

Supplementary Materials for

Biodiversity intervention enhances immune regulation and health-associated commensal microbiota among daycare children

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Published 14 October 2020, *Sci. Adv.* **6**, eaba2578 (2020)
DOI: 10.1126/sciadv.aba2578

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Table S1. Characteristics of study participants.

Kruskal-Wallis tests showed that there are no differences in days spent in daycare centers or summer cottage, sick days, outdoor recreation (Home yard, neighborhood or nature), animal contact, nutrition (berries and vegetables) and number of siblings between children in intervention, standard and nature-oriented daycares.

Days spend in daycares, summer cottage, and siblings and sick days during the intervention period are presented as mean \pm standard deviation (sd).

Outdoor recreation and animal contact during the intervention period are presented at nominal scale (median \pm confidence level 95% = cl):

1 = not at all, 2 = rarely, 3 = weekly, 4 = almost daily and 5 = daily.

Nutrition is presented at nominal scale (median \pm confidence level 95% = cl):

1 = not at all, 2 = occasionally, 3 = frequently.

	Intervention		Standard		Nature-oriented		Kruskal-Wallis test	
	Mean	sd	Mean	sd	Mean	sd	Chi-squared	P value
Daycare days	15.800	3.398	14.333	2.664	14.864	3.468	2.169	0.338
Sick days	0.567	0.898	1.233	2.499	0.591	0.908	0.086	0.958
Days in summer cottage	0.967	1.866	0.600	0.910	1.364	1.814	2.176	0.337
Siblings	1.133	0.937	0.867	0.834	1.455	0.963	3.768	0.152
	Median	cl	Median	cl	Median	cl	Chi-squared	P value
Outdoor recreation home yard	4	0.431	4	0.700	5	0.265	4.166	0.125
Outdoor recreation surrounding neighbourhood	4	0.300	4	0.478	3	0.333	4.822	0.090
Outdoor recreation nature	3	0.305	2	0.412	2.5	0.380	1.543	0.462
Animal contact	2	0.261	2	0.532	2	0.237	2.605	0.272
Nutrition berries	2	0.194	2	0.310	2	0.202	0.160	0.923
Nutrition vegetables	2	0.300	2	0.390	2	0.271	1.533	0.465

Table S2. Bacterial community composition (PERMANOVA) comparisons in soil (A-C), on skin (D) and in the gut (E and F) between daycare groups. Differences in surface soil bacterial communities (A) between intervention and standard daycare centers after the intervention and (B) before-after comparisons at class level. (C) Comparison of beta-diversity of surface soil bacterial communities between intervention and standard (PERMDISP) daycare soils at genus level after the intervention period. Community composition of bacteria (D) on skin at OTU level differed between children in nature-oriented and children in standard daycares at baseline. (E) Gut Ruminococcaceae community differed between nature-oriented and standard at baseline, and after intervention period also between children in intervention and standard daycares. (F) *Faecalibacterium* community composition in the gut differed between children in nature-oriented and standard daycares after the intervention period. The leftmost panel indicates groups and time points compared. Both relative abundance (left) and presence absence data (right) were compared. PERMANOVA statistics are reported as F statistics, R2 = Coefficient of determination, probability P value and Benjamini-Hochberg adjusted P value and PERMDISP results as F statistics, mean \pm sd, P value and Benjamini-Hochberg adjusted P value.

A) Soil: Intervention vs Standard	ABUNDANCE DATA				PRESENCE/ABSENCE DATA			
	F value	R2	P value	P adjust*	F value	R2	P value	P adjust*
OTU level	2.053	0.079	0.024	0.048	2.226	0.085	0.017	0.102
Genus level	2.368	0.090	0.023	0.048	1.729	0.067	0.083	0.166
Family level	2.500	0.094	0.021	0.048	1.486	0.058	0.163	0.244
Order level	2.126	0.081	0.049	0.074	1.321	0.052	0.244	0.244
Class level	1.840	0.071	0.072	0.086	1.476	0.058	0.227	0.244
Phylum level	1.945	0.075	0.101	0.101	2.827	0.105	0.059	0.166
Phylum Proteobacteria	2.179	0.083	0.018	0.030	2.083	0.080	0.022	0.037
Phylum Bacteroidetes	2.014	0.077	0.011	0.030	2.393	0.091	0.017	0.037
Phylum Actinobacteria	2.240	0.085	0.014	0.030	2.720	0.102	0.015	0.037
Phylum Acidobacteria	2.016	0.077	0.024	0.030	1.991	0.077	0.039	0.049
Phylum Verrucomicrobia	1.544	0.060	0.084	0.084	1.615	0.063	0.111	0.111
Class Actinobacteria	2.229	0.085	0.02	0.055	2.693	0.101	0.011	0.044
Class Sphingobacteriia	1.874	0.072	0.038	0.084	2.391	0.091	0.021	0.046
Class Alphaproteobacteria	2.291	0.087	0.015	0.055	2.232	0.085	0.025	0.046
Class Betaproteobacteria	1.945	0.075	0.102	0.121	2.827	0.105	0.047	0.054
Class Gammaproteobacteria	1.497	0.059	0.138	0.138	2.159	0.083	0.025	0.046
Class Flavobacteriia	1.945	0.075	0.101	0.121	2.827	0.105	0.056	0.056
Class Cytophagia	2.512	0.095	0.007	0.055	2.515	0.095	0.012	0.044
Class Acidobacteria Gp4	1.945	0.075	0.082	0.121	2.827	0.105	0.047	0.054
Class Deltaproteobacteria	1.794	0.070	0.01	0.055	2.059	0.079	0.011	0.044
Class Spartobacteria	1.945	0.075	0.089	0.121	2.827	0.105	0.044	0.054

Class Gemmatimonadetes	1.945	0.075	0.11	0.121	2.827	0.105	0.049	0.054						
Order Actinomycetales	2.202	0.084	0.021	0.063	2.763	0.103	0.008	0.038						
Order Sphingobacteriales	1.874	0.072	0.037	0.067	2.391	0.091	0.015	0.038						
Order Flavobacteriales	0.987	0.040	0.456	0.497	1.704	0.066	0.073	0.110						
Order Cytophagales	2.512	0.095	0.006	0.063	2.515	0.095	0.016	0.038						
Order Xanthomonadales	1.627	0.063	0.075	0.090	2.303	0.088	0.030	0.051						
Order Rhizobiales	2.398	0.091	0.013	0.063	2.292	0.087	0.012	0.038						
Order Rhodospirillales	2.395	0.091	0.017	0.063	2.985	0.111	0.007	0.038						
Order Spartobacteria unclassified	1.829	0.071	0.039	0.067	2.356	0.089	0.024	0.048						
	ABUNDANCE DATA				PRESENCE/ABSENCE DATA									
B) Soil: Intervention before vs after	F value	R2	P value	P adjust*	F value	R2	P value	P adjust*						
Class Actinobacteria	2.319	0.072	0.011	0.030	2.243	0.070	0.016	0.059						
Class Sphingobacteriia	2.255	0.070	0.011	0.030	2.037	0.064	0.043	0.079						
Class Alphaproteobacteria	2.073	0.065	0.018	0.040	1.872	0.059	0.035	0.077						
Class Betaproteobacteria	1.603	0.051	0.153	0.153	2.046	0.064	0.133	0.147						
Class Gammaproteobacteria	2.250	0.070	0.008	0.030	2.329	0.072	0.011	0.059						
Class Flavobacteriia	1.603	0.051	0.148	0.153	2.046	0.064	0.132	0.147						
Class Cytophagia	1.706	0.054	0.042	0.077	2.128	0.066	0.025	0.069						
Class Acidobacteria Gp4	1.603	0.051	0.143	0.153	2.046	0.064	0.147	0.147						
Class Deltaproteobacteria	2.059	0.064	0.002	0.022	2.741	0.084	0.001	0.011						
Class Spartobacteria	1.603	0.051	0.137	0.153	2.046	0.064	0.147	0.147						
Class Gemmatimonadetes	1.603	0.051	0.149	0.153	2.046	0.064	0.136	0.147						
C) PERMDISP	ABUNDANCE DATA							PRESENCE/ABSENCE DATA						
SOIL		Standard		Intervention				Standard		Intervention				
Intervention vs Standard	F	Mean	sd	Mean	sd	P value	P adjust*	F	Mean	sd	Mean	sd	P value	P adjust*
Actinomycetales unclassified	23.2	0.6	0.1	0.4	0.1	0.001	0.026	28.7	0.6	0.1	0.3	0.1	0.001	0.026
Hymenobacter	12.7	0.5	0.2	0.3	0.1	0.003	0.035	6.5	0.5	0.2	0.3	0.1	0.008	0.069
Noviherbaspirillum	13.6	0.5	0.2	0.2	0.1	0.004	0.035	0.1	0.1	0.3	0.1	0.2	0.718	0.849
Sphingomonas	7.5	0.5	0.2	0.3	0.1	0.009	0.047	3.4	0.4	0.2	0.3	0.1	0.076	0.395
Marmoricola	6.7	0.4	0.2	0.2	0.2	0.009	0.047	0.1	0.1	0.2	0.1	0.2	0.806	0.873
Massilia	6.1	0.4	0.2	0.3	0.1	0.016	0.069	0.6	0.2	0.2	0.2	0.1	0.54	0.78
Chitinophagaceae unclassified	1.4	0.5	0.1	0.5	0.2	0.277	0.463	1.4	0.5	0.2	0.4	0.1	0.262	0.56
Nocardioides	1.3	0.5	0.2	0.4	0.2	0.282	0.463	0.5	0.4	0.2	0.3	0.1	0.489	0.748
Arthrobacter	1.8	0.4	0.2	0.3	0.2	0.198	0.463	1.9	0.2	0.1	0.2	0.2	0.186	0.56
Intrasporangiaceae unclassified	1.5	0.4	0.3	0.3	0.2	0.223	0.463	1.3	0.3	0.2	0.2	0.1	0.276	0.56
Halomonas	1.5	0.4	0.3	0.3	0.2	0.214	0.463	1.3	0.3	0.2	0.2	0.1	0.28	0.56
Chryseobacterium	1.2	0.6	0.2	0.6	0.2	0.307	0.463	0.2	0.4	0.2	0.4	0.3	0.682	0.849
Burkholderiales unclassified	1.2	0.4	0.2	0.3	0.2	0.298	0.463	5.1	0.4	0.1	0.3	0.2	0.032	0.208
Xanthomonadaceae unclassified	1.7	0.5	0.2	0.6	0.2	0.201	0.463	0	0.5	0.1	0.5	0.2	0.989	0.989
Microbacteriaceae unclassified	0.9	0.4	0.2	0.3	0.1	0.338	0.463	15.6	0.4	0.1	0.3	0.1	0.004	0.052
Sphingomonadaceae unclassified	1.1	0.6	0.1	0.6	0.2	0.305	0.463	1.4	0.6	0.2	0.5	0.5	0.249	0.56
Betaproteobacteria unclassified	2.5	0.6	0.1	0.6	0.2	0.135	0.463	1.0	0.5	0.2	0.5	0.2	0.353	0.612
Comamonadaceae unclassified	0.7	0.5	0.2	0.4	0.2	0.396	0.515	0.7	0.3	0.2	0.3	0.2	0.404	0.657

Pseudomonas	0.5	0.5	0.2	0.6	0.2	0.488	0.604	0.3	0.3	0.3	0.4	0.4	0.605	0.828
Pedobacter	0.1	0.5	0.1	0.5	0.1	0.7	0.827	1.6	0.4	0.2	0.3	0.1	0.215	0.56
Ferruginibacter	0.1	0.6	0.1	0.6	0.2	0.757	0.856	0.1	0.5	0.2	0.4	0.2	0.801	0.873
Flavobacterium	0.0	0.5	0.2	0.5	0.2	0.851	0.899	0.8	0.5	0.2	0.4	0.2	0.352	0.612
Spartobacteria unclassified	0.0	0.6	0.1	0.6	0.2	0.864	0.899	1.5	0.5	0.1	0.5	0.2	0.262	0.56

D) Skin: OTU level. baseline	Abundance data			Presence/absence data			P adjust
	F value	R2	P value	F value	R2	P value	
Intervention vs standard	0.986	0.021	0.456	0.946	0.020	0.555	0.555
Intervention vs nature-oriented	1.321	0.025	0.060	1.388	0.026	0.070	0.105
Nature-oriented vs standard	1.241	0.040	0.102	1.474	0.047	0.025	0.075

E) Gut: Family Ruminococcaceae	Abundance data				Presence/absence data			
	F value	R2	P value	P adjust*	F value	R2	P value	P adjust*
Intervention before vs after	0.528	0.010	0.871		0.389	0.007	0.919	
Standard before vs after	0.530	0.021	0.895		0.532	0.021	0.808	
Nature before vs after	0.174	0.005	0.997		0.357	0.010	0.903	
Nature vs Standard before	1.928	0.057	0.047	0.101	2.448	0.071	0.020	0.079
Intervention vs Standard before	1.473	0.036	0.143		1.052	0.026	0.409	
Nature vs Intervention before	0.511	0.011	0.881		1.757	0.037	0.093	
Nature vs Standard after	2.265	0.070	0.010	0.030	1.222	0.039	0.301	
Intervention vs Standard after	1.942	0.049	0.027	0.081	1.463	0.037	0.163	
Nature vs Intervention after	0.644	0.014	0.793		0.613	0.014	0.777	

F) Gut: Genus Faecalibacterium**	Abundance data			Presence/absence data		
	F value	R2	P value	F value	R2	P value
Intervention before vs after	0.158	0.003	0.943	-0.027	-0.001	0.876
Standard before vs after	1.293	0.049	0.261	1.122	0.043	0.474
Nature before vs after	0.310	0.008	0.813	0.543	0.014	0.792
Nature vs Standard before	1.705	0.051	0.185	1.554	0.046	0.272
Intervention vs Standard before	1.705	0.041	0.136	1.362	0.033	0.317
Nature vs Intervention before	0.492	0.011	0.770	-0.692	-0.015	0.968
Nature vs Standard after	3.233	0.097	0.030	0.150	0.005	1.000
Intervention vs Standard after	2.288	0.057	0.086	0.240	0.006	0.795
Nature vs Intervention after	0.669	0.015	0.592	0.116	0.003	0.950

*Benjamini-Hochberg correction

***Faecalibacterium* was the sole genus analyzed

Table S3. Comparison of bacterial (A) relative abundance at genus level between intervention and standard daycare center surface soils and (B) between baseline and day-28 at intervention daycare yards (Wilcoxon signed-rank test). (C) Comparison of bacterial richness and diversity between intervention and standard daycare center surface soils at total bacterial and class levels (t-test). Data is shown as mean \pm standard deviation = sd.

A) Intervention vs standard, Degrees of Freedom = 23	Mean standard	sd	Mean intervention	sd	Statistic	P value	P adjust
Flavobacterium	134.3	102.0	233.0	168.4	108.0	0.147	0.491
Chitinophagaceae unclassified	261.1	123.6	202.1	72.9	51.5	0.140	0.484
Sphingomonas	208.6	47.3	159.8	108.3	50.5	0.126	0.484
Massilia	231.4	96.9	141.9	114.9	45.0	0.069	0.365
Hymenobacter	193.7	76.1	114.9	125.2	45.0	0.069	0.365
Ferruginibacter	185.9	110.5	112.4	82.2	46.0	0.077	0.367
Mucilaginibacter	18.3	28.3	94.6	164.6	106.0	0.178	0.494
Pedobacter	82.4	62.3	90.4	83.2	82.0	0.937	1.000
Spartobacteria unclassified	22.7	18.0	81.9	90.5	118.0	0.048	0.300
Nocardioides	84.6	37.3	76.1	51.6	74.0	0.772	0.963
Gammaproteobacteria unclassified	8.3	6.1	64.7	77.2	137.0	0.003	0.052
Arthrobacter	71.3	61.0	63.3	79.4	58.0	0.257	0.578
Betaproteobacteria unclassified	28.6	21.9	61.8	92.7	81.5	0.958	1.000
Burkholderiales unclassified	58.3	23.6	61.0	33.0	77.0	0.895	1.000
Chryseobacterium	122.1	263.5	55.1	76.2	87.5	0.712	0.942
Intrasporangiaceae unclassified	63.9	27.3	50.6	30.2	60.5	0.317	0.678
Xanthomonadaceae unclassified	47.7	69.6	49.8	37.3	103.5	0.225	0.563
Microbacteriaceae unclassified	43.1	19.1	47.4	36.5	78.5	0.958	1.000
Comamonadaceae unclassified	48.4	17.0	45.5	23.4	77.0	0.895	1.000
Actinomycetales unclassified	46.5	22.6	40.6	36.9	51.5	0.140	0.484
Luteolibacter	27.4	23.3	40.1	39.0	91.5	0.561	0.901
Cryobacterium	40.8	26.7	39.8	45.8	63.0	0.384	0.780
Aridibacter unclassified	50.8	30.4	39.7	35.8	63.5	0.399	0.780
Sphingomonadaceae unclassified	47.4	23.1	39.0	18.9	66.0	0.476	0.874
Myxococcales unclassified	25.4	21.8	35.8	35.7	89.0	0.654	0.942
Gp6 unclassified	17.8	12.3	35.6	55.8	80.0	1.000	1.000
Oxalobacteraceae unclassified	46.4	27.0	34.4	26.6	58.0	0.257	0.578
Mycobacterium	7.1	4.7	33.8	31.4	140.5	0.002	0.052
Bacteroidetes unclassified	21.6	20.5	33.1	28.4	102.5	0.246	0.578
Granulicella unclassified	6.7	16.5	32.9	76.1	79.0	0.975	1.000
Clostridium sensu stricto	5.7	4.9	32.9	56.0	120.5	0.035	0.240
Bradyrhizobium	14.0	8.6	32.8	22.1	121.0	0.033	0.240
Acidobacteria Gp1 unclassified	2.8	3.1	31.4	63.3	74.0	0.762	0.963
Marmoricola	49.5	15.0	30.1	27.6	38.5	0.031	0.240
Gemmatimonas	56.0	42.5	30.0	30.6	53.5	0.170	0.494
Noviherbaspirillum	80.0	34.3	29.9	32.7	20.5	0.002	0.052
Rhizobiales unclassified	24.5	18.0	28.5	26.2	74.5	0.792	0.963
Burkholderia	4.0	6.1	26.2	53.7	92.0	0.537	0.901

Polaromonas	22.3	20.7	24.8	21.1	85.5	0.792	0.963
Alphaproteobacteria unclassified	5.9	6.0	24.5	31.3	110.0	0.119	0.484
Novosphingobium	24.0	15.3	23.7	23.3	70.5	0.635	0.942
Spirosoma	46.0	31.2	23.6	25.8	50.5	0.124	0.484
Gp3 unclassified	12.1	10.7	23.4	28.6	88.5	0.672	0.942
Pseudomonas	27.7	30.7	22.9	30.9	68.0	0.544	0.901
Actinobacteria unclassified	11.4	8.5	22.7	16.0	117.5	0.050	0.300
Arenimonas	26.0	18.3	22.6	22.1	71.5	0.672	0.942
Sinobacteraceae unclassified	14.4	19.3	21.9	28.6	89.0	0.653	0.942
Planctomycetaceae unclassified	4.2	4.4	20.6	19.6	123.0	0.025	0.227
Brevundimonas	15.2	29.2	20.2	23.0	100.0	0.303	0.665
Proteobacteria unclassified	19.0	7.2	20.1	17.9	73.5	0.751	0.963
Gaiella	8.5	6.2	18.3	29.2	79.0	0.979	1.000
candidate division WPS.1 unclassified	8.7	10.0	18.0	17.8	106.5	0.169	0.494
Acidobacteria Gp4 unclassified	23.8	16.7	17.9	15.5	64.0	0.413	0.791
Blastocatella unclassified	20.1	8.0	16.8	35.2	33.0	0.014	0.159
Duganella	21.4	14.3	16.7	15.7	58.0	0.256	0.578
Terrimonas	7.7	6.1	16.6	19.4	96.5	0.398	0.780
Nakamurella	19.2	13.8	16.5	9.2	77.5	0.916	1.000
Subdivision3 unclassified	6.7	7.2	15.4	18.5	93.0	0.501	0.874
Opiritatus	6.3	6.1	14.9	21.0	98.5	0.340	0.712
Cytophagales unclassified	16.1	10.9	14.6	20.2	54.0	0.178	0.494
Roseomonas	24.5	13.9	14.4	14.7	47.5	0.091	0.410
Acidobacteria Gp3 unclassified	0.8	8.8	14.1	20.5	76.0	0.853	1.000
Dyadobacter	10.1	15.1	13.7	18.6	93.5	0.489	0.874
Cellvibrio	20.4	29.9	13.6	12.7	78.0	0.937	1.000
Acetobacteraceae unclassified	35.4	58.8	13.6	20.7	55.5	0.205	0.526
Gp4 unclassified	4.6	8.2	13.3	40.4	76.5	0.869	1.000
Rhizobium	11.2	6.0	12.2	9.7	78.0	0.937	1.000
Rhodopseudomonas	17.6	7.0	12.1	10.4	51.5	0.140	0.484
Nocardoidaceae unclassified	10.9	9.8	12.1	10.2	80.5	1.000	1.000
Flavisolibacter	38.3	21.4	12.0	13.7	22.5	0.003	0.052
Caulobacter	11.3	6.4	11.5	9.7	78.0	0.937	1.000
Conexibacter	8.2	8.6	11.1	15.1	72.0	0.690	0.942
Chryseolinea unclassified	7.8	7.5	11.1	12.3	89.5	0.634	0.942
Roseiarcus	0.1	0.3	10.4	23.7	99.0	0.187	0.494
Pseudoxanthomonas	1.5	2.3	10.0	8.6	134.5	0.004	0.052
Deltaproteobacteria unclassified	7.8	5.2	9.9	13.2	73.5	0.751	0.963
Cellulomonas	6.5	5.3	8.6	6.9	91.5	0.560	0.901
Phenylobacterium	7.5	5.9	8.1	9.6	72.0	0.691	0.942
Chloroflexi unclassified	2.6	2.9	7.5	8.5	105.5	0.183	0.494
Micromonosporaceae unclassified	17.0	9.2	7.5	9.4	24.0	0.003	0.052
Actinoplanes	5.2	5.7	7.2	14.6	67.0	0.505	0.874
Gp7 unclassified	5.0	7.6	7.1	19.0	70.0	0.592	0.935
Hyphomicrobium	2.0	1.8	6.6	10.7	112.0	0.076	0.367
Cytophaga	9.9	14.6	6.5	10.4	72.5	0.708	0.942

Segetibacter	33.6	30.8	5.8	7.9	25.0	0.004	0.052
Moraxellaceae unclassified	3.1	6.6	5.7	14.7	87.5	0.674	0.942
Sphingomonadales unclassified	14.4	9.7	5.7	7.7	37.5	0.025	0.227
Rhizomicrobium unclassified	0.0	0.0	5.3	11.7	95.0	0.169	0.494
Solirubrobacterales unclassified	9.0	11.3	5.2	7.1	66.0	0.473	0.874
Solirubrobacter	2.8	2.6	5.1	7.9	78.0	0.935	1.000
B) Intervention yards baseline vs day-28, Degrees of Freedom = 31	Mean baseline	sd	Mean day-28	sd	Statistic	P value	P adjust
Flavobacterium	171.8	145.6	233.0	168.4	80.0	0.552	0.869
Chitinophagaceae unclassified	213.6	72.3	202.1	72.9	48.5	0.326	0.606
Bacteria unclassified	206.0	157.4	180.2	66.4	57.0	0.587	0.870
Sphingomonas	155.8	77.2	159.8	108.3	73.0	0.816	0.970
Massilia	86.9	46.8	141.9	114.9	91.0	0.245	0.584
Hymenobacter	100.1	72.5	114.9	125.2	68.0	1.000	1.000
Ferruginibacter	123.7	69.6	112.4	82.2	60.0	0.698	0.933
Mucilaginibacter	7.9	9.1	94.6	164.6	102.0	0.018	0.279
Pedobacter	65.1	44.7	90.4	83.2	70.5	0.570	0.869
Spartobacteria unclassified	27.3	53.0	81.9	90.5	111.0	0.028	0.280
Nocardioides	123.5	60.6	76.1	51.6	24.0	0.024	0.279
Gammaproteobacteria unclassified	14.9	12.3	64.7	77.2	109.0	0.036	0.290
Arthrobacter	89.4	67.4	63.3	79.4	43.0	0.205	0.566
Betaproteobacteria unclassified	30.2	40.5	61.8	92.7	83.0	0.453	0.740
Burkholderiales unclassified	56.1	29.7	61.0	33.0	64.5	0.877	0.970
Chryseobacterium	34.9	60.9	55.1	76.2	95.5	0.162	0.500
Intrasporangiaceae unclassified	98.3	70.3	50.6	30.2	26.0	0.032	0.283
Xanthomonadaceae unclassified	65.5	45.2	49.8	37.3	46.5	0.277	0.584
Microbacteriaceae unclassified	44.3	36.7	47.4	36.5	79.5	0.569	0.869
Comamonadaceae unclassified	45.6	20.8	45.5	23.4	68.5	1.000	1.000
Actinomycetales unclassified	53.3	64.1	40.6	36.9	62.5	0.796	0.970
Luteolibacter	24.9	21.6	40.1	39.0	91.5	0.234	0.584
Cryobacterium	28.4	30.7	39.8	45.8	72.0	0.856	0.970
Aridibacter unclassified	44.0	24.2	39.7	35.8	55.5	0.820	0.970
Sphingomonadaceae unclassified	48.6	25.0	39.0	18.9	50.5	0.379	0.632
Myxococcales unclassified	22.3	23.5	35.8	35.7	78.5	0.306	0.584
Gp6 unclassified	21.4	18.3	35.6	55.8	66.0	0.938	0.988
Oxalobacteraceae unclassified	25.7	18.6	34.4	26.6	93.5	0.196	0.560
Mycobacterium	8.1	5.0	33.8	31.4	129.0	0.002	0.114
Bacteroidetes unclassified	39.9	24.2	33.1	28.4	45.5	0.255	0.584
Granulicella unclassified	0.8	4.6	32.9	76.1	18.0	0.142	0.455
Clostridium sensu stricto	11.6	20.0	32.9	56.0	84.0	0.052	0.326
Bradyrhizobium	16.0	10.1	32.8	22.1	102.5	0.079	0.370
Acidobacteria Gp1 unclassified	12.1	46.4	31.4	63.3	46.0	0.264	0.584
Marmoricola	73.8	41.7	30.1	27.6	7.0	0.003	0.114
Gemmatimonas	43.3	25.1	30.0	30.6	50.0	0.365	0.632
Noviherbaspirillum	57.7	85.2	29.9	32.7	50.5	0.379	0.632
Burkholderia	1.6	2.3	26.2	53.7	80.5	0.016	0.279
Polaromonas	23.2	12.5	24.8	21.1	71.0	0.897	0.970

Novosphingobium	32.6	25.5	23.7	23.3	50.5	0.379	0.632
Spirosoma	45.1	28.9	23.6	25.8	33.0	0.074	0.370
Gp3 unclassified	18.4	39.1	23.4	28.6	89.0	0.289	0.584
Pseudomonas	64.4	74.5	22.9	30.9	28.0	0.041	0.299
Actinobacteria unclassified	22.8	12.1	22.7	16.0	57.5	0.605	0.880
Arenimonas	33.9	23.4	22.6	22.1	35.5	0.098	0.392
Sinobacteraceae unclassified	5.6	5.9	21.9	28.6	88.0	0.118	0.411
Planctomycetaceae unclassified	16.0	28.2	20.6	19.6	91.0	0.245	0.584
Brevundimonas	15.8	12.6	20.2	23.0	72.5	0.836	0.970
Proteobacteria unclassified	18.6	13.4	20.1	17.9	67.5	1.000	1.000
Gaiella	11.9	12.3	18.3	29.2	63.0	0.887	0.970
candidate division WPS.1 unclassified	6.6	6.0	18.0	17.8	105.5	0.056	0.326
Acidobacteria Gp4 unclassified	26.2	27.4	17.9	15.5	41.0	0.170	0.505
Blastocatella unclassified	17.1	46.0	16.8	35.2	36.0	0.103	0.392
Duganella	22.7	7.5	16.7	15.7	93.0	0.065	0.345
Terrimonas	13.6	6.8	16.6	19.4	90.0	0.093	0.392
Subdivision3 unclassified	4.4	5.9	15.4	18.5	88.0	0.118	0.411
Opitutus	15.9	6.4	14.9	21.0	59.0	0.706	0.933
Cytophagales unclassified	16.1	12.5	14.6	20.2	46.0	0.266	0.584
Roseomonas	23.2	9.9	14.4	14.7	59.0	0.660	0.924
Acidobacteria Gp3 unclassified	8.1	17.3	14.1	20.5	79.0	0.293	0.584
Dyadobacter	14.2	12.0	13.7	18.6	56.0	0.842	0.970
Cellvibrio	36.9	31.5	13.6	12.7	43.2	0.023	0.279
Acetobacteraceae unclassified	14.3	18.7	13.6	20.7	68.0	0.670	0.924
Gp4 unclassified	1.1	1.9	13.3	40.4	53.5	0.270	0.584
Rhizobium	16.0	10.9	12.2	9.7	67.0	0.712	0.933
Rhodopseudomonas	17.3	7.7	12.1	10.4	64.5	0.877	0.970
Nocardoidaceae unclassified	14.4	11.0	12.1	10.2	53.5	0.733	0.946
Flavisolibacter	23.3	15.0	12.0	13.7	26.0	0.057	0.326
Conexibacter	24.6	40.5	11.1	15.1	49.0	0.338	0.615
Chryseolinea unclassified	7.0	6.6	11.1	12.3	54.0	0.576	0.869
Roseiarcus	0.4	1.1	10.4	23.7	43.2	0.128	0.425
Pseudoxanthomonas	10.2	11.5	10.0	8.6	67.0	0.979	1.000
Cellulomonas	9.9	9.4	8.6	6.9	66.0	0.938	0.988
Phenylobacterium	9.0	3.6	8.1	9.6	62.0	0.776	0.970
Phycococcus	15.3	9.5	7.6	6.4	19.0	0.012	0.279
Actinoplanes	7.5	6.3	7.2	14.6	55.5	0.875	0.970
Gp7 unclassified	8.2	15.1	7.1	19.0	45.0	0.305	0.584
Hyphomicrobium	2.1	2.7	6.6	10.7	72.5	0.217	0.578
Solirubrobacterales unclassified	20.0	30.2	5.2	7.1	36.0	0.103	0.392
Solirubrobacter	5.7	4.0	5.1	7.9	60.5	0.637	0.910

C) Intervention vs standard, Day 28 Degrees of Freedom = 23	Mean standard	sd	Mean intervention	sd	Statistic	P value	P adjust*
Observed OTU richness	484.0	99.7	588.7	128.7	2.3	0.029	0.04
Chao richness index	533.0	134.9	670.9	156.9	2.4	0.027	0.04
Shannon total bacterial community	5.2	0.4	5.6	0.4	2.2	0.040	0.04
CLASS LEVEL RICHNESS							
Actinobacteria richness	72.5	16.2	75.8	16.4	0.5	0.626	0.939
Sphingobacteriia richness	67.4	17.8	74.4	17.2	1.0	0.333	0.777
Alphaproteobacteria richness	80.5	16.3	83.5	14.6	0.5	0.640	0.939
Betaproteobacteria richness	13.0	2.2	12.9	1.6	-0.1	0.939	0.939
Gammaproteobacteria richness	19.3	5.9	28.3	6.5	3.6	0.002	0.024
Flavobacteriia richness	13.0	2.2	12.9	1.6	-0.1	0.939	0.939
Cytophagia richness	39.7	15.5	28.6	16.7	-1.7	0.101	0.364
Acidobacteria Gp4 richness	13.0	2.2	12.9	1.6	-0.1	0.939	0.939
Deltaproteobacteria richness	9.8	5.1	14.1	8.0	1.7	0.104	0.364
Spartobacteria richness	13.0	2.2	12.9	1.6	-0.1	0.939	0.939
Bacilli richness	1.2	1.9	1.6	2.2	0.4	0.665	0.939
Gemmatimonadetes richness	13.0	2.2	12.9	1.6	-0.1	0.939	0.939
Acidobacteria Gp1 richness	3.4	3.5	11.3	20.3	1.5	0.150	0.419
Verrucomicrobiae richness	3.4	2.4	6	4.1	2.1	0.050	0.349
CLASS LEVEL DIVERSITY							
Actinobacteria Shannon	3.6	0.2	3.6	0.3	-0.2	0.850	0.850
Sphingobacteriia Shannon	3.3	0.3	3.6	0.3	2.7	0.016	0.074
Alphaproteobacteria Shannon	3.7	0.2	3.8	0.2	0.8	0.416	0.529
Betaproteobacteria Shannon	1.5	0.1	1.6	0.2	1.9	0.077	0.135
Gammaproteobacteria Shannon	2.3	0.3	2.6	0.3	2.6	0.016	0.074
Flavobacteriia Shannon	1.5	0.1	1.6	0.2	1.9	0.077	0.135
Cytophagia Shannon	3.1	0.4	2.6	0.6	-2.8	0.010	0.074
Acidobacteria Gp4 Shannon	1.5	0.1	1.6	0.2	1.9	0.077	0.135
Deltaproteobacteria Shannon	1.8	0.7	2.2	0.6	1.5	0.163	0.253
Spartobacteria Shannon	1.5	0.1	1.6	0.2	1.9	0.077	0.135
Bacilli Shannon	0.3	0.6	0.4	0.6	0.6	0.579	0.676
Gemmatimonadetes Shannon	1.5	0.1	1.6	0.2	1.9	0.077	0.135
Acidobacteria Gp1 Shannon	0.8	0.8	0.9	1.4	0.4	0.689	0.742
Verrucomicrobiae Shannon	0.9	0.6	1.2	0.7	1.0	0.316	0.442

*Benjamini-Hochberg correction

Table S4. Comparisons of bacterial richness and Shannon diversity on skin before the intervention period (Baseline A-C) and after the intervention period (Day-28 D-F) among three daycare groups (Intervention, standard and nature-oriented). Data is shown at total bacterial community, phylum and class levels (mean±standard deviation = sd). Analysis of covariance (ANCOVA) or Kruskal-Wallis test was used depending on Shapiro-Wilk test of normality. Tukey’s HSD post hoc test was used when normality assumption was true and Dunn’s test when normality assumption was violated. The upper part shows (A) **baseline** ANCOVA and Kruskal-Wallis tests and (B) post-hoc tests Tukey HSD and (C) Dunn’s test. The lower part shows (D) **day-28** ANCOVA and Kruskal-Wallis tests and (E) post-hoc tests Tukey HSD and (F) Dunn’s test. **Bold font** indicates significant differences ($p \leq 0.05$).

A) Analysis of covariance	Intervention		Standard		Nature		ANCOVA ^a		Shapiro-Wilk		Kruskal-Wallis	
	mean	sd	mean	sd	mean	sd	F	P value	W	P value	X ²	P value
Baseline												
Total bacterial community												
Observed OTU richness	52.8	29.3	56.6	31.7	65.9	39.6	1.1	0.347	1.0	0.099	1.3	0.531
Chao richness index	60.3	42.2	61.9	40.4	78.5	55.9	1.1	0.333	0.9	0.005	1.0	0.592
shannon	3.3	0.7	3.6	0.6	3.6	0.7	1.2	0.306	0.9	0.001	1.6	0.445
DIVERSITY PHYLUM												
Firmicutes Shannon	1.9	0.9	2.2	0.8	2.2	0.6	0.8	0.455	1.0	0.967	1.3	0.509
Proteobacteria Shannon	2.1	0.8	2.2	0.7	2.4	0.9	0.6	0.540	0.9	0.007	1.3	0.527
Actinobacteria Shannon	2.0	0.8	2.2	0.6	2.3	0.9	1.4	0.243	1.0	0.247	1.8	0.407
Bacteroidetes Shannon	1.2	0.9	1.2	0.9	1.4	1.0	0.3	0.736	0.9	0.000	0.7	0.722
DIVERSITY CLASS												
Bacilli Shannon	1.7	0.8	1.9	0.6	1.9	0.4	1.1	0.333	1.0	0.756	1.5	0.472
Gammaproteobacteria Shannon	1.5	0.7	1.4	0.7	1.3	0.6	0.6	0.572	1.0	0.011	1.2	0.547
Betaproteobacteria Shannon	1.0	0.8	1.2	0.7	1.5	0.7	2.2	0.117	1.0	0.053	3.4	0.179
Alphaproteobacteria Shannon	0.5	0.8	0.6	0.9	1.2	1.1	3.6	0.033	0.9	0.000	5.9	0.053
Clostridia Shannon	0.4	0.8	0.6	0.9	0.4	0.8	0.4	0.665	0.7	0.000	1.1	0.575
Bacteroidia Shannon	0.7	0.8	0.8	0.8	0.8	0.8	0.1	0.881	0.9	0.000	0.3	0.844
Fusobacteriia Shannon	0.4	0.6	0.5	0.7	0.4	0.7	0.1	0.899	0.7	0.000	0.4	0.832
Negativicutes Shannon	0.3	0.5	0.4	0.5	0.2	0.4	0.5	0.585	0.8	0.000	0.9	0.642
RICHNESS PHYLUM												
Firmicutes richness	16.3	15.3	16.0	10.4	14.9	8.8	0.1	0.936	0.9	0.000	0.3	0.852
Proteobacteria richness	14.2	10.7	13.4	8.2	20.1	14.6	2.2	0.120	0.9	0.007	2.4	0.295
Actinobacteria richness	12.1	8.8	14.4	12.0	18.3	14.6	2.0	0.147	0.9	0.002	1.3	0.512
Bacteroidetes richness	5.5	4.7	5.6	4.7	7.6	6.5	1.0	0.365	0.9	0.001	1.0	0.600
CLASS												
Bacilli richness	11.5	9.8	11.5	7.8	10.8	5.5	0.0	0.958	0.9	0.000	0.4	0.833
Gammaproteobacteria richness	6.9	4.8	5.6	3.5	6.0	3.6	0.5	0.599	1.0	0.115	0.2	0.897
Betaproteobacteria richness	4.5	3.7	4.5	2.4	7.1	5.2	3.1	0.050	0.9	0.003	3.8	0.149
Alphaproteobacteria richness	2.6	4.1	2.9	4.6	6.6	8.0	3.8	0.027	0.9	0.000	4.1	0.130
Clostridia richness	3.5	11.1	2.7	3.6	2.8	5.9	0.1	0.949	0.4	0.000	1.8	0.406

Bacteroidia richness	3.0	3.7	3.1	3.3	3.4	3.1	0.1	0.941	0.9	0.000	0.4	0.829
Fusobacteriia richness	1.6	2.0	2.2	3.1	1.6	2.0	0.4	0.661	0.8	0.000	0.0	0.989
Negativicutes richness	1.2	1.6	1.5	1.7	1.2	1.5	0.2	0.811	0.9	0.000	0.5	0.764
B) POST-HOC TUKEY'S HSD												
	Intervention vs Standard				Nature vs Standard				Nature vs Intervention			
Baseline	Mean difference		P value		Mean difference		P value		Mean difference		P value	
Total bacterial community												
Observed OTU richness	-3.843		0.922		9.302		0.688		13.144		0.329	
Chao richness index	16.587		0.993		16.587		0.542		18.192		0.332	
Shannon index	-0.546		0.561		0.056		0.973		0.284		0.350	
DIVERSITY PHYLUM												
Firmicutes Shannon	-0.239		0.618		0.013		0.999		0.251		0.531	
Proteobacteria Shannon	-0.087		0.935		0.162		0.832		0.249		0.522	
Actinobacteria Shannon	-0.226		0.632		0.139		0.871		0.365		0.247	
Bacteroidetes Shannon	-0.011		0.999		0.187		0.834		0.199		0.737	
DIVERSITY CLASS												
Bacilli Shannon	-0.209		0.595		0.060		0.967		0.269		0.365	
Gammaproteobacteria Shannon	0.148		0.752		-0.040		0.984		-0.188		0.582	
Betaproteobacteria Shannon	-0.157		0.774		0.282		0.524		0.439		0.101	
Alphaproteobacteria Shannon	-0.090		0.946		0.589		0.164		0.678		0.030	
Clostridia Shannon	-0.235		0.654		-0.217		0.751		0.018		0.997	
Bacteroidia Shannon	-0.074		0.951		0.036		0.991		0.110		0.877	
Fusobacteriia Shannon	-0.094		0.889		-0.069		0.952		0.025		0.990	
Negativicutes Shannon	-0.130		0.649		-0.164		0.584		-0.034		0.966	
RICHNESS PHYLUM												
Firmicutes richness	0.314		0.997		-1.056		0.972		-1.370		0.930	
Proteobacteria richness	0.771		0.972		6.683		0.200		5.911		0.151	
Actinobacteria richness	-2.214		0.795		3.921		0.569		6.135		0.132	
Bacteroidetes richness	-0.157		0.995		1.913		0.559		2.070		0.361	
RICHNESS CLASS												
Bacilli richness	-0.543		1.000		-0.722		0.969		-0.679		0.959	
Gammaproteobacteria richness	1.243		0.618		0.357		0.969		-0.886		0.747	
Betaproteobacteria richness	-0.514		1.000		2.611		0.142		2.625		0.054	
Alphaproteobacteria richness	-0.329		0.978		3.683		0.124		4.011		0.027	
Clostridia richness	0.743		0.962		0.063		1.000		-0.679		0.962	
Bacteroidia richness	-0.114		0.994		0.246		0.979		0.360		0.933	
Fusobacteriia richness	-0.643		0.650		-0.603		0.742		0.040		0.998	
Negativicutes richness	-0.829		0.791		-0.278		0.876		0.051		0.993	
C) POST-HOC DUNN'S TEST												
	Intervention vs Standard				Nature vs Standard				Nature vs Intervention			
Baseline	Dunn statistic		P value		Dunn statistic		P value		Dunn statistic		P value	
Total bacterial community												
Observed OTU richness	0.425		0.671		0.659		0.671		1.268		0.614	
Chao richness index	0.374		0.708		0.629		0.708		1.177		0.708	
Shannon index	0.748		0.649		0.455		0.649		1.371		0.511	
DIVERSITY PHYLUM												
Firmicutes Shannon	1.154		0.405		0.125		0.900		1.104		0.405	

Firmicutes richness	14.8	9.9	14.2	8.3	15.9	12.2	0.1	0.899	0.9	0.011	0.0	0.989
Proteobacteria richness	19.9	15.4	9.1	4.6	19.8	13.0	3.4	0.042	0.9	0.000	9.4	0.009
Actinobacteria richness	15.6	16.7	9.3	4.8	19.4	13.1	2.1	0.136	0.8	0.000	3.9	0.142
Bacteroidetes richness	6.4	5.7	3.5	2.4	7.0	4.8	2.1	0.138	0.9	0.000	3.3	0.188
RICHNESS CLASS												
Bacilli richness	12.2	8.4	10.8	7.5	10.5	7.8	0.4	0.681	0.9	0.000	0.6	0.756
Gammaproteobacteria richness	7.8	5.3	4.5	2.8	6.9	5.1	1.8	0.168	0.9	0.001	4.4	0.111
Betaproteobacteria richness	6.6	5.9	3.3	2.2	7.0	4.5	2.5	0.090	0.9	0.000	8.0	0.018
Alphaproteobacteria richness	5.0	7.2	1.0	2.5	5.4	7.5	2.0	0.144	0.8	0.000	7.4	0.025
Clostridia richness	1.4	2.7	2.4	3.8	3.9	7.4	1.5	0.235	0.6	0.000	1.9	0.381
Bacteroidia richness	2.9	2.8	2.1	1.9	3.1	3.6	0.5	0.615	0.9	0.000	0.5	0.791
Fusobacteriia richness	2.5	2.5	1.4	1.3	2.0	2.8	0.9	0.409	0.9	0.000	1.8	0.401
Negativicutes richness	1.1	1.3	1.0	1.2	1.3	1.2	0.2	0.825	0.9	0.000	0.6	0.755
E) POST-HOC TUKEY's HSD day 28												
	Intervention vs Standard		Nature vs Standard		Nature vs Intervention							
Total bacterial community	Mean difference	P value	P adjust*	Mean difference	P value	P adjust*	Mean difference	P value	Mean difference	P value	Mean difference	P value
Observed OTU richness	21.541	0.168		28.283	0.075	0.113	6.742		6.742		0.794	
Chao richness index	39.563	0.224		39.563	0.159	0.159	6.589		6.589		0.924	
shannon	0.066	0.087		0.585	0.024	0.072	0.153		0.153		0.665	
DIVERSITY PHYLUM												
Firmicutes Shannon	0.182	0.736	0.736	0.141	0.853	0.853	-0.041		-0.041		0.980	
Proteobacteria Shannon	0.645	0.027	0.108	0.720	0.021	0.084	0.075		0.075		0.934	
Actinobacteria Shannon	0.228	0.724	0.736	0.599	0.157	0.209	0.371		0.371		0.342	
Bacteroidetes Shannon	0.379	0.357	0.714	0.555	0.154	0.209	0.176		0.176		0.748	
DIVERSITY CLASS												
Bacilli Shannon	0.282	0.410		0.024	0.994		-0.257		-0.257		0.386	
Gammaproteobacteria Shannon	0.551	0.053		0.369	0.309		-0.181		-0.181		0.651	
Betaproteobacteria Shannon	0.400	0.245		0.617	0.061		0.217		0.217		0.582	
Alphaproteobacteria Shannon	0.731	0.064		0.792	0.063		0.060		0.060		0.975	
Clostridia Shannon	-0.218	0.681		0.123	0.900		0.341		0.341		0.307	
Bacteroidia Shannon	0.120	0.890		0.102	0.929		-0.017		-0.017		0.997	
Fusobacteriia Shannon	0.161	0.716		0.034	0.987		-0.127		-0.127		0.766	
Negativicutes Shannon	-0.032	0.970		0.069	0.890		0.101		0.101		0.689	
RICHNESS PHYLUM												
Firmicutes richness	0.528	0.987	0.987	1.664	0.897		1.136		1.136		0.927	
Proteobacteria richness	10.785	0.038	0.152	10.713	0.060		-0.073		-0.073		1.000	
Actinobacteria richness	6.244	0.363	0.484	10.061	0.110		3.817		3.817		0.613	
Bacteroidetes richness	2.841	0.199	0.398	3.462	0.130		0.621		0.621		0.903	
RICHNESS CLASS												
Bacilli richness	1.403	0.859		-0.296	0.994		-1.699		-1.699		0.752	
Gammaproteobacteria richness	3.220	0.117		2.409	0.348		-0.811		-0.811		0.834	
Betaproteobacteria richness	3.313	0.116		3.692	0.100		0.379		0.379		0.963	
Alphaproteobacteria richness	4.034	0.156		4.421	0.147		0.387		0.387		0.978	

Clostridia richness	-0.971	0.825	1.563	0.652	2.534	0.196		
Bacteroidia richness	0.854	0.667	0.976	0.635	0.122	0.989		
Fusobacteriia richness	1.098	0.378	0.615	0.766	-0.483	0.783		
Negativicutes richness	0.069	0.985	0.263	0.832	0.194	0.861		
F) POST HOC DUNN'S TEST Day 28	Intervention vs Standard			Nature vs Standard			Nature vs Intervention	
Total bacterial community	Dunn statistic	P value	P adjust*	Dunn statistic	P value	P adjust*	Dunn statistic	P value
Observed OTU richness	1.608	0.162		2.112	0.104		0.758	0.449
Chao richness index	1.505	0.199		2.064	0.117		0.816	0.415
shannon	1.664	0.144		2.321	0.061		0.949	0.342
DIVERSITY PHYLUM								
Firmicutes Shannon	0.815	0.415	0.415	0.857	0.392	0.392	0.123	0.902
Proteobacteria Shannon	2.579	0.010	0.040	2.920	0.004	0.014	0.644	0.520
Actinobacteria Shannon	0.914	0.361	0.415	2.069	0.039	0.077	1.489	0.136
Bacteroidetes Shannon	1.173	0.241	0.415	1.632	0.103	0.137	0.664	0.507
DIVERSITY CLASS								
Bacilli Shannon	1.236	0.216	0.413	0.337	0.736	0.954	0.987	0.324
Gammaproteobacteria Shannon	2.530	0.005	0.041	1.465	0.142	0.378	1.075	0.169
Betaproteobacteria Shannon	1.652	0.099	0.263	2.927	0.003	0.027	1.701	0.089
Alphaproteobacteria Shannon	2.379	0.017	0.069	2.293	0.022	0.087	0.106	0.915
Clostridia Shannon	1.130	0.258	0.413	0.187	0.852	0.954	1.507	0.132
Bacteroidia Shannon	0.433	0.665	0.665	0.246	0.806	0.954	0.190	0.849
Fusobacteriia Shannon	0.637	0.524	0.665	0.057	0.954	0.954	0.790	0.429
Negativicutes Shannon	0.439	0.660	0.665	0.528	0.598	0.954	1.141	0.254
RICHNESS PHYLUM								
Firmicutes richness	0.146	0.884	0.884	0.064	0.949	0.949	0.088	0.930
Proteobacteria richness	2.717	0.007	0.026	2.829	0.005	0.019	0.378	0.705
Actinobacteria richness	0.965	0.334	0.446	1.941	0.052	0.094	1.276	0.202
Bacteroidetes richness	1.359	0.174	0.349	1.808	0.071	0.094	0.669	0.504
RICHNESS CLASS								
Bacilli richness	0.412	0.680	0.777	0.216	0.829	0.864	0.729	0.466
Gammaproteobacteria richness	2.096	0.036	0.103	1.300	0.194	0.516	0.785	0.432
Betaproteobacteria richness	2.068	0.039	0.103	2.802	0.005	0.041	1.080	0.280
Alphaproteobacteria richness	2.607	0.009	0.073	2.262	0.024	0.095	0.189	0.850
Clostridia richness	0.486	0.627	0.777	0.688	0.491	0.849	1.390	0.165

^a Degree of Freedom between groups = 2 and within groups = 58

*Benjamini-Hochberg correction

Table S5. Before-after comparisons (Wilcoxon-Signed Rank test) for bacterial diversity on skin at total bacterial community, and phylum and class levels among children in **(A)** intervention (n = 29), **(B)** standard (n = 14) and **(C)** nature-oriented daycare centers (n = 19) (Mean \pm Standard Deviation = sd). **Bold** font indicates significant differences ($p \leq 0.05$).

A) Intervention	Mean before	sd before	Mean after	sd after	Statistic	P value	P adjust*
Observed OTU richness	53.3	29.1	63.5	41.0	239	0.110	
Chao richness index	59.9	40.1	77.5	75.8	257	0.105	
Shannon	3.3	0.8	3.6	0.6	268	0.059	
DIVERSITY PHYLUM							
Firmicutes Shannon	1.9	0.8	2.1	0.6	231	0.319	
Proteobacteria Shannon	2.2	0.8	2.4	0.7	233	0.296	
Actinobacteria Shannon	2.0	0.9	2.0	1.0	187	0.780	
Bacteroidetes Shannon	1.1	0.9	1.4	0.9	198	0.346	
Fusobacteria Shannon	0.5	0.6	0.6	0.7	102	0.486	
DIVERSITY CLASS							
Bacilli Shannon	1.8	0.8	1.9	0.5	225	0.394	0.879
Gammaproteobacteria Shannon	1.6	0.6	1.6	0.7	182	0.879	0.879
Betaproteobacteria Shannon	1.2	0.7	1.3	0.8	208	0.657	0.879
Alphaproteobacteria Shannon	0.6	0.9	1.0	1.0	127	0.018	0.144
Clostridia Shannon	0.3	0.4	0.3	0.6	42	0.844	0.879
Bacteroidia Shannon	0.6	0.7	0.7	0.8	97	0.632	0.879
Fusobacteriia Shannon	0.5	0.6	0.6	0.7	102	0.486	0.879
Negativicutes Shannon	0.2	0.4	0.2	0.4	37	0.756	0.879
B) Standard	Mean before	sd before	Mean after	sd after	Statistic	P value	
Observed OTU richness	58.9	37.3	44.3	14.7	28	0.235	
Chao richness index	69.2	52.8	47.8	19.1	28	0.235	
Shannon	3.5	0.8	3.2	0.5	36	0.529	
DIVERSITY PHYLUM							
Firmicutes Shannon	2.1	1.1	2.1	0.4	40	0.727	
Proteobacteria Shannon	2.1	0.8	2.0	0.6	48	0.889	
Actinobacteria Shannon	2.0	0.9	1.6	0.8	27	0.367	
Bacteroidetes Shannon	1.2	0.9	1.1	0.7	30	0.505	
Fusobacteria Shannon	0.5	0.7	0.6	0.6	25	0.813	
DIVERSITY CLASS							
Bacilli Shannon	1.6	0.9	1.8	0.6	50	0.780	
Gammaproteobacteria Shannon	1.2	0.6	1.3	0.6	57	0.442	
Betaproteobacteria Shannon	1.2	0.7	1.2	0.5	48	0.889	
Alphaproteobacteria Shannon	0.4	0.9	0.1	0.3	3	0.281	
Clostridia Shannon	0.8	1.2	0.4	0.7	12	0.441	
Bacteroidia Shannon	0.7	0.9	0.7	0.7	24	0.906	

Fusobacteriia Shannon	0.5	0.7	0.6	0.6	25	0.813
Negativicutes Shannon	0.3	0.4	0.2	0.4	12	0.800
C) Nature-oriented	Mean before	sd before	Mean after	sd after	Statistic	P value
Observed OTU richness	69.5	41.1	73.7	39.2	64	0.864
Chao richness index	84.1	58.5	88.2	56.1	62	0.932
Shannon	3.7	0.8	3.8	0.6	69	0.629
DIVERSITY PHYLUM						
Firmicutes Shannon	2.2	0.6	2.2	0.9	57	0.887
Proteobacteria Shannon	2.4	1.0	2.6	0.8	68	0.670
Actinobacteria Shannon	2.3	0.9	2.6	0.8	74	0.443
Bacteroidetes Shannon	1.5	1.0	1.6	0.8	71	0.551
Fusobacteria Shannon	0.4	0.7	0.5	0.5	30	0.878
DIVERSITY CLASS						
Bacilli Shannon	1.9	0.4	1.7	0.8	49	0.551
Gammaproteobacteria Shannon	1.4	0.6	1.6	0.7	73	0.478
Betaproteobacteria Shannon	1.5	0.7	1.5	0.7	55	0.900
Alphaproteobacteria Shannon	1.3	1.1	1.1	1.1	34	0.724
Clostridia Shannon	0.5	0.8	0.7	1.1	34	0.541
Bacteroidia Shannon	0.8	0.7	0.8	0.9	29	0.756
Fusobacteriia Shannon	0.4	0.7	0.5	0.5	30	0.878
Negativicutes Shannon	0.3	0.5	0.3	0.4	28	0.594

Table S6. Gut bacterial before-after comparisons (Paired t-test) among A) intervention, B) nature-oriented and C) standard daycare children. Relative abundances are shown at order level and diversities at family level [Mean ± Standard Deviation (sd)].

A) Intervention	Mean before	sd before	Mean after	sd after	Statistic	P value	P adjust*
ORDER RELATIVE ABUNDANCE							
Bacteroidales	1165.3	689.9	1490.9	503.2	2.2	0.039	0.256
Bifidobacteriales	181.1	98.1	190.2	167.8	0.2	0.820	0.888
Burkholderiales	17.5	26.7	17.0	15.1	-0.1	0.941	0.941
Clostridiales	2014.3	490.7	1717.7	401.1	-3.3	0.003	0.043
Coriobacteriales	45.1	35.3	63.1	66.2	1.6	0.127	0.383
Desulfovibrionales	1.3	3.5	2.8	4.7	1.2	0.236	0.383
Enterobacteriales	14.7	25.9	10.1	18.2	-0.9	0.404	0.478
Erysipelotrichales	11.3	12.5	23.0	29.0	1.8	0.083	0.358
Firmicutes_unclassified	41.1	75.2	22.8	36.2	-1.3	0.201	0.383
Lactobacillales	27.9	40.7	41.8	87.6	0.9	0.404	0.478
Pasteurellales	73.5	274.4	9.7	16.6	-1.1	0.277	0.401
Selenomonadales	94.1	99.5	143.8	166.5	1.3	0.225	0.383
Verrucomicrobiales	136.7	244.1	101.8	170.4	-1.3	0.219	0.383
FAMILY DIVERSITIES							

Bacteroidaceae Shannon	1.4	0.5	1.5	0.5	0.4	0.726	0.726
Bifidobacteriaceae Shannon	0.6	0.4	0.6	0.3	0.8	0.456	0.570
Lachnospiraceae Shannon	2.3	0.3	2.4	0.3	1.7	0.096	0.222
Prevotellaceae Shannon	0.2	0.4	0.1	0.3	-1.6	0.133	0.222
Ruminococcaceae Shannon	1.6	0.5	1.8	0.4	2.8	0.010	0.050
B) Nature-oriented Degrees of Freedom = 19	Mean before	sd before	Mean after	sd after	Statistic	P value	P adjust*
ORDER RELATIVE ABUNDANCE							
Bacteroidales	1487.3	629.8	1764.3	840.9	1.6	0.133	
Bifidobacteriales	210.5	191.8	147.5	120.6	-2.1	0.051	
Burkholderiales	12.3	20.6	14.9	17.7	0.5	0.616	
Clostridiales	1910.3	515.2	1664.2	659.9	-1.5	0.147	
Coriobacteriales	42.7	37.8	37.6	39.0	-0.8	0.432	
Desulfovibrionales	1.5	3.9	1.7	3.9	0.2	0.819	
Enterobacteriales	4.4	9.3	15.7	47.2	1.0	0.312	
Erysipelotrichales	17.3	27.3	9.5	9.7	-1.7	0.109	
Firmicutes_unclassified	29.3	53.4	39.4	68.6	1.4	0.177	
Lactobacillales	29.1	44.7	18.9	36.4	-0.7	0.469	
Pasteurellales	17.2	32.8	8.6	12.8	-1.1	0.300	
Selenomonadales	42.8	73.5	65.1	109.0	1.0	0.347	
Verrucomicrobiales	31.9	60.9	39.4	79.8	0.5	0.602	
FAMILY DIVERSITY							
Bacteroidaceae Shannon	1.5	0.3	1.5	0.4	0.0	0.990	
Bifidobacteriaceae Shannon	0.6	0.3	0.5	0.4	-1.8	0.088	
Lachnospiraceae Shannon	2.5	0.4	2.5	0.3	1.2	0.244	
Prevotellaceae Shannon	0.2	0.4	0.3	0.4	1.2	0.254	
Ruminococcaceae Shannon	1.7	0.4	1.8	0.3	1.1	0.292	
C) Standard Degrees of Freedom = 12	Mean before	sd before	Mean after	sd after	Statistic	P value	P adjust*
ORDER RELATIVE ABUNDANCE							
Bacteroidales	1060.2	791.1	1489.3	630.1	2.0	0.069	0.179
Bifidobacteriales	367.3	233.3	213.6	182.0	-2.1	0.059	0.179
Burkholderiales	1.4	2.6	13.6	11.8	3.4	0.007	0.091
Clostridiales	2123.5	642.3	1907.5	623.0	-1.2	0.260	0.483
Coriobacteriales	77.3	71.5	42.5	46.5	-2.6	0.029	0.179
Desulfovibrionales	1.3	4.2	2.1	3.3	0.7	0.502	0.670
Enterobacteriales	11.3	15.2	6.9	20.0	-0.5	0.617	0.670
Erysipelotrichales	24.5	25.9	12.6	12.3	-1.7	0.113	0.245
Firmicutes_unclassified	35.6	34.2	44.7	78.4	0.5	0.618	0.670
Lactobacillales	31.4	48.3	4.6	8.3	-2.1	0.060	0.179
Pasteurellales	2.5	5.2	3.1	4.4	0.4	0.718	0.718
Selenomonadales	39.0	65.1	55.1	115.5	0.6	0.580	0.670
Verrucomicrobiales	53.6	120.9	30.4	74.1	-0.5	0.614	0.670
FAMILY DIVERSITY							
Bacteroidaceae Shannon	1.3	0.3	1.5	0.4	2.3	0.044	0.219
Bifidobacteriaceae Shannon	0.5	0.3	0.5	0.3	0.1	0.902	0.902
Lachnospiraceae Shannon	2.5	0.2	2.4	0.4	-0.5	0.634	0.793

Prevotellaceae Shannon	0.0	0.1	0.1	0.2	1.3	0.227	0.567
Ruminococcaceae Shannon	1.9	0.4	1.8	0.3	-0.6	0.560	0.793

*Benjamini-Hochberg correction

Table S7. Correlations between diversity changes on skin microbiota and plasma cytokine concentrations.

(A) Increase in skin Gammaproteobacterial Alpha diversity during the 28-day intervention period was associated with rise in plasma TGF- β 1 concentration when all children (n = 26) were analyzed with linear mixed model. Among the intervention daycare center children (n = 12), (B) this diversity increase was also associated with a decrease in plasma IL-17A levels and (C) increase in total Treg cell levels. (D) Among the nature-oriented daycare center children (n = 10), the high Gammaproteobacterial diversity was associated with an increase in IL-10 expression, while Proteobacterial diversity was associated with a decrease in IL-10 expression. (E) Among the standard daycare center children (n = 6), the Gammaproteobacterial diversity change was mostly negative, and it was associated with TGF- β 1 plasma concentration. Thus, the change in TGF- β 1 plasma concentration was mostly negative. (F) Linear mixed-effect model showed that among children in nature-oriented daycare centers (n = 14), the higher abundance of *Faecalibacterium prausnitzii* (Otu000007) was associated with lower plasma levels of IL-17A and Otu000008 (unknown *Faecalibacterium*) with higher plasma levels of IL-17A. (G) The latter association was observed also within standard daycare children (n = 8). (H) Linear mixed-effect model showed that among nature-oriented daycare center children (n = 10), the decrease in IL-17A during the trial was associated with the decrease in the relative abundance of *Dorea* and *Romboutsia*, and increase in *Anaerostipes*.

A) All children: TGF-β1	Estimate	Std. Error	t value	P value	P adjusted*
Gammaproteobacteria	2.601	0.904	2.877	0.004	0.012
B) Intervention children: IL-17A	Estimate	Std. Error	t value	P value	P adjusted*
Gammaproteobacteria	-0.885	0.283	-3.132	0.002	0.003
C) Treg cells	Estimate	Std. Error	t value	P value	
Intervention: Gammaproteobacteria	1.238	0.513	2.413	0.016	
Standard: Gammaproteobacteria	-0.281	0.300	-0.936	0.349	

D) Nature-oriented children: IL-10					
	Estimate	Std. Error	t value	P value	P adjusted*
Gammaproteobacteria	3.539	0.715	4.947	7.5 x 10⁻⁷	2.3 x 10⁻⁶
Proteobacteria	-2.278	0.644	-3.536	0.0004	0.0006
E) Standard children: TGF-β1					
	Estimate	Std. Error	t value	P value	P adjusted*
Gammaproteobacteria	4.388	1.598	2.745	0.006	0.012
F) Nature-oriented children					
	Estimate	Std. Error	t value	P value	P adjusted*
Otu000007	-0.023	0.006	-3.885	0.000	0.004
Otu000008	0.013	0.004	3.470	0.001	0.009
G) Standard children					
	Estimate	Std. Error	t value	P value	P adjusted*
Otu000008	0.033	0.012	3.067	0.002	0.038
H) Nature-oriented children: IL-17A					
	Estimate	Std. Error	t value	P value	P adjusted*
Dorea	0.033	0.011	2.953	0.003	0.027
Romboutsia	0.0381	0.008	4.794	1.6 x 10⁻⁶	5.7 x 10⁻⁵
Anaerostipes	-0.013	0.004	-3.324	0.0009	0.010

*Benjamini-Hochberg correction

Table S8. Correlation between stool *Faecalibacterium* community composition and plasma cytokine levels.

Community composition of *Faecalibacterium* was associated with the IL-17A concentration in the plasma (n = 55) after but not before the 28-day intervention. No other associations between the measured plasma cytokines and *Faecalibacterium* community composition were found. Non-metric multidimensional scaling (NMDS) with Bray-Curtis metric was used to score the OTUs onto an ordination (NMDS coordinates 1 and 2) and correlation with corresponding cytokine expression levels was assessed using function *envfit* in *vegan* package in R computing environment.

Before	NMDS 1	NMDS 2	r²	P-value	
IL-10	0.292	0.956	0.011	0.794	
IL-17A	-0.517	0.856	0.076	0.179	
TGF-β1	-0.302	0.953	0.045	0.362	
After	NMDS 1	NMDS 2	r²	P-value	P adjust*
IL-10	-0.950	-0.311	0.048	0.354	0.531
IL-17A	-0.884	-0.468	0.214	0.015	0.045
TGF-β1	0.918	0.397	0.025	0.585	0.585

*Benjamini-Hochberg correction

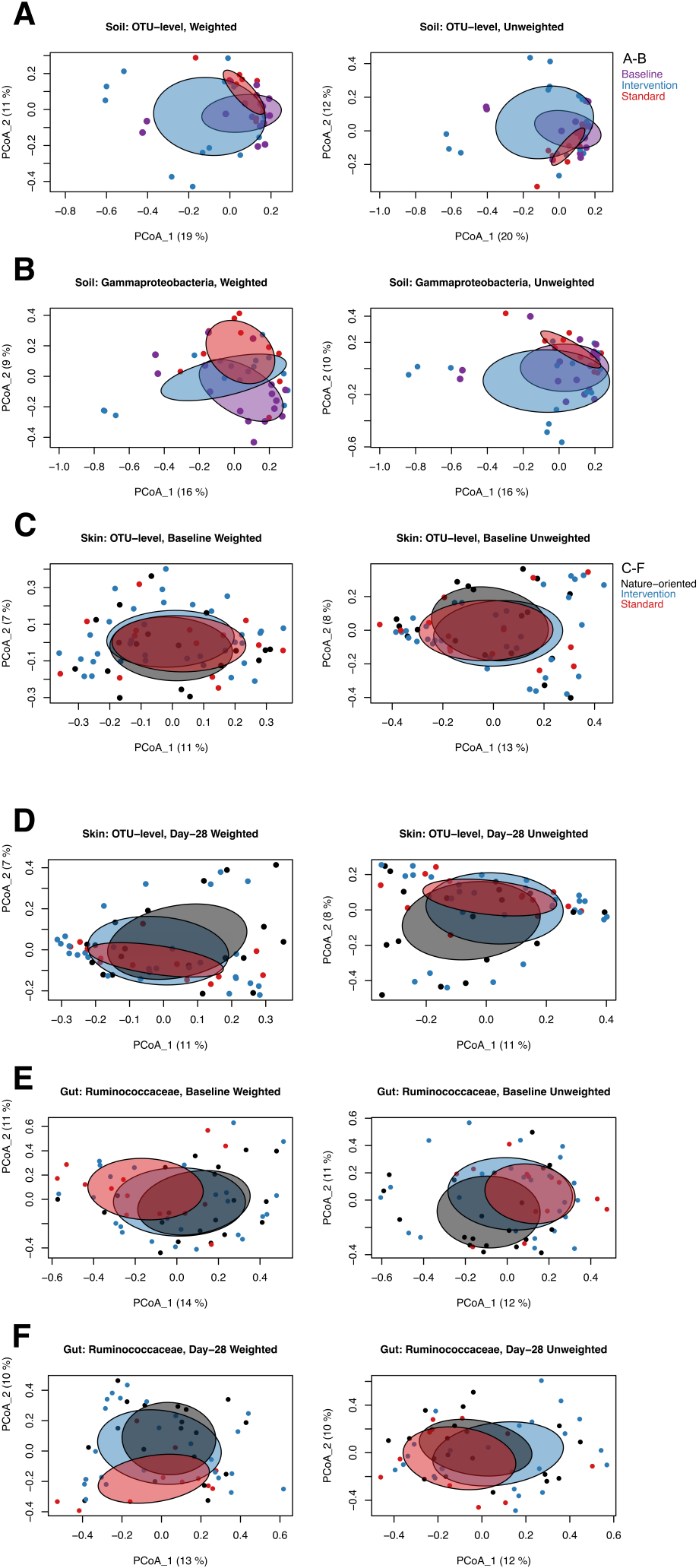


Fig. S1. Principal Coordinate Analysis (PCoA) for differences and shifts in bacterial communities A-B) in soil, B-C) on skin and E-F) in the gut. PCoA plots were calculated with dissimilarity indices with Bray-Curtis metric for weighted (abundance data) and unweighted (presence/absence data) data sets. For details of significant differences, please see Table S2. After the intervention period, **(A)** standard and intervention yards had different weighted and unweighted total bacterial and **(B)** Gammaproteobacterial community composition in soil. **(C)** Skin total bacterial community differed before intervention period between children in nature-oriented and standard daycares, **(D)** but not after intervention. In the gut, the sole difference at baseline was found **(E)** between nature-oriented and standard children in Ruminococcaceae community before intervention, while **(F)** after the intervention period Ruminococcaceae community was different additionally between the intervention and standard children.

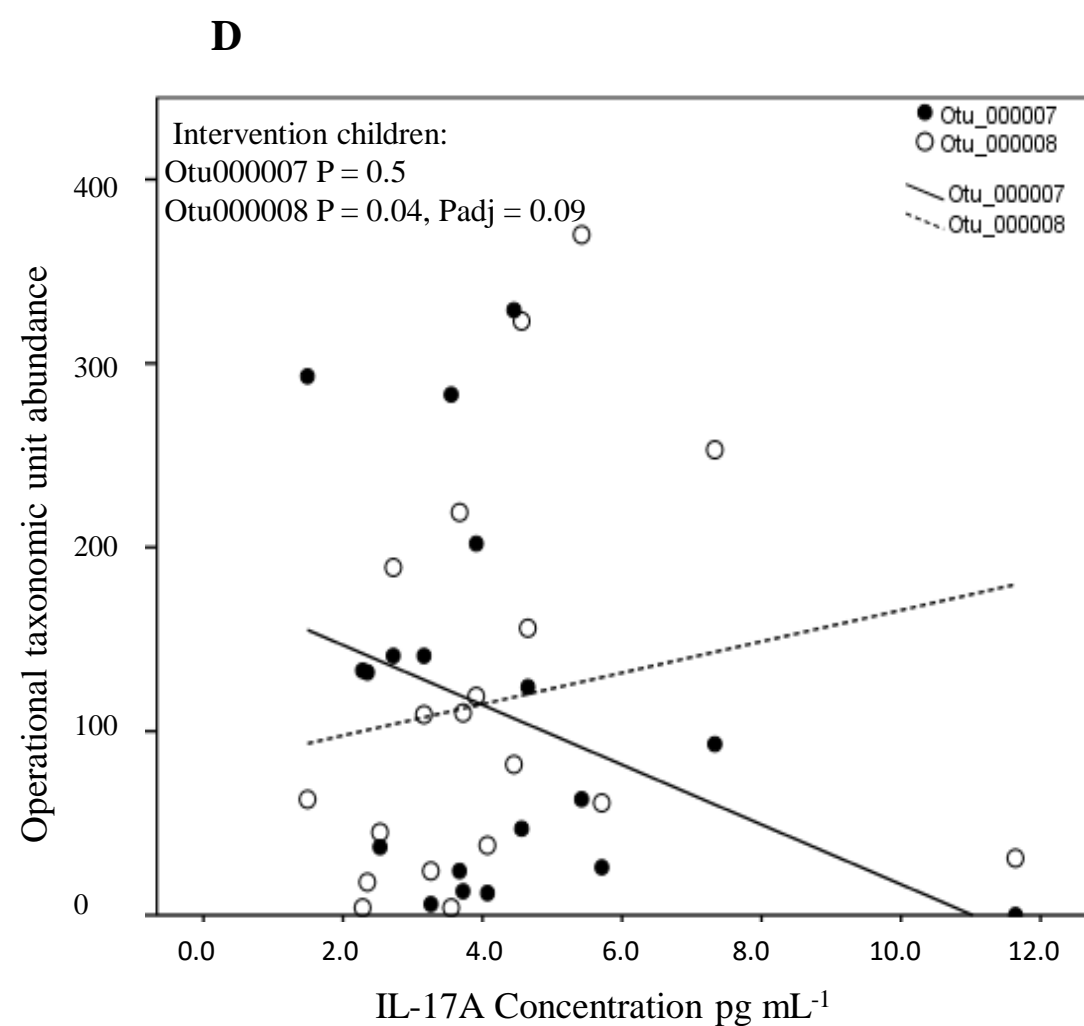
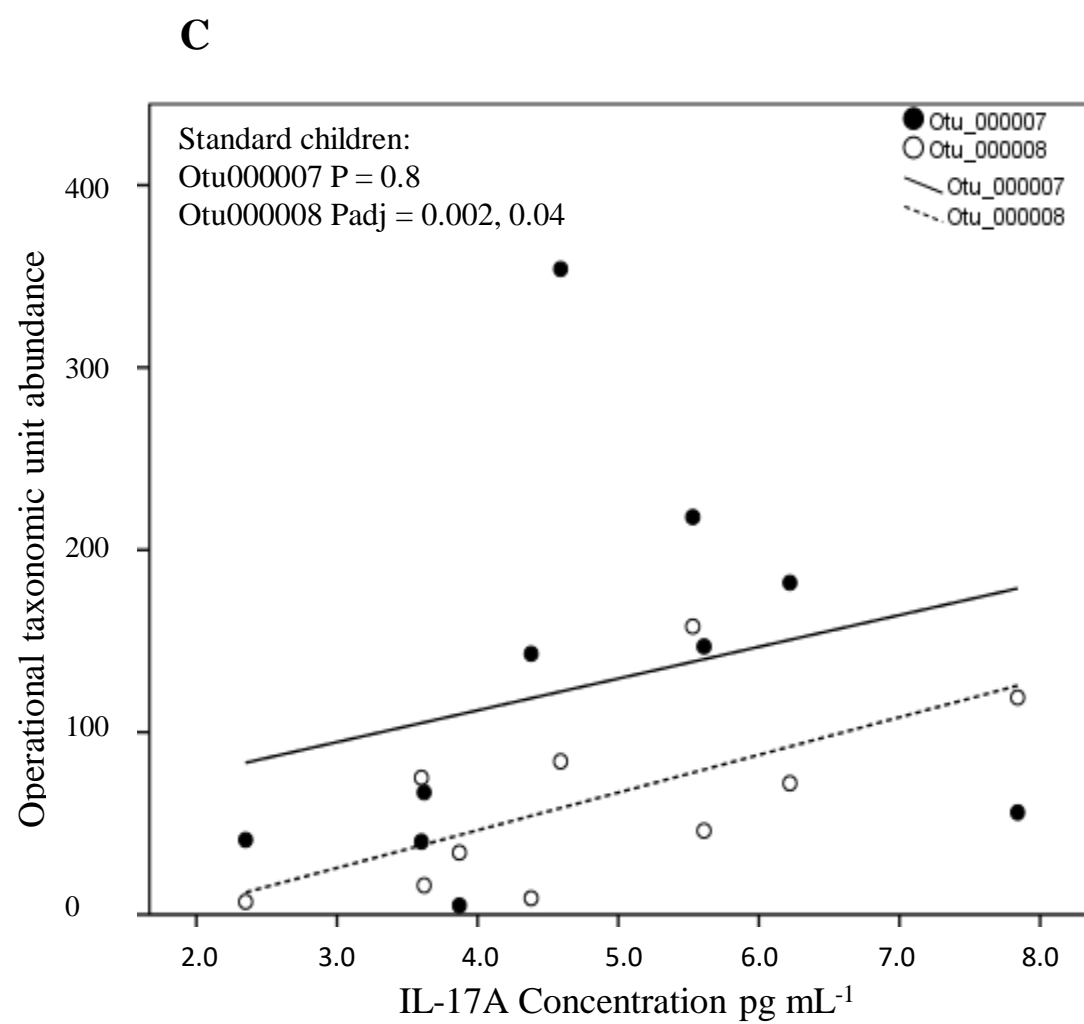
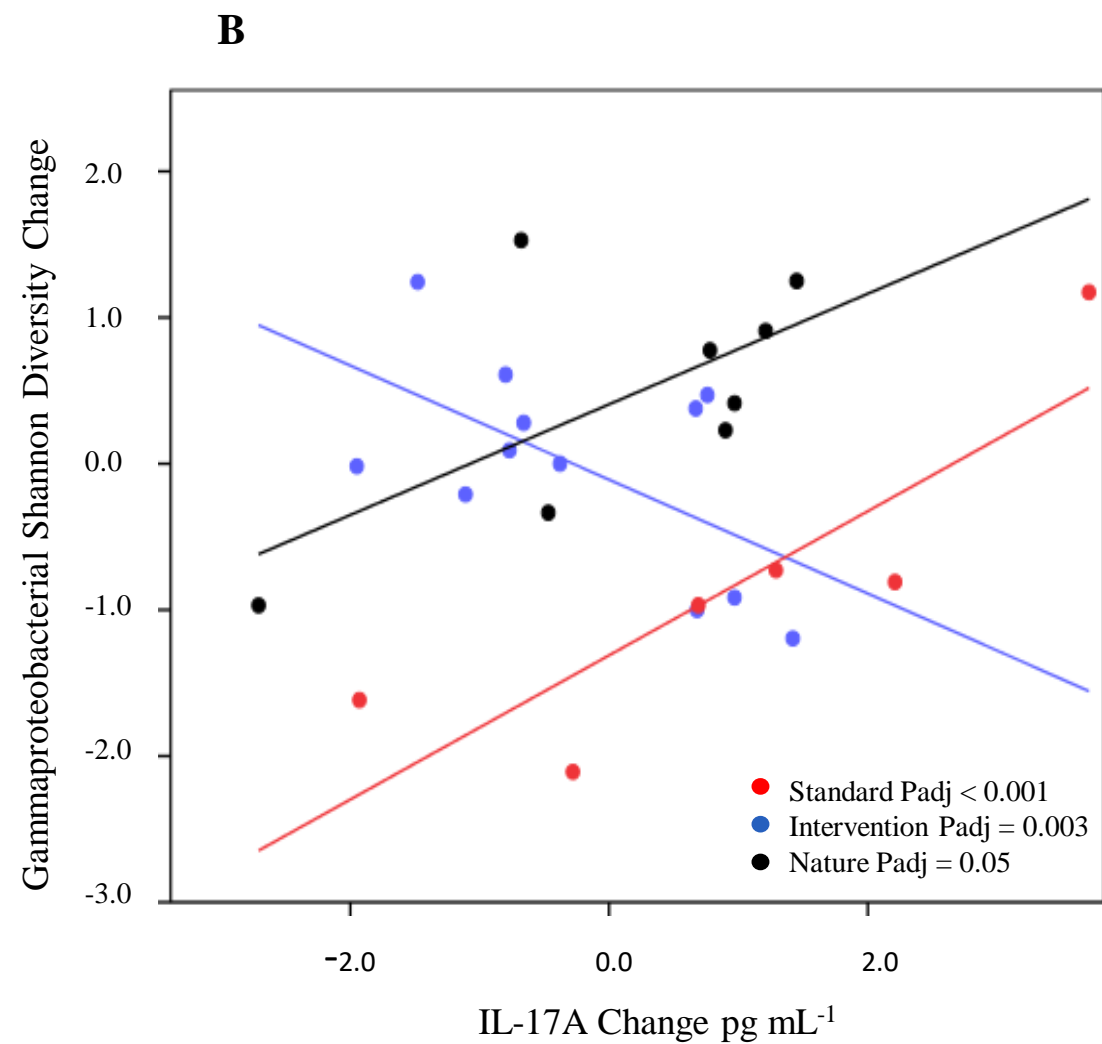
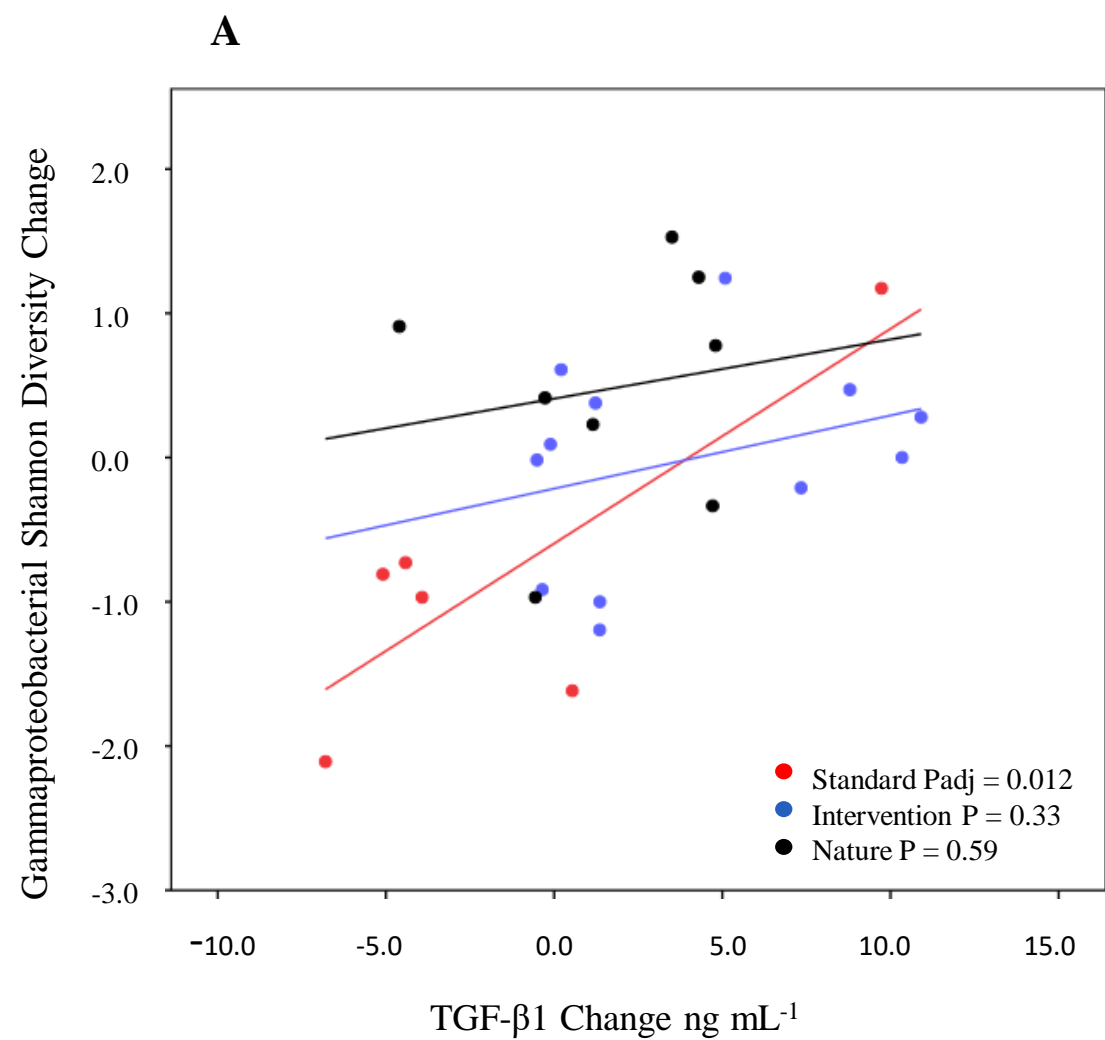


Fig. S2. Associations between change in A-B) skin Gammaproteobacterial diversity and C-D) gut Faecalibacterium OTUs, and immune markers. Skin Gammaproteobacterial diversity change was associated with plasma (A) TGF- β 1 (ng mL⁻¹) and (B) IL-17A (pg mL⁻¹) change among children in standard, intervention and nature-oriented daycares. (C) Among standard and (D) intervention children, *Faecalibacterium* Otu000008 was associated with an increase in IL-17A concentration (results from end of trial). Skin alpha diversity (Shannon index) and immune markers were measured on days 0 and 28 (change = day 28 – day 0).