Gut microbiome disturbances of altricial Blue and Great tit nestlings are countered by continuous microbial inoculations from parental microbiomes

David Diez-Méndez[1*a], Kasun H. Bodawatta[2*], Inga Freiberga[1], Irena Klečková[1], Knud A. Jønsson[2], Michael Poulsen[3], and Katerina Sam[1,4]

[1] Biology Centre of Czech Academy of Sciences, Institute of Entomology, České Budějovice, Czech Republic

[2] Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

[3] Section for Ecology and Evolution, Department of Biology, University of Copenhagen, Copenhagen, Denmark

[4] Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

[*] These authors contributed equally to this work

[a] Corresponding author: david.diezmendez@gmail.com

Table S1: Great tit data from the efficacy experiment showing the number of the metallic ring, the sex, and the treatment of an individual. Weight data shows the change in body mass since the day they were mist-netted (day 0) until the day they were released back to their habitat (day 8).

			Weight (g)					
Ring no.	**Sex**	**Treatment**	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 8**
N941680	Male	Antibiotics	18.32	16.54	16.54	16.09	16.28	15.48
N941682	Male	Probiotics	18.48	17.10	16.69	16.61	16.40	15.94
N941677	Male	Antibiotics	19.38	17.39	17.45	16.72	16.75	15.97
N941678	Female	Probiotics	17.78	16.15	16.00	15.86	15.51	14.95
N941676	Female	Antibiotics	17.15	15.80	15.41	15.35	15.52	14.30
N941698	Male	Probiotics	19.03	18.19	17.25	17.05	16.56	15.72
N941699	Male	Antibiotics	17.87	17.53	16.51	16.30	16.33	16.39
N941697	Male	Probiotics	17.53	16.37	15.87	15.74	15.55	15.70
N930804	Male	Antibiotics	17.50	17.60	17.27	17.15	17.07	16.59
N930805	Male	Probiotics	16.99	16.93	16.73	16.60	16.87	16.16

Table S2: Doses applied to the experimental hatchlings during the field experiment. Antibiotic and probiotic doses were diluted in a water volume similar to the indicated for control doses.

	Day 1	**Day 4**	**Day 7**	**Day 10**	**Day 13**
Great tits					
Expected mean weight per chick (g)	1	5	10	15	18
Antibiotic (mg)	0.5	2.5	5.0	7.5	9.0
Probiotic (mg)	6.7	33.5	67.0	100.5	120.6
Control (ml)	0.1	0.15	0.175	0.2	0.2
Blue tits					
Expected mean weight per chick (g)	1	3	6	9	11
Antibiotic (mg)	0.5	1.5	3.0	4.5	5.5
Probiotic (mg)	6.7	20.1	40.2	60.3	73.7
Control (ml)	0.1	0.15	0.175	0.2	0.2

Table S3. Estimates resulting from the linear mixed-effect models of antibiotic and probiotic treatments, day of experiment and host sex on alpha diversities of gut microbiomes in the efficacy experiment. The categorical variable *treatment* was assessed in reference to the probiotic treatment. Individual ID was a random factor. Significant results are indicated in bold font.

	Estimate	SE	t value	p
Observed ASV richness				
$R^2_{fixed} = 0.29$, $R^2_{fixed+random\ effects} = 0.63$				
Intercept	246.90	86.965	2.839	
Treatment	-5.18	63.072	-0.082	0.937
Day	**-485.06**	**181.48**	**-2.673**	**0.011**
Day^2	86.68	140.495	0.617	0.541
Weight	-1.60	35.375	-0.045	0.964
Sex	-57.56	94.743	-0.608	0.556
Treatment*Day	-12.62	186.789	-0.068	0.947
Treatment*Day^2	190.49	189.013	1.008	0.321
Faith's PD				
$R^2_{fixed} = 0.27$, $R^2_{fixed+random\ effects} = 0.46$				
Intercept	11.14	1.882	5.922	
Treatment	0.09	1.290	0.069	0.947
Day	**-13.97**	**4.941**	**-2.827**	**0.007**
Day^2	5.30	4.027	1.315	0.197
Weight	-0.38	0.904	-0.419	0.678
Sex	-0.56	2.097	-0.265	0.796
Treatment*Day	2.97	5.391	0.551	0.586
Treatment*Day^2	0.30	5.444	0.055	0.957
Shannon's diversity index				
$R^2_{fixed} = 0.38$, $R^2_{fixed+random\ effects} = 0.60$				
Intercept	4.86	0.709	6.861	
Treatment	-0.79	0.496	-1.589	0.156
Day	-2.31	1.714	-1.346	0.186
Day^2	-0.78	1.369	-0.573	0.571
Weight	0.48	0.322	1.48	0.149
Sex	-1.03	0.784	-1.319	0.213
Treatment*Day	-1.48	1.827	-0.812	0.423
Treatment*Day^2	1.33	1.847	0.722	0.476
Relative dominance (log transformed)				
$R^2_{fixed} = 0.38$, $R^2_{fixed+random\ effects} = 0.60$				
Intercept	-2.52	0.445	-5.673	
Treatment	0.63	0.307	2.048	0.080
Day	0.65	1.132	0.579	0.566
Day^2	1.10	0.916	1.198	0.239
Weight	-0.39	0.210	-1.853	0.074
Sex	0.57	0.494	1.145	0.275
Treatment*Day	0.57	1.225	0.462	0.647
Treatment*Day^2	-0.60	1.237	-0.486	0.630

Table S4: Estimates resulting from the linear mixed-effect models of control, antibiotic and probiotic treatments and day of experiment on alfa diversities of gut microbiomes in wild chicks (field experiment). The categorical variable *treatment* was assessed in reference to the control treatment. Brood ID was a random intercept factor and chick ID was a random slope factor within day of experiment. Significant results are indicated with bold font. Table is attached as a separate excel file.

Great tits	Estimate	SE	t value	p
Observed ASV richness (square root transf.)				
$R^2_{fixed} = 0.40$, $R^2_{fixed+random\ effects} = 0.50$				
Intercept	13.24	0.574	23.074	
Antibiotic	1.01	0.548	1.841	**0.067**
Probiotic	0.55	0.552	0.99	0.323
Day	**44.25**	**7.362**	**6.011**	**<0.001**
Day^2	**-35.48**	**6.838**	**-5.188**	**<0.001**
Antibiotic*Day	3.67	10.309	0.356	0.723
Antibiotic *Day^2	-3.22	10.309	-0.312	0.756
Probiotic*Day	-2.81	9.547	-0.295	0.769
Probiotic *Day^2	4.26	9.550	0.446	0.656
Faith's PD				
$R^2_{fixed} = 0.32$, $R^2_{fixed+random\ effects} = 0.39$				
Intercept	10.49	0.427	24.576	
Antibiotic	**0.86**	**0.429**	**2.005**	**0.046**
Probiotic	0.52	0.433	1.211	0.227
Day	**29.46**	**5.292**	**5.567**	**<0.001**
Day^2	**-24.15**	**5.239**	**-4.61**	**<0.001**
Antibiotic*Day	-0.38	7.425	-0.051	0.959
Antibiotic *Day^2	-7.02	7.417	-0.947	0.345
Probiotic*Day	-3.07	7.322	-0.419	0.675
Probiotic *Day^2	4.29	7.336	0.584	0.560
Shannon's diversity index (log transf.)				
$R^2_{fixed} = 0.38$, $R^2_{fixed+random\ effects} = 0.60$				
Intercept	18.58	0.966	19.245	
Antibiotic	-0.40	0.803	-0.497	0.620
Probiotic	0.04	0.804	0.045	0.965
Day	**44.48**	**10.636**	**4.182**	**<0.001**
Day^2	**-58.66**	**11.138**	**-5.266**	**<0.001**
Antibiotic*Day	2.29	15.153	0.151	0.880
Antibiotic *Day^2	-7.25	14.978	-0.484	0.630
Probiotic*Day	1.25	15.928	0.078	0.938
Probiotic *Day^2	8.72	15.785	0.553	0.584
Relative dominance (log transf.)				
$R^2_{fixed} = 0.38$, $R^2_{fixed+random\ effects} = 0.60$				
Intercept	-2.33	0.090	-25.967	
Antibiotic	0.06	0.08	0.749	0.455
Probiotic	-0.00	0.083	-0.022	0.982
Day	-2.02	1.204	-1.677	0.099
Day^2	**4.11**	**1.211**	**3.393**	**0.001**
Antibiotic*Day	1.45	1.699	0.856	0.396
Antibiotic *Day^2	1.17	1.704	0.689	0.494
Probiotic*Day	-0.33	1.701	-0.196	0.845
Probiotic *Day^2	-0.61	1.710	-0.357	0.722

Blue tits	Estimate	SE	t value	p
Observed ASV richness (square root transf.)				
$R^2_{fixed} = 0.41$, $R^2_{fixed+random\ effects} = 0.61$				
Intercept	12.83	0.641	20.020	
Antibiotic	-0.32	0.713	-0.447	0.658
Probiotic	-0.77	0.720	-1.063	0.295
Day	**38.25**	**7.224**	**5.295**	**<0.001**
Day^2	**-20.88**	**6.222**	**-3.357**	**0.001**
Antibiotic*Day	6.053	9.826	0.616	0.542
Antibiotic *Day^2	6.012	10.270	0.585	0.562
Probiotic*Day	-2.83	8.552	-0.331	0.742
Probiotic *Day^2	-8.77	8.831	-0.993	0.323
Faith's PD				
$R^2_{fixed} = 0.32$, $R^2_{fixed+random\ effects} = 0.39$				
Intercept	10.98	0.495	22.198	
Antibiotic	-0.40	0.581	-0.696	0.490
Probiotic	-0.70	0.587	-1.184	0.244
Day	**29.30**	**5.310**	**5.519**	**<0.001**
Day^2	**-14.00**	**5.032**	**-2.783**	**0.006**
Antibiotic*Day	2.08	7.246	0.288	0.775
Antibiotic *Day^2	-1.14	7.581	-0.150	0.881
Probiotic*Day	-6.61	6.899	-0.958	0.340
Probiotic *Day^2	-3.56	7.149	-0.498	0.619
Shannon's diversity index				
$R^2_{fixed} = 0.32$, $R^2_{fixed+random\ effects} = 0.61$				
Intercept	4.16	0.140	29.589	
Antibiotic	-0.08	0.138	-0.612	0.545
Probiotic	-0.17	0.138	-1.238	0.225
Day	**6.54**	**1.442**	**4.533**	**<0.001**
Day^2	**-2.88**	**1.219**	**-2.361**	**0.021**
Antibiotic*Day	0.98	1.961	0.499	0.621
Antibiotic *Day^2	0.44	2.047	0.213	0.833
Probiotic*Day	-0.76	1.675	-0.454	0.651
Probiotic *Day^2	-1.97	1.726	-1.143	0.257
Relative dominance (log transf.)				
$R^2_{fixed} = 0.14$, $R^2_{fixed+random\ effects} = 0.60$				
Intercept	-2.33	0.134	-17.387	
Antibiotic	0.06	0.108	0.583	0.564
Probiotic	0.02	0.102	0.197	0.845
Day	-1.95	1.340	-1.453	0.156
Day^2	0.86	0.895	0.965	0.337
Antibiotic*Day	-3.01	1.812	-1.661	0.107
Antibiotic *Day^2	-1.95	1.895	-1.029	0.311
Probiotic*Day	0.12	1.226	0.099	0.921
Probiotic *Day^2	0.36	1.276	0.284	0.777

Table S5: Nest-box coordinates in decimal degrees where experimental Great tits (GT) and Blue tits (BT) bred.

Nest-box ID	Species	Latitude	Longitude
6	BT	48.975208	14.427872
7	GT	48.975376	14.427562
13	GT	48.97437	14.425271
14	BT	48.973945	14.425397
18	BT	48.973658	14.424246
20	GT	48.973796	14.42289
23	GT	48.973734	14.421232
29	GT	48.972894	14.416866
32	GT	48.9739	14.415584
37	GT	48.975645	14.415549
36	GT	48.976257	14.416205
34	BT	48.976951	14.416727
74	GT	48.978074	14.417141
78	GT	48.978969	14.415103
70	BT	48.97913	14.414627
90	BT	48.984384	14.416846
C15	BT	48.98453	14.4207
68	BT	48.984724	14.421852
93	BT	48.984421	14.423256
100	BT	48.983528	14.426514

Table S6: Estimates resulting from the linear mixed-effect models of control, antibiotic and probiotic treatments and day of experiment on body mass gain in wild chicks. Brood ID is random intercept factor. Chick ID is a random slope intercept factor within day of experiment. The categorical variable *treatment* was assessed in reference to the control treatment. Significant results are indicated with bold font.

Great tits	Estimate	SE	t value	p
$R^2_{fixed} = 0.97$, $R^2_{fixed+random\ effects} = 0.98$				
Intercept	9.07	0.140	64.992	
Antibiotic	0.08	0.173	0.449	0.656
Probiotic	0.18	0.175	1.015	0.316
Day	**95.71**	**1.392**	**68.732**	**<0.001**
Day^2	**-12.72**	**1.416**	**-8.982**	**<0.001**
Antibiotic*Day	-2.15	1.971	-1.089	0.279
Antibiotic *Day^2	-0.15	1.992	-0.074	0.941
Probiotic*Day	-1.97	2.014	-0.977	0.331
Probiotic*Day^2	-0.37	2.036	-0.182	0.856
Blue tits				
$R^2_{fixed} = 0.91$, $R^2_{fixed+random\ effects} = 0.98$				
Intercept	4.78	0.197	24.304	
Antibiotic	0.07	0.270	0.255	0.800
Probiotic	0.19	0.272	0.698	0.489
Day	**48.01**	**1.998**	**24.03**	**<0.001**
Day^2	-2.93	1.762	-1.662	0.103
Antibiotic*Day	0.78	2.725	0.287	0.775
Antibiotic *Day^2	1.14	2.813	0.405	0.688
Probiotic*Day	-0.72	2.411	-0.299	0.766
Probiotic*Day^2	-2.43	2.467	-0.984	0.329

Table S7: Estimates resulting from the linear mixed-effect models of control, antibiotic and probiotic treatments and untreated chicks on body size (tarsus length) at day 16. Brood ID is random intercept factor. The categorical variable *treatment* was assessed in reference to the control treatment. Significant results are indicated with bold font.

Great tits	Estimate	SE	t value	p
$R^2_{fixed} = 0.06$, $R^2_{fixed+random\ effects} = 0.23$				
Intercept	19.88	0.114	174.967	
Controls	0.23	0.138	1.685	0.097
Antibiotics	0.01	0.138	0.037	0.970
Probiotics	0.27	0.140	1.933	0.057
Blue tits				
$R^2_{fixed} = 0.01$, $R^2_{fixed+random\ effects} = 0.39$				
Intercept	16.55	0.200	82.558	
Controls	0.09	0.229	0.390	0.699
Antibiotics	-0.13	0.209	-0.613	0.543
Probiotics	-0.02	0.244	-0.073	0.942

Table S8: Estimates resulting from the linear mixed-effect models of control, antibiotic and probiotic treatments and untreated chicks, together with nest and parents on alpha diversities at day 16. Brood ID was a random intercept factor. The categorical variable *treatment* was assessed in reference to the control treatment. Significant results are indicated with bold font. Table is attached as a separate excel file.

Great tits	Estimate	SE	t value	p
Observed ASV richness				
$R^2_{fixed} = 0.16$, $R^2_{fixed+random\ effects} = 0.32$				
Intercept	247.82	25.485	9.724	
Antibiotic	33.64	30.276	1.111	0.270
Probiotic	11.78	30.698	0.384	0.702
Untreated	4.62	28.015	0.165	0.869
Nest	**103.38**	**35.801**	**2.888**	**0.005**
Female	78.97	51.234	1.541	0.127
Male	**-75.24**	**34.772**	**-2.164**	**0.033**
Faith's PD				
$R^2_{fixed} = 0.05$, $R^2_{fixed+random\ effects} = 0.16$				
Intercept	11.85	0.631	18.786	
Antibiotic	0.58	0.805	0.717	0.475
Probiotic	0.16	0.816	0.191	0.849
Untreated	0.05	0.745	0.066	0.947
Nest	0.99	0.953	1.041	0.301
Female	2.15	1.359	1.579	0.118
Male	-0.68	0.925	-0.737	0.463
Shannon's diversity index				
$R^2_{fixed} = 0.16$, $R^2_{fixed+random\ effects} = 0.35$				
Intercept	4.28	0.223	19.169	
Antibiotic	0.05	0.258	0.190	0.850
Probiotic	0.08	0.261	0.306	0.760
Untreated	-0.07	0.239	-0.285	0.776
Nest	0.53	0.305	1.733	0.087
Female	-0.46	0.436	-1.056	0.294
Male	**-1.05**	**0.296**	**-3.541**	**<0.001**

Blue tits	Estimate	SE	t value	p
Observed ASV richness				
$R^2_{fixed} = 0.28$, $R^2_{fixed+random\ effects} = 0.32$				
Intercept	274.26	42.528	6.449	
Antibiotic	-10.31	53.460	-0.193	0.848
Probiotic	-28.35	58.901	-0.481	0.632
Untreated	-9.05	48.709	-0.186	0.853
Nest	**210.50**	**58.282**	**3.612**	**<0.001**
Female	-56.54	58.651	-0.964	0.339
Male	-26.93	63.546	-0.424	0.673
Faith's PD				
$R^2_{fixed} = 0.16$, $R^2_{fixed+random\ effects} = 0.29$				
Intercept	13.82	1.090	12.673	
Antibiotic	-1.15	1.306	-0.883	0.381
Probiotic	-1.71	1.446	-1.183	0.242
Untreated	-0.73	1.191	-0.615	0.541
Nest	2.75	1.418	1.937	0.058

Female	-1.85	1.439	-1.284	0.204
Male	-1.52	1.561	-0.975	0.334
Shannon's diversity index				
$R^2_{fixed} = 0.29$, $R^2_{fixed+random\ effects} = 0.44$				
Intercept	4.80	0.295	16.292	
Antibiotic	-0.13	0.342	-0.369	0.713
Probiotic	-0.41	0.379	-1.090	0.280
Untreated	-0.08	0.312	-0.244	0.808
Nest	0.40	0.371	1.078	0.286
Female	**-1.35**	**0.378**	**-3.574**	**<0.001**
Male	**-1.20**	**0.410**	**-2.915**	**0.005**

Table S9: Pairwise comparisons of chick, adult and nest microbiome composition on final day based on Bray-Curtis distances. Significant results are indicated with bold font.

	F statistic	R^2	Adjusted p
Great tits			
Male vs. Control chicks	**4.003**	**0.1291**	**0.002**
Male vs. Antibiotic chicks	**4.129**	**0.1371**	**0.002**
Male vs. Probiotic chicks	**4.097**	**0.1408**	**0.002**
Male vs. Untreated chicks	**4.536**	**0.1209**	**0.002**
Male vs. Female	0.809	0.0632	0.999
Male vs. Nest	**3.353**	**0.1571**	**0.002**
Female vs. Control chicks	1.843	0.0807	0.067
Female vs. Antibiotic chicks	2.002	0.0911	0.050
Female vs. Probiotic chicks	2.025	0.0963	0.069
Female vs. Untreated chicks	**2.066**	**0.0023**	**0.048**
Female vs. Nest	1.774	0.1288	0.092
Control vs. Antibiotic chicks	0.533	0.0151	0.999
Control vs. Probiotic chicks	0.449	0.0131	0.999
Control vs. Untreated chicks	0.621	0.0146	0.999
Control chicks vs. Nest	0.847	0.0304	0.999
Antibiotic vs. Probiotic chicks	0.441	0.0132	0.999
Antibiotic vs. Untreated chicks	0.649	0.0156	0.999
Antibiotic vs. Nest	0.884	0.0329	0.999
Probiotic vs. Untreated chicks	0.585	0.0144	0.999
Probiotic chicks vs. Nest	0.873	0.0338	0.999
Untreated chicks vs. Nest	1.271	0.0371	0.999
Blue tits			
Male vs. Control chicks	**2.123**	**0.1503**	**0.025**
Male vs. Antibiotic chicks	**2.334**	**0.1273**	**0.004**
Male vs. Probiotic chicks	**2.081**	**0.1478**	**0.023**
Male vs. Untreated chicks	**2.445**	**0.0891**	**0.019**
Male vs. Female	0.799	0.0624	0.999
Male vs. Nest	**2.359**	**0.1643**	**0.011**
Female vs. Control chicks	1.934	0.1214	0.265
Female vs. Antibiotic chicks	2.035	0.1016	0.050
Female vs. Probiotic chicks	1.702	0.1084	0.905
Female vs. Untreated chicks	**2.175**	**0.0745**	**0.036**
Female vs. Nest	2.117	0.1313	0.130
Control vs. Antibiotic chicks	0.847	0.0449	0.999
Control vs. Probiotic chicks	0.872	0.0586	0.999
Control vs. Untreated chicks	0.763	0.0275	0.999
Control chicks vs. Nest	0.997	0.0665	0.999
Antibiotic vs. Probiotic chicks	0.838	0.0445	0.999
Antibiotic vs. Untreated chicks	0.721	0.0227	0.999
Antibiotic vs. Nest	0.994	0.0523	0.999
Probiotic vs. Untreated chicks	0.768	0.0277	0.999
Probiotic chicks vs. Nest	0.961	0.0643	0.999
Untreated chicks vs. Nest	1.017	0.0363	0.999

Table S10: Estimates resulting from the linear mixed-effect models of nest and chick categories and day of experiment on alpha diversities. Brood ID is random intercept factor. The categorical variable *chicks* was assessed in reference to *nests*. Significant results are indicated with bold font.

Great tits	Estimate	SE	t value	p
Observed ASV richness				
$R^2_{fixed} = 0.48$, $R^2_{fixed+random\ effects} = 0.52$				
Intercept	304.70	30.36	10.038	
Day of experiment	46.50	40.92	1.136	0.258
Chicks	**-223.86**	**32.26**	**-6.939**	**<0.001**
Day of experiment*Chicks	**131.55**	**44.55**	**2.953**	**0.004**
Faith's PD				
$R^2_{fixed} = 0.43$, $R^2_{fixed+random\ effects} = 0.48$				
Intercept	12.26	0.785	15.625	
Day of experiment	0.58	1.067	0.541	0.589
Chicks	**-4.85**	**0.841**	**-5.764**	**<0.001**
Day of experiment*Chicks	**4.01**	**1.161**	**3.456**	**<0.001**
Shannon's diversity index				
$R^2_{fixed} = 0.29$, $R^2_{fixed+random\ effects} = 0.43$				
Intercept	4.44	0.236	18.763	
Day of experiment	0.37	0.298	1.248	0.215
Chicks	**-1.08**	**0.235**	**-4.585**	**<0.001**
Day of experiment*Chicks	0.55	0.325	1.686	0.094

Blue tits	Estimate	SE	t value	p
Observed ASV richness				
$R^2_{fixed} = 0.63$, $R^2_{fixed+random\ effects} = 0.66$				
Intercept	379.70	31.55	12.033	
Day of experiment	**101.91**	**45.56**	**2.237**	**0.028**
Chicks	**-309.60**	**33.70**	**-9.187**	**<0.001**
Day of experiment*Chicks	86.05	49.71	1.731	0.087
Faith's PD				
$R^2_{fixed} = 0.61$, $R^2_{fixed+random\ effects} = 0.65$				
Intercept	13.74	0.787	17.455	
Day of experiment	**2.58**	**1.121**	**2.304**	**0.023**
Chicks	**-6.47**	**0.829**	**-7.805**	**<0.001**
Day of experiment*Chicks	**2.84**	**1.223**	**2.323**	**0.022**
Shannon's diversity index				
$R^2_{fixed} = 0.58$, $R^2_{fixed+random\ effects} = 0.60$				
Intercept	4.99	0.193	25.852	
Day of experiment	0.15	0.282	0.538	0.592
Chicks	**-1.67**	**0.209**	**-7.995**	**<0.001**
Day of experiment*Chicks	**1.16**	**0.308**	**3.751**	**<0.001**

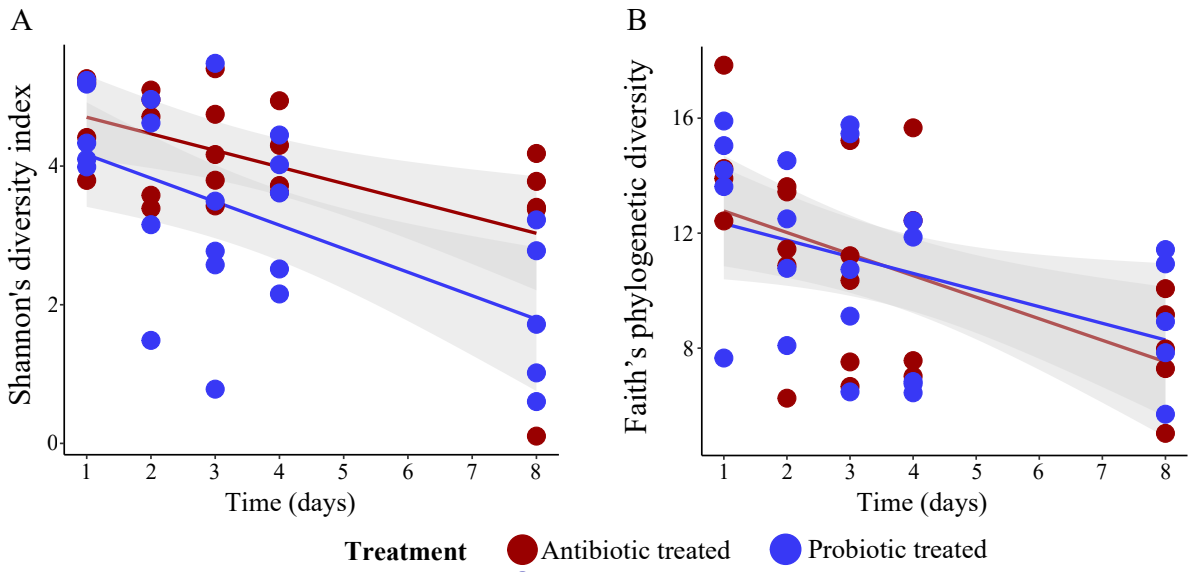

Fig S1. Alfa diversity patterns during the efficacy experiment: Shannon's diversity (A) and Faith's PD (B) in gut microbiomes of antibiotic and probiotic treated individuals.

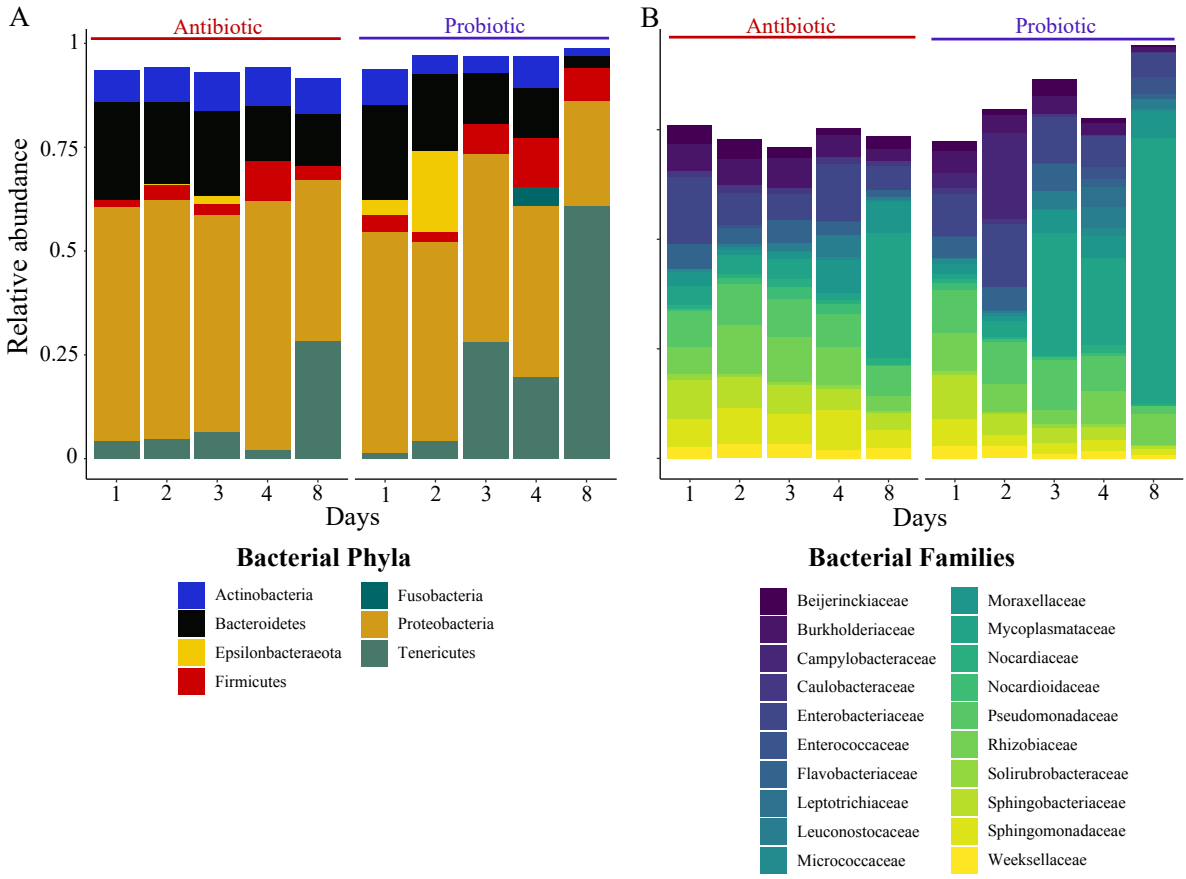

Fig S2. Relative abundance of main bacterial phyla (A) and the 20 most abundant bacterial families (B) in microbiomes of antibiotic and probiotic treated individuals across sampling days. Low abundant bacterial phyla and families are not shown in the figure.

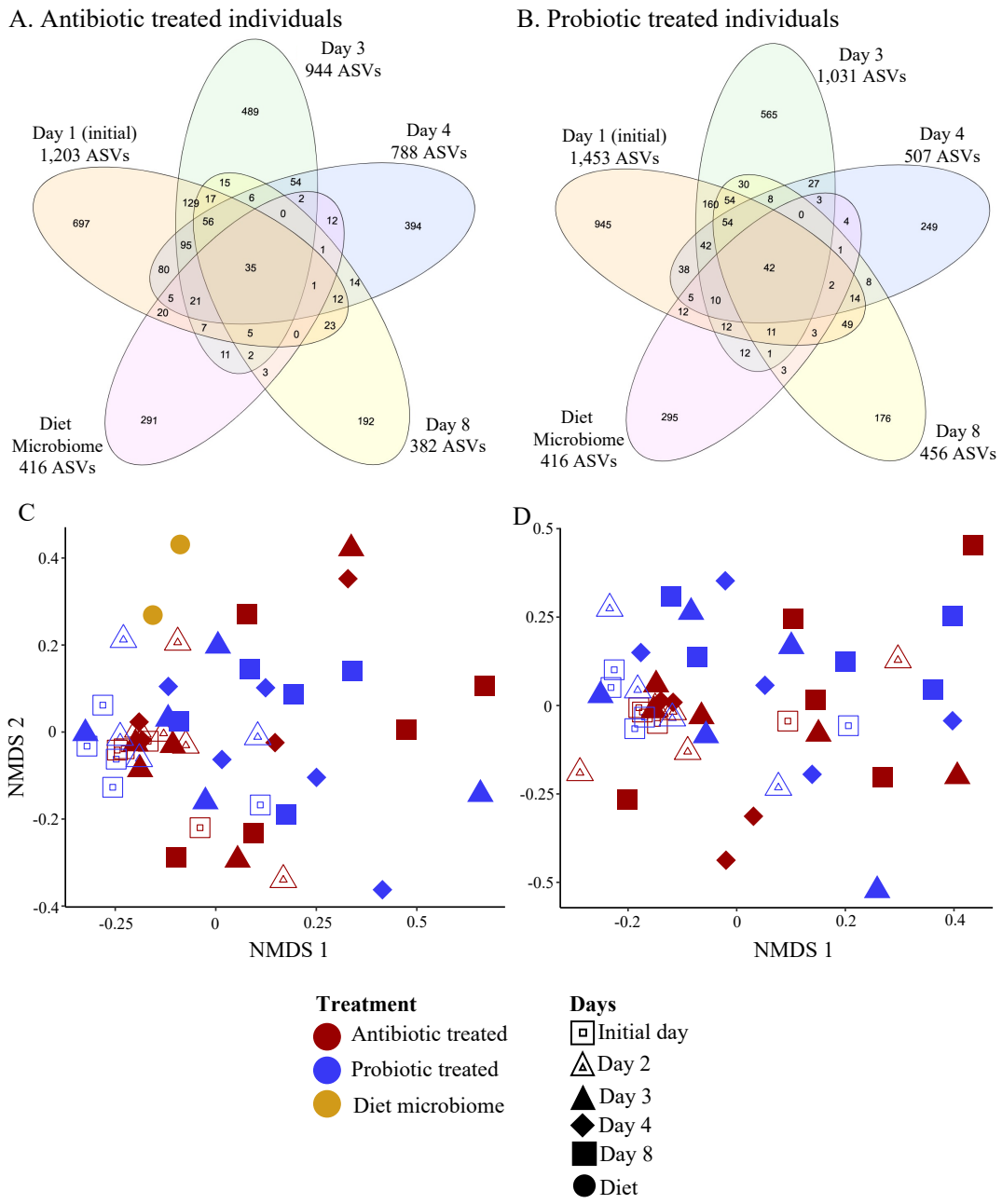

Fig S3. Flower plots depicting the shared ASVs in microbiomes of individuals during the treatment period (**A**: antibiotic treated, **B**: probiotic treated) and food microbiome in the efficacy experiment. NMDS plots showing the compositional similarity (based on Bray-Curtis distances) of gut microbiomes of treated individuals and the food microbiomes (stress = 0.2519) (**C**) and the compositional similarity of gut microbiomes after removing any ASV found in the food microbiome (stress = 0.2304) (**D**). The removal of food-born microbes from the analyses did not influence the statistical outcome of the effect of antibiotic/probiotic treatment over time (PERMANOVAs: Antibiotic $_{(Bray\text{-}Curtis)}$: $F = 1.063$, $R^2 = 0.1911$, $p = 0.2069$; Probiotic $_{(Bray\text{-}Curtis)}$: $F = 1.209$, $R^2 = 0.2029$, $p = 0.0997$).

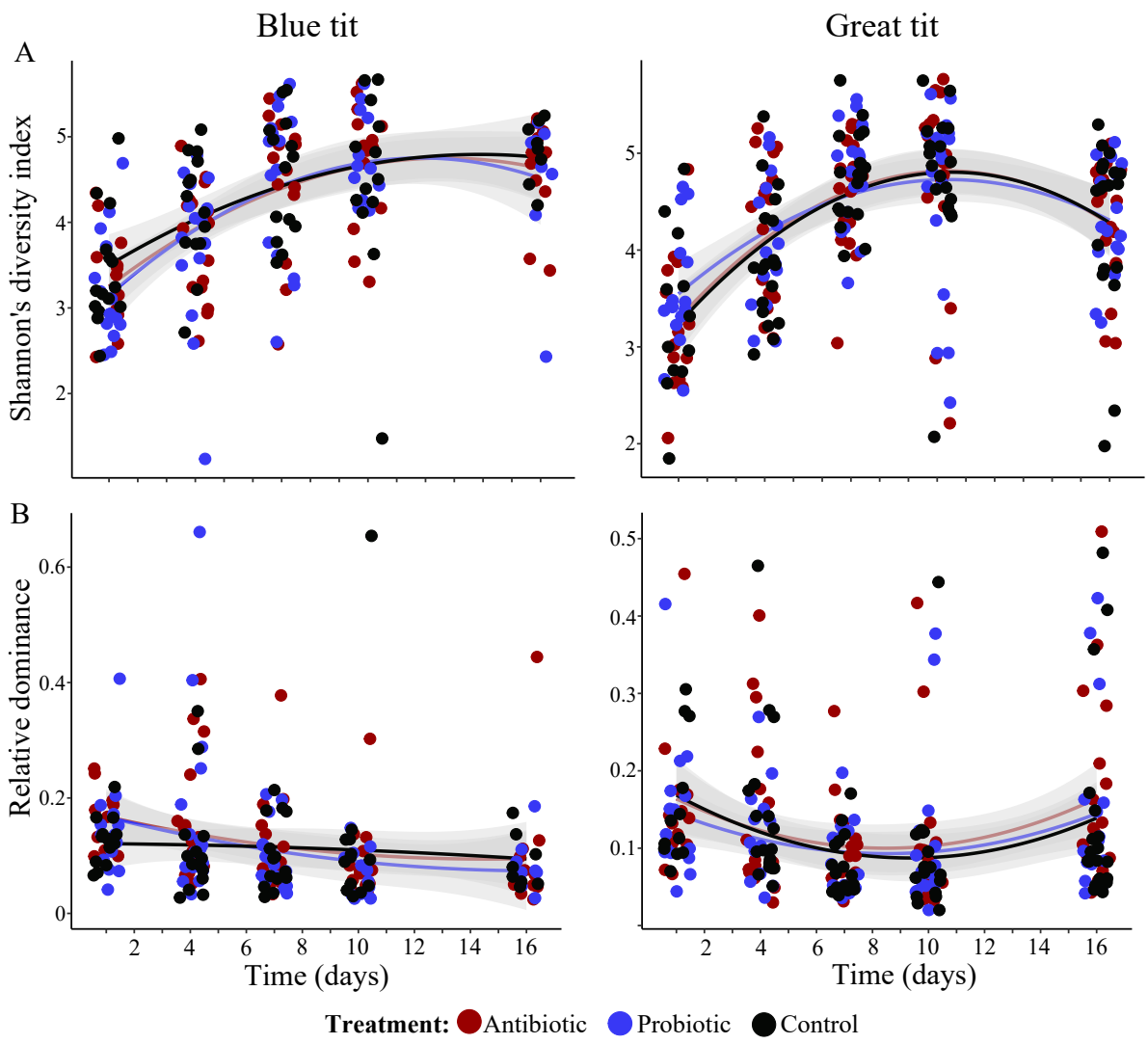

Fig S4. Association between days and Shannon's diversity index (A), and relative dominance (B) of gut microbial communities of developing chicks of *P. major* and *C. caeruleus*.

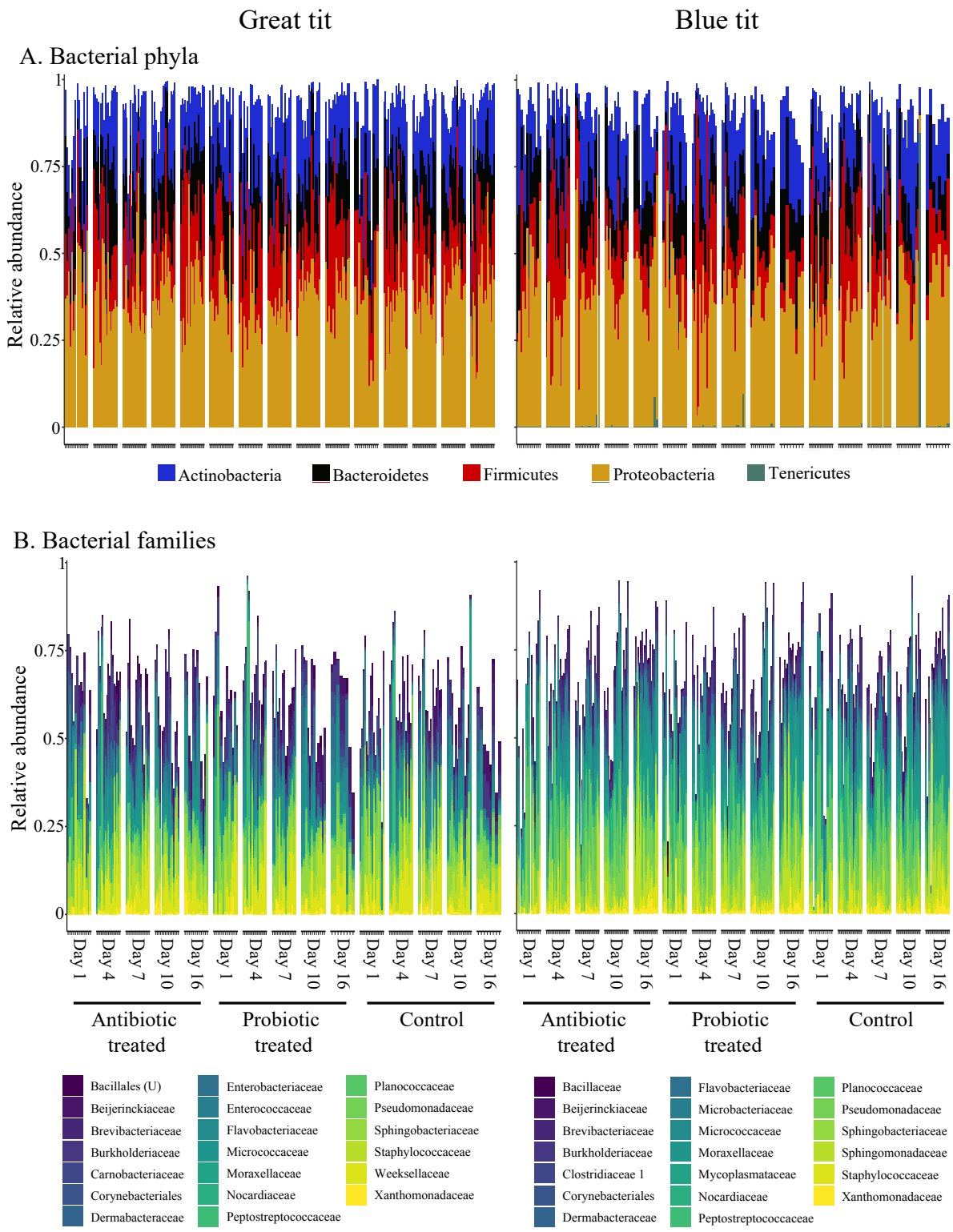

Fig S5. Relative abundance of main bacterial phyla (A) and 20 most abundant bacterial families (B) in microbiomes of chicks during the manipulation period. Each bar represent an individual and empty areas above each bar represent the relative abundance of other bacterial phyla and families.

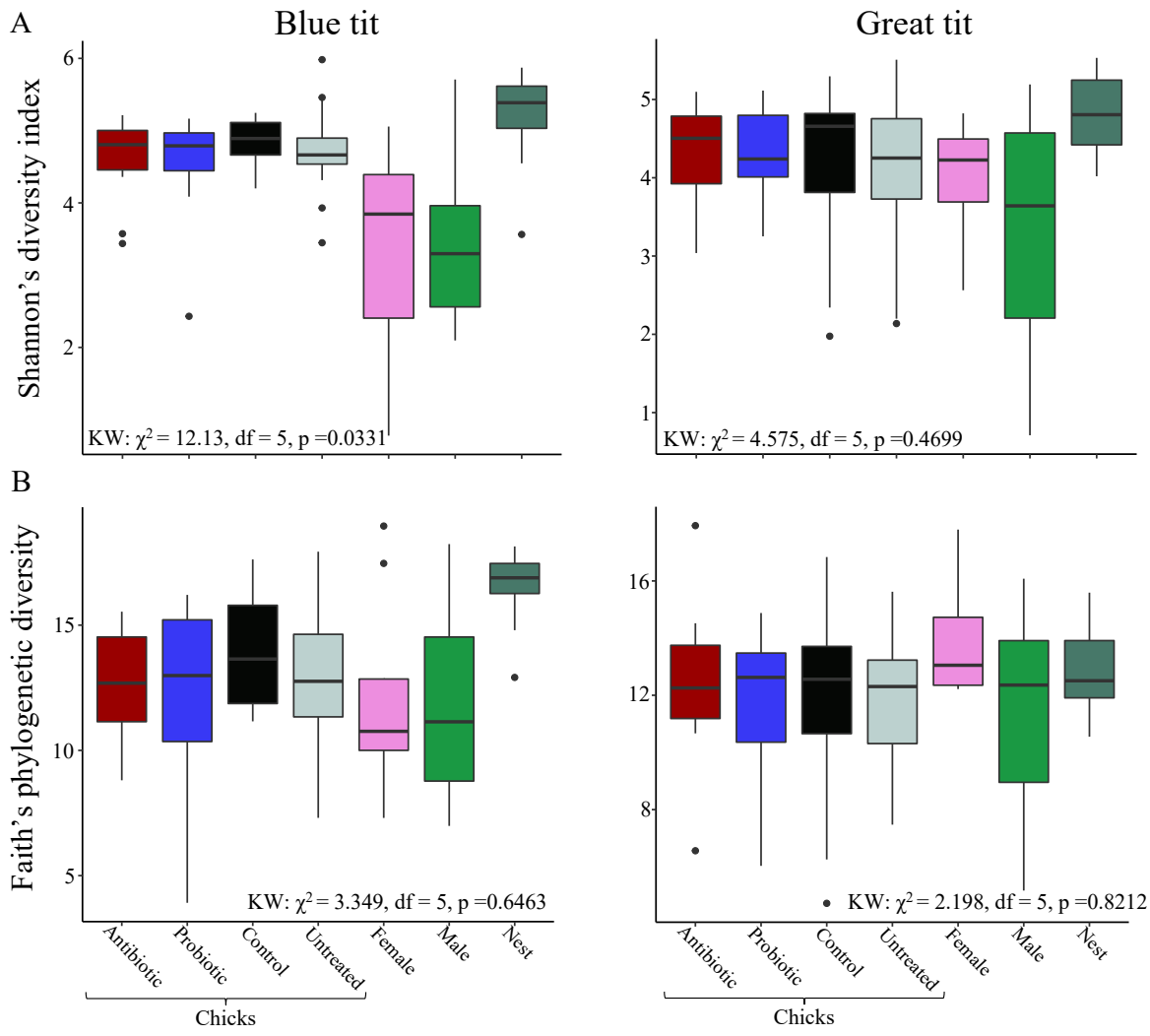

Fig. S6. Box plots depicting Shannon's diversity index (A) and Faith's phylogenetic diversity (B) of gut microbiomes of chicks and adults in the last sampling day.

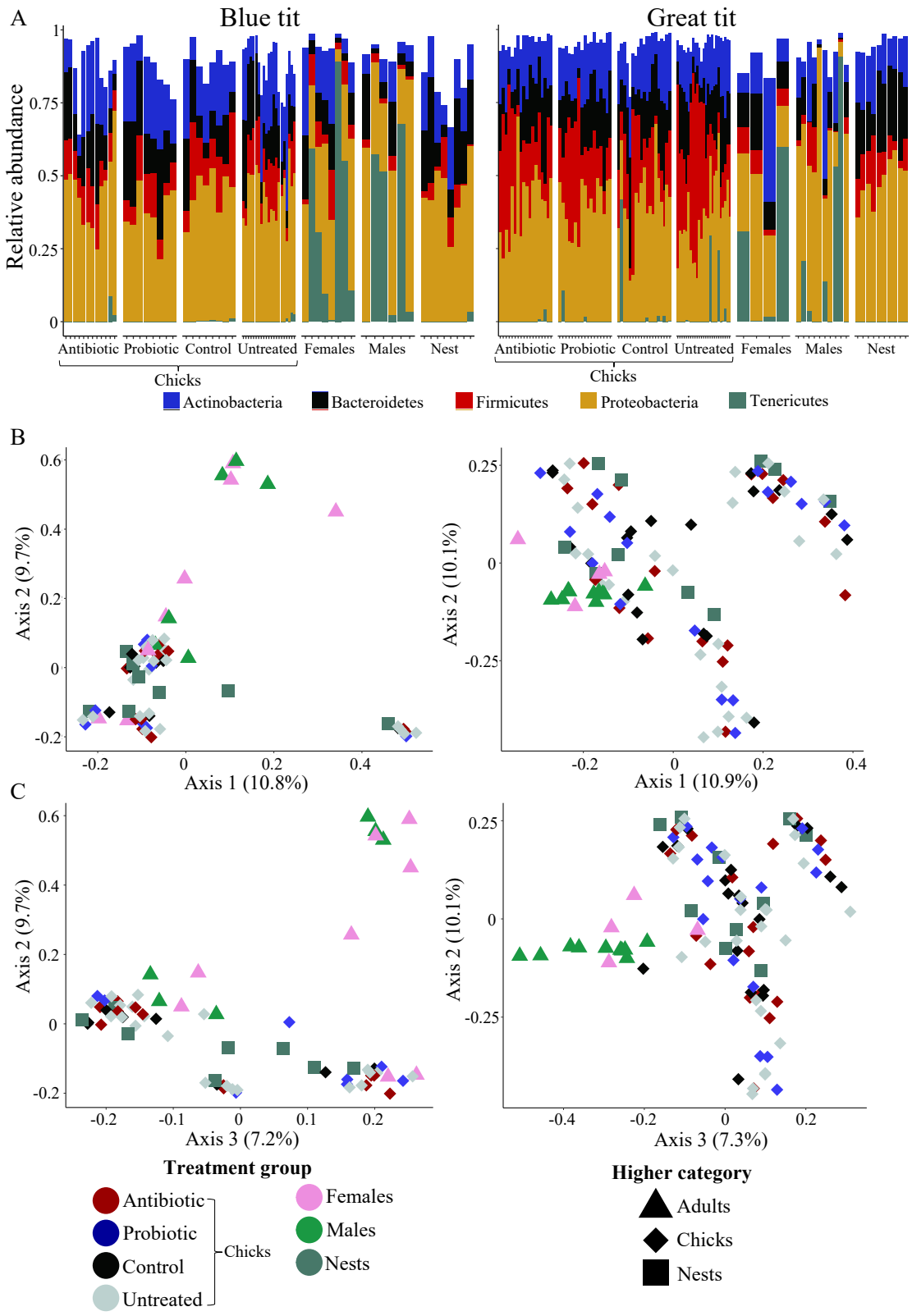

Fig. S7. (A) Relative abundance of dominant bacterial phyla in microbiomes of different treatment groups during the last sampling day. (B) Principal Coordinate Analyses (PCoA) plots (A: axis 1 and 2; B: Axis 2 and 3) depicting the microbial community similarity (Bray-Curtis distances) of different treatment groups during the last sampling day.

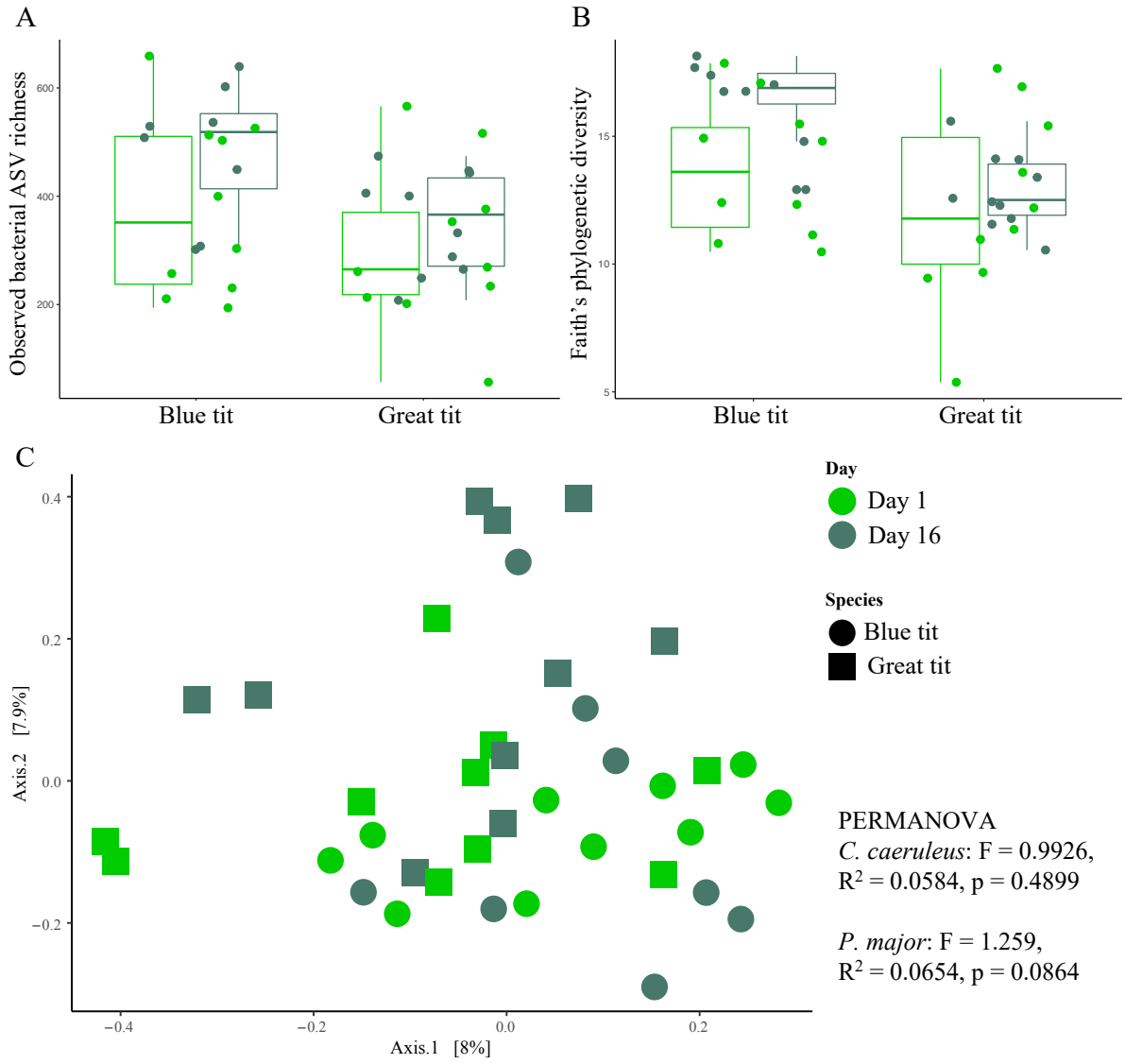

Fig S8. Observed ASV richness (A) and Faiths phylogenetic diversity (B) of nest microbiomes during 1st and last sampling days. (C) Nest bacterial community composition (measured with Bray-Curtis distances) during the two sampling time points.