. Similar to the concept of registry of standard parts in synthetic biology, a standard registry of traits detailing the characterization of their biological mechanisms and ecological consequences constitutes a major step towards predictable dietary responses and ultimately personalized nutrition. By inventing a new application of gLV with uncertainty assessment to infer primary fiber degraders and associated interaction network, we provided a generalizable computational approach that facilitate ecological characterization of gut microbial community from human longitudinal data. To improve the robustness of ecological characterization, large-scale human cohorts with dense longitudinal sampling are needed to cover the substantial inter-individual variation of human gut microbiome.

**Methods**

**Animal experiments.** Specific-pathogen-free (SPF) female C57BL/6J mice were obtained at 6 weeks of age from four different vendors, including 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)) and Shanghai (SLAC Laboratory Animal Co., Ltd., Shanghai, China). Mice were maintained in 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 (n = 5), resistant starch group (n = 5), 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 S5). 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 approved by the Institutional Animal Care and Use Committee (IACUC) of the Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences.

**Quantification of fecal SCFA concentration by GC-MS.** The SCFAs of mice fecal samples were analyzed by GC-MS [75]. 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.** DNA of mice fecal samples 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 density were determined using qPCR as described recently [76]. The absolute abundance of a bacterial taxon was estimated by multiplication of its relative abundance and the total bacterial load.

**16S rRNA amplicon sequencing and shotgun metagenomic sequencing.** 16S rRNA gene sequencing was performed as previously described with modifications [77]. Library preparation was done using a two-step PCR method. During the first step of PCR, primers S-D-Bact-0341-b-S-17 (forward) and S-D-Bact-0785-a-A-21 (reverse) were used to target and amplify the v3-4 region [78], 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 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).

**Bioinformatics analysis**. The 16S rRNA sequencing reads were analyzed by QIIME 2-2020.2 software [79]. 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 [80]. 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 [81]. 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. S16), indicated sufficient sequencing depth for majority of the analyzed samples. Estimated alpha diversity metrics, beta diversity metrics (Aitchison distance [27]) 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. Unless specified, ASVs are grouped to the lowest classified taxonomy level for further analysis.

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 removed. Kraken2 was run against genome taxonomy database (GTDB\_r89\_54k) with default parameters [82]. 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 [83]. The gene abundance was analyzed and calculated as previously described with modifications [84]. Putative genes were then predicted on contigs longer than 200 base pairs using Prodigal under metagenome mode (-p meta) [85]. A non-redundant gene catalogue was constructed with CD-HIT using the parameters “-c 0.95 –aS 0.9” [86]. 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) [87]. For all the predicted genes, CAZymes were annotated using hmmsearch against the dbCAN2 database V9 (e value <1 × 10−10; coverage >0.3) [88]. 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 [89]. The binning results were refined based on the bin quality assessment (completeness >75, and contamination <15) of different binners from CheckM [90]. Taxonomic classification of each bin was determined by GTDB-tk [91], and subjected to prediction of polysaccharide utilization loci (PULz) using pipeline PULpy [92].

**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

|  |  |
| --- | --- |
|  | 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.** The generalized Lotka-Volterra (gLV) model describes how the absolute abundance of bacterial species change over time

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

where is the number of bacterial taxa, is the absolute abundance of taxon () , 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 . Bayesian regression techniques were used to parameterize the generalized Lotka-Volterra (gLV) model, as similarly used in Morjaria et al [93]. For each mice (), Eq. (1) can be transformed into a matrix form that incorporates all discrete time points of measurements (, )

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| --- | --- | --- |
|  |  | 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.

Since gLV models the absolute abundance of bacterial taxa, we multiplied the bacterial load by their relative abundance to calculate absolute abundance. The time-series data from all mice were simultaneously fed into the gLV model based on the premise that ecological forces driving microbiome dynamics are largely host-independent and universal [34]. We used uninformative priors for all gLV parameters and Stan program [94] 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 [95] 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 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. For all significance tests that require multiple test correction, the Benjamini-Hochberg procedure [96] was used for controlling the false discovery rate in multiple test correction.

**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.

**Data availability**

Sequencing data, desposit to SRA

SCFA and other data: supplementary tables

**Code availability**

All scripts will be available on Github.

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