Analyses of rarified bacterial abundances

To assess the impact of sequencing depth on our data and findings, we re-calculated the relative abundance of each ASV at the same sequencing depth using the MicrobiomeAnalyst pipeline (Chong et al., 2020). ASVs were filtered based on abundance (minimum median count of 2) and all samples were rarified to the smallest sample size after filtering (n= 1361) using default settings (**Fig. S1**). Rarefaction did not alter the number of ASVs per tadpole; however, it slightly decreased the value of Shannon diversity index (**Fig. S2**). Nevertheless, there was a strong positive correlation between Shannon diversity calculated from the rarified data and Shannon diversity calculated from the non-rarified data (Pearson correlation: $r= 0.96$, $p< 0.001$, $n= 41$). Accordingly, the effect of habitat remained non-significant on Shannon diversity when calculated from the rarified data instead of the non-rarified data (GEE model: $p= 0.942$ for all 3 pairwise comparisons), while bacterial community composition remained different among the three habitats types (**Fig. S3**). Similarly, the Spearman correlations of Shannon diversity with corticosterone variables remained qualitatively unchanged when calculated from the rarified data instead of the non-rarified data: there was a significant positive correlation with baseline corticosterone release rate ($r= 0.34$, $p= 0.03$, $n= 41$) but no significant correlation with stress response ($r= -0.014$, $p= 0.9$, $n= 41$) and negative feedback ($r= -0.046$, $p= 0.8$, $n= 41$).

Reference:

Chong, J., Liu, P., Zhou, G. et al. 2020. Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nat. Protoc. 15, 799–821. https://doi.org/10.1038/s41596-019-0264-1

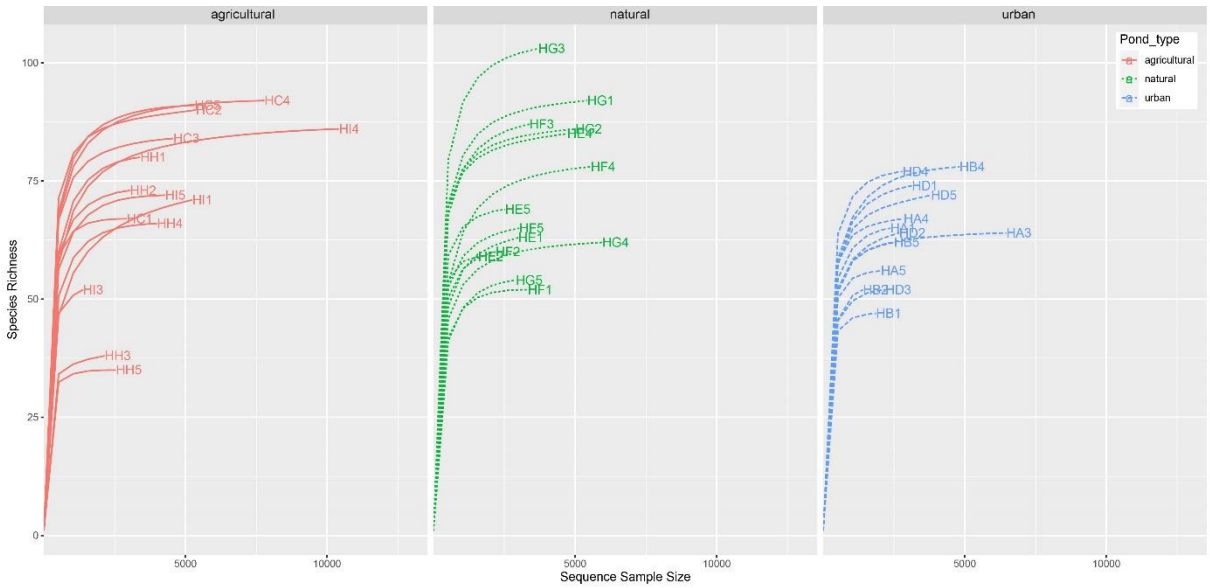

Figure S1. Rarefaction curves of the 41 samples.

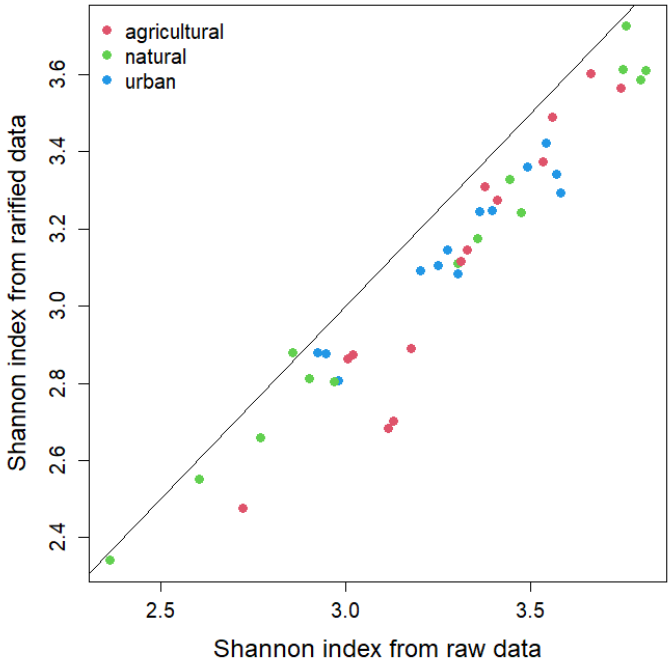

Figure S2. Relationship between Shannon diversity indices calculated with and without rarefaction of microbial data. The line represents equality between the two measures.

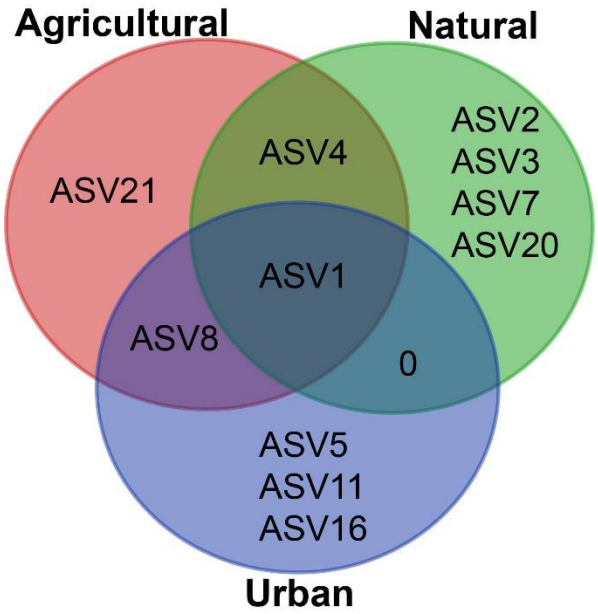

Figure S3. Venn diagram showing the overlap of ASVs with minimum relative abundance of 0.01% and prevalence of at least 60% of samples in each habitat type.

Table S2. Pairwise comparisons (linear contrast estimates with standard error, SE) of tadpole gut microbiome diversity metrics between 3 habitat types from GEE models. P-values are corrected with the false discovery rate method for each dependent variable.

Dependent variable	Contrast	Estimate	SE	z	p
	agricultural-natural	0.780	6.730	0.116	0.908
A) species richness	agricultural-urban	8.280	6.290	1.317	0.282
	natural-urban	7.500	3.680	2.039	0.124
	agricultural-natural	0.056	0.190	0.294	0.946
B) Shannon diversity	agricultural-urban	-0.009	0.132	-0.068	0.946
	natural-urban	-0.065	0.148	-0.438	0.946
	natural-agricultural	0.447	0.390	1.146	0.756
C) ln(Firmicutes/Bacteroidota)	natural-urban	0.100	0.411	0.243	0.808
	agricultural-urban	-0.347	0.546	-0.636	0.788
D) Simpson diversity	agricultural-natural	0.019	0.024	0.815	0.622
	agricultural-urban	-0.004	0.011	-0.344	0.731
	natural-urban	-0.023	0.023	-1.021	0.623
E) Chao diversity	agricultural-natural	0.567	6.96	0.081	0.935
	agricultural-urban	8.456	6.62	1.277	0.302
	natural-urban	7.890	3.80	2.078	0.1131

Table S3. Relationships between aspects of the corticosterone profile *versus* measures of gut microbiome diversity and composition, controlling for habitat type. In each cell of the table, the χ^2 statistic and p-value come from the analysis-of-deviance table of a GEE model that includes habitat type and another predictor (as specified in the table). The results refer to these predictors and are not shown for habitat type which was merely controlled for. In part "A" of the table, corticosterone variables were used as predictors and microbiome aspects as dependent variables, whereas in part "B" the predictor and dependent variable were swapped. For these analyses, to ensure model fit we transformed baseline corticosterone release rate and Firmicutes/Bacteroidota ratio to their natural logarithm, and the rate of negative feedback using the following formula (based on Bókony et al. 2022): (negative feedback + 480)^4.

A)	**Species richness**	**Shannon diversity**	**Firmicutes/Bacteroidota ratio**
Baseline corticosterone release rate	$\chi^2_1 = 10.63$ $p = 0.001$	$\chi^2_1 = 60.0$ $p < 0.001$	$\chi^2_1 = 0.20$ $p = 0.655$
Stress response	$\chi^2_1 = 0.480$ $p = 0.490$	$\chi^2_1 = 0.167$ $p = 0.680$	$\chi^2_1 = 3.310$ $p = 0.069^*$
Negative feedback	$\chi^2_1 = 0.080$ $p = 0.780$	$\chi^2_1 = 1.052$ $p = 0.310$	$\chi^2_1 = 0.056$ $p = 0.810$
B)	Baseline corticosterone release rate	Stress response	Negative feedback
Species richness	$\chi^2_1 = 5.88$ $p = 0.015$	$\chi^2_1 = 0.078$ $p = 0.780$	$\chi^2_1 = 0.210$ $p = 0.650$
Shannon diversity	$\chi^2_1 = 0.441$ $p = 0.510$	$\chi^2_1 = 0.224$ $p = 0.640$	$\chi^2_1 = 0.140$ $p = 0.710$
Firmicutes/Bacteroidota ratio	$\chi^2_1 = 0.975$ $p = 0.320$	$\chi^2_1 = 5.530$ $p = 0.019^†$	$\chi^2_1 = 0.070$ $p = 0.790$

*This marginally significant result is due to a single outlier (tadpole 'HH5' from an agricultural site; this individual had extremely low abundance of Bacteroidota). Excluding the outlier yields p=0.464

†This significant result is due to a single outlier (same individual as above). Excluding the outlier yields p=0.740