

SUPPLEMENTAL TABLES

Table S1. Number of samples collected of each sample type for this study. Only one sample of each sample type was collected per animal, however multiple sample types were often collected from the same animal. A total of 128 *Hirudo verbana* and 46 *Macrobdella decora* animals were used in this study.

DaF*	ILF			Intestinum		Bladder	
	<i>H. verbana</i> S1†	<i>H. verbana</i> S2	<i>M. decora</i>	<i>H. verbana</i>	<i>M. decora</i>	<i>H. verbana</i>	<i>M. decora</i>
0	–	20	10	–	10	–	6
1	–	5	2	–	2	–	1
2	13	17	1	–	1	2	–
4	5	9	4	–	3	–	2
7	11	11	5	1	3	–	2
30+	–	5	3	–	3	–	2
90+	19	8	5	11	–	8	–
TOTAL	48	75	30	12	22	10	13

* Days after laboratory-administered, sheep blood meal.

† S2 = supplier 2, S1 = supplier 1.

Table S2: Permutational multivariate analysis of variance (PERMANOVA), using Bray-Curtis and Binary Ochiai metrics, comparing ILF microbiome of *Macrobdella decora* and *Hirudo verbana* when bacterial ASVs are clustered at various taxonomic levels.

Taxonomic Level	Bray-Curtis			Binary Ochiai		
	Pseudo-F	R ²	p*	Pseudo-F	R ²	p
Phylum	57.35	0.22	0.001	11.37	0.05	0.001
Class	55.51	0.21	0.001	9.46	0.04	0.001
Order	51.77	0.20	0.001	9.95	0.05	0.001
Family [†]	248.54	0.54	0.001	37.46	0.15	0.001
Genus	214.30	0.51	0.001	48.33	0.19	0.001
ASV	186.02	0.47	0.001	53.30	0.20	0.001

* Bold values indicate significant differences ($p \leq 0.01$). p values are based on 999 permutations.

† Taxonomic levels below the grey line have R² value ~ double of those above the line.

Table S3: Permutational multivariate analysis of variance (PERMANOVA) between leech-derived samples based on binary Ochiai dissimilarities. Variables were tested and listed in order of decreasing R².

Metadata Variable	Df*	Pseudo-F	R ²	p [†]
<i>Hirudo verbana</i>				
Organ	2	13.44	0.16	0.001
Supplier	1	23.13	0.14	0.001
Feeding	4	2.71	0.06	0.001
Shipment Lot	3	3.64	0.06	0.001
Blood Meal Lot‡	5	1.09	0.03	0.279
<i>Macrobdella decora</i>				
Organ	2	9.55	0.17	0.001
Feeding	6	2.34	0.13	0.001
Month Collected	5	2.35	0.11	0.001
Collection Site	3	2.42	0.07	0.001
Collection/Blood Lot§	3	1.43	0.04	0.045

* Df = degrees of freedom

† Bold values indicate significant differences ($p \leq 0.01$). p values are based on 999 permutations.

‡ Blood Meal Lot indicates the fresh shipment of blood on which the animals fed in the laboratory.

§ Because of the extended time between collection events and the short shelf-life of blood products, each blood lot was only fed to one collection lot of *M. decora*. The variation caused by either of these metadata variables can therefore not be separated for *M. decora* samples.

Table S4: See Excel file

Table S5: List of leech host-specific ASVs from genera present in both *Hirudo verbana* and *Macrobdella decora* *.

Class	Closest Genus	Organ [†]	<i>H. verbana</i>	<i>M. decora</i>
<i>Bacteroidia</i>	<i>Bacteroides</i>	ILF	ASV34	ASV3, ASV5, ASV28[‡]
	<i>Mucinivorans</i>	Intestinum	ASV1 , ASV23, ASV63, ASV35	ASV49
	<i>Rikenella</i>	Intestinum	<i>ASV15</i> , ASV31, ASV46	
<i>Clostridia</i>	<i>Proteocatella</i>	ILF	ASV22	ASV4
	<i>Ensifer</i>	Bladder	ASV53	ASV68
<i>Alphaproteobacteria</i>	<i>Phreatobacter</i>	Bladder	ASV81	ASV42
	<i>Insolitispirillum</i>	Intestinum	ASV11 , ASV62	ASV38, ASV52
<i>Betaproteobacteria</i>	<i>Comamonas</i>	Bladder	ASV12	<i>ASV21</i>
<i>Deltaproteobacteria</i>	<i>Desulfovibrio</i>	Intestinum	ASV32, <i>ASV33</i>	ASV25

* Only ASVs present in $\geq 30\%$ of samples in a sample group are listed. See Table S3 for a full list of ASVs and prevalences in all sample groups.

[†] Organ site in which comparison is made.

[‡] Core ASVs (present in $\geq 90\%$ of samples) are written in bold. Common ASVs (present in 70 – 90% of samples) are italicized. All other ASVs are transient (present in $< 70\%$ of samples).

Table S6: List of ASVs that exhibit specificity between *Hirudo verbana** suppliers.

Class	Closest Genus	Supplier 1	Supplier 2
<i>Bacteroidia</i>	<i>Bacteroides</i>		ASV34, ASV51
	<i>Mucinivorans</i>	ASV93, ASV35	ASV10
	<i>Rikenella</i>		ASV15
<i>Clostridia</i>	<i>Proteiniclasticum</i>		ASV7
	<i>Proteocatella</i>	ASV113, ASV4	ASV22, ASV67
<i>Negativicutes</i>	<i>Veillonella</i>	ASV110	
<i>Fusobacteriia</i>	<i>Fusobacterium</i>		ASV8
<i>Deltaproteobacteria</i>	<i>Desulfovibrio</i>		ASV50, ASV71
<i>Gammaproteobacteria</i>	<i>Aeromonas</i>	ASV108, ASV120	
	<i>Morganella</i>		ASV55
	<i>Proteus</i>		ASV40

* Only ASVs present in $\geq 30\%$ of samples in a sample group are listed. See Table S3 for a full list of ASVs and prevalences in all sample groups.

Table S7: Calculated PERMANOVA p values (above diagonal line) and Bonferroni-adjusted Wilcoxon rank-sum p values (below diagonal line) for comparison of *Macrobodella decora* and *Hirudo verbana* ILF microbiomes as a function of days after feeding (DaF). See Figure 4 for a plot of Bray-Curtis distances between these microbial communities.

	DaF	0	1	2	4	7	30+	90+
<i>Hirudo verbana</i>	0		0.048	0.002*	0.012	0.017	0.756	0.266
	1	3×10^{-20}		0.028	0.041	0.092	0.151	0.334
	2	3×10^{-53}	1		0.626	0.944	0.121	0.184
	4	4×10^{-13}	0.506	2×10^{-6}		0.646	0.212	0.490
	7	1×10^{-37}	1	1	0.001		0.243	0.232
	30+	3×10^{-13}	1	0.599	1	1		0.663
	90+	1×10^{-12}	0.038	9×10^{-13}	1	9×10^{-8}	0.379	
	<i>Macrobodella decora</i>	0		0.054	---	0.022	0.052	0.293
1		1		---	0.135	0.134	0.099	0.887
2		---	---		---	---	---	---
4		1×10^{-4}	0.943	---		0.687	0.122	0.018
7		7×10^{-5}	0.579	---	1		0.151	0.021
30+		1	0.613	---	0.003	0.006		0.281
90+		0.021	2×10^{-4}	---	3×10^{-6}	8×10^{-6}	0.729	

* $p \leq 0.05$ are in bold and $p \leq 0.01$ are highlighted in grey.

Table S8: Wald test adjusted p-values for all ASVs significantly different in *Hirudo verbana* and *Macrobdella decora* ILF between days after feeding. Core and common ASVs are included as comparisons.

	Class	Genus	ASV*	Days After Feeding					
				1 [†]	2	4	7	30+	90+
<i>Hirudo verbana</i>	<i>Bacteroidia</i>	<i>Mucinivorans</i>	ASV1	1	0.25	0.04	0.10	1	0.04
		<i>Rikenella-like</i>	ASV15	1	0.04	0.26	0.02	0.70	0.38
	<i>Clostridia</i>	<i>Proteocatella</i>	ASV22	1	0.13	0.79	0.09	3x10⁻¹⁵	0.29
	<i>Fusobacteriia</i>	<i>Fusobacterium</i>	ASV8	1	0.72	0.99	0.85	0.03	0.53
	<i>Deltaproteobacteria</i>	<i>Desulfovibrio</i>	ASV50	0.38	4.5x10⁻⁵	4x10⁻⁵	4x10⁻⁶	0.01	0.02
		<i>Desulfovibrio</i>	ASV71	1	0.03	0.12	0.01	0.70	0.01
		<i>Desulfovibrio</i>	ASV33	1	0.005	0.01	0.02	0.91	0.01
	<i>Gammaproteobacteria</i>	<i>Aeromonas</i>	ASV2	0.38	0.06	0.80	0.94	0.25	0.53
		<i>Pseudomonas</i>	ASV36	1	1x10⁻²⁶	4x10⁻⁵	6x10⁻¹⁵	1	3.0x10⁻⁶
		<i>Salmonella</i>	ASV16	3x10⁻⁵	0.81	0.68	0.01	0.25	0.56
<i>Macrobdella decora</i>	<i>Bacteroidia</i>	<i>Bacteroides-like</i>	ASV3	1	---	0.81	0.43	1	0.95
		<i>Bacteroides-like</i>	ASV44	1	---	0.93	0.89	1	1x10⁻¹²
		<i>Bacteroides-like</i>	ASV5	0.60	---	0.21	0.13	1	0.88
	<i>Clostridia</i>	<i>Butyricicoccus-like</i>	ASV6	0.60	---	1x10⁻⁴	0.002	0.89	1
		<i>Proteocatella</i>	ASV4	0.60	---	0.31	0.13	1	0.95
		<i>Sporobacter-like</i>	ASV13	0.60	---	0.26	0.36	1	0.95
		<i>Tyzzarella</i>	ASV20	0.52	---	0.002	0.49	1	0.88
		unk <i>Clostridiales</i>	ASV26	0.01	---	0.001	0.001	0.06	2x10⁻⁵
	<i>Gammaproteobacteria</i>	<i>Aeromonas</i>	ASV2	0.79	---	1x10⁻⁴	0.36	1	0.95

* All ASVs that differ significantly in abundance are shown. ASV8, ASV16, ASV22, ASV26, ASV36, ASV44, ASV 50, ASV71 are transient.

[†] Adjusted p values ≤ 0.001 are highlighted in grey. Adjusted p values ≤ 0.05 are in bold.

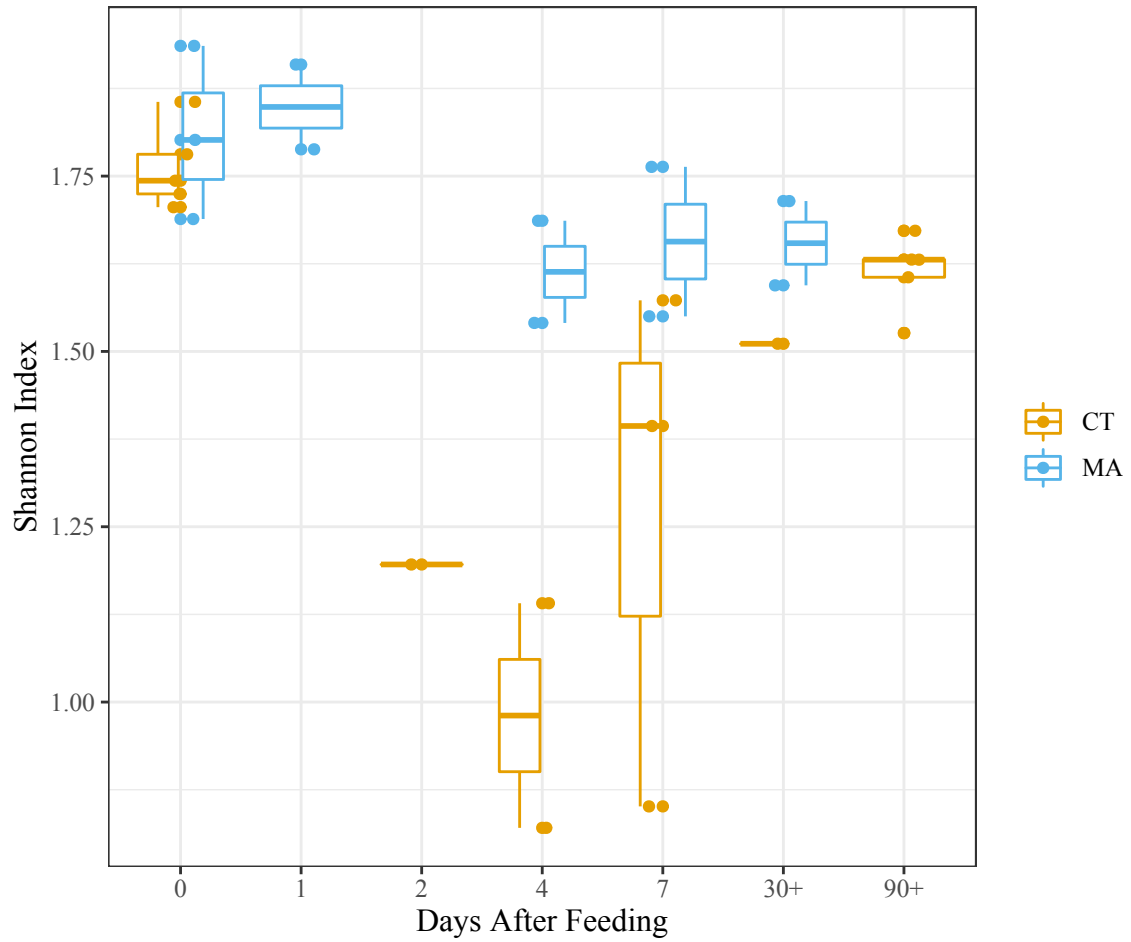


Figure S1: Shannon diversity index of *Macrobodella decora* ILF samples as a factor of days after feeding (DaF) and collection site. Orange = *M. decora* collected from CT, Cyan = *M. decora* collected from MA. Statistics for community comparisons are recorded in Table S5. A similar pattern is observed in Chao-1 and Inverse Simpson metrics.

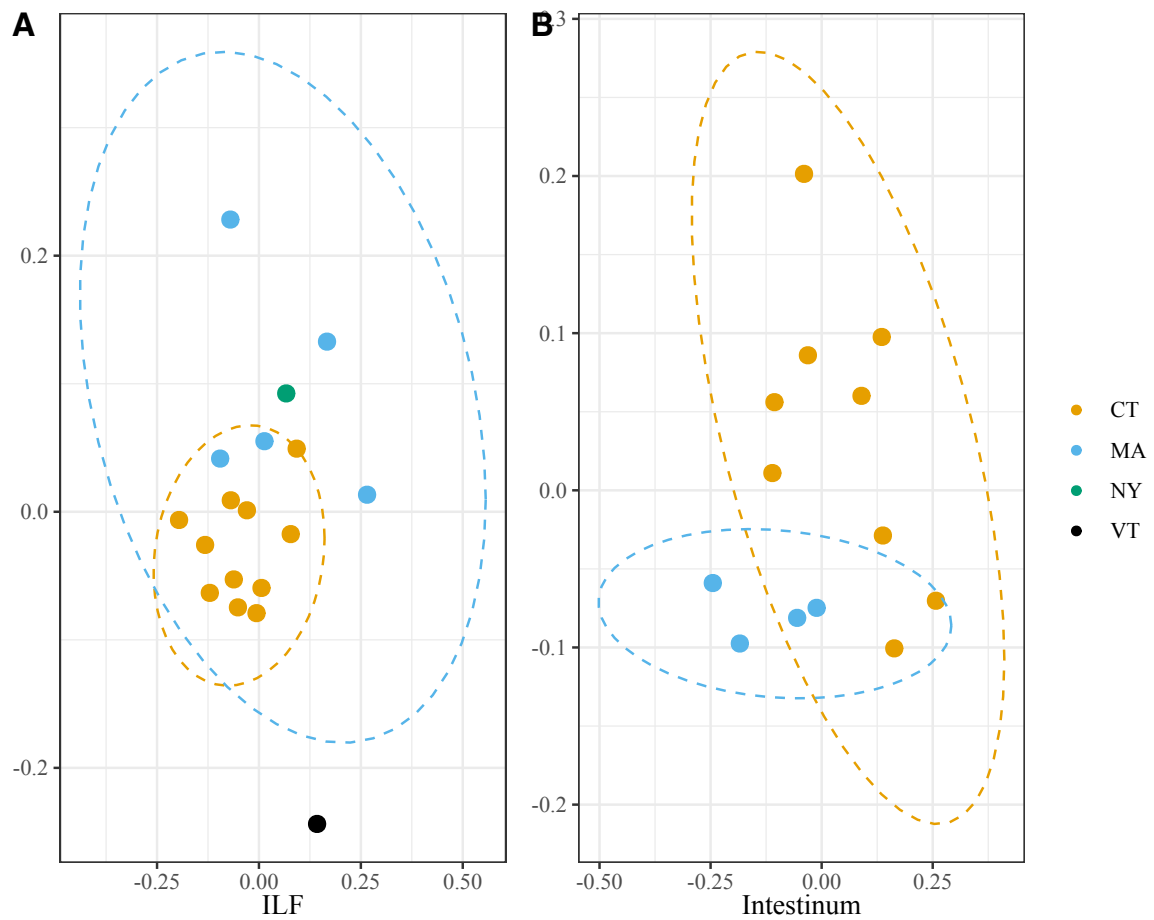


Figure S2: Bray-Curtis NMDS plot of wild-caught *Macrobdeella decora* - associated microbiota. Animals were collected from four states of the Northeastern U.S.A.: Connecticut (orange), Massachusetts (cyan), New York (green), and Vermont (black). A) ILF samples show no significant difference between ILF of *M. decora* from CT, MA, NY, and VT ($p \geq 0.222$). B) Intestinum samples from CT and MA are significantly different from each other ($p = 0.006$). Data points do not include fed animals. Ellipses drawn at 95% confidence interval. Stress < 0.12.

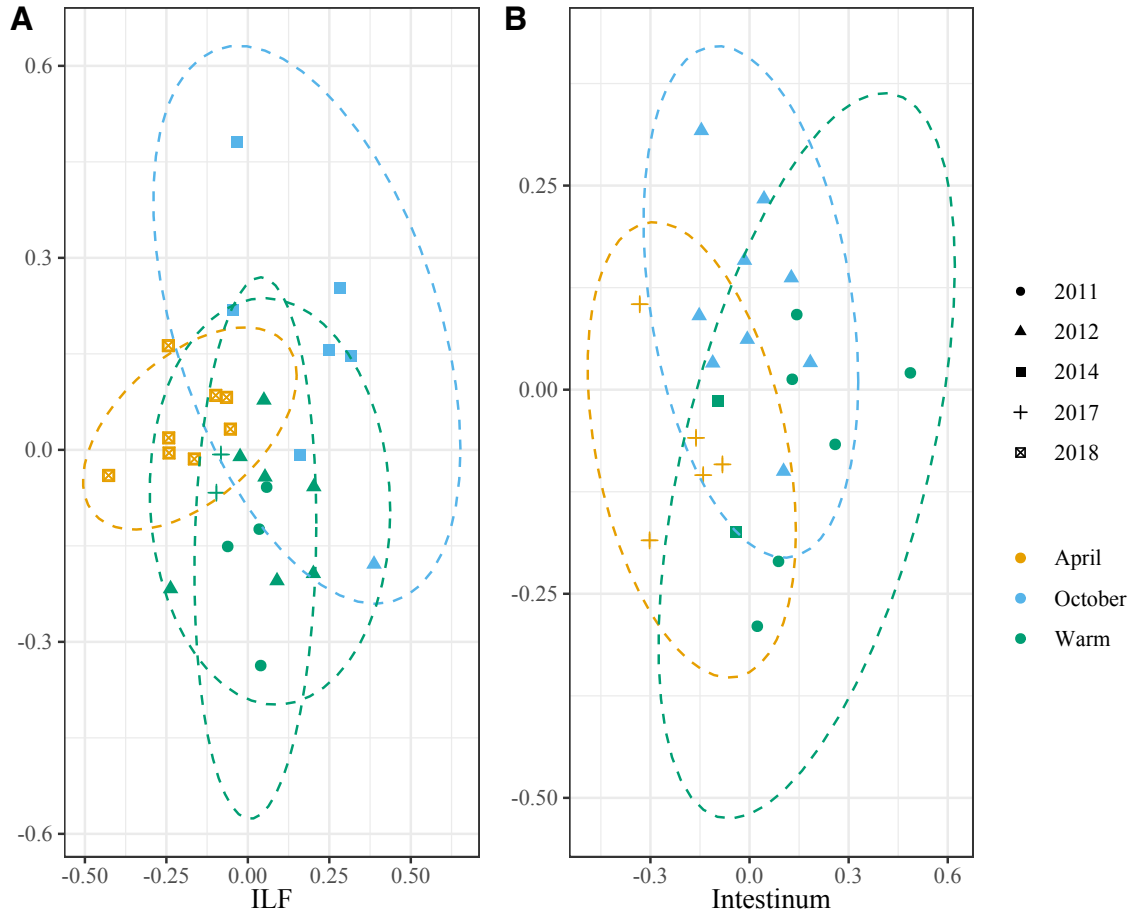


Figure S3: Binary Ochiai NMDS plot of wild-caught *Macrobdella decora* - associated microbiota shows that month of collection affects gut microbiome. Animals were collected in three seasons: late cold (April - orange), warm (June - September - green), and early cold (October - cyan). A) ILF samples. ILF microbiota from animals collected in October were significantly different from that of animals collected in April or warm months ($p \leq 0.024$). B) Intestinum samples. Intestinum microbiota were not significantly different between animals collected over different seasons ($p \geq 0.096$). Data points include fed and unfed animals. Ellipses drawn at 95% confidence interval. Stress < 0.2.

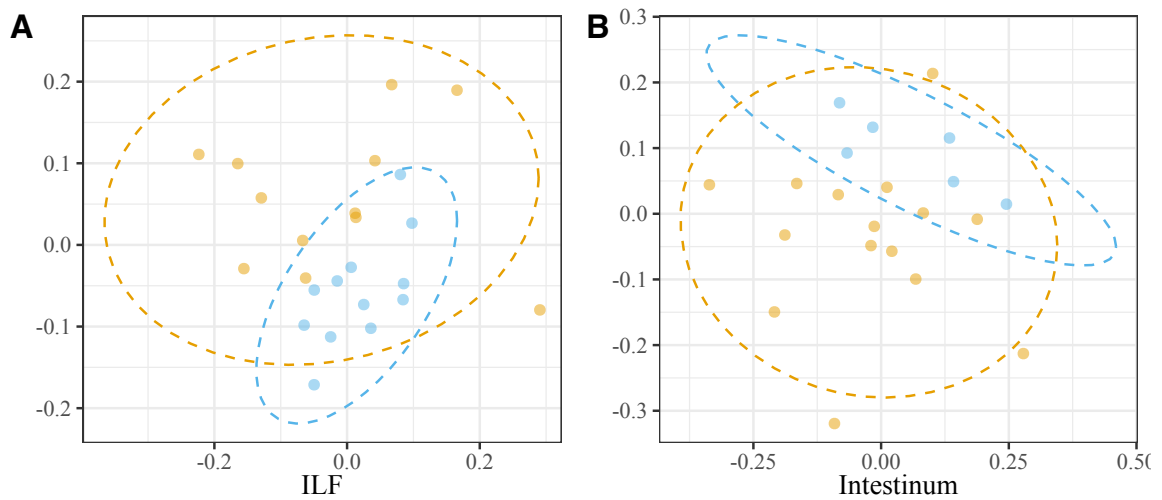


Figure S4: Bray-Curtis NMDS plots of *Macrobodella decora* A) ILF and B) intestinum - associated microbiota shows that extraction method did not affect observed microbiome. Sample extracted using the bead beating method are in orange. MasterPure extracted samples are in cyan. A) ILF samples. B) Intestinum samples. Ellipses drawn at 95% confidence interval. Stress < 0.15.