Supplementary Materials: Supplemental Material for Byproduct Cross Feeding and Community Stability in an In Silico Biofilm Model of the Gut Microbiome

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1. Establishing Species Coexistence in the Multispecies Biofilm

We eliminated all byproduct uptakes from the model to determine if cross feeding was necessary as well as sufficient for community stability. Figure S1 shows the predicted dynamics of the species concentrations, growth rates and byproduct concentrations in the middle of a 40 μm biofilm. *F. prausnitzii* was quickly wiped out due to its relatively low growth rate, presumably due to the lack of acetate and succinate cross feeding. *B. thetaiotaomicron* remained competitive for much longer but was eventually eliminated due to its lower growth rate than *E. coli*, presumably due to a lack of ethanol cross feeding. As a result, the steady state consisted of just *E. coli* biomass with high levels of its primary byproducts acetate, ethanol and formate and low levels of succinate.

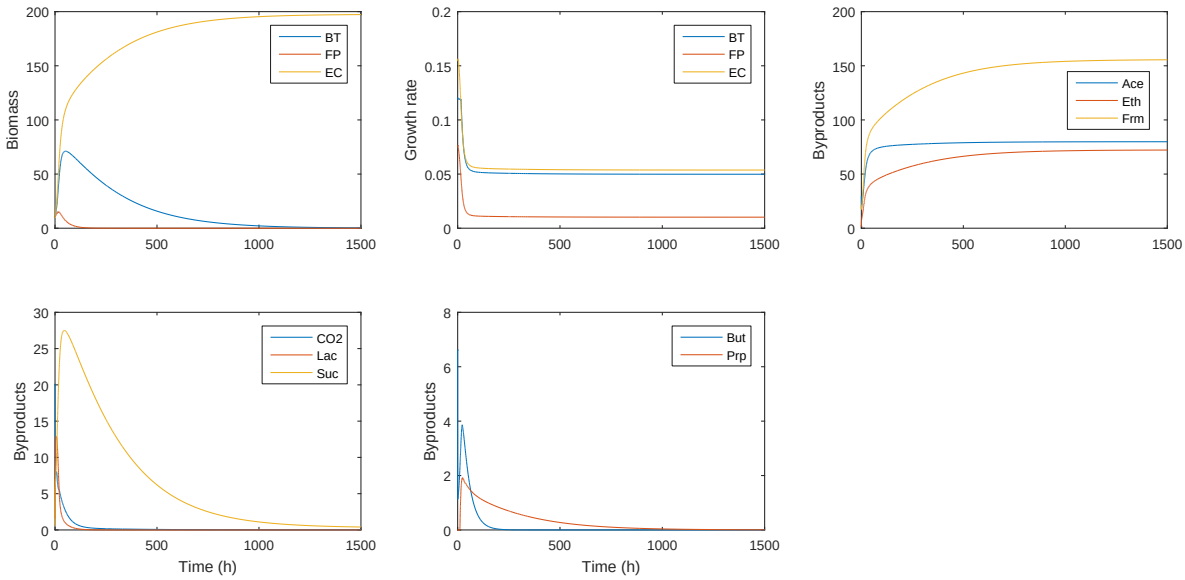

Figure S1. Time profiles predicted in the middle of the multispecies biofilm without byproduct crossfeeding. Units are g/L for biomass, h^{-1} for growth rates and mmol/L for the byproducts.

To better understand the effects of byproduct cross feeding on community stability, we performed biofilm simulations for all three possible combinations of two species. As before, CO_2 uptake was excluded for *B. thetaiotaomicron* to favor propionate synthesis and the ATP maintenance value in the *E. coli* reconstruction was decreased to 6.75 mmol ATP/gDW/h to achieve coexistence of the three species community. Each two species, biofilm simulation was performed with a fixed thickness of 40 μm and bulk nutrient concentrations of 5 mmol/L glucose and 0.5 mmol/L each amino acid. The initial conditions corresponded to spatially uniform biomass concentrations of 10 g/L for each species.

The *B. thetaiotaomicron–F. prausnitzii* biofilm was predicted to yield coexistence of the two species with *B. thetaiotaomicron* producing higher biomass concentrations throughout the biofilm (Figure S2). Each species established a unique metabolic niche, with *B. thetaiotaomicron* having higher growth rates in the nutrient rich region and *F. prausnitzii* having higher growth rates in the nutrient lean

region. In the absence of cross feeding, *B. thetaiotaomicron* growth was dominant throughout the biofilm and *F. prausnitzii* was wiped out (not shown). In the *B. thetaiotaomicron–E. coli* biofilm, the two species were predicted to coexist with *B. thetaiotaomicron* dominating due to its higher growth rate in the nutrient rich region. The removal of byproduct cross feeding destabilized the community and *B. thetaiotaomicron* was wiped out (not shown). By contrast, the *F. prausnitzii–E. coli* biofilm was not stable as the relatively high growth rates of *F. prausnitzii* in the nutrient lean region were not sufficient to avoid its extinction. Collectively, these results suggest that byproduct cross feeding was an important element of two species stability and that *B. thetaiotaomicron* was necessary for *F. prausnitzii* to coexist in the presence of *E. coli*.

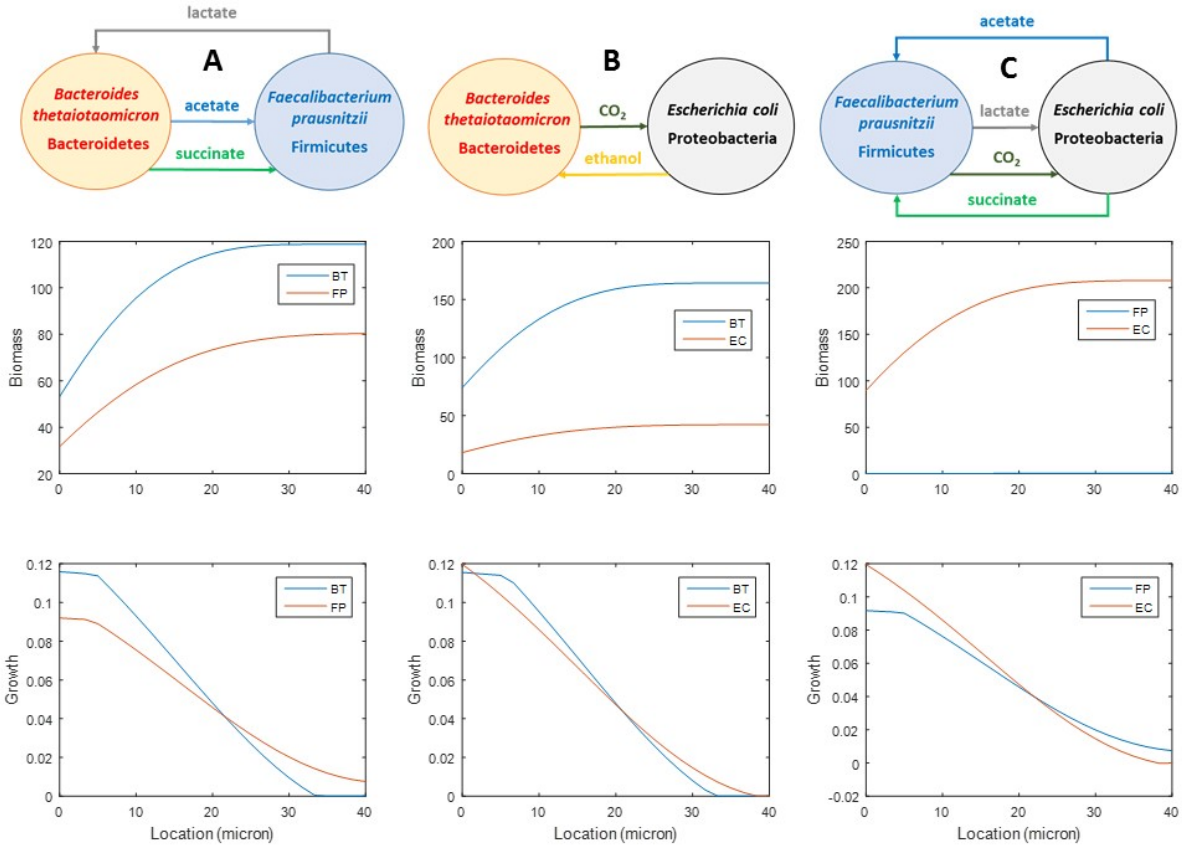

Figure S2. Steady-state spatial profiles predicted by two species biofilm models. (A) *B. thetaiotaomicron–F. prausnitzii* biofilm. (B) *B. thetaiotaomicron–E. coli* biofilm. (C) *F. prausnitzii–E. coli* biofilm. Units are g/L for biomass and h^{-1} for growth.

1.1. Robustness of the Cross-Feeding Strategy

Six nutrient levels (1 mmol/L glucose, 0.1 mmol/L each amino acid; 2.5 mmol/L glucose, 0.25 mmol/L each amino acid; 5 mmol/L glucose, 0.5 mmol/L each amino acid; 7.5 mmol/L glucose, 0.75 mmol/L each amino acid; 10 mmol/L glucose, 1 mmol/L each amino acid; 20 mmol/L glucose, 2 mmol/L each amino acid) were investigated for the nominal thickness L = 40 μm. As expected, the total biomass concentration was predicted to exhibit a sharp increasing trend with increasing nutrient levels (Figure S3). Based on measured biomass densities in bacterial biofilms, the value of 927 g/L obtained for the highest nutrient levels appeared to an unrealistic artifact of the constant biofilm thickness assumption. The three species coexisted except for the highest nutrient concentrations, where *F. prausnitzii* was eliminated due to its reduced competitiveness in nutrient rich environments. For this case, the other two species coexisted with abundances of 65.5% *B. thetaiotaomicron* and

34.5% *E. coli*. The model predicted small variations in the abundances for the other five cases: *B. thetaiotaomicron*: 53.9-58.1%; *F. prausnitzii*: 25.0-32.8%; *E. coli*: 12.9-16.9%.

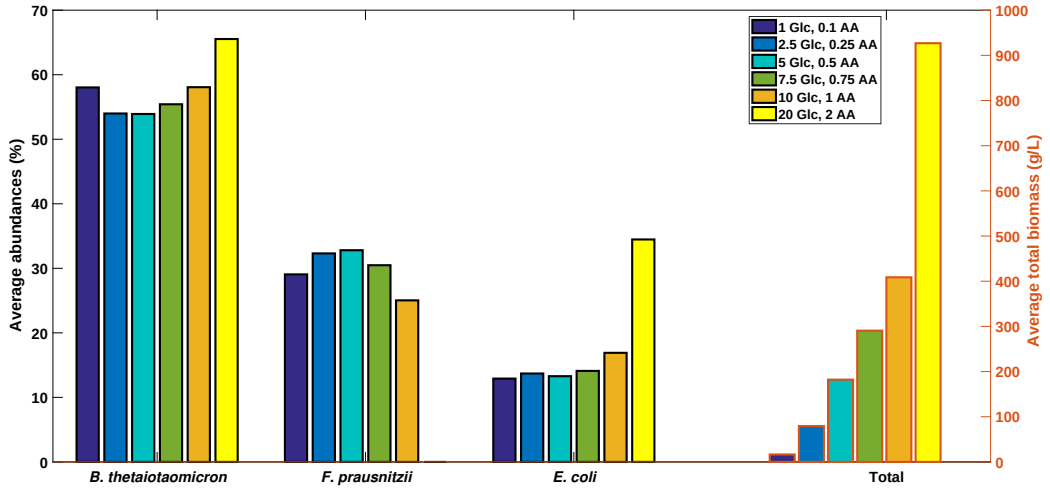

Figure S3. Effect of the bulk nutrient concentrations on the abundance of each species and the total biomass concentration averaged across the biofilm. Glc denotes glucose and AA denotes each amino acid.

1.2. Flexibility of the Cross-Feeding Strategy

We performed simulations by modulating the *F. prausnitzii* maximum succinate uptake rate instead of the acetate uptake rate (Figure S4). The most sensitive range was predicted to be succinate v_{max} values of 3.25–4.00 mmol/gDW/h, suggesting that *F. prausnitzii* was more dependent on acetate uptake than succinate uptake to sustain its growth rate. Over the sensitive range, the *F. prausnitzii* abundance increased from 5% to 27%, the acetate level decreased from 75% to 10% and the butyrate level increased from 13% to 78%. By contrast, the propionate level was approximately constant and the total SCFA concentration showed a non-monotonic but weaker trend. The SCFA profile was predicted to be particularly sensitive over the small v_{max} range of 3.25–3.50 mmol/gDW/h.

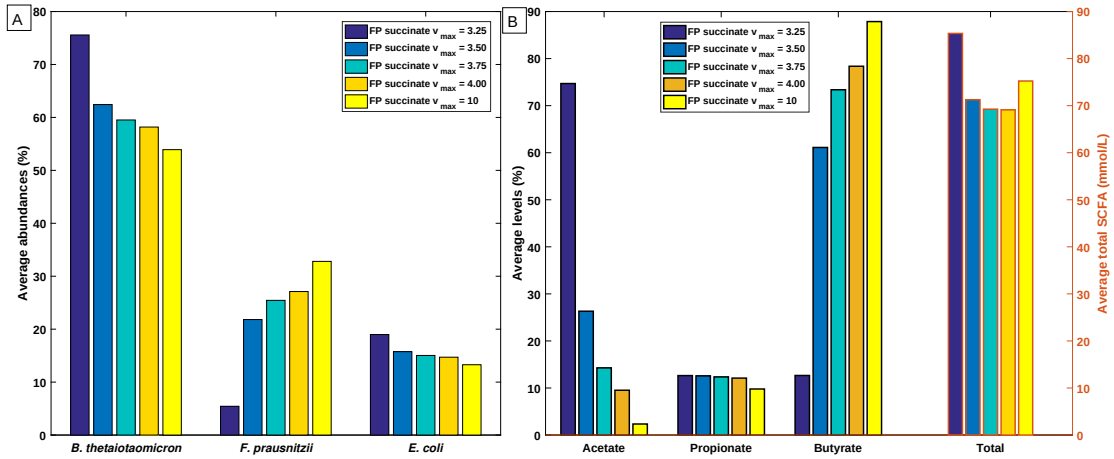

Figure S4. Effect of the *F. prausnitzii* maximum succinate uptake rate v_{max} on (A) species abundances and (B) acetate, propionate and butyrate levels and the total SCFA concentration.

The sensitive range for the *B. thetaiotaomicron* maximum ethanol uptake rate v_{max} was predicted to be 0.75–0.90 mmol/gDW/h (Figure S5), demonstrating that only a small ethanol uptake rate was needed to elevate the *B. thetaiotaomicron* growth rate such that community stability was maintained. Below this range, both *B. thetaiotaomicron* and *F. prausnitzii* were wiped out by *E. coli* due to a lack of ethanol uptake by *B. thetaiotaomicron* followed by a lack of succinate cross feeding between *B. thetaiotaomicron* and *F. prausnitzii*. Over the sensitive range, v_{max} had a substantial effect on both species abundances (*B. thetaiotaomicron*: 7–41%, *F. prausnitzii*: 8–30%, *E. coli*: 13–85%) and SCFA levels (acetate: 4–78%, propionate: 1–8%, butyrate: 21–87%). The total SCFA concentration showed a weak, non-monotonic trend.

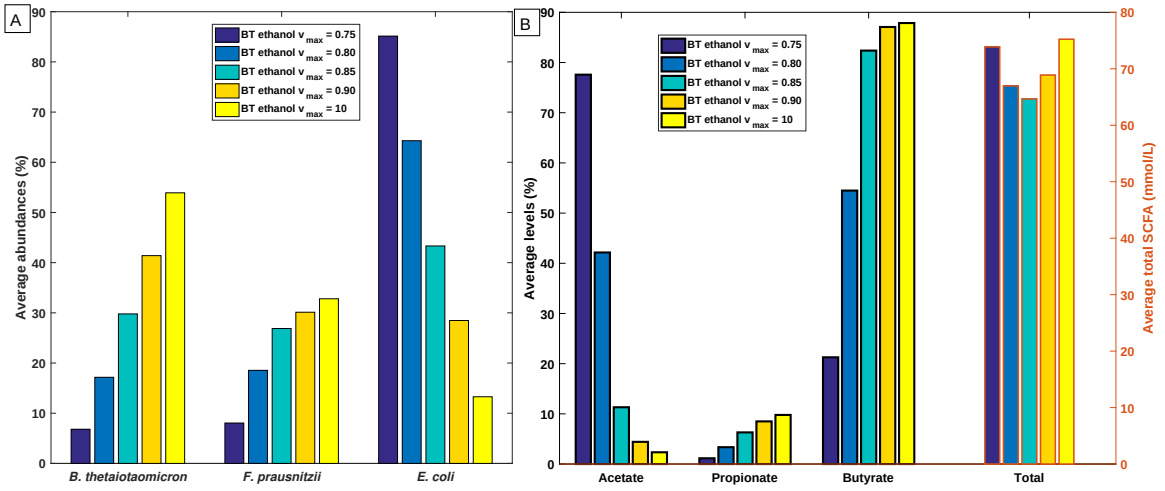

Figure S5. Effect of the *B. thetaiotaomicron* maximum ethanol uptake rate v_{max} on (A) species abundances and (B) acetate, propionate and butyrate levels and the total SCFA concentration.