Supplementary Materials:

Supplementary methods:

Additional details on bioinformatic analysis

We followed the analysis pipeline outlined in Caporaso *et al*., [1], with the exception of the *denoiser* step, which was omitted as it is not required for Illumina data. Sequence reads were excluded if there was more than a one base pair error in the barcode. The pipeline began with trimming and demultiplexing of paired reads. Default cutoff values were used for quality score and read length. Chimeric sequences were then identified using ChimeraSlayer [2] and removed before performing downstream analyses. OTUs were picked using a 0.97 similarity threshold and the default parameters of the *pick_open_reference_otus* pipeline from MacQIIME (an open reference method). Reads identified as belonging to chloroplasts were removed from the resulting OTU table using the *filter_taxa_from_otu_table.py* script (and -n c__chloroplast parameter). The relative abundance of each taxa was estimated from the final OTU table, summarized at the level of order and plotted.

Microbial function assignments were done using PICRUSt version 1.1.0 [3]. Since the OTUs for the above analyses were picked using an open reference it was necessary to filter the OTU table so that it only contained OTUs found in the Green Genes database [4]. Filtering of the OTU table was done against Green Genes 13.5 release with 97% sequence similarity threshold. The filtered OTU table was normalized by copy number, then used to predict the meta-genome with estimation of NSTI scores. The mean and median NSTI score for our samples was 0.08 (range 0.06 - 0.11 across populations). We were then able to categorize the OTUs by biological function, yielding KEGG annotations.

Supplementary Table 1: Sampling Information

Location	Population Types	Number of Individuals
Little Quarry, Nelson Island	Benthic and Limnetic	5 Benthic 5 Limnetic
Paxton Lake, Texada Island	Benthic and Limnetic	5 Benthic 5 Limnetic
Priest Lake, Texada Island	Benthic and Limnetic	5 Benthic 5 Limnetic
Enos Lake, Vancouver Island	Hybrid	5
Oyster Lagoon	Marine	5

Supplementary Table 2: Sequencing Depth Information

Sample Name	**Number of Reads**

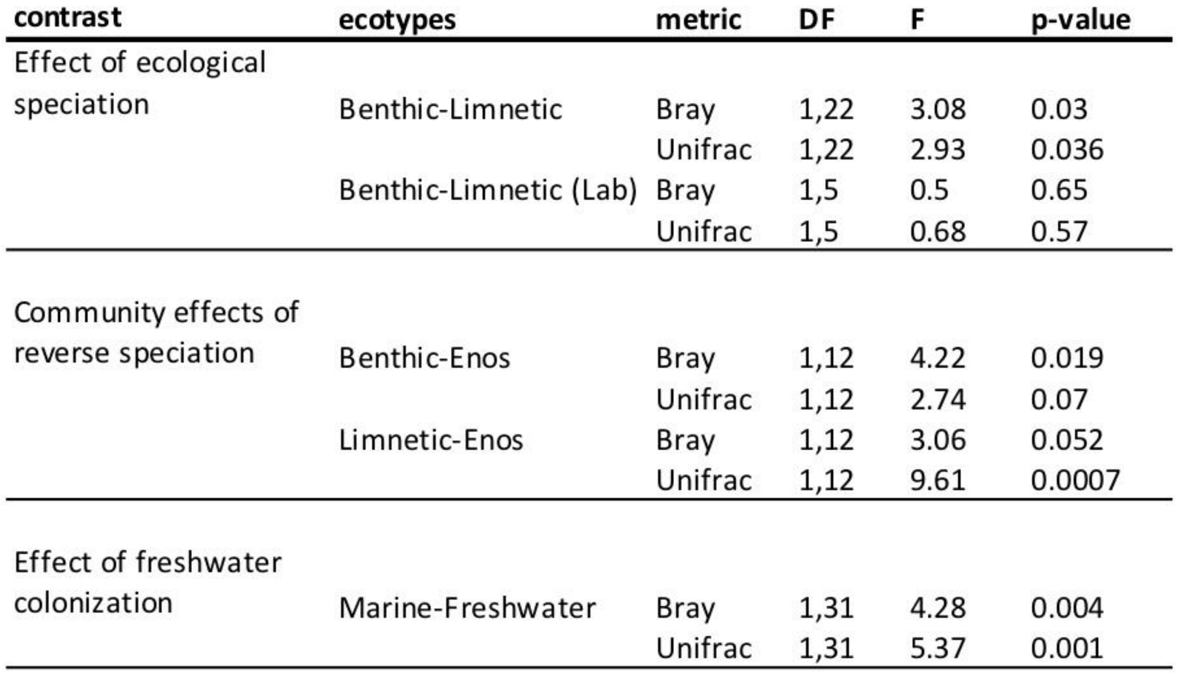
Enos_1	263785
Enos_2	162060
Enos_3	221898
Enos_4	52433
Enos_5	119908
F1_1	249198
F1_2	286382
F1_3	258084
Lab_Benthic_1	199326
Lab_Benthic_2	254145
Lab_Benthic_3	174560
Lab_Limnetic_1	229710
Lab_Limnetic_2	338744
Lab_Limnetic_3	135637
LQ_Benthic_1	386895
LQ_Benthic_2	193048

LQ_Benthic_3	317028
LQ_Benthic_4	354577
LQ_Benthic_5	8517
LQ_Limnetic_1	311149
LQ_Limnetic_2	279873
LQ_Limnetic_3	210474
LQ_Limnetic_4	282915
LQ_Limnetic_5	130182
Oyster_1	177604
Oyster_2	255892
Oyster_3	351335
Oyster_4	211424
Oyster_5	98692
Pax_Benthic_1	295771
Pax_Benthic_2	269054
Pax_Benthic_3	206654

Pax_Benthic_4	333619
Pax_Benthic_5	350804
Pax_Limnetic_1	206183
Pax_Limnetic_2	212249
Pax_Limnetic_3	175808
Pax_Limnetic_4	353157
Pax_Limnetic_5	193084
Pri_Benthic_1	293703
Pri_Benthic_2	253183
Pri_Benthic_3	283444
Pri_Benthic_4	136522
Pri_Benthic_5	293976
Pri_Limnetic_1	245312
Pri_Limnetic_2	279793
Pri_Limnetic_3	301799
Pri_Limnetic_4	286803

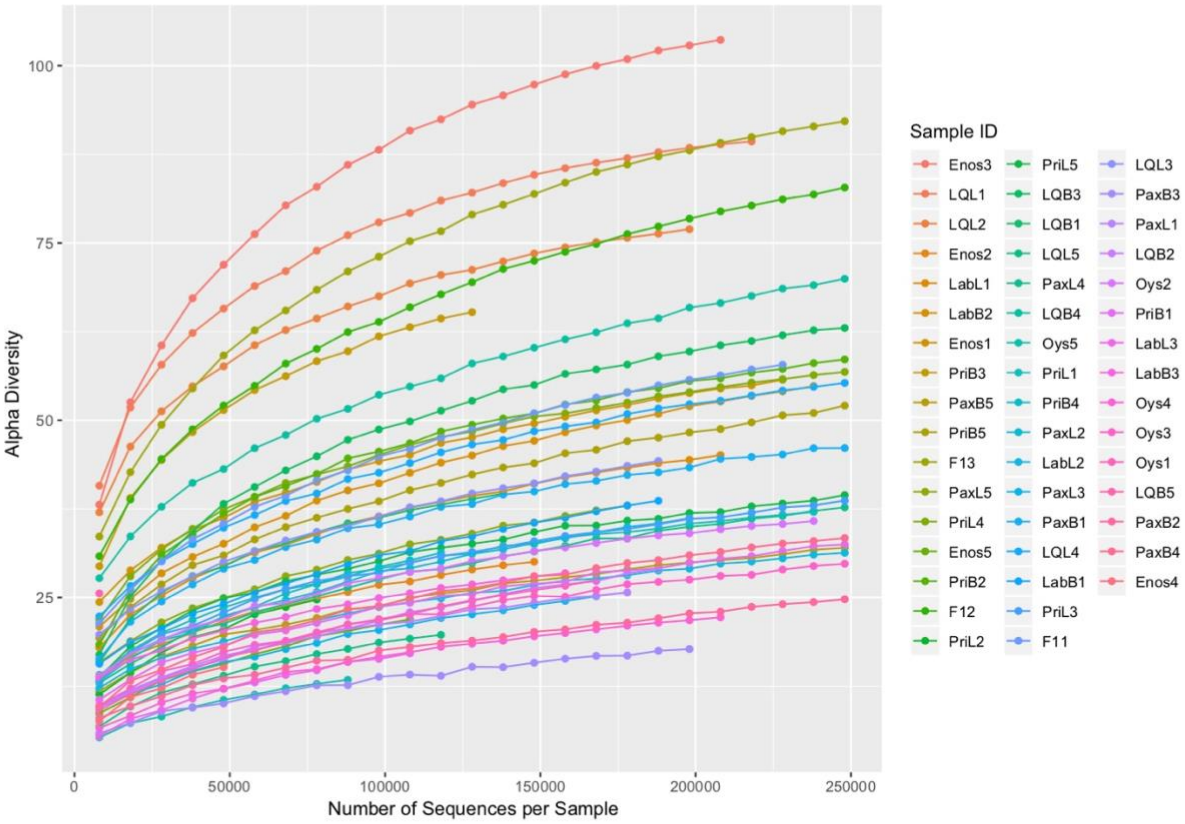
Pri_Limnetic_5	357676

Supplementary Table 3: Results from community composition analysis using both Bray-Curtis and weighted Unifrac diversity metrics. To match with analyses of parallelism all tests were conducted MANOVAs on the first 5 NMDS axes.

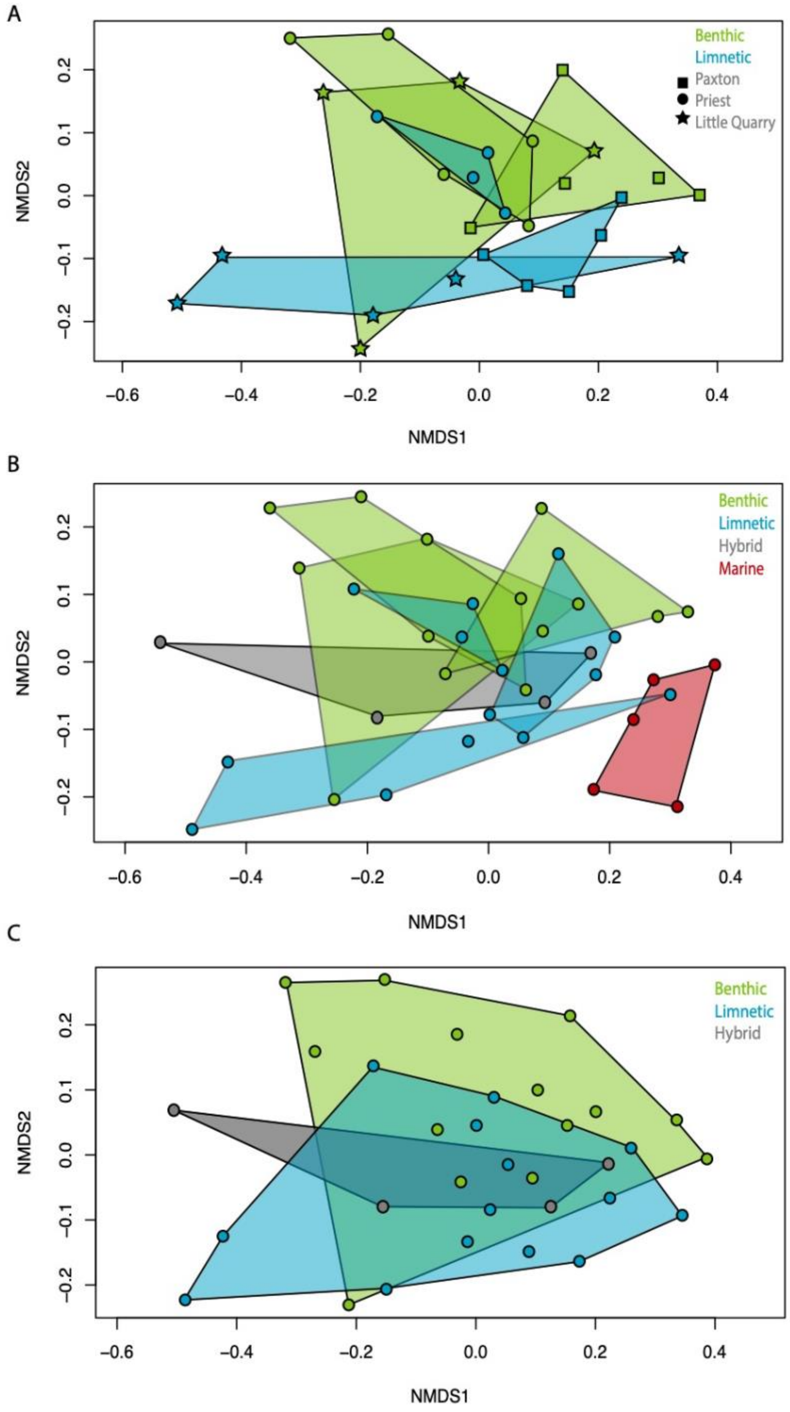


contrast	ecotypes	metric	DF	F	p-value
Effect of ecological speciation	Benthic-Limnetic	Bray	1,22	3.08	0.03
		Unifrac	1,22	2.93	0.036
	Benthic-Limnetic (Lab)	Bray	1,5	0.5	0.65
		Unifrac	1,5	0.68	0.57
Community effects of reverse speciation	Benthic-Enos	Bray	1,12	4.22	0.019
		Unifrac	1,12	2.74	0.07
	Limnetic-Enos	Bray	1,12	3.06	0.052
		Unifrac	1,12	9.61	0.0007
Effect of freshwater colonization	Marine-Freshwater	Bray	1,31	4.28	0.004
		Unifrac	1,31	5.37	0.001

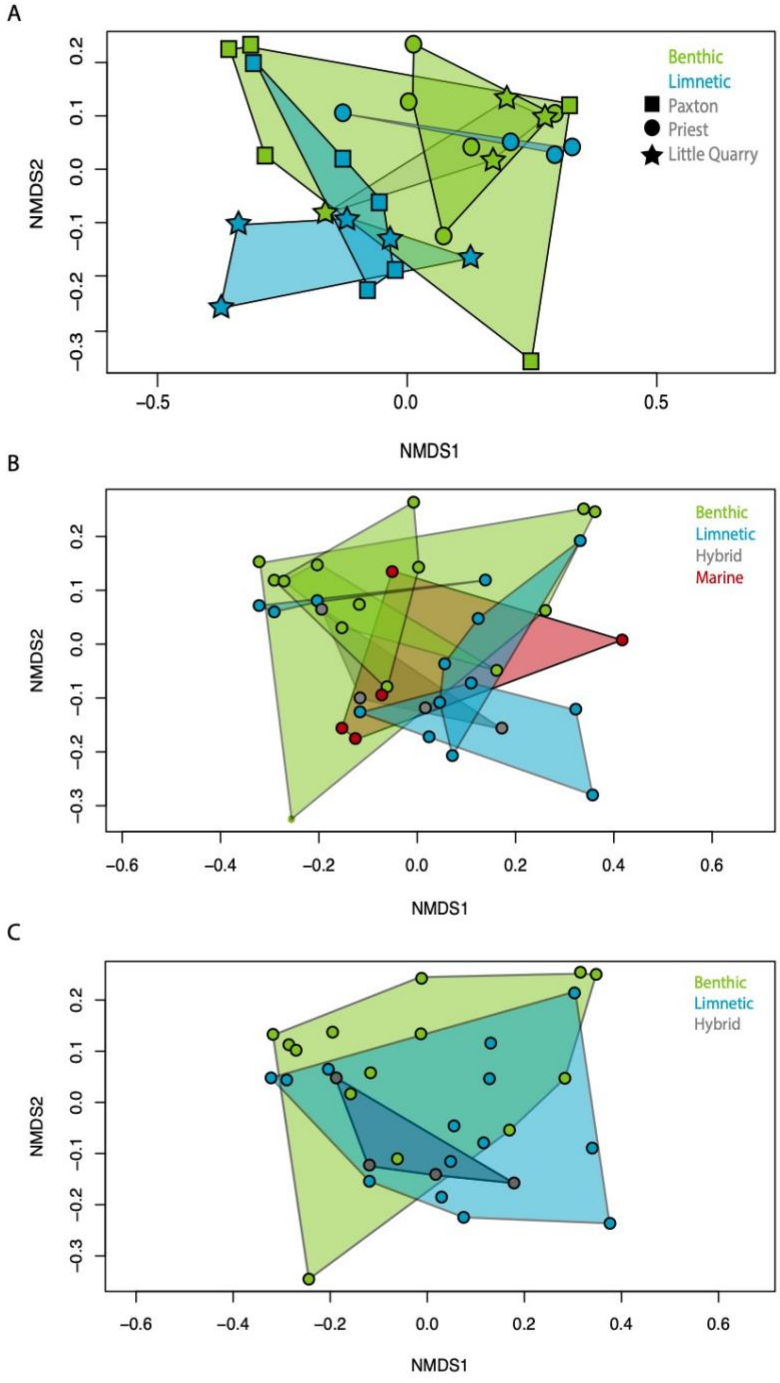
Supplementary Figure 1. Rarefaction plots for each individual indicating estimated alpha diversity for the number of sampled sequencing reads.



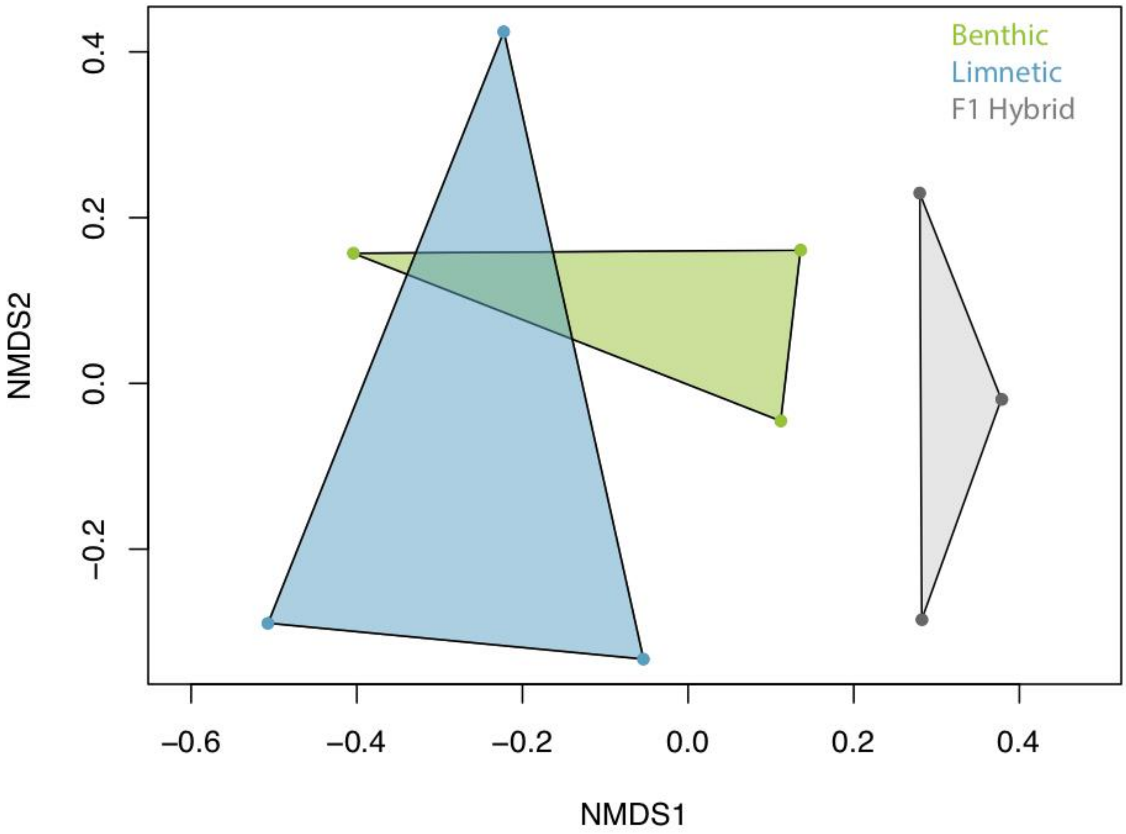
Supplementary Figure 2: Differentiation of the taxonomic composition based on unweighted UniFrac distances for (a) the gut microbiome of benthic and limnetic threespine stickleback from Paxton, Priest and Little Quarry, (b) freshwater ecotypes and marine individuals, and (c) hybrid threespine stickleback from Enos lake relative to benthic and limnetic stickleback from Paxton, Priest and Little Quarry.



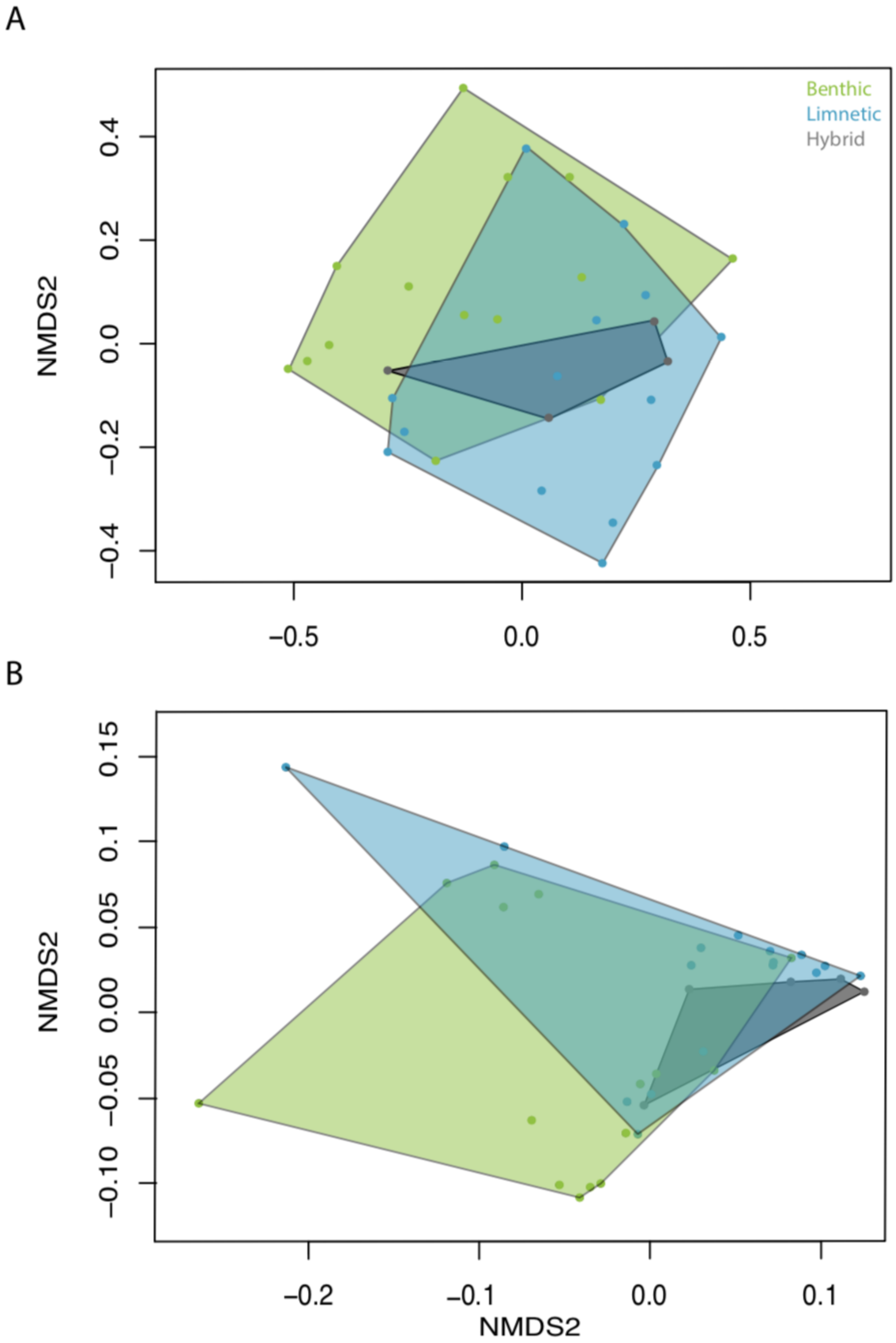
Supplementary Figure 3: Differentiation of the taxonomic composition based on weighted UniFrac distances for (a) the gut microbiome of benthic and limnetic threespine stickleback from Paxton, Priest and Little Quarry, (b) freshwater ecotypes and marine individuals, and (c) hybrid threespine stickleback from Enos lake relative to benthic and limnetic stickleback from Paxton, Priest and Little Quarry.



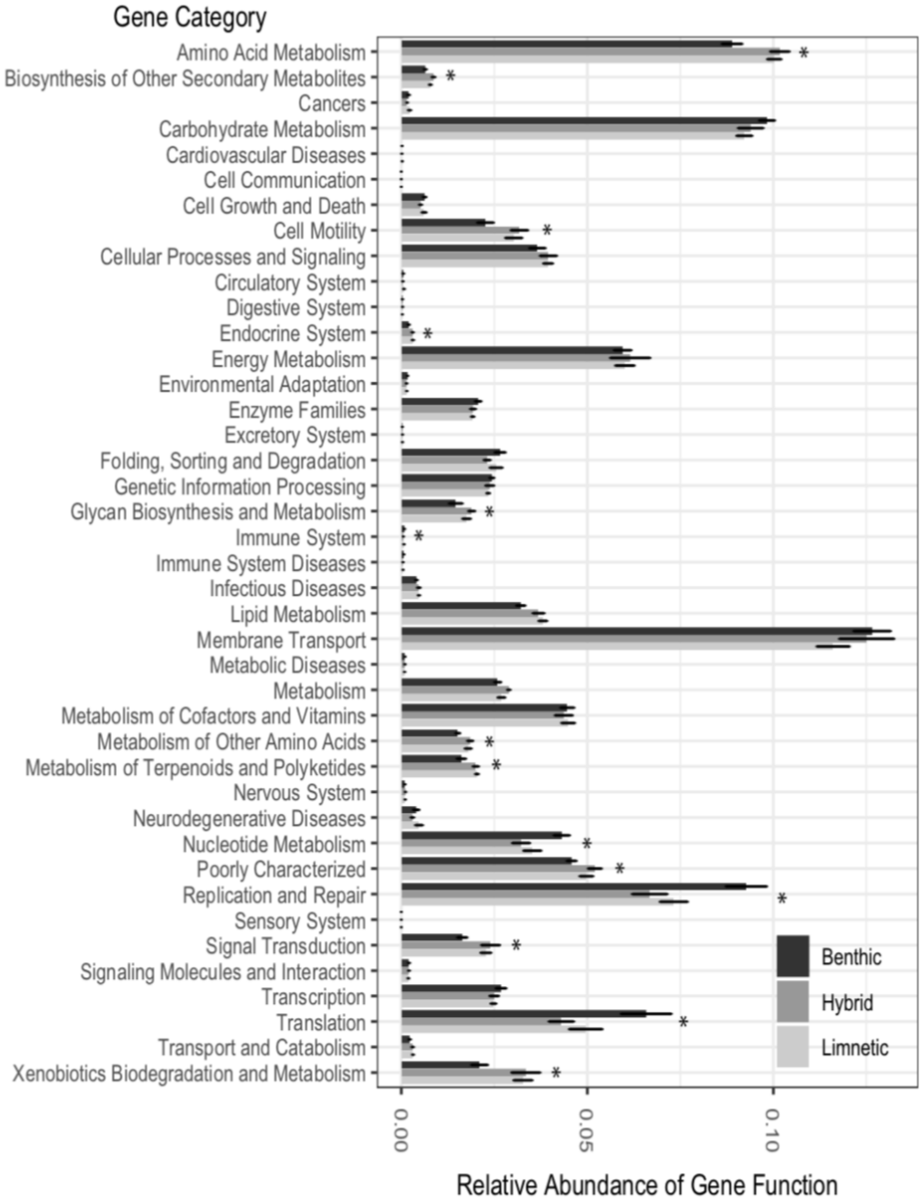
Supplementary Figure 4: Differentiation of the composition of the gut microbiome of lab reared benthic, limnetic, and F1 benthic-limnetic hybrid threespine stickleback. Multidimensional diversity is estimated using the Bray Curtis beta diversity metric.



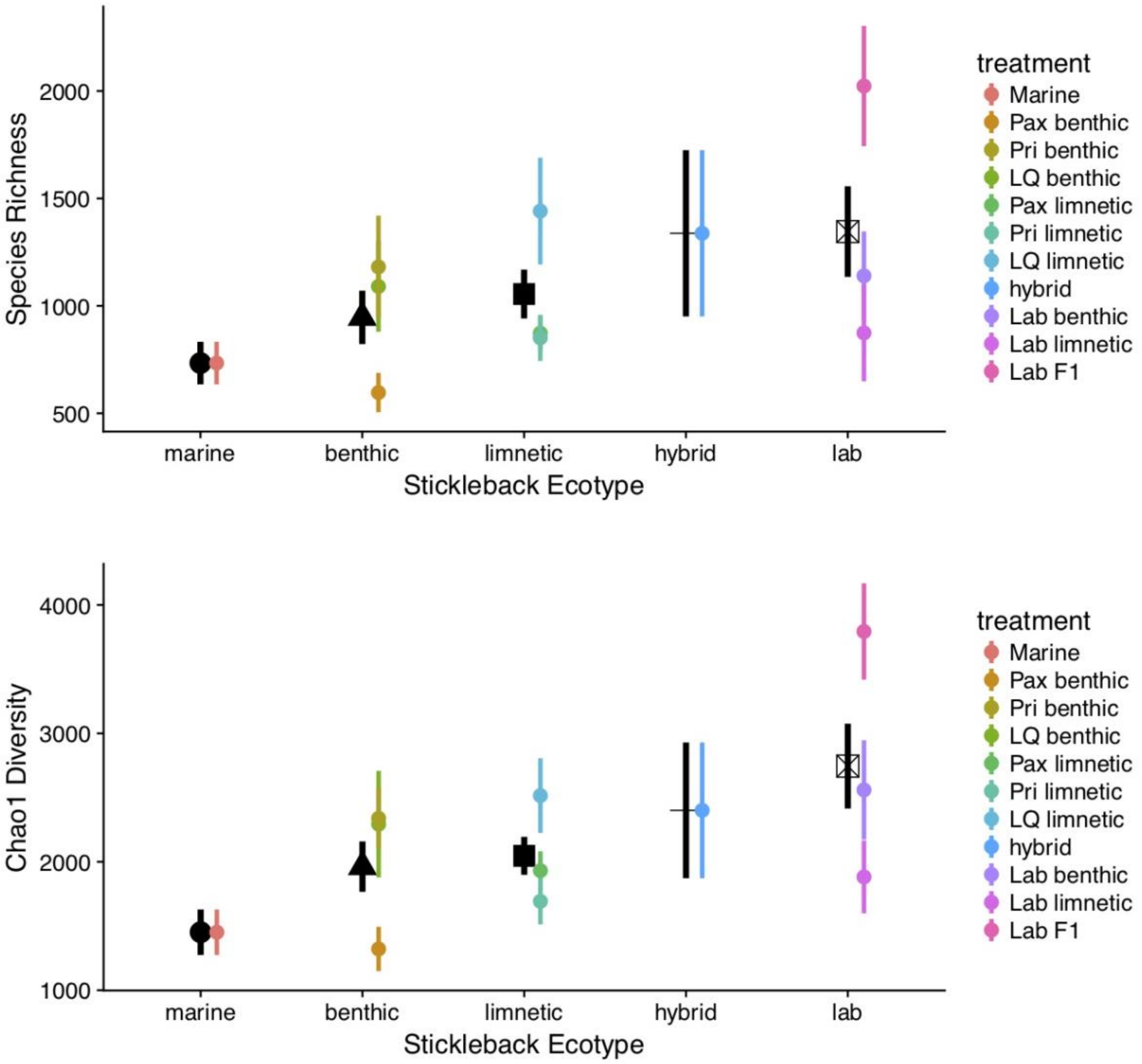
Supplementary Figure 5: Differentiation of the (a) taxonomic composition and (b) functional composition of the gut microbiome of hybrid threespine stickleback from Enos lake relative to benthic and limnetic stickleback from Paxton, Priest and Little Quarry based on Bray-Curtis dissimilarity.

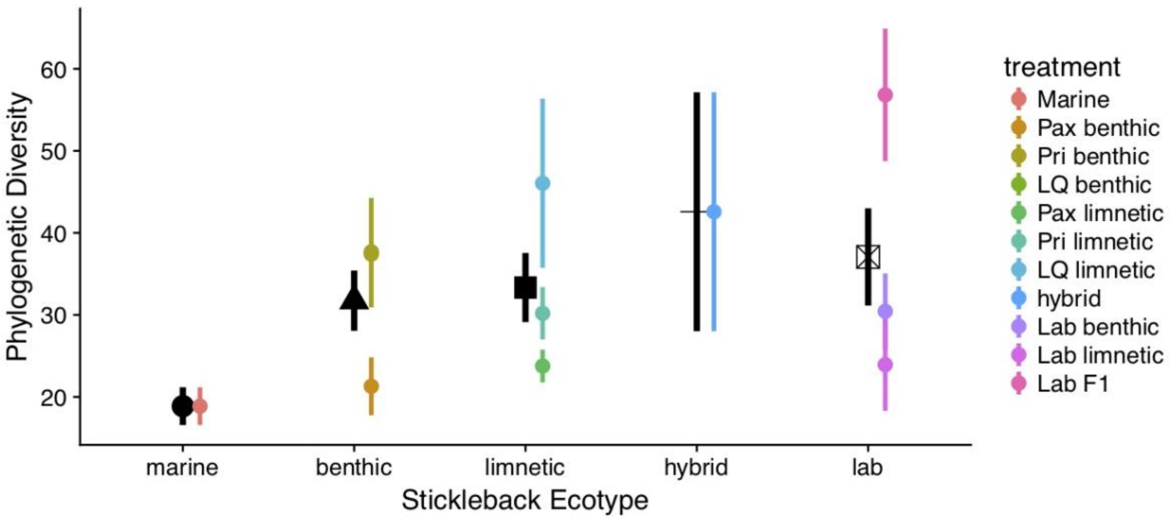


Supplementary Figure 6: Results of PICRUSt analysis showing relative abundance of KEGG orthologs for pure benthic and limnetic ecotypes relative to the hybrid swarm from Enos lake.



Supplementary Figure 7: Alpha diversity metrics for gut microbial communities from each stickleback ecotype. Black points show the means (+/-SEM) for each ecotype, colored points show the mean (+/-SEM) for each population within each ecotype. Panel A - species richness, Panel B - Chao1, Panel C - Phylogenetic diversity.





Literature Cited

1. Caporaso JG *et al.* 2010 QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**, 335–336.

2. Haas BJ *et al.* 2011 Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res.* **21**, 494–504.

3. Langille MGI *et al.* 2013 Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* **31**, 814–821.

4. DeSantis TZ *et al.* 2006 Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Appl. Environ. Microbiol.* **72**, 5069–5072.