Supplementary materials

The influence of caging, bedding, and diet on the composition of the microbiota in different regions of the mouse gut

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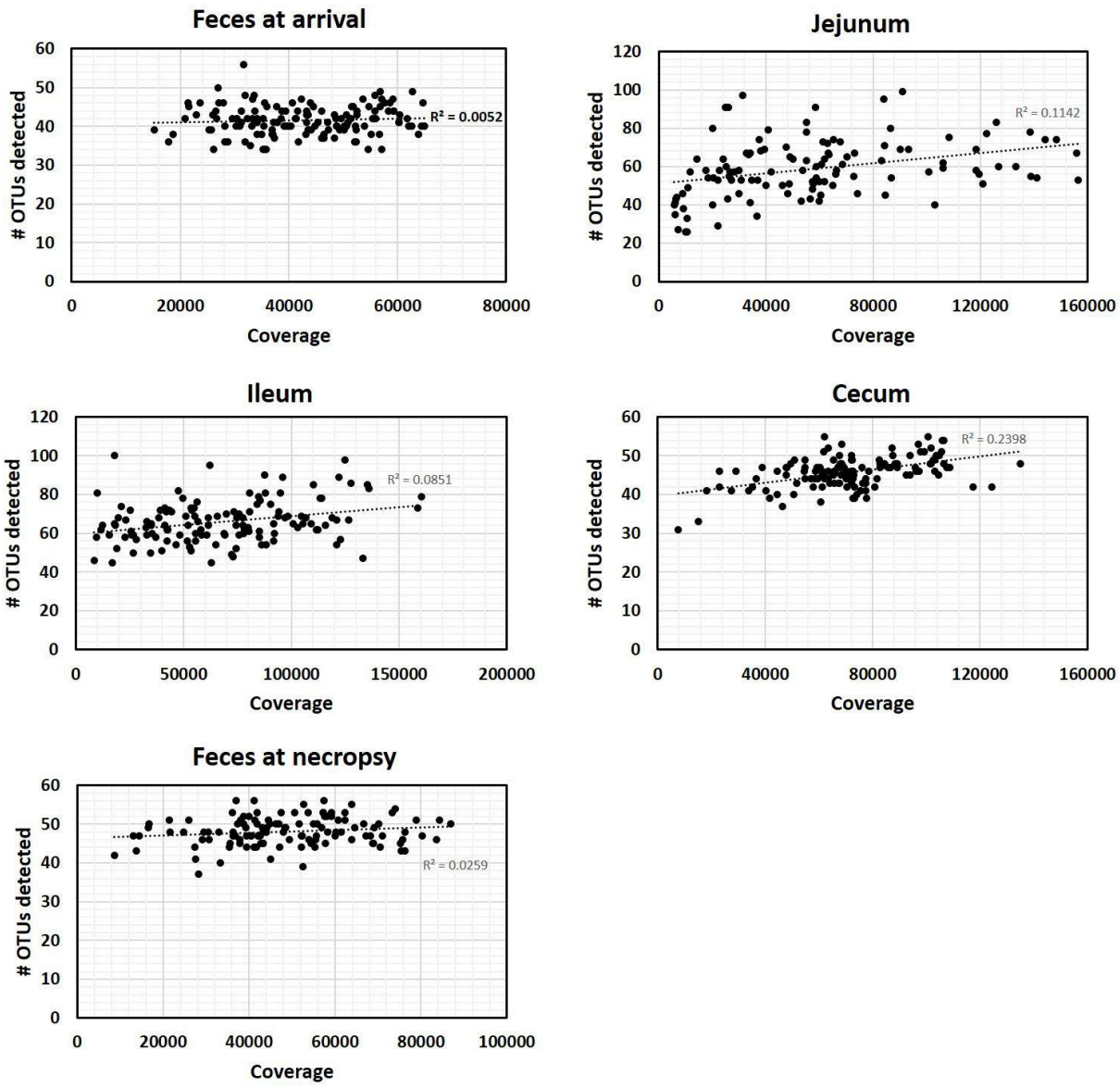
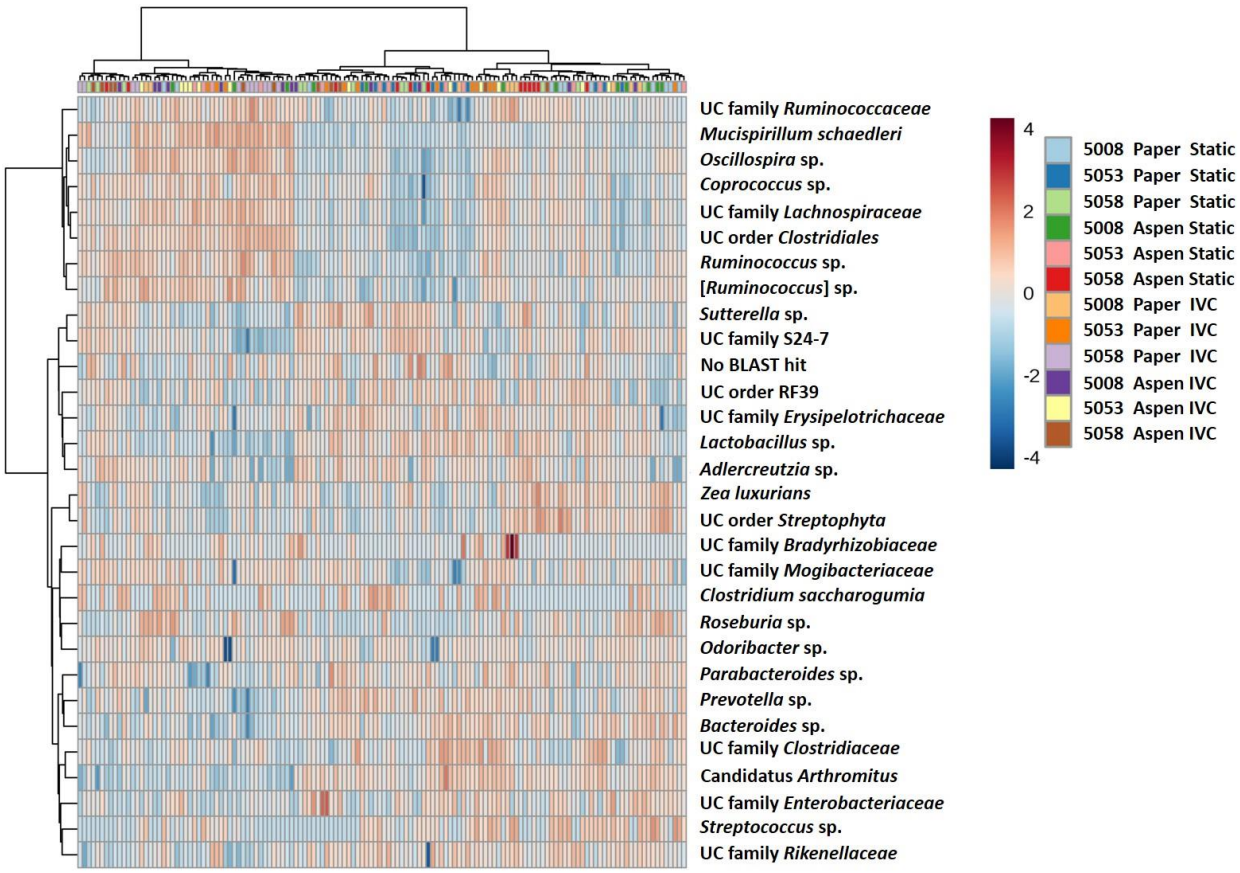
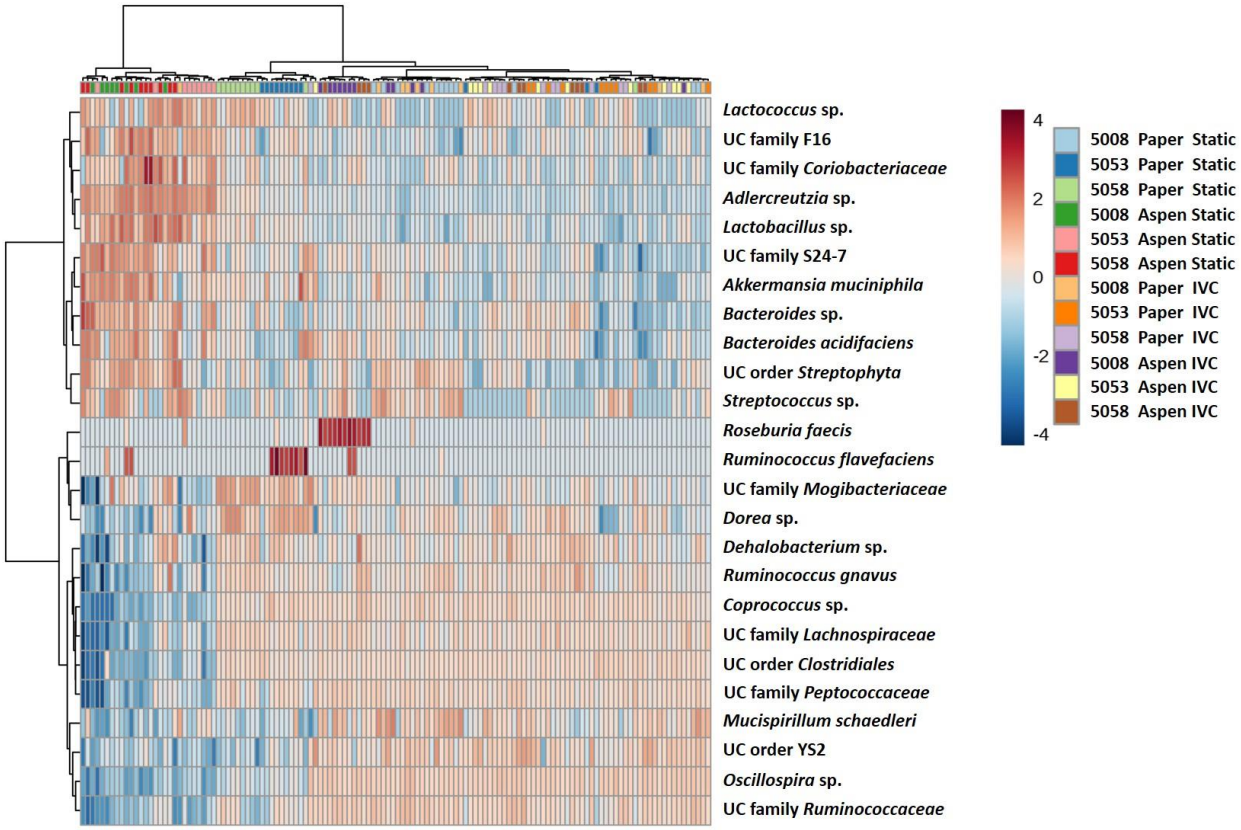


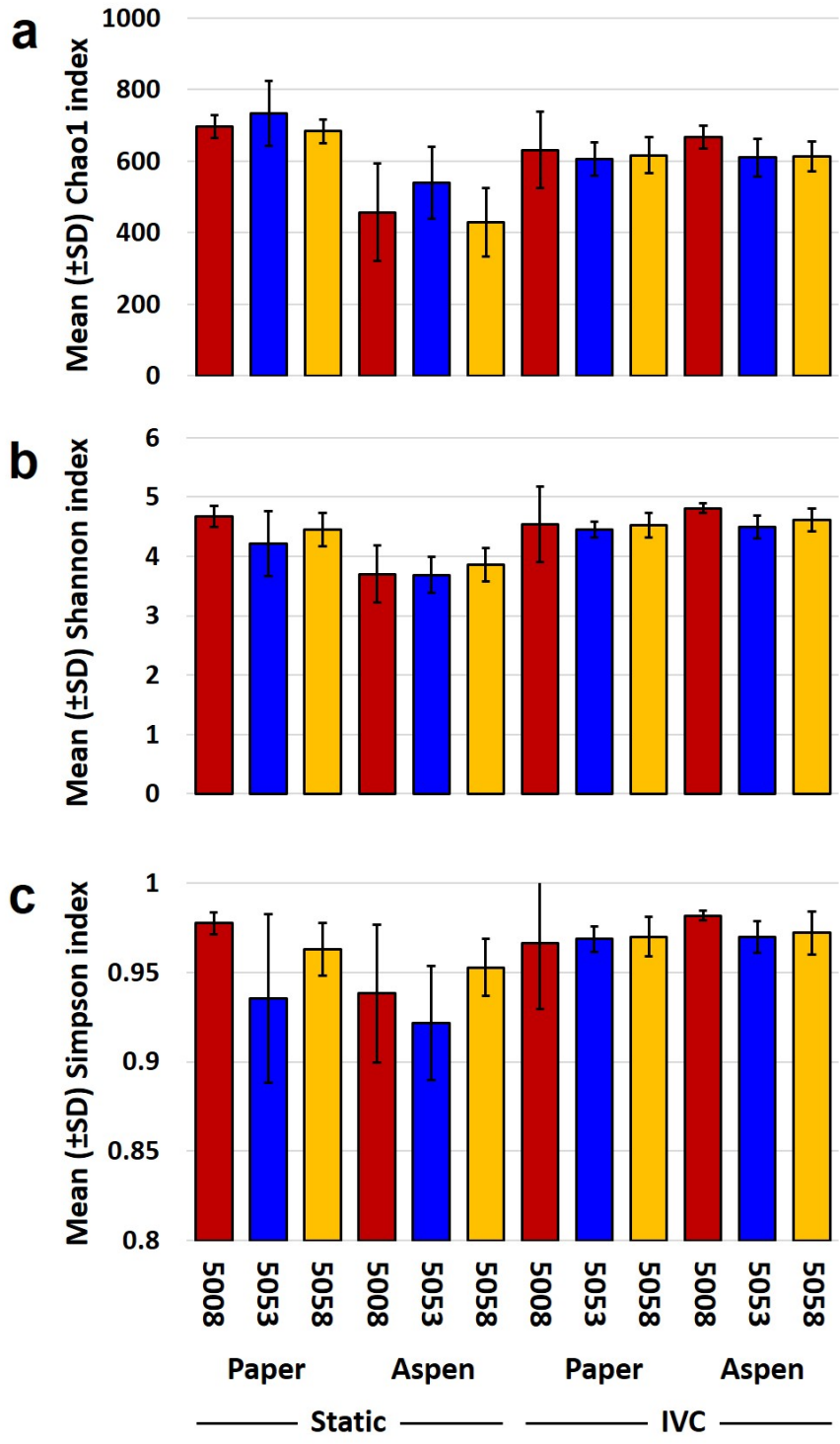
Supplementary Figure S1. Rarefaction of samples collected from each site, showing consistency of detected microbial richness for samples with coverage greater than 5000 sequences per sample.



Supplementary Figure S2. Heatmap of log-fold relative abundance in baseline feces of top 25 differentially abundant operational taxonomic units (OTUs) as determined via analysis of variance, arranged via agglomerative hierarchical clustering of Euclidean distances between samples (x-axis) and OTUs (y-axis).



Supplementary Figure S3. Heatmap of log-fold relative abundance in endpoint cecal microbiota of top 25 differentially abundant operational taxonomic units (OTUs) as determined via analysis of variance, arranged via agglomerative hierarchical clustering of Euclidean distances between samples (x-axis) and OTUs (y-axis).



Supplementary Figure S4. Bar charts showing mean ± SD Chao1 (a), Shannon (b), and Simpson (c) diversity indices of cecal contents.

Supplementary Video S1. Rotating three-dimensional principal coordinates analysis of unweighted UniFrac distances with samples grouped according to their combination of housing and bedding. I = IVC, S = static, P = paper, and A = aspen.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		0.018	0.016	0.531	0.604	0.025	0.032	0.503	**<0.001**	0.228	0.008	0.112
Static	**Paper**	**5053**	3.37		0.251	0.025	0.025	0.065	0.047	0.026	**<0.001**	**<0.001**	**<0.001**	0.003
Static	**Paper**	**5058**	3.63	1.27		0.009	0.021	0.995	0.004	0.005	**<0.001**	0.002	**<0.001**	0.008
Static	**Aspen**	**5008**	0.74	3.33	4.52		0.547	0.013	0.343	0.844	**0.001**	0.213	0.103	0.345
Static	**Aspen**	**5053**	0.64	3.06	3.69	0.67		0.028	0.086	0.681	0.004	0.573	0.164	0.425
Static	**Aspen**	**5058**	3.18	2.54	0.01	4.46	3.38		0.003	0.009	**<0.001**	0.005	**<0.001**	0.016
IVC	**Paper**	**5008**	2.86	2.70	5.17	1.05	2.21	6.15		0.479	**<0.001**	0.008	0.009	0.078
IVC	**Paper**	**5053**	0.82	2.86	4.46	0.37	0.56	4.32	0.90		**0.001**	0.204	0.066	0.310
IVC	**Paper**	**5058**	14.18	24.44	25.48	11.05	7.23	22.20	16.95	11.15		0.006	0.011	0.011
IVC	**Aspen**	**5008**	1.40	6.89	6.80	1.42	0.63	5.75	4.56	1.50	7.16		0.179	0.453
IVC	**Aspen**	**5053**	4.07	9.53	11.86	2.16	1.66	10.40	5.02	2.29	4.73	1.57		0.306
IVC	**Aspen**	**5058**	2.02	4.86	4.82	1.03	0.88	4.09	2.50	1.14	5.89	0.80	1.15	

Supplementary Table S1. Results of PERMANOVA testing of Bray-Curtis distance matrices between the fecal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation, one week after arrival. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing and maintained $p < 0.05$. Associated F values shown in lower left.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		0.010	**<0.001**	0.064	**<0.001**	**<0.001**	0.050	**<0.001**	0.002	0.005	**<0.001**	0.002
Static	**Paper**	**5053**	2.34		0.023	**<0.001**	0.086	0.002	0.001	**<0.001**	**<0.001**	**<0.001**	0.063	0.011
Static	**Paper**	**5058**	2.90	2.03		**<0.001**	0.049	0.060	0.090	0.003	0.007	0.255	0.030	0.888
Static	**Aspen**	**5008**	1.81	3.95	3.52		0.008	0.004	0.268	**<0.001**	0.003	0.013	0.002	0.018
Static	**Aspen**	**5053**	2.82	1.62	1.76	2.39		0.029	0.122	0.265	0.121	0.017	0.727	0.063
Static	**Aspen**	**5058**	2.84	2.50	1.72	2.65	1.86		0.010	**<0.001**	0.004	0.037	0.010	0.223
IVC	**Paper**	**5008**	1.79	3.01	1.56	1.25	1.45	2.12		0.019	0.003	0.409	0.006	0.094
IVC	**Paper**	**5053**	3.31	2.96	2.37	2.93	1.24	3.23	1.87		0.070	0.026	0.751	0.021
IVC	**Paper**	**5058**	2.73	2.90	2.36	2.78	1.51	2.42	2.39	1.60		0.005	0.971	0.049
IVC	**Aspen**	**5008**	2.54	3.24	1.26	2.30	2.01	1.84	1.06	1.81	2.15		0.072	0.407
IVC	**Aspen**	**5053**	2.70	1.73	1.91	2.96	0.76	2.12	2.25	0.74	0.32	1.64		0.067
IVC	**Aspen**	**5058**	2.70	2.18	0.49	2.24	1.68	1.31	1.62	2.06	1.85	1.05	1.72	

Supplementary Table S2. Results of PERMANOVA testing of Jaccard distance matrices between the fecal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation, one week after arrival. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing and maintained $p < 0.05$. Associated F values shown in lower left.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		0.096	0.334	0.223	0.011	0.354	0.191	0.269	0.015	0.008	0.001	0.228
Static	**Paper**	**5053**	2.18		0.100	0.205	0.050	0.048	0.152	0.303	0.049	0.011	0.002	0.412
Static	**Paper**	**5058**	1.08	2.01		0.463	0.003	0.494	0.554	0.154	0.095	0.046	0.005	0.495
Static	**Aspen**	**5008**	1.41	1.46	0.81		0.010	0.513	0.451	0.159	0.092	0.059	0.003	0.907
Static	**Aspen**	**5053**	4.73	2.58	4.99	4.20		**<0.001**	0.011	0.364	0.007	0.001	0.002	0.010
Static	**Aspen**	**5058**	1.05	2.55	0.83	0.79	6.60		0.231	0.064	0.009	0.002	**0.001**	0.697
IVC	**Paper**	**5008**	1.67	1.78	0.52	0.73	4.68	1.42		0.139	0.491	0.375	0.037	0.505
IVC	**Paper**	**5053**	1.23	1.17	1.84	1.77	0.99	2.46	2.06		0.050	0.021	0.017	0.183
IVC	**Paper**	**5058**	4.76	2.69	2.19	2.21	4.60	4.48	0.76	3.01		0.943	0.322	0.096
IVC	**Aspen**	**5008**	6.03	3.95	3.01	2.68	7.33	6.40	0.94	4.33	0.25		0.102	0.042
IVC	**Aspen**	**5053**	10.41	6.50	6.58	7.18	7.70	12.53	3.64	5.21	1.14	2.07		0.003
IVC	**Aspen**	**5058**	1.42	0.98	0.77	0.27	4.09	0.55	0.71	1.66	2.22	2.91	7.37	

Supplementary Table S3. Results of PERMANOVA testing of Bray-Curtis distance matrices between the fecal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation for 13 weeks. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing and maintained $p < 0.05$. Associated F values shown in lower left.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		0.002	0.012	0.034	**<0.001**	**<0.001**	0.028	**<0.001**	0.001	0.044	**<0.001**	0.032
Static	**Paper**	**5053**	2.98		0.368	0.003	0.034	0.006	0.006	**<0.001**	0.001	0.007	0.002	0.014
Static	**Paper**	**5058**	2.44	1.09		0.008	0.052	0.744	0.007	0.001	0.051	0.001	0.001	0.035
Static	**Aspen**	**5008**	2.12	3.49	2.93		**<0.001**	**<0.001**	0.017	**<0.001**	**0.001**	0.043	0.004	0.036
Static	**Aspen**	**5053**	4.78	2.15	1.85	5.71		0.001	0.001	**<0.001**	**<0.001**	**<0.001**	0.002	**<0.001**
Static	**Aspen**	**5058**	3.63	2.68	0.68	4.73	3.44		0.010	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
IVC	**Paper**	**5008**	2.18	3.03	2.45	2.39	4.05	2.48		**0.001**	**<0.001**	0.011	**<0.001**	0.006
IVC	**Paper**	**5053**	4.14	4.02	4.00	5.24	5.06	6.96	4.68		**<0.001**	0.004	0.008	0.003
IVC	**Paper**	**5058**	3.70	3.72	2.05	3.47	5.38	4.67	4.57	3.69		0.010	0.036	0.112
IVC	**Aspen**	**5008**	2.08	2.73	3.16	2.17	4.05	5.25	2.85	3.93	2.64		0.034	0.247
IVC	**Aspen**	**5053**	3.65	3.39	3.08	3.08	2.92	5.51	3.94	2.39	2.16	2.30		0.019
IVC	**Aspen**	**5058**	2.08	2.43	1.92	2.02	4.04	3.20	2.91	2.98	1.62	1.31	2.32	

Supplementary Table S4. Results of PERMANOVA testing of Jaccard distance matrices between the fecal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation for 13 weeks. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing and maintained $p < 0.05$. Associated F values shown in lower left.

Caging	Bedding	Diet	OTU count	Chao1	Shannon	Simpson
Static	**Paperchip**	**5008 (*n* = 12)**	48.5 ± 1.9	540 ± 48	4.34 ± 0.24	0.969 ± 0.009
Static	**Paperchip**	**5053 (*n* = 12)**	50.3 ± 4.2	591 ± 77	4.40 ± 0.32	0.964 ± 0.015
Static	**Paperchip**	**5058 (*n* = 7)**	48.4 ± 3.6	547 ± 52	4.38 ± 0.38	0.968 ± 0.015
Static	**Aspen**	**5008 (*n* = 10)**	46.6 ± 2.5	561 ± 81	4.47 ± 0.23	0.972 ± 0.007
Static	**Aspen**	**5053 (*n* = 12)**	50.1 ± 2.9	591 ± 74	4.30 ± 0.23	0.958 ± 0.012
Static	**Aspen**	**5058 (*n* = 12)**	48.6 ± 3.8	567 ± 60	4.42 ± 0.22	0.969 ± 0.008
IVC	**Paperchip**	**5008 (*n* = 6)**	47.3 ± 3.1	551 ± 92	4.58 ± 0.28	0.976 ± 0.004
IVC	**Paperchip**	**5053 (*n* = 7)**	44.1 ± 4.1	494 ± 98	4.02 ± 0.23	0.951 ± 0.012
IVC	**Paperchip**	**5058 (*n* = 10)**	45.8 ± 4.5	543 ± 66	4.29 ± 0.31	0.958 ± 0.016
IVC	**Aspen**	**5008 (*n* = 12)**	48.9 ± 4.5	603 ± 39	4.65 ± 0.12	0.976 ± 0.003
IVC	**Aspen**	**5053 (*n* = 11)**	46.6 ± 2.0	535 ± 46	4.28 ± 0.22	0.953 ± 0.015
IVC	**Aspen**	**5058 (*n* = 11)**	48.2 ± 3.5	543 ± 36	4.41 ± 0.15	0.970 ± 0.009

Supplementary Table S5. Mean ± standard deviation number of operational taxonomic units (OTU count), Chao1, Shannon, and Simpson diversity indices of the fecal microbiota of adult mice housed under various combinations of caging, bedding, and dietary formulations for 13 weeks. IVC = individually ventilated cages. Results of statistical testing for main effects and interactions provided in **Table 1** of the main text.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		0.090	0.920	0.260	0.326	0.004	0.095	0.098	0.032	0.017	0.015	0.018
Static	**Paper**	**5053**	2.35		0.122	0.110	0.042	**<0.001**	0.001	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Paper**	**5058**	0.19	1.75		0.473	0.320	0.005	0.067	0.111	0.044	0.013	0.011	0.014
Static	**Aspen**	**5008**	1.31	1.96	0.76		0.031	**<0.001**	0.003	0.004	**0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Aspen**	**5053**	1.01	2.99	1.12	3.76		0.046	0.270	0.302	0.163	0.059	0.049	0.057
Static	**Aspen**	**5058**	6.56	17.95	7.34	20.55	3.01		0.774	0.362	0.968	0.487	0.055	0.213
IVC	**Paper**	**5008**	2.69	9.32	3.71	11.03	1.34	0.37		0.737	0.978	0.603	0.177	0.332
IVC	**Paper**	**5053**	2.55	8.59	2.64	9.03	1.15	0.92	0.26		0.634	0.438	0.172	0.394
IVC	**Paper**	**5058**	4.14	11.78	4.40	13.18	1.76	0.09	0.05	0.38		0.543	0.127	0.243
IVC	**Aspen**	**5008**	5.12	16.95	6.41	18.50	2.82	0.77	0.53	0.75	0.57		0.305	0.635
IVC	**Aspen**	**5053**	5.27	16.10	6.41	18.47	2.87	2.78	1.74	1.73	2.10	1.14		0.528
IVC	**Aspen**	**5058**	4.96	16.18	6.08	17.36	2.95	1.45	1.05	0.83	1.37	0.44	0.62	

Supplementary Table S6. Results of PERMANOVA testing of Bray-Curtis distances between the jejunal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation for 13 weeks. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing with adjusted $p < 0.05$. Associated F values shown in lower left.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		0.034	0.260	0.180	0.045	0.004	0.021	**<0.001**	0.011	**<0.001**	**<0.001**	**<0.001**
Static	**Paper**	**5053**	1.86		0.243	0.054	0.115	0.018	0.008	**<0.001**	0.003	**<0.001**	**<0.001**	**<0.001**
Static	**Paper**	**5058**	1.14	1.20		0.158	0.800	0.075	0.038	0.016	0.175	0.002	**0.001**	**0.001**
Static	**Aspen**	**5008**	1.28	1.59	1.31		0.017	0.013	0.055	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Aspen**	**5053**	1.62	1.41	0.71	1.91		0.020	0.002	**<0.001**	0.004	**<0.001**	**<0.001**	**<0.001**
Static	**Aspen**	**5058**	2.89	1.94	1.56	1.86	2.03		0.052	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
IVC	**Paper**	**5008**	2.51	2.34	1.94	1.76	3.25	1.70		0.004	0.004	**0.001**	**<0.001**	**<0.001**
IVC	**Paper**	**5053**	3.13	3.45	2.11	3.81	2.89	4.13	3.27		0.176	0.201	0.058	0.021
IVC	**Paper**	**5058**	1.94	2.64	1.30	2.88	2.41	3.16	2.90	1.30		0.197	**<0.001**	0.004
IVC	**Aspen**	**5008**	2.93	3.82	2.60	3.72	3.25	4.99	4.05	1.23	1.23		0.010	0.017
IVC	**Aspen**	**5053**	4.44	5.05	3.33	6.06	3.65	6.99	5.14	1.65	2.82	1.99		0.001
IVC	**Aspen**	**5058**	3.94	4.42	2.73	4.57	3.76	4.69	3.88	1.81	2.07	1.76	2.30	

Supplementary Table S7. Results of PERMANOVA testing of Jaccard distance matrices between the jejunal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation for 13 weeks. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing with adjusted $p < 0.05$. Associated F values shown in lower left.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		0.296	0.453	0.202	0.035	0.003	0.250	0.323	0.087	0.118	0.062	0.200
Static	**Paper**	**5053**	1.14		0.229	0.027	0.083	0.003	0.386	0.389	0.167	0.019	0.078	0.172
Static	**Paper**	**5058**	0.85	1.38		0.173	0.258	0.078	0.312	0.885	0.407	0.678	0.284	0.346
Static	**Aspen**	**5008**	1.50	3.84	1.61		0.004	0.003	0.029	0.133	0.041	0.102	0.058	0.039
Static	**Aspen**	**5053**	2.98	2.16	1.34	5.06		0.143	0.121	0.183	0.283	0.016	0.222	0.112
Static	**Aspen**	**5058**	4.34	3.79	2.02	5.55	1.70		0.031	0.106	0.477	0.017	0.172	0.078
IVC	**Paper**	**5008**	1.26	1.00	1.16	3.25	1.78	2.33		0.562	0.576	0.154	0.273	0.465
IVC	**Paper**	**5053**	1.09	0.96	0.27	1.97	1.58	1.95	0.73		0.818	0.398	0.620	0.317
IVC	**Paper**	**5058**	2.01	1.59	0.99	2.56	1.21	0.89	0.77	0.45		0.215	0.987	0.610
IVC	**Aspen**	**5008**	1.97	3.42	0.60	2.10	3.44	2.99	1.62	0.97	1.41		0.188	0.191
IVC	**Aspen**	**5053**	2.58	2.26	1.23	2.86	1.42	1.61	1.26	0.60	0.13	1.58		0.300
IVC	**Aspen**	**5058**	1.44	1.50	1.09	2.92	1.97	2.06	0.92	1.14	0.69	1.47	1.20	

Supplementary Table S8. Results of PERMANOVA testing of Bray-Curtis distance matrices between the ileal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation for 13 weeks. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing with adjusted $p < 0.05$. Associated F values shown in lower left.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		0.031	0.021	0.020	0.006	**0.001**	0.002	**<0.001**	**<0.001**	0.026	0.002	**<0.001**
Static	**Paper**	**5053**	1.82		0.336	0.078	0.133	0.018	0.005	**<0.001**	0.002	0.051	0.037	0.001
Static	**Paper**	**5058**	1.82	1.08		0.003	0.119	0.136	**<0.001**	**<0.001**	0.003	0.009	0.033	0.047
Static	**Aspen**	**5008**	1.82	1.57	2.14		**<0.001**	0.001	0.003	**<0.001**	**<0.001**	0.070	0.091	**0.001**
Static	**Aspen**	**5053**	2.22	1.40	1.35	2.65		0.020	**0.001**	**<0.001**	**<0.001**	0.009	**0.001**	**0.001**
Static	**Aspen**	**5058**	2.25	1.88	1.29	2.29	1.79		**<0.001**	**<0.001**	0.001	0.004	**0.001**	0.015
IVC	**Paper**	**5008**	1.97	2.23	2.51	1.96	2.81	2.37		**<0.001**	0.001	0.032	0.013	0.002
IVC	**Paper**	**5053**	3.33	3.61	3.17	3.69	3.64	2.70	2.31		0.001	**<0.001**	**<0.001**	**<0.001**
IVC	**Paper**	**5058**	2.90	2.69	2.06	2.41	3.28	2.08	2.10	2.39		0.008	0.063	0.533
IVC	**Aspen**	**5008**	1.97	1.81	2.16	1.59	2.39	2.30	1.70	3.35	2.28		0.095	0.010
IVC	**Aspen**	**5053**	2.03	1.62	1.53	1.39	2.23	2.12	1.63	2.30	1.40	1.41		0.114
IVC	**Aspen**	**5058**	2.48	2.51	1.55	2.35	2.63	1.72	2.10	2.94	0.93	2.11	1.33	

Supplementary Table S9. Results of PERMANOVA testing of Jaccard distance matrices between the ileal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation for 13 weeks. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing with adjusted $p < 0.05$. Associated F values shown in lower left.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.551	**<0.001**	0.028	0.099	0.013	0.631
Static	**Paper**	**5053**	8.30		0.398	**<0.001**	**<0.001**	**<0.001**	0.030	0.016	0.002	**0.003**	0.008	**0.002**
Static	**Paper**	**5058**	10.64	0.98		**<0.001**	**<0.001**	**<0.001**	0.005	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Aspen**	**5008**	50.87	27.89	26.90		0.039	0.225	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Aspen**	**5053**	35.51	17.73	17.26	3.71		0.020	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Aspen**	**5058**	56.71	33.12	32.02	1.38	4.20		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
IVC	**Paper**	**5008**	0.87	2.77	3.22	19.04	13.9	22.41		0.308	0.226	0.591	0.268	0.276
IVC	**Paper**	**5053**	5.84	4.14	5.91	39.18	25.43	44.58	1.18		0.102	0.010	0.168	0.022
IVC	**Paper**	**5058**	2.31	5.41	6.01	38.94	28.57	45.16	1.36	1.85		0.193	0.589	0.636
IVC	**Aspen**	**5008**	1.82	7.28	8.35	41.24	29.66	46.84	0.77	3.69	1.48		0.015	0.176
IVC	**Aspen**	**5053**	3.74	4.66	6.06	45.89	31.1	51.81	1.26	1.62	0.77	3.04		0.149
IVC	**Aspen**	**5058**	2.09	6.94	7.55	43.35	31.65	49.27	1.20	3.17	0.69	1.55	1.63	

Supplementary Table S10. Results of PERMANOVA testing of Bray-Curtis distance matrices between the cecal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation for 13 weeks. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing with adjusted $p < 0.05$. Associated F values shown in lower left.

Caging			Static	Static	Static	Static	Static	Static	IVC	IVC	IVC	IVC	IVC	IVC
	Bedding		Paper	Paper	Paper	Aspen	Aspen	Aspen	Paper	Paper	Paper	Aspen	Aspen	Aspen
		Diet	5008	5053	5058	5008	5053	5058	5008	5053	5058	5008	5053	5058
Static	**Paper**	**5008**		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Paper**	**5053**	3.92		0.063	0.003	0.003	**<0.001**	0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Paper**	**5058**	6.21	1.94		0.002	0.032	0.002	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Aspen**	**5008**	5.21	3.20	2.92		0.025	0.246	0.007	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Static	**Aspen**	**5053**	6.56	2.86	2.21	2.17		0.011	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.002	**<0.001**
Static	**Aspen**	**5058**	5.24	4.95	3.59	1.30	2.38		0.002	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.002
IVC	**Paper**	**5008**	3.53	4.09	4.91	2.57	5.07	3.41		**0.001**	0.080	0.097	0.016	**<0.001**
IVC	**Paper**	**5053**	6.35	6.64	7.90	4.58	3.83	4.41	3.86		**<0.001**	**<0.001**	0.175	0.022
IVC	**Paper**	**5058**	5.58	3.85	4.15	5.21	5.81	5.15	1.81	6.11		0.003	0.032	0.068
IVC	**Aspen**	**5008**	5.06	4.05	5.86	4.36	5.06	4.71	1.71	4.68	3.00		0.019	0.005
IVC	**Aspen**	**5053**	4.22	4.32	4.51	4.21	3.10	3.72	2.61	1.43	2.23	2.62		0.029
IVC	**Aspen**	**5058**	5.32	5.21	4.87	3.35	5.46	3.06	2.40	4.69	1.89	3.27	2.40	

Supplementary Table S11. Results of PERMANOVA testing of Jaccard distance matrices between the cecal microbiota of groups of mice housed under various combinations of caging (static microisolators versus individually ventilated cages (IVC)), bedding (compressed paper versus aspen chip), and dietary formulation for 13 weeks. Unadjusted *p* values shown in upper right; bold values withstood correction for multiple testing with adjusted $p < 0.05$. Associated F values shown in lower left.

Caging	Bedding	Diet	OTU count	Chao1	Shannon	Simpson
Static	**Paperchip**	**5008 (*n* = 12)**	48.2 ± 1.8	696 ± 32	4.67 ± 0.17	0.978 ± 0.006
Static	**Paperchip**	**5053 (*n* = 12)**	49.4 ± 4.5	734 ± 90	4.21 ± 0.54	0.936 ± 0.047
Static	**Paperchip**	**5058 (*n* = 11)**	48.7 ± 2.1	783 ± 34	4.45 ± 0.28	0.963 ± 0.015
Static	**Aspen**	**5008 (*n* = 8)**	43.9 ± 5.6	457 ± 137	3.70 ± 0.48	0.938 ± 0.039
Static	**Aspen**	**5053 (*n* = 9)**	46.4 ± 3.0	540 ± 101	3.69 ± 0.30	0.922 ± 0.032
Static	**Aspen**	**5058 (*n* = 10)**	42.8 ± 5.4	429 ± 96	3.86 ± 0.28	0.953 ± 0.016
IVC	**Paperchip**	**5008 (*n* = 9)**	44.3 ± 3.8	631 ± 108	4.54 ± 0.64	0.967 ± 0.037
IVC	**Paperchip**	**5053 (*n* = 11)**	43.3 ± 3.3	605 ± 47	4.45 ± 0.14	0.969 ± 0.007
IVC	**Paperchip**	**5058 (*n* = 12)**	45.6 ± 1.8	616 ± 50	4.52 ± 0.20	0.970 ± 0.011
IVC	**Aspen**	**5008 (*n* = 12)**	46.8 ± 2.6	667 ± 32	4.81 ± 0.08	0.982 ± 0.003
IVC	**Aspen**	**5053 (*n* = 12)**	43.4 ± 1.8	610 ± 54	4.49 ± 0.19	0.970 ± 0.009
IVC	**Aspen**	**5058 (*n* = 12)**	44.0 ± 4.5	614 ± 42	4.62 ± 0.19	0.972 ± 0.012

Supplementary Table S12. Mean ± standard deviation number of operational taxonomic units (OTU count), Chao1, Shannon, and Simpson diversity indices of the cecal microbiota of adult mice housed under various combinations of caging, bedding, and dietary formulations for 13 weeks. IVC = individually ventilated cages. Results of statistical testing for main effects and interactions provided in **Table 2** of the main text.