

Appendix 1

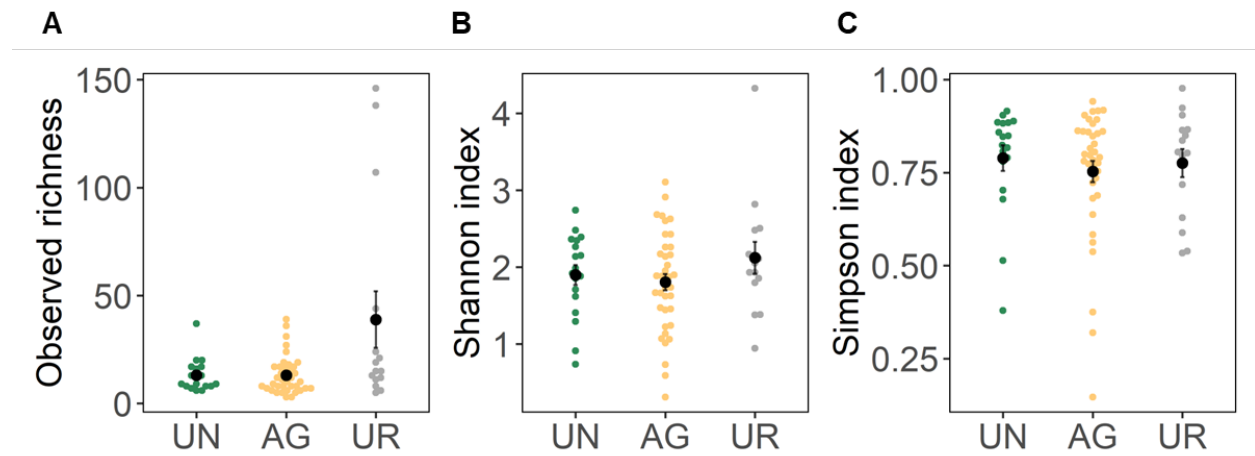


Fig. S1. Effect of land use type (green = undeveloped (UN), yellow = agricultural (AG), gray = urban (UR)) on alpha diversity of small and medium ground finch fecal microbiotas. Panels depict (A) Observed ASV richness, (B) Shannon index, and (C) Simpson index. Points represent individual birds. Black circles denote the mean values (\pm SE) of birds from each treatment.

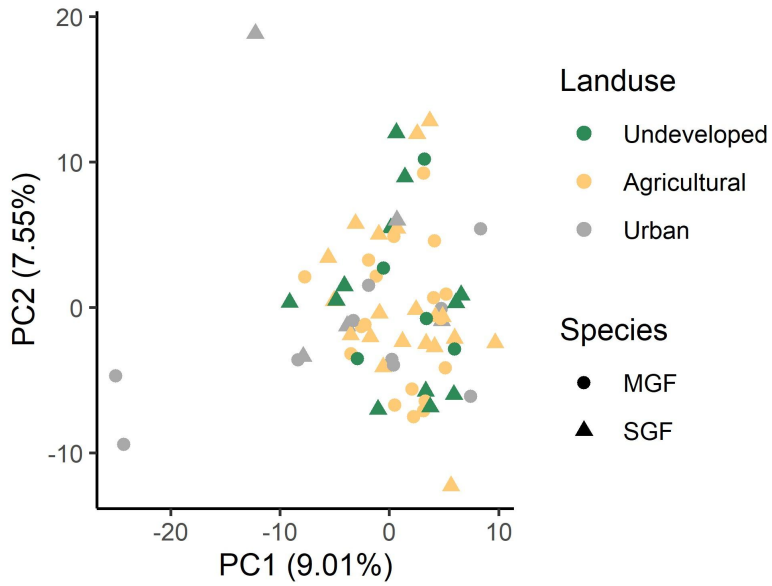


Fig S2. PC1 and PC2 scores derived from relative abundances of bacterial amplicon sequence variants (ASVs) from fecal samples of medium and small ground finches. Colors indicate land use type (green = undeveloped, yellow = agricultural, gray = urban) and shape denotes species (circles = medium ground finches, triangles = small ground finches).

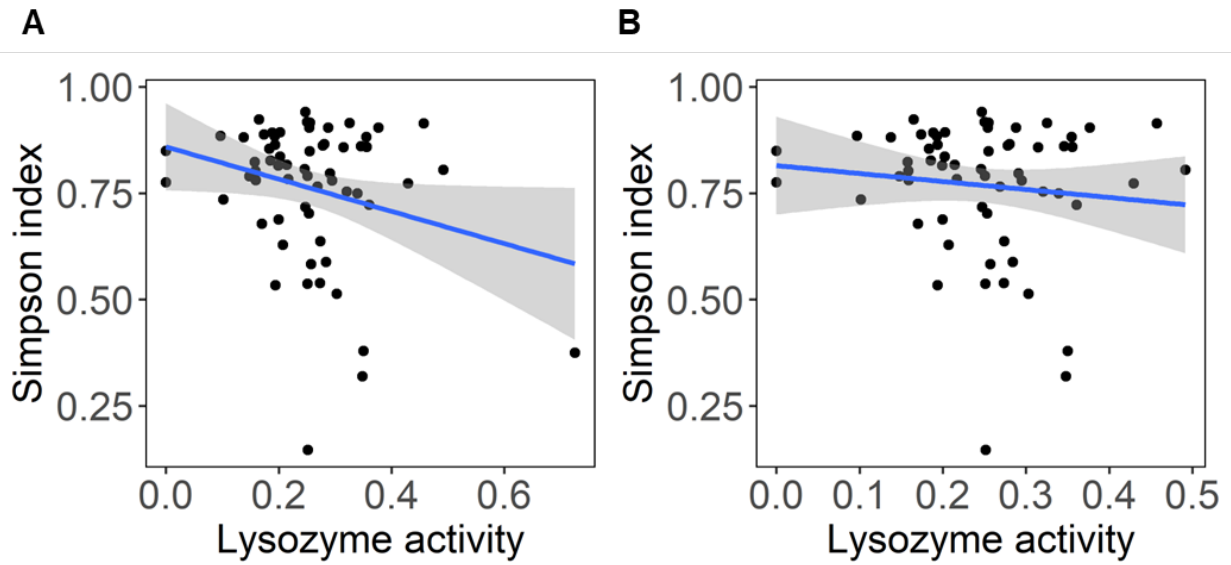


Fig. S3. Correlation between Simpson index and lysozyme activity in medium and small ground finches (A) with all data points included and (B) when the individual with the highest lysozyme activity (outlier) is removed. Each point represents an individual bird.

Table S1. GPS locations for each field site and the number of individuals caught for each field site.

Land use type	Field site name	Latitude	Longitude	Elevation (m)	# individuals <i>G. fortis</i>	# individuals <i>G. fuliginosa</i>
Urban	Puerto Ayora	-0.743101	-90.310493	7	10	5
Agricultural	El Chato	-0.682552	-90.432501	202	6	7
	Finca	-0.633890	-90.433617	376	12	4
	Media Luna	-0.672168	-90.323153	405	0	10
Undeveloped	El Garrapatero	-0.689600	-90.222585	21	5	8
	Los Gemelos	-0.625779	-90.385080	605	0	6
Total					33	40

Table S2. ANOVA results from the generalized linear models examining the effect of land use type, finch species, and their interaction on bacterial diversity metrics calculated from the rarefied dataset.

	χ^2	df	P-value
Observed richness			
Land use type	15.92	2	0.0003
Species	0.23	1	0.63
Interaction	2.87	2	0.24
Shannon index			
Land use type	2.55	2	0.28
Species	1.67	1	0.20
Interaction	2.89	2	0.24
Simpson index			
Land use type	0.52	2	0.77
Species	1.48	1	0.22
Interaction	2.46	2	0.29

Table S3. ANOVA results from the generalized linear models examining the effect of land use type, finch species, and their interaction on Shannon index and Simpson index calculated from the zero-replaced dataset.

	χ^2	df	P-value
Shannon index			
Land use type	0.56	2	0.75
Species	0.95	1	0.33
Interaction	4.04	2	0.13
Simpson index			
Land use type	0.43	2	0.81
Species	1.62	1	0.20
Interaction	3.36	2	0.19

Table S4. ANOVA results from the generalized linear models examining the effect of land use type, finch species, and their interaction on the first two principal components of a PCA calculated from the centered log-ratio (CLR) transformed and zero-replaced feature table.

	χ^2	df	P-value
PC1			
Species	0.01	1	0.94
Land use type	4.01	2	0.13
Interaction	0.48	2	0.79
PC2			
Species	2.23	1	0.14
Land use type	0.07	2	0.97
Interaction	3.28	2	0.19

Table S5. Results of ANCOM-BC analysis, including log fold change (LFC), standard errors (SE), and adjusted P-values (P_{adj}). Differential abundance in bacterial genera between undeveloped, agricultural (Ag.), and urban land use types in medium ground finches.

Genus	Ag. - Undeveloped			Urban - Undeveloped			Ag. - Urban		
	LFC	SE	P_{adj}	LFC	SE	P_{adj}	LFC	SE	P_{adj}
<i>Citrobacter</i>	-0.11	0.57	1	5.09	0.00	0	-5.22	0.00	0
<i>Afipia</i>	-0.28	2.01	1	-0.61	2.20	1	0.30	1.73	1
<i>Pajaroellobacter</i>	-2.72	2.19	1	-2.25	2.50	1	-0.49	1.75	1
<i>Bradyrhizobium</i>	-0.84	1.86	1	-0.76	2.15	1	-0.11	1.71	1
<i>Staphylococcus</i>	-0.08	1.99	1	1.48	2.23	1	-1.59	1.61	1
<i>Brucella</i>	1.84	0.00	0	0.31	0.00	0	1.50	1.16	1
<i>Sphingomonas</i>	-0.34	1.72	1	-0.23	1.92	1	-0.13	1.54	1
<i>Methylobacterium-Methylorubrum</i>	1.13	0.00	0	2.66	0.00	0	-1.55	1.64	1
<i>Paucibacter</i>	-2.21	2.30	1	-1.82	2.45	1	-0.42	1.28	1
<i>Haemophilus</i>	-0.66	1.89	1	-1.52	1.96	1	0.83	1.19	1
<i>Stenotrophomonas</i>	-1.32	1.73	1	-2.14	1.83	1	0.79	1.22	1
<i>Achromobacter</i>	-0.73	1.50	1	-0.45	1.63	1	-0.31	1.16	1
<i>Streptococcus</i>	-1.68	1.73	1	-2.06	1.86	1	0.36	0.97	1
<i>Corynebacterium</i>	-0.10	1.82	1	-0.44	1.94	1	0.32	1.24	1
<i>Escherichia-Shigella</i>	0.16	0.00	0	2.36	0.00	0	-2.22	1.54	1
<i>Klebsiella</i>	-1.48	1.27	1	-1.45	1.43	1	-0.06	0.74	1
<i>Cutibacterium</i>	0.11	0.65	1	0.21	0.82	1	-0.13	0.85	1

Table S6. Results of ANCOM-BC analysis, including log fold change (LFC), standard errors (SE), and adjusted P-values (P_{adj}). Differential abundance in bacterial genera between undeveloped, agricultural (Ag.), and urban land use types in small ground finches.

Genus	Ag. - Undeveloped			Urban - Undeveloped			Ag. - Urban		
	LFC	SE	P_{adj}	LFC	SE	P_{adj}	LFC	SE	P_{adj}
<i>Citrobacter</i>	-0.30	1.28	1	1.65	2.46	1	-2.70	2.34	1
<i>Afiptia</i>	-1.19	1.63	1	-2.28	2.45	1	0.34	2.29	1
<i>Pajaroellobacter</i>	0.72	1.55	1	1.72	2.21	1	-1.75	2.13	1
<i>Bradyrhizobium</i>	0.07	1.51	1	0.33	2.28	1	-1.00	2.13	1
<i>Staphylococcus</i>	0.88	1.51	1	0.65	2.39	1	-0.52	2.37	1
<i>Brucella</i>	0.87	1.08	1	-1.55	0.00	0	1.67	0.00	0
<i>Sphingomonas</i>	0.85	1.44	1	-0.45	2.40	1	0.54	2.34	1
<i>Methylobacterium-Methylorubrum</i>	0.27	1.54	1	-1.78	1.97	1	1.30	1.82	1
<i>Paucibacter</i>	-1.23	1.29	1	-3.13	0.00	0	1.14	0.00	0
<i>Haemophilus</i>	0.14	0.83	1	0.38	1.34	1	-0.99	1.30	1
<i>Stenotrophomonas</i>	-0.28	1.02	1	-1.82	0.00	0	0.78	0.00	0
<i>Achromobacter</i>	-2.03	1.12	1	-3.04	0.00	0	0.26	0.00	0
<i>Enhydrobacter</i>	0.46	1.02	1	0.64	1.34	1	-0.93	1.40	1
<i>Streptococcus</i>	0.15	1.18	1	-0.70	1.43	1	0.10	1.45	1
<i>Lawsonella</i>	-0.32	1.04	1	1.54	1.91	1	-2.61	1.84	1
<i>Corynebacterium</i>	-0.16	0.77	1	0.28	1.15	1	-1.19	1.16	1
<i>Delftia</i>	-0.76	1.00	1	-1.97	0.00	0	0.47	0.00	0
<i>Gemella</i>	-0.34	1.01	1	-0.53	1.18	1	-0.56	1.03	1
<i>Kocuria</i>	0.40	0.97	1	-0.26	1.02	1	-0.09	0.97	1
<i>Klebsiella</i>	-0.38	0.87	1	0.56	1.44	1	-1.70	1.32	1
<i>Cutibacterium</i>	0.71	0.00	0	0.07	0.00	0	-0.10	0.75	1

Table S7. The results of GLMMs on the effect of land use type, finch species, and their interaction on immune metrics.

	Land use type	Species	Interaction
Haptoglobin	$\chi^2 = 3.27, df = 2,$ $P = 0.19$	$\chi^2 = 0.15, df = 1,$ $P = 0.70$	$\chi^2 = 1.45, df = 2,$ $P = 0.48$
Lysozyme	$\chi^2 = 7.64, df = 2,$ $P = \mathbf{0.02}$	$\chi^2 = 3.63, df = 1,$ $P = 0.06$	$\chi^2 = 4.53, df = 2,$ $P = 0.10$
Complement antibodies	$\chi^2 = 9.41, df = 2,$ $P = \mathbf{0.01}$	$\chi^2 = 0.37, df = 1,$ $P = 0.55$	$\chi^2 = 0.24, df = 2,$ $P = 0.89$
Natural antibodies	$\chi^2 = 1.72, df = 2,$ $P = 0.42$	$\chi^2 = 0.06, df = 1,$ $P = 0.80$	$\chi^2 = 3.03, df = 2,$ $P = 0.22$

Table S8. Mean \pm SE of each immune metric across land use types and finch species. Sample size in parenthesis.

	Undeveloped	Agricultural	Urban
Small ground finches			
Haptoglobin	0.97 \pm 0.16 (13)	0.94 \pm 0.08 (20)	0.52 \pm 0.14 (3)
Lysozyme	0.23 \pm 0.02 (13)	0.25 \pm 0.02 (21)	0.24 \pm 0.03 (3)
Complement antibodies	3.17 \pm 0.55 (12)	5.00 \pm 0.48 (20)	5.50 \pm 0.50 (2)
Natural antibodies	1.21 \pm 0.28 (12)	1.00 \pm 0.18 (20)	1.50 \pm 1.0 (2)
Medium ground finches			
Haptoglobin	0.81 \pm 0.18 (5)	0.89 \pm 0.09 (18)	0.74 \pm 0.07 (10)
Lysozyme	0.31 \pm 0.02 (5)	0.31 \pm 0.05 (14)	0.20 \pm 0.03 (9)
Complement antibodies	3.40 \pm 0.51 (5)	5.53 \pm 0.53 (17)	5.10 \pm 0.74 (10)
Natural antibodies	0.60 \pm 0.29 (5)	1.32 \pm 0.19 (17)	1.50 \pm 0.37 (10)

Table S9. Correlation coefficients for bacterial diversity metrics (observed richness, Shannon index, Simpson index) and immune metrics (lysozyme activity, and haptoglobin, complement antibody, and natural antibody levels).

Dataset	Alpha diversity metric	Haptoglobin levels	Lysozyme activity	Complement antibodies (agglutination)	Natural antibodies (lysis)
Rarefied	Observed richness	-0.09	-0.04	0.09	0.07
Rarefied	Shannon index	-0.17	-0.21	0.11	0.04
Rarefied	Simpson index	-0.10	-0.26	0.09	0.04
Zero-replaced	Shannon index	-0.10	-0.19	0.07	-0.05
Zero-replaced	Simpson index	-0.03	-0.22	0.05	-0.01

Table S10. The results of GLMMs on the effect of land use type, finch species, and their interaction on body mass and size.

	Land use type	Species	Interaction
Body mass	$\chi^2 = 3.88$, df = 2, $P = 0.14$	$\chi^2 = 353.03$, df = 1, $P < 0.0001$	$\chi^2 = 6.60$, df = 2, $P = 0.04$
Tarsus length	$\chi^2 = 3.14$, df = 2, $P = 0.21$	$\chi^2 = 92.54$, df = 1, $P < 0.0001$	$\chi^2 = 2.26$, df = 2, $P = 0.32$
Scaled mass index	$\chi^2 = 0.51$, df = 2, $P = 0.78$	$\chi^2 = 5.57$, df = 1, $P = 0.02$	$\chi^2 = 0.10$, df = 2, $P = 0.95$

Table S11. Mean (\pm SE) of each body mass (g) and tarsus length (mm) across land use types and finch species. Sample size in parenthesis.

	Undeveloped	Agricultural	Urban
Small ground finches			
Body mass	14.07 \pm 0.30 (14)	14.43 \pm 0.23 (21)	14.36 \pm 0.61 (5)
Tarsus length	21.12 \pm 0.31 (14)	21.41 \pm 0.22 (21)	21.30 \pm 0.34 (5)
Scaled mass index	14.27 \pm 0.26 (14)	14.33 \pm 0.23 (21)	14.37 \pm 0.62 (5)
Medium ground finches			
Body mass	19.36 \pm 1.17 (5)	22.32 \pm 0.50 (18)	22.44 \pm 0.58 (10)
Tarsus length	22.88 \pm 0.56 (5)	24.12 \pm 0.27 (18)	23.93 \pm 0.31 (10)
Scaled mass index	21.33 \pm 1.23 (5)	21.86 \pm 0.47 (18)	22.39 \pm 0.65 (10)