

SUPPLEMENTARY INFORMATION

LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Figure 1: Sialic acid (Neu5Ac) quantification curves of each experiment. Quantification was performed using the Sigma-Aldrich quantification kit.

Supplementary Figure 2: Measurements in mmol/L of acetate (A), lactate (B), butyrate (C), and succinate (D) for exponential and stationary growth times (10 and 18 hours, respectively). Values represent the average of two biological replicates. Quantification was performed by HPLC.

Supplementary Figure 3: Principal component analysis of metatranscriptomics data.

Supplementary Figure 4: Adjustment of the proposed mathematical model. The eight experiments adjusted to the mathematical model are presented, where it is possible to appreciate the growth of the culture, the utilization of HMOs, the dynamics of sialic acid and fucose, as well as the production and consumption of simple sugars such as lactose, galactose, and glucose. Experimental data correspond to the average of the biological replicates and are shown as dots, while the model prediction is indicated as a solid line. Fitting and visualization were performed using MATLAB 2023a software.

Supplementary Table 1: log₁₀ fold change in absolute abundance for each microorganism in each experiment.

	Time	<i>B. bifidum</i>	<i>B. infantis</i>	<i>B. breve</i>	<i>B. thetaiotaomicron</i>	<i>L. symbiosum</i>	<i>E. coli</i>	<i>P. acidilactici</i>
All	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	10	0,38	-0,78	0,09	-1,02	-0,52	0,05	0,28
	18	2,01	-0,05	0,47	-1,00	-0,10	0,10	2,31
ΔECOL	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	10	1,88	-0,55	0,86	-1,64	-1,94	-0,90	1,62
	18	1,89	0,18	0,97	-1,93	-1,55	-1,12	1,65
ΔBINF	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	10	2,16	-0,28	-0,58	-0,35	0,13	0,85	0,43
	18	0,95	0,54	-0,16	-1,36	-1,51	-1,07	1,22
ΔBTHE	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	10	1,00	-0,29	-0,90	0,42	-1,04	-0,58	1,34
	18	1,10	0,39	0,23	0,31	-1,31	-0,58	1,83
ΔBBIF	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	10	-0,57	-0,20	-0,62	0,12	0,29	-0,15	-2,96
	18	0,72	-0,25	-0,85	0,71	2,79	0,81	-0,14
ΔBBRE	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	10	-0,90	-0,94	-1,78	-0,96	-1,07	-0,01	-1,20
	18	-0,11	-0,16	-0,94	0,10	-0,28	0,12	1,21
ΔPACI	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	10	2,05	-0,48	-2,30	0,56	0,83	1,14	0,27
	18	1,45	1,15	-0,90	-0,16	-0,32	-0,25	2,02
ΔLSYM	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	10	0,79	-0,31	-2,19	-0,05	0,55	-0,24	-0,62
	18	0,97	0,08	-1,53	-0,16	0,71	-0,17	-0,42

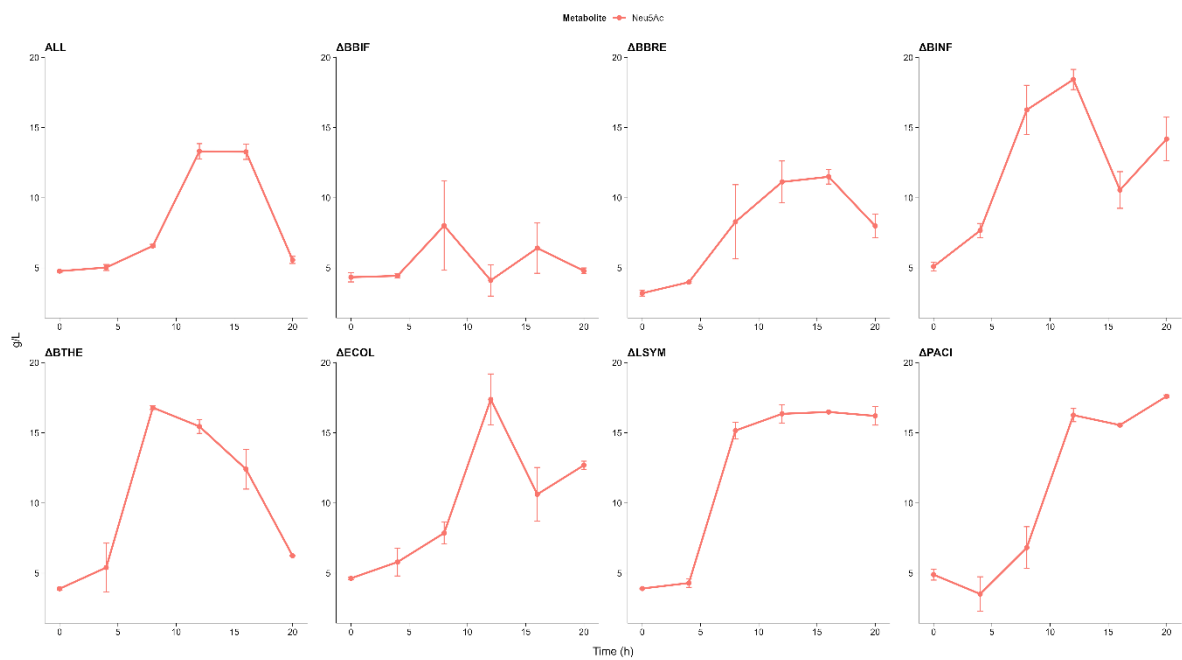
Supplementary Table 2: Total reads of each metatranscriptome RNA gene sequencing. Samples are named after the deleted species, each of the biological replicates, and later, each technical replicate.

Sample	Pre-processed (M)	Post-processed (M)
all_r1_1	4.78	3.52
all_r1_2	4.78	3.52
all_r2_1	5.76	4.04
all_r2_2	5.76	4.04
all_r3_1	8.93	6.56
all_r3_2	8.93	6.56
bbif_r1_1	10.53	6.50
bbif_r1_2	10.53	6.50
bbif_r2_1	24.20	13.74
bbif_r2_2	24.20	13.74
bbif_r3_1	28.89	20.54
bbif_r3_2	28.89	20.54
bbre_r1_1	25.81	19.18
bbre_r1_2	25.81	19.18
bbre_r2_1	11.43	9.08
bbre_r2_2	11.43	9.08
bbre_r3_1	15.95	12.02
bbre_r3_2	15.95	12.02
binf_r1_1	5.58	2.46
binf_r1_2	5.58	2.46
binf_r2_1	23.66	13.68
binf_r2_2	23.66	13.68
binf_r3_1	14.01	7.92
binf_r3_2	14.01	7.92
bthe_r1_1	7.49	4.15
bthe_r1_2	7.49	4.15
bthe_r2_1	12.06	6.04
bthe_r2_2	12.06	6.04
bthe_r3_1	11.05	6.21
bthe_r3_2	11.05	6.21
ecol_r1_1	9.61	3.23
ecol_r1_2	9.61	3.23
ecol_r2_1	31.00	16.16
ecol_r2_2	31.00	16.16
ecol_r3_1	28.18	15.78
ecol_r3_2	28.18	15.78
lsym_r1_1	28.06	21.05

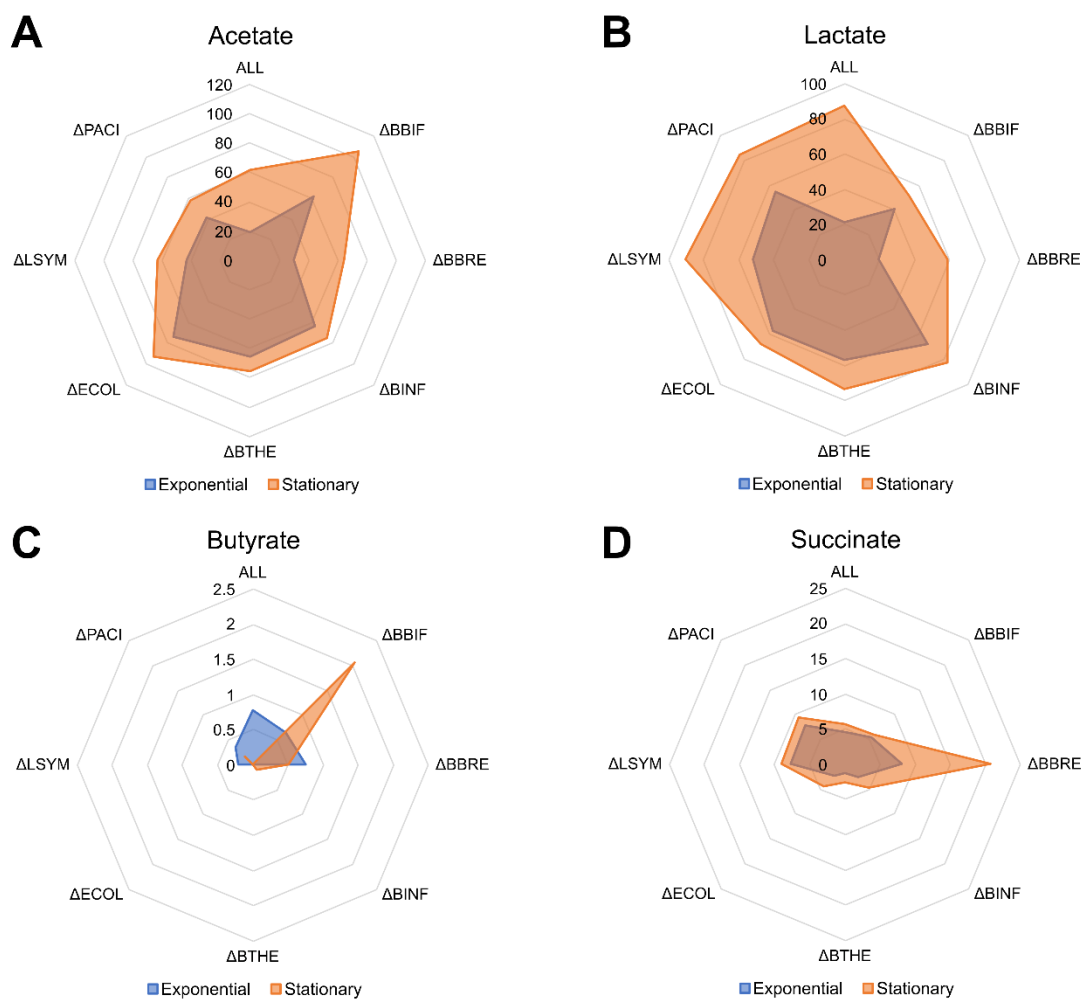
lsym_r1_2	28.06	21.05
lsym_r2_1	10.45	7.06
lsym_r2_2	10.45	7.06
lsym_r3_1	11.97	8.09
lsym_r3_2	11.97	8.09
paci_r1_1	30.47	21.65
paci_r1_2	30.47	21.65
paci_r2_1	29.15	24.58
paci_r2_2	29.15	24.58
paci_r3_1	29.18	22.74
paci_r3_2	29.18	22.74

Supplementary Table 3: Primers used in this study.

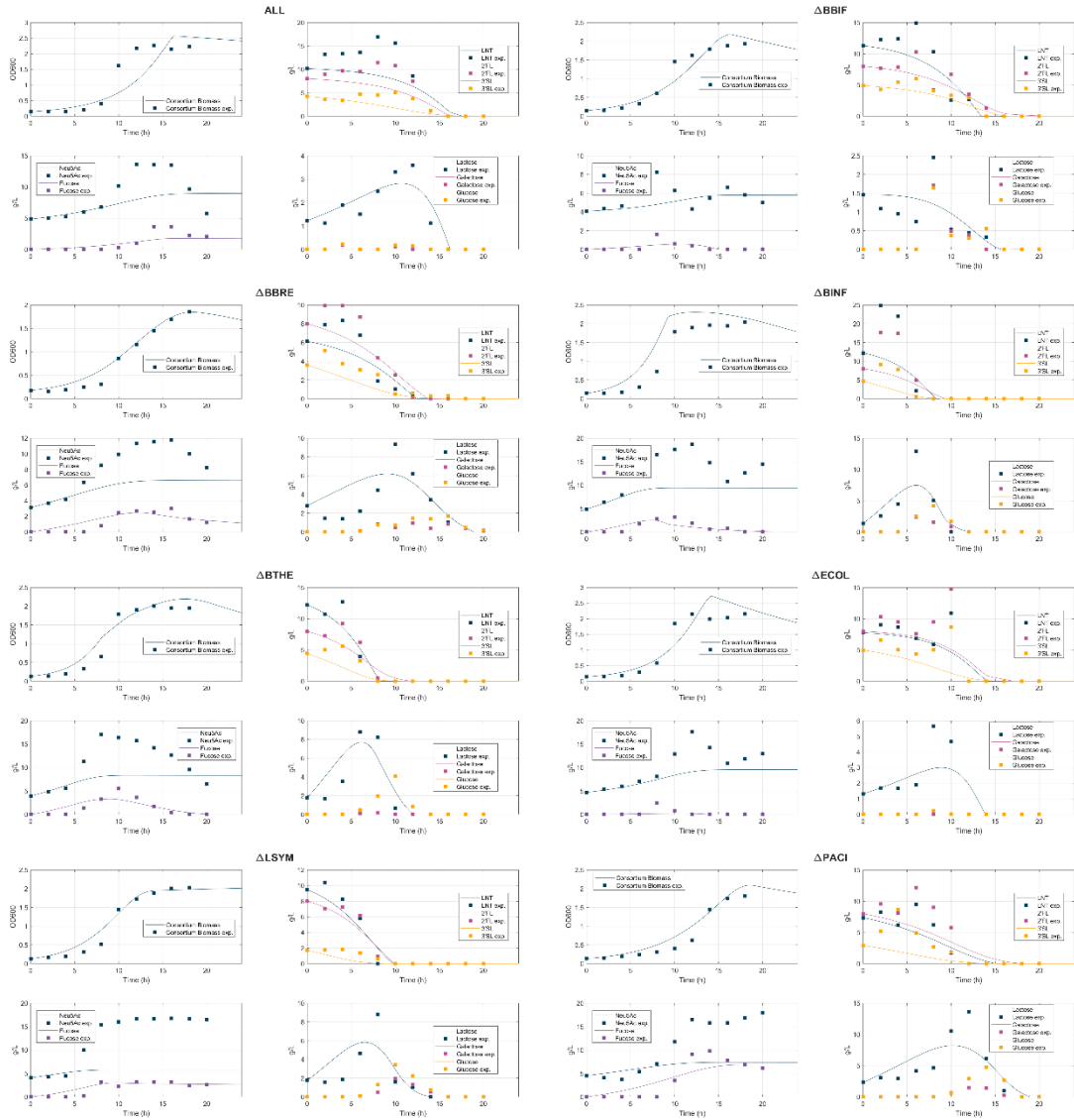
Microorganism	Primer	Sequency (5'-3')	Tm	Amplicon size
<i>B. bifidum</i> JCM 1254	B.BIF-F	GGGAACGAACAAGAGAGGAAGG	60,61	122
<i>B. bifidum</i> JCM 1254	B.BIF-R	CGCACCATACCGCCATAGAA	60,25	122
<i>B. breve</i> I1	B.BRE-F	TAAACCACAGCCCGACCA	58,44	131
<i>B. breve</i> I1	B.BRE-R	AGCACGCCCATCACCAT	58,91	131
<i>B. infantis</i> ATCC 15697	B.INF-F	CTGGATGTAGTGGGCGTGAG	60,18	125
<i>B. infantis</i> ATCC 15697	B.INF-R	TGGCTTGTGGGCGATTGAT	60,00	125
<i>B. thetaiotaomicron</i> VPI 5482	B.THE-F	TTGTCTGCGTTCCTTCCTGA	59,24	135
<i>B. thetaiotaomicron</i> VPI 5482	B.THE-R	GGCGTATGTTCCGGTGATGC	59,36	135
<i>L. symbiosum</i> WAL-14673	C.SYM-F	GCAGGGAGATGGAGGATAAGC	60,00	120
<i>L. symbiosum</i> WAL-14673	C.SYM-R	CAAGAGCAAAGGCAAGACGC	60,39	120
<i>E. coli</i> K-12 MG1655	E.COL-F	TCGGCTGTCTCTTCTCTCGT	60,32	125
<i>E. coli</i> K-12 MG1655	E.COL-R	GCTCTGGTTGTGGTGGGTTA	59,89	125
<i>P. acidilactici</i> PT22	P.ACI-F	CGGCGCGATTATTTGCATGA	60,11	199
<i>P. acidilactici</i> PT22	P.ACI-R	GAACGTAGCCTTCACCACCA	60,03	199



SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2



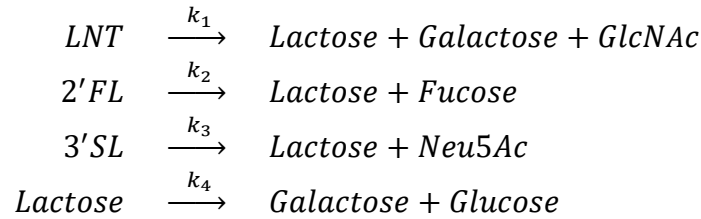
SUPPLEMENTARY FIGURE 4

Mathematical Model of HMO degradation

Rationale

This mathematical model simulates the degradation of three HMOs (LNT, 2'FL and 3'SL) by a microbial community. It is based on 10 ordinary differential equations, corresponding to mass balances of degradation and production of metabolites related to HMO consumption. These equations simulate four general chemical reactions of HMO degradation. In order to reduce the number of parameters and better adjust the model to experimental data, a community biomass (X_{COM}) term was used in the model instead of individual biomass for each member of the consortium. Data from each substrate concentration (S_i), community biomass (X_{COM}), and growth rates (μ_i) were used to run these simulations and determine parameter values, minimizing the square error between theoretical and experimental data.

Reactions (4)



ODEs (10)

$$\begin{aligned}
 \frac{dX_{COM}}{dt} &= X_{COM}(\mu - k_d) \\
 \frac{dS_{LNT}}{dt} &= -X_{COM} \left(k_1 S_{LNT} + \frac{\mu}{Y_{X_{COM}S_{LNT}}} \right) \\
 \frac{dS_{2'FL}}{dt} &= -X_{COM} \left(k_2 S_{2'FL} + \frac{\mu}{Y_{X_{COM}S_{2'FL}}} \right) \\
 \frac{dS_{3'SL}}{dt} &= -X_{COM} \left(k_3 S_{3'SL} + \frac{\mu}{Y_{X_{COM}S_{3'SL}}} \right) \\
 \frac{dS_{Lactose}}{dt} &= X_{COM} \left(k_1 S_{LNT} + k_2 S_{2'FL} + k_3 S_{3'SL} - k_4 S_{Lactose} - \frac{\mu}{Y_{X_{COM}S_{Lactose}}} \right) \\
 \frac{dS_{Galactose}}{dt} &= X_{COM} \left(k_1 S_{LNT} + k_4 S_{Lactose} - \frac{\mu}{Y_{X_{COM}S_{Galactose}}} \right) \\
 \frac{dS_{Glucose}}{dt} &= X_{COM} \left(k_4 S_{Lactose} - \frac{\mu}{Y_{X_{COM}S_{Glucose}}} \right)
 \end{aligned}$$

$$\begin{aligned}\frac{dS_{Neu5Ac}}{dt} &= X_{COM} \left(k_3 S_{3'SL} - \frac{\mu}{Y_{X_{COM}S_{Neu5Ac}}} \right) \\ \frac{dS_{Fucose}}{dt} &= X_{COM} \left(k_2 S_{2'FL} - \frac{\mu}{Y_{X_{COM}S_{Fucose}}} \right) \\ \frac{dS_{GlcNAc}}{dt} &= X_{COM} k_1 S_{GlcNAc}\end{aligned}$$

Algebraic equations (1)

$$\begin{aligned}\mu = \mu_{max} & \left[\left(\frac{S_{LNT}}{K_{S_{LNT}} + S_{LNT}} \right) + \left(\frac{S_{2'FL}}{K_{S_{2'FL}} + S_{2'FL}} \right) + \left(\frac{S_{3'SL}}{K_{S_{3'SL}} + S_{3'SL}} \right) + \left(\frac{S_{Lactose}}{K_{S_{Lactose}} + S_{Lactose}} \right) \right. \\ & + \left(\frac{S_{Galactose}}{K_{S_{Galactose}} + S_{Galactose}} \right) + \left(\frac{S_{Glucose}}{K_{S_{Glucose}} + S_{Glucose}} \right) + \left(\frac{S_{Neu5Ac}}{K_{S_{Neu5Ac}} + S_{Neu5Ac}} \right) \\ & \left. + \left(\frac{S_{Fucose}}{K_{S_{Fucose}} + S_{Fucose}} \right) \right] \frac{1}{8}\end{aligned}$$

Parameters (22)

μ_{max} : Maximum growth rate

k_d : Death/Dilution rate

$Y_{X_{COM}S,i}$: Biomass yield per substrate (8)

k_j : Reaction conversion rate (4)

$K_{S,i}$: Monod's constant (8)

Supplementary Results

The model successfully reproduced experimental growth curves and sugar consumption patterns, including the early accumulation of lactose detected by TLC (Figure 4; Supplementary Figure 4). Some monosaccharide dynamics (e.g., glucose, galactose, sialic acid) were less well captured, likely because the model assumes near-instantaneous consumption compared with the slower kinetics observed experimentally.

Additional simulations further revealed that absence of *B. bifidum* not only reduced HMO degradation rates but also increased lactose degradation rates, suggesting that when extracellular hydrolysis is impaired, metabolic activity shifts toward universally accessible sugars. The degradation of 2'FL and 3'SL in the model resulted in the production of fucose and sialic acid, respectively: while fucose dynamics were well fitted, sialic acid dynamics deviated from experimental measurements, indicating additional biological processes not accounted for by the model.