Predicting the spatially differential gut microbiota composition using genome-scale metabolic modeling

Supplementary Methods

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Data and parameters used

Genome-scale metabolic models

The community metabolic model used consisted of the previously published genome-scale metabolic models listed in Table S1.

Table S1. Organisms in the community model

Organism	Phylum	Ref.
Bacteroides thetaiotaomicron (*B. thetaiotaomicron*)	Bacteroidetes	[1,2]
Eubacterium rectale (*E. rectale*)	Firmicutes	[3]
Faecalibacterium prausnitzii (*F. prausnitzii*)	Firmicutes	[4]
Escherichia coli (*E. coli*)	Proteobacteria	[5]
Corynebacterium glutamicum (*C. glutamicum*)	Actinobacteria	[6]

Diet

Nutrients from a chow diet (7012, Teklad LM-485) for mice that are available to the gut microbes were estimated (see the supplementary file SI_chow_diet.xlsx).

Mucosal biomass density

The mucosal biomass density X_{muc}^{total} for each section of the intestines was estimated by multiplying the average number of cells per gram from the experimental data in ref. [7] by 10^{-13} gdw per cell, which is based on the weight of an *E. coli* cell growing at 100 min doubling time (http://book.bionumbers.org/ how-big-is-an-e-coli-cell-and-what-is-its-mass/). See Table S2 for the estimated values (the default row) and other values tested in the sensitivity analysis.

Table S2. Sets of values tested for the mucosal biomass density X_{muc}^{total}.

X_{muc}^{total} (gdw g^{-1})	SI.P	SI.M	SI.D	Cecum	LI.P	LI.M	LI.D
Default	6.75e-6	2.7e-6	1.5e-5	1.95e-4	5.25e-4	7.5e-6	9e-6
Test 1	1e-6	1e-6	1e-6	1e-6	1e-6	1e-6	1e-6
Test 2	1e-5	1e-5	1e-5	1e-5	1e-5	1e-5	1e-5
Test 3	1e-4	1e-4	1e-4	1e-4	1e-4	1e-4	1e-4

Bowel transit time

Table S3 shows the transit time in mice for each section of the intestines estimated from ref. [8].

Table S3. Estimated transit time.

Transit time (h)	SI.P	SI.M	SI.D	Cecum	LI.P	LI.M	LI.D
	2	2	2	3	3	3	3

Total oxygen available

From the experimental data, the oxygen uptake rate by aerobes is given as 2666.67 molecules per cell per sec
This was converted to mmol $gdw^{-1}h^{-1}$ by:

2666.67 / (6.022×10^{20} molecules/mmol) / (10^{-13} gdw/cell) x (3600 sec/h) = 0.16 mmol $gdw^{-1}h^{-1}$

This value is used to estimate the maximum level of oxygen flux that the gut microbiota can have. The geometric mean of the mucosal biomass density is on the order of 10^{-5} gdw/gram material. A large portion of oxygen is expected to be consumed by the mucosal microbiota, so the total oxygen flux available to the gut microbiota r_{oxygen}^{total} was estimated to be 0.16×10^{-5} mmol h^{-1}(gram material)$^{-1}$. Note that both the mucosal biomass density and the oxygen available were converted using the same factor of the dry weight of one cell. The relative magnitude $r_{oxygen}^{total}/X_{muc}^{total}$, i.e. the maximum oxygen uptake per unit of mucosal biomass, is therefore independent of the conversion factor for cell dry weight. Five sets of values around the estimated value for this parameter were tested (Table S4):

Default: The estimated average, constant in each section of the intestines;
Test 1: Half of the estimated average, constant;
Test 2: Double of the estimated average, constant;
Test 3: The estimated average steadily decreased by 5% (from 100% to 70%), from the proximal small intestine to the distal large intestine, and;
Test 4: The estimated average steadily decreased by 10% (from 100% to 40%)

Table S4. Sets of values tested for the total oxygen available r_{oxygen}^{total}.

r_{oxygen}^{total} (mmol $h^{-1}g^{-1}$)	SI.P	SI.M	SI.D	Cecum	LI.P	LI.M	LI.D
Default	1.6e-6	1.6e-6	1.6e-6	1.6e-6	1.6e-6	1.6e-6	1.6e-6
Test 1	0.8e-6	0.8e-6	0.8e-6	0.8e-6	0.8e-6	0.8e-6	0.8e-6
Test 2	3.2e-6	3.2e-6	3.2e-6	3.2e-6	3.2e-6	3.2e-6	3.2e-6
Test 3	1.6e-6	1.52e-6	1.44e-6	1.36e-6	1.28e-6	1.2e-6	1.12e-6
Test 4	1.6e-6	1.44e-6	1.28e-6	1.12e-6	0.96e-6	0.8e-6	0.64e-6

Specific oxygen uptake rate by aerobes

While 0.16 mmol $gdw^{-1}h^{-1}$ was used to estimate a level for the oxygen available to the microbiota. This value was not used to directly constrain the maximum specific oxygen uptake rate (OUR) by individual aerobes, i.e. LB_{OUR}^{k} in the model. The OUR should vary with the oxygen concentration as well as the microbial growth rate, so without specific OURs measured experimentally in a similar environment we have left this value to be chosen by the model by only constraining a physiologically feasible bound. For adaptively evolved *E. coli* in the laboratory, the OUR ranges from 10 to 20 mmol $gdw^{-1}h^{-1}$ at growth rates ranging from 0.5 to 0.9 h^{-1} [9]. For *C. glutamicum*, the OUR ranges from 5 to 10 mmol $gdw^{-1}h^{-1}$ for growth rates ranging from 0.2 to 0.5 h^{-1}[10,11]. These data were obtained under laboratory conditions with aeration and agitation. Considering (1) the slower growth rate (0.02 to 0.2 h^{-1}) for gut microbes [12], (2) the near-four-fold decrease in oxygen partial pressure, i.e. around 150 mmHg for air under atmospheric pressure compared to the maximum of 40 mmHg at the intestinal mucosal wall [13], and (3) the far less intense agitation in the intestines, the maximum possible OURs for aerobes, LB_{OUR}^{k}, in the model were defaulted at 2 mmol $gdw^{-1}h^{-1}$. The parameters were also varied from 1, 2, 5, 10 to 20 mmol $gdw^{-1}h^{-1}$.

Oxygen available to the mucosal and luminal microbiota

Another parameter regarding oxygen is the fraction of the total oxygen available to luminal microbiota α_{oxygen}^{lum}. The oxygen available to the luminal microbiota is equal to $r_{oxygen}^{total}\alpha_{oxygen}^{lum}$ and the oxygen available to the mucosal microbiota is $r_{oxygen}^{total}\left(1-\alpha_{oxygen}^{lum}\right)$. The default set of values in Table S5 is a rough estimate on the change in surface area and radius between the different sections of the intestine (from ref. [7] and personal communications). A larger proportion of oxygen is taken up by the luminal microbiota in the small intestines compared to the large intestines because of the smaller radius and larger surface area inherent in the small intestines. The cecum has particularly low amount of oxygen diffused into the lumen because of the larger radius.

Table S5. Sets of values tested for the fraction of oxygen available to the luminal microbiota α_{oxygen}^{lum}.

α_{oxygen}^{lum}	SI.P	SI.M	SI.D	Cecum	LI.P	LI.M	LI.D
Default	0.2	0.15	0.15	0.01	0.05	0.05	0
Test 1	0.4	0.3	0.3	0.05	0.1	0.1	0
Test 2	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Test 3	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Test 4	0	0	0	0	0	0	0

Steady-state mucosal microbiota

In general, the change in the biomass of organism *k* in the mucosal microbiota, X_{muc}^k, can be described by:

$$\frac{dX_{muc}^k}{dt} = r_{muc}^k + r_{lum}^k - D^k X_{muc}^k , \qquad \forall k \in \mathbf{K} \qquad \text{(A1)}$$

where r_{muc}^k is the rate of increase of the mucosal biomass due to the growth , r_{lum}^k is the rate of increase of the mucsoal due to the attachment of luminal biomass, and D^k is the dilution rate of the biomass due to flushing to luminal contents and epithelial cell shedding, etc. For stabilized steady-state mucosal microbiota, we assume that the attachment of luminal biomass to the mucosa is negligible:

$$r_{lum}^k \approx 0 , \qquad \forall k \in \mathbf{K}$$

Also, the total biomass is constant over time, denoted by X_{muc}^{total}, and the net change in the total mucosal biomass is therefore zero in average:

$$\sum_{k\in\mathbf{K}} X_{muc}^k = X_{muc}^{total} \qquad \text{(A2)}$$

$$\frac{d}{dt}\sum_{k\in\mathbf{K}} X_{muc}^k = \sum_{k\in\mathbf{K}} r_{muc}^k - \sum_{k\in\mathbf{K}} D^k X_{muc}^k = 0$$

$$\Rightarrow \sum_{k\in\mathbf{K}} r_{muc}^k = \sum_{k\in\mathbf{K}} D^k X_{muc}^k \qquad \text{(A3)}$$

Under this condition, the 'vacancy' in the mucosal microbiota due to flushing or shedding is 'filled up' according to r_{muc}^k, the increase due to the growth of mucosal biomass that remains on the mucosa. Assume that r_{muc}^k is proportional to the produced biomass of organism *k*, i.e.

$$r_{muc}^k = C\mu_{muc}^k X_{muc}^k$$

where *C* is a constant of proportionality and μ_{muc}^k is the growth rate of organism *k* in the mucosal microbiota. This implies that an organism with a higher overall growth rate and abundance can retain more biomass in the mucosa. To be consistent with equation (A3):

$$C\sum_{k\in\mathbf{K}} \mu_{muc}^k X_{muc}^k = \sum_{k\in\mathbf{K}} D^k X_{muc}^k$$

$$\Rightarrow r_{muc}^k = \mu_{muc}^k X_{muc}^k \frac{\sum_{q\in\mathbf{K}} D^q X_{muc}^q}{\sum_{q\in\mathbf{K}} \mu_{muc}^q X_{muc}^q}$$

$$= \text{produced biomass of } k \times \frac{\text{total biomass shed into the lumen}}{\text{total produced biomass}}$$

Note that $\sum_{q\in\mathbf{K}} D^q X^q_{muc} \leq \sum_{q\in\mathbf{K}} \mu^q_{muc} X^q_{muc}$ to ensure that the mucosal microbiota is not washed away over time. Therefore persistently faster-growing organisms will increase in their abundances over time (because of higher μ^k_{muc}). The change in the mucosal biomass of organism *k* is now given by:

$$\frac{dX^k_{muc}}{dt} = \left(\mu^k_{muc} X^k_{muc} \frac{\sum_{q\in\mathbf{K}} D^q X^q_{muc}}{\sum_{q\in\mathbf{K}} \mu^q_{muc} X^q_{muc}}\right) - D^k X^k_{muc}$$

For a steady-state mucosal microbiota, we have:

$$\mu^k_{muc} = D^k \frac{\sum_{q\in\mathbf{K}} \mu^q_{muc} X^q_{muc}}{\sum_{q\in\mathbf{K}} D^q X^q_{muc}}, \quad \forall k \in \mathbf{K}$$

Therefore the growth rate is proportional to the dilution rate for each organism and co-growth of all mucosal microbes is ensured as long as they are subject to dilution. In the absence of organism-specific dilution rates, assume the unbiased uniform case:

$$D^k = D, \quad \forall k \in \mathbf{K}$$

Then we have:

$$\mu^k_{muc} = \sum_{q\in\mathbf{K}} \left(\frac{X^q_{muc}}{X^{total}_{muc}}\right) \mu^q_{muc}, \quad \forall k \in \mathbf{K}$$

This means that all organisms have their growth rates equal to the average growth rate of the community, thus identical. Otherwise if there is an organism on the mucosa persistently growing at a higher rate over time than all other members, it will colonize more rapidly the 'vacancy' created by the shedding and the steady-state is violated. This condition of identical growth rate applies not to every time point but an average over time. Therefore, fast-growing organisms may grow quickly over a period of time, however they will become nutrient limited allowing for other microbes to catch-up. Note also that at steady-state, the rate of shedding of the mucosal biomass of organism *k* into the lumen is equal to the biomass production rate $\mu^k_{muc} X^k_{muc}$. Figure A1 visualizes four different cases. Figure S1A shows the steady-state as a result of identical dilution rate and growth rate. Figure S1D shows a case of steady-state as a result of the growth rate of each organism proportional to its organism-specific dilution rate. Figure S1B – S1C shows the corresponding cases of non-steady-state caused by unequal growth rates and unproportional growth rates respectively.

Note that this effect of steady-state stabilization of the growth rates in response to the dilution rates can be a long-term effect (months to years). Therefore even small dilution rates compared to the growth rates of the mucosal microbes can alter the steady-state mucosal microbiota with correlated growth rates of microbes in the long run. This hypothesis of a steady-state mucosal microbiota along with the

proposed model dynamics is consistent with the observation that the gut microbiome is in general stable over time [14].

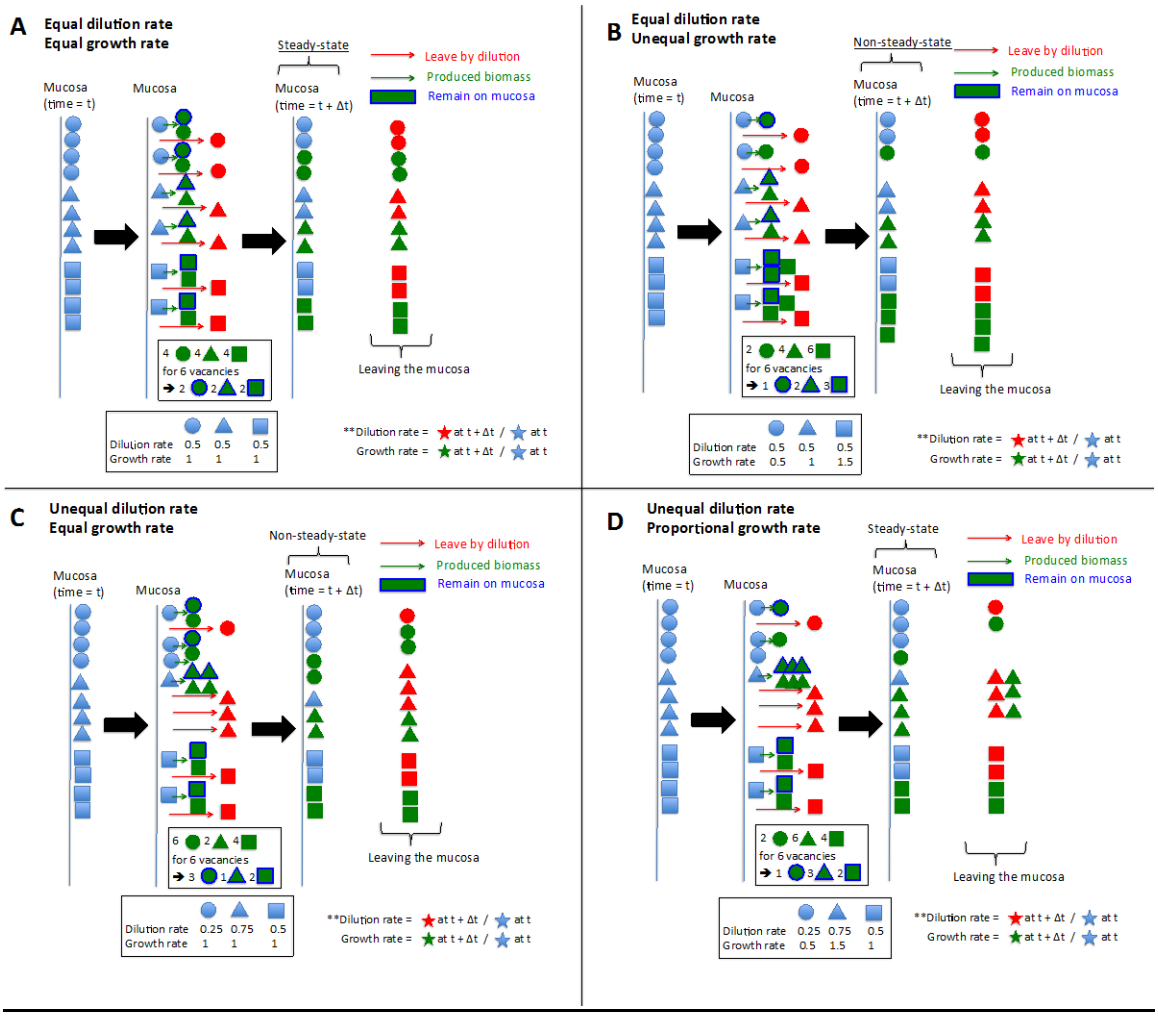

Fig. S1. Four cases of identical/non-identical dilution rates and growth rates leading to steady-state/non-steady-state mucosal microbiota. Given an identical dilution rate for each organism, (A) an identical growth rate leads to steady-state while (B) unequal growth rates do not lead to steady-state. Given organism-specific unequal dilution rates, (C) equal growth rates do not lead to steady-state while (D) growth rates proportional to dilution rates lead to steady-state.