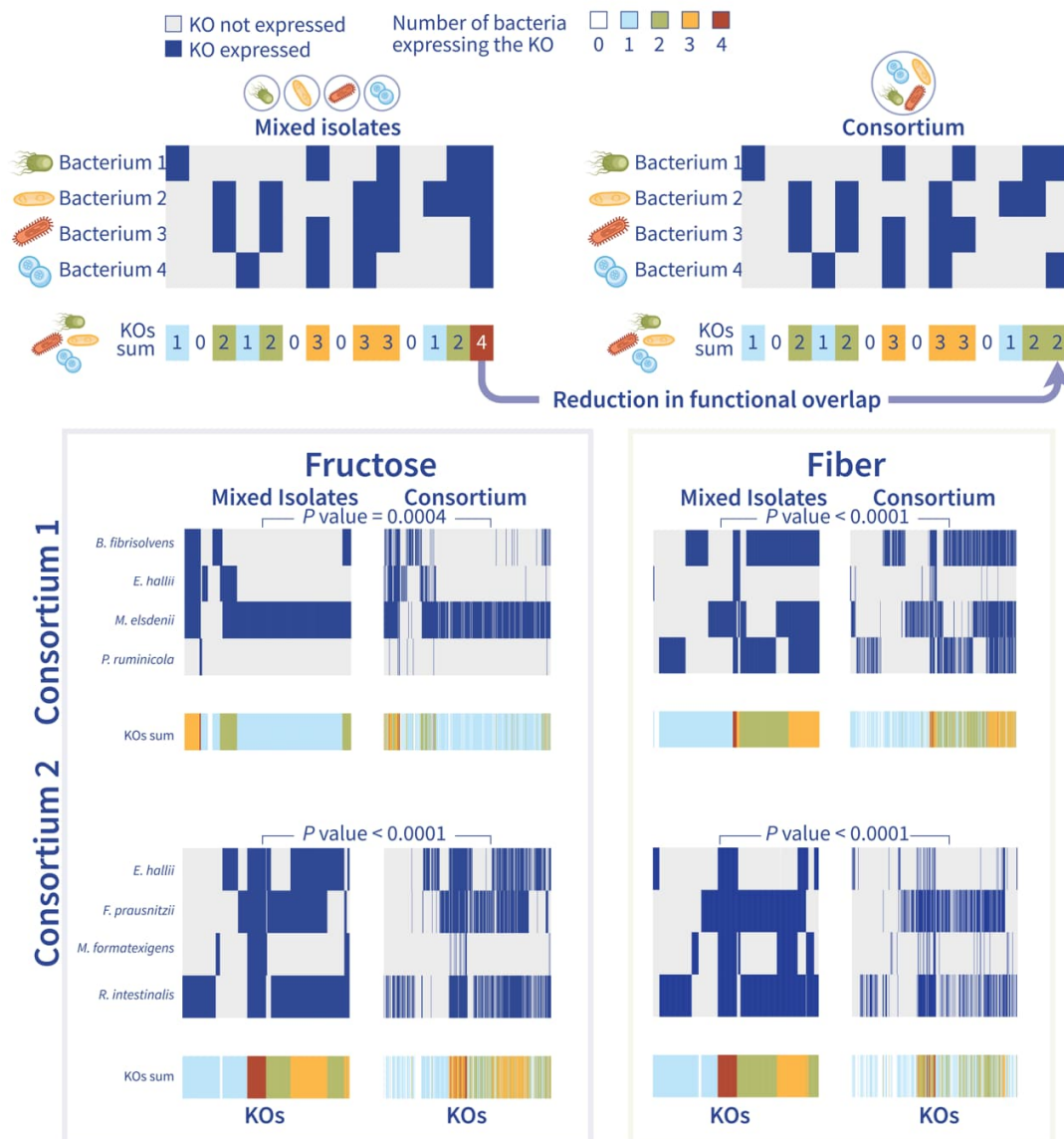
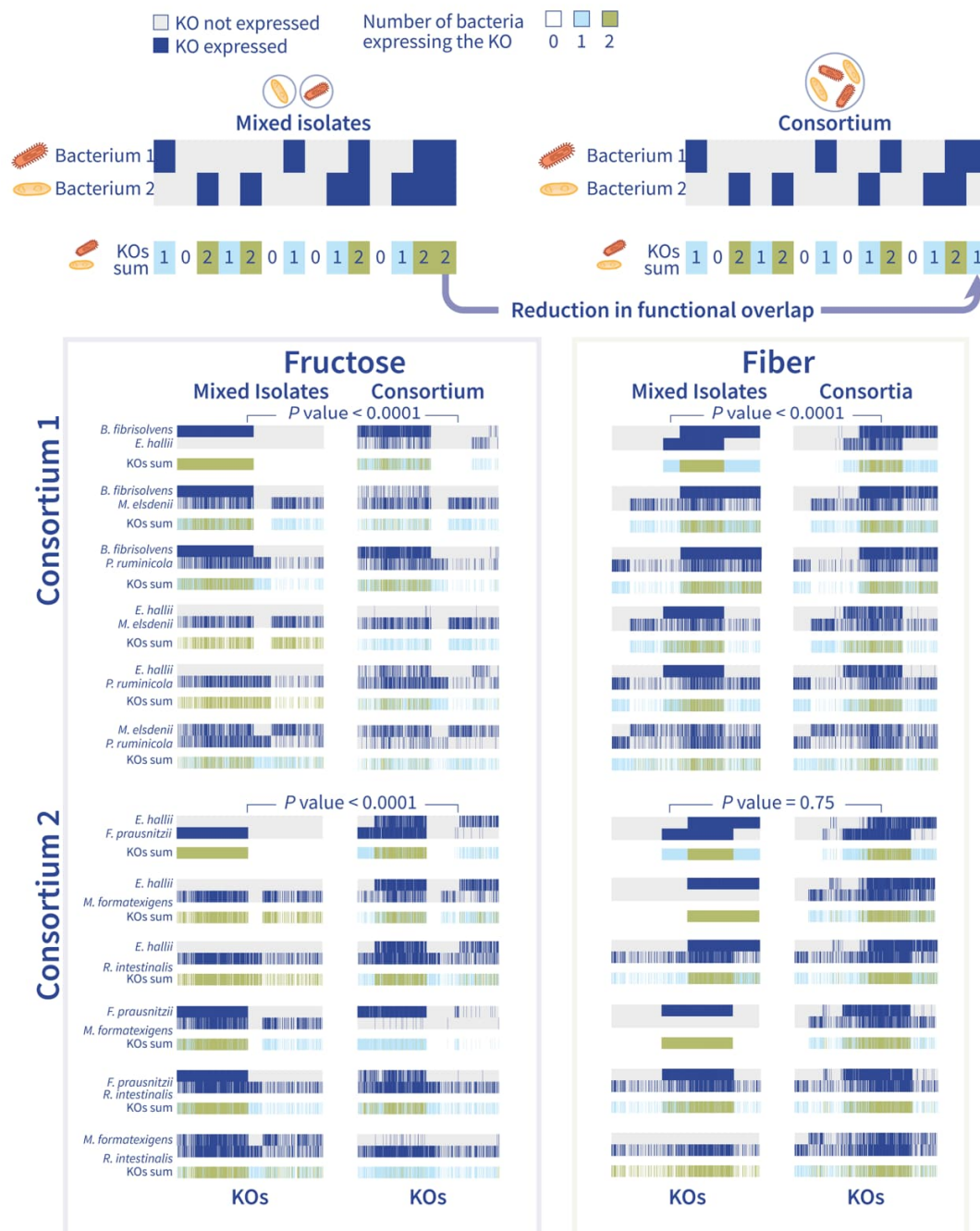

Community context reshapes microbial proteomes and reduces functional overlap

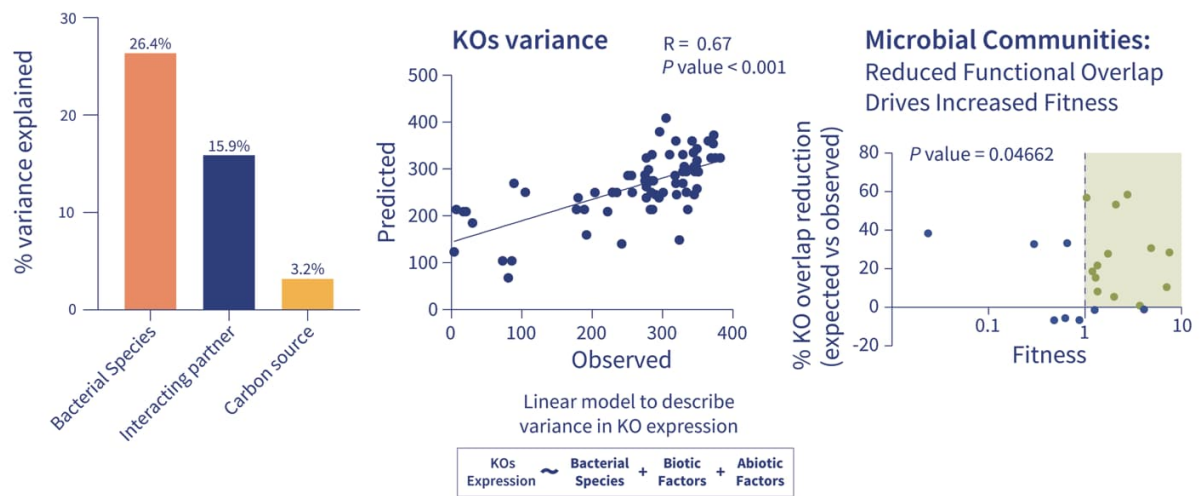
In the format provided by the
authors and unedited



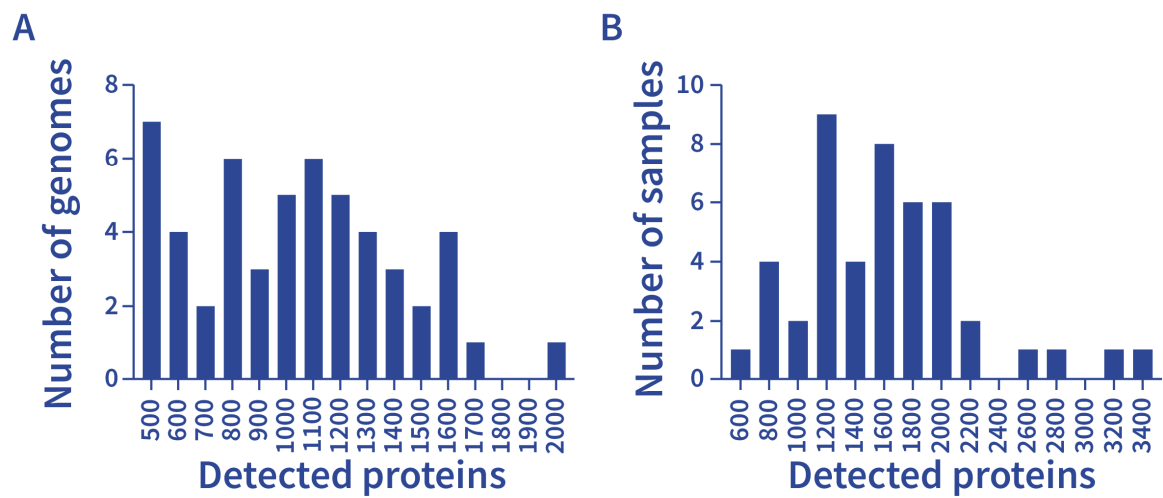
Supplementary Figure 1: Reduction in functional redundancy within four-species consortia compared to mixed isolates controls (NSAF presence-absence). Heatmaps of KOs within metabolic pathways (map01100) for two different four-species consortia grown on either fructose or fiber, alongside their corresponding mixed-isolate controls. Each heatmap represents the number of detected KOs per species in at least one of the three biological replicates (4 rows) and the total number of KOs detected across the entire consortium (bottom row). Comparisons reveal a marked reduction in functional redundancy within the grown consortia, as they exhibit fewer shared KOs than expected from the mixed isolates proteomic analyses. Chi-square test ((two-sided) P values are indicated).



Supplementary Figure 2: Reduction in functional redundancy within pairwise consortia compared to isolates (NSAF presence-absence). Heatmaps of KOs within metabolic pathways (map01100) for all pairwise consortia grown on either fructose or fiber, alongside their corresponding isolates. Each heatmap represents the number of detected KOs per species in at least one of the three biological replicates (2 rows) and the total number of KOs detected across the entire consortium (bottom row). Comparisons reveal a marked reduction in functional redundancy within the grown consortia, as they exhibit fewer shared KOs than expected from the mixed isolates proteomic analyses. Chi-square test (two-sided) P values are indicated.



Supplementary Figure 3: Left: Bar plot displaying the proportion of variance in KO expression explained by bacterial species (LFQ presence-absence), environmental conditions, and microbial community composition. Middle: Linear regression model depicting the contribution of each factor to KO variance. Statistical significance was assessed using ANOVA (two-sided). Right: Scatter plot showing the relationship between percent KO overlap reduction (y-axis, LFQ presence-absence) and community productivity (x-axis). Communities exhibiting reduced functional redundancy alongside increased productivity are highlighted in green; Odds ratio (OR) = 9.75; Wald test one-sided P value = 0.04662. Each dot represent the mean of three biological replicates.



Supplementary Figure 4: Histograms of number of detected proteins per genome (A) or samples (B) of the data analysed in the manuscript.

Supplementary Table 1: Pearson correlation coefficients are given for correlations between species-specific protein abundances, comparing the mixed isolates to the corresponding single-species cultures or mixed isolates to the corresponding consortium.

	<i>Pearson R (mixed isolates vs isolate)</i>	<i>Pearson R (mixed isolates vs consortium)</i>
<i>B. fibrisolvens</i> fiber cytosolic	0.92	0.716
<i>B. fibrisolvens</i> fiber secreted	0.797	0.572
<i>E. hallii</i> fructose cytosolic	0.862	0.78
<i>E. hallii</i> fructose secreted	0.587	0.408
<i>F. prausnitzii</i> fructose cytosolic	0.892	0.776
<i>F. prausnitzii</i> fructose secreted	0.747	0.388
<i>F. prausnitzii</i> fiber cytosolic	0.894	0.867
<i>F. prausnitzii</i> fiber secreted	0.642	0.55
<i>M. elsdenii</i> fructose cytosolic	0.944	0.86
<i>M. elsdenii</i> fructose secreted	0.889	0.643
<i>M. elsdenii</i> fiber cytosolic	0.914	0.704
<i>M. elsdenii</i> fiber secreted	0.507	0.389
<i>P. ruminicola</i> fiber cytosolic	0.892	0.76
<i>P. ruminicola</i> fiber secreted	0.604	0.585
<i>R. intestinalis</i> fiber cytosolic	0.807	0.762
<i>R. intestinalis</i> fiber secreted	0.439	0.349

Supplementary Table 2: List of Kos and corresponding KEGG modules identified as reduced or maintained in functional overlap

Remained KOs	Module_ID	Module_Name	Reoccurrence
K00134	md:M00001	Glycolysis (Embden-Meyerhof pathway), glucose => pyruvate	61
K00134	md:M00002	Glycolysis, core module involving three-carbon compounds	61
K00134	md:M00003	Gluconeogenesis, oxaloacetate => fructose-6P	61
K00134	md:M00165	Reductive pentose phosphate cycle (Calvin cycle)	61
K00134	md:M00308	Semi-phosphorylative Entner-Doudoroff pathway, gluconate => glycerate-3P	61
K00134	md:M00552	D-galactonate degradation, De Ley-Doudoroff pathway, D-galactonate => glycerate-3P	61
K03737	md:M00173	Reductive citrate cycle (Arnon-Buchanan cycle)	60
K03737	md:M00307	Pyruvate oxidation, pyruvate => acetyl-CoA	60
K01610	md:M00003	Gluconeogenesis, oxaloacetate => fructose-6P	47
K01610	md:M00170	C4-dicarboxylic acid cycle, phosphoenolpyruvate carboxykinase type	47
K00248	md:M00013	Malonate semialdehyde pathway, propanoyl-CoA => acetyl-CoA	36
K00249	md:M00036	Leucine degradation, leucine => acetoacetate + acetyl-CoA	36
K00249	md:M00087	beta-Oxidation	36
K02110	md:M00157	F-type ATPase, prokaryotes and chloroplasts	20
K00290	md:M00030	Lysine biosynthesis, AAA pathway, 2-oxoglutarate => 2-aminoadipate => lysine	14
Reduced KOs	Module_ID	Module_Name	Reoccurrence
K00287	md:M00126	Tetrahydrofolate biosynthesis, GTP => THF	14
K00287	md:M00840	Tetrahydrofolate biosynthesis, mediated by ribA and trpF, GTP => THF	14
K01495	md:M00126	Tetrahydrofolate biosynthesis, GTP => THF	13
K01495	md:M00841	Tetrahydrofolate biosynthesis, mediated by PTPS, GTP => THF	13
K01495	md:M00842	Tetrahydrobiopterin biosynthesis, GTP => BH4	13
K01495	md:M00843	L-threo-Tetrahydrobiopterin biosynthesis, GTP => L-threo-BH4	13
K01495	md:M00988	PreQ1 biosynthesis, GTP => 7-Aminomethyl-7-deazaguanine	13
K00859	md:M00120	Coenzyme A biosynthesis, pantothenate => CoA	10
K03149	md:M00127	Thiamine biosynthesis, prokaryotes, AIR (+ DXP/tyrosine) => TMP/TPP	10
K03149	md:M00895	Thiamine biosynthesis, prokaryotes, AIR (+ DXP/glycine) => TMP/TPP	10

Supplementary Table 3: Composition of the mixed-isolates consortia.

Strain	Pellet fiber cultivations		Pellet fructose cultivations		Supernatant fiber cultivations		Supernatant fructose cultivations	
Consortium 1	NSAF [%]	protein [µg]	NSAF [%]	protein [µg]	NSAF [%]	sample volume [µL]	NSAF [%]	sample volume [µL]
<i>P. ruminicola</i>	17.99	3.6	2.69E-03	5.37E-04	22.19	44.37	1.34E-02	3.00E-02
<i>M. elsdenii</i>	40.68	8.14	97.33	19.47	34.41	68.83	97.43	194.87
<i>B. fibrisolvens</i>	40.61	8.12	1.51	0.3	43.08	86.16	1.18	2.35
<i>E. hallii</i>	0.72	0.14	1.16	0.23	0.32	0.64	1.38	2.75
Total	100	20	100	20	100	200	100	200
Consortium 2	NSAF [%]	protein [µg]	NSAF [%]	protein [µg]	NSAF [%]	sample volume [µL]	NSAF [%]	sample volume [µL]
<i>E. hallii</i>	5.36	1.07	22.12	4.42	5.8	11.6	17.89	35.79
<i>R. intestinalis</i>	23.21	4.65	33.14	6.63	33.61	67.22	29.53	59.07
<i>M. formatexigens</i>	5.02	1.00	1.78	0.36	4.44	8.88	1.06	2.1
<i>F. prausnitzii</i>	66.41	13.28	42.96	8.59	56.15	112.3	51.52	103.04
Total	100	20	100	20	100	200	100	200

Supplementary Table 4: Primers used in this study

	Forward	Reverse	Reference
<i>Prevotella ruminicola</i> PreRum1F and PreRum1R	GAAAGTCGGATTAATGCTCTATGTTG	CATCCTATAGCGGTAAACCTTTGG	Stevenson et al, 2007
<i>Megasphaera elsdenii</i> MegEls2F and MegEls2R	AGATGGGGACAACAGCTGGA	CGAAAGCTCCGAAGAGCCT	Stevenson et al, 2007
<i>Butyrivibrio fibrisolvens</i> ButFib2F and ButFib2R	ACCGCATAAGCGCACGGA	CGGGTCCATCTTGTACCGATAAAT	Stevenson et al, 2007
<i>Eubacterium hallii</i> Eh1F and Eh1R	GCGTAGGTGGCAGTGCAA	GCACCGRAGCCTATAGGG	Ramirez-Farias et al, 2009
<i>Faecalibacterium prauzitinii</i> Fprau-F and Fprau-R	ggaggaagaaggtcttcgg	Aattccgcctacctctgcact	Vital et al, 2013
<i>Roseburia intestinalis</i> R.intF and R.intR	cgKactagagtgtcggagg	agtttYattcttgcgaacg	Vital et al, 2013
<i>Marvinbryantia formatexigens</i> MarvinF and MarvinR	ATGACGAATGACGGTACCTGACCAAGA	GGCACTCAAGCCATACAGTT	Rey et al, 2010

Supplementary Table 5: Overview of used liquid chromatography systems and mass spectrometers including used settings.

Sample (Fraction)	Liquid chromatography (gradient)	Mass spectrometer (measurement parameters)
Single cultivations (pellets)	EASY nLC II (non-binary 100 min 1-99% acetonitrile in 0.1% (v/v) acetic acid)	LTQ Orbitrap Velos Pro (full scan; resolution 30,000; mass range 300 to 2,000 m/z; TopN = 20)
Single cultivations (supernatants)	EASY nLC II (non-binary 100 min 1-99% acetonitrile in 0.1% (v/v) acetic acid)	LTQ Orbitrap XL / LTQ Orbitrap (full scan; resolution 30,000; mass range 300 to 2,000 m/z; TopN = 5)
Consortia and mixed-isolates (pellets)	EASYnLC1200 (non-binary 100 min 0-95% acetonitrile in 0.1% (v/v) acetic acid)	LTQ Orbitrap Elite (full scan; resolution 60,000; mass range 300 to 1,700 m/z; TopN = 20)
Consortia and mixed-isolates (supernatants)	EASYnLC1200 (non-binary 180 min 0-95% acetonitrile in 0.1% (v/v) acetic acid)	LTQ Orbitrap Elite (full scan; resolution 60,000; mass range 300 to 1,700 m/z; TopN = 20)
Pairwise cultivations (pellets)	EASYnLC1200 (non-binary 180 min 0-95% acetonitrile in 0.1% (v/v) acetic acid)	LTQ Orbitrap Elite (full scan; resolution 60,000; mass range 300 to 1,700 m/z; TopN = 20)
Pairwise cultivations (supernatants)	EASY nLC II (non-binary 100 min 1-99% acetonitrile in 0.1% (v/v) acetic acid)	LTQ Orbitrap XL / LTQ Orbitrap (full scan; resolution 30,000; mass range 300 to 2,000 m/z; TopN = 5)