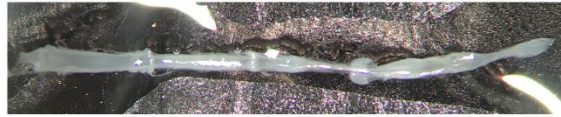


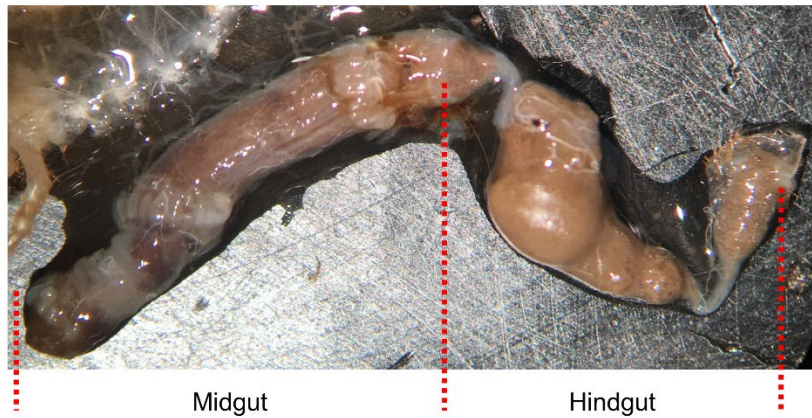
Supplementary Material

1 Supplementary Figures and Tables

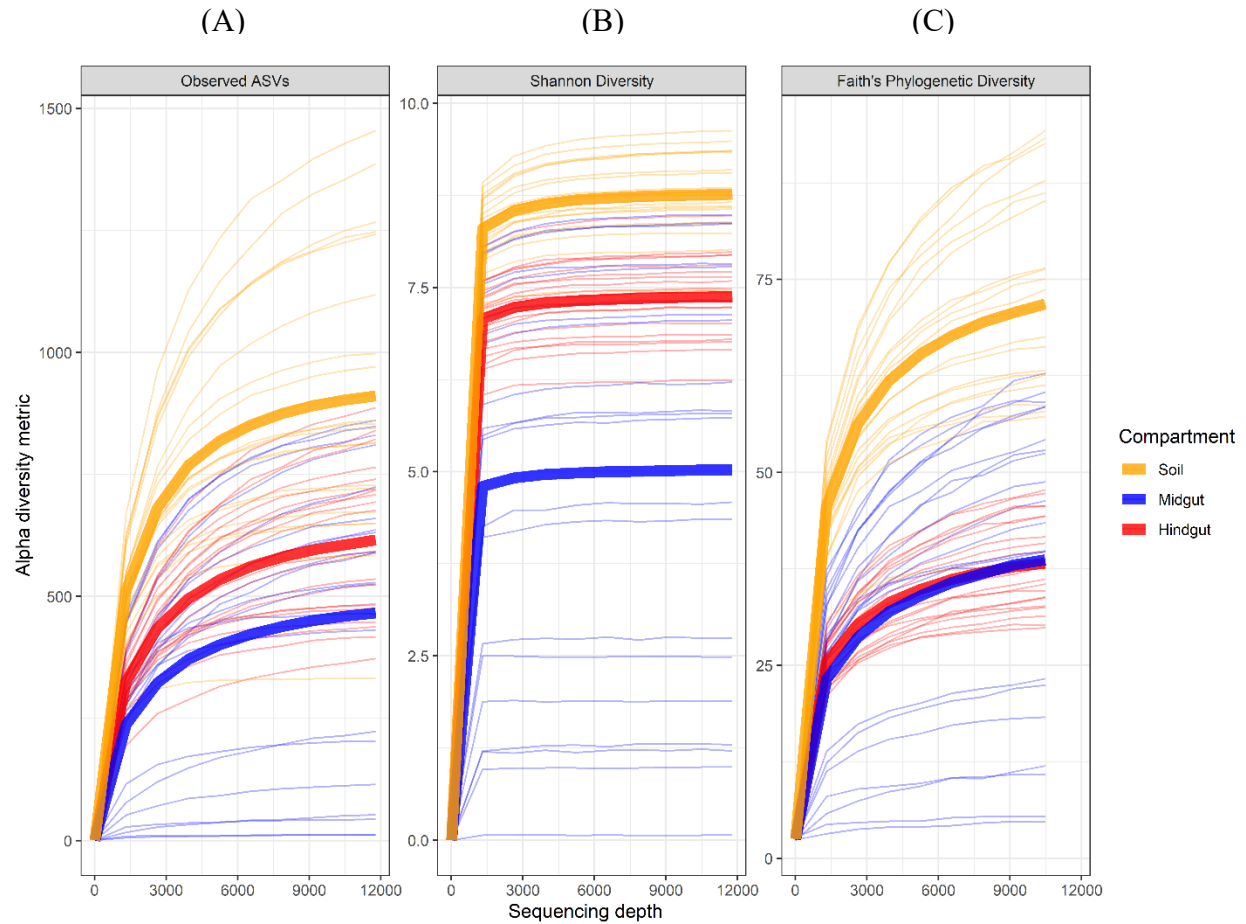
(A)



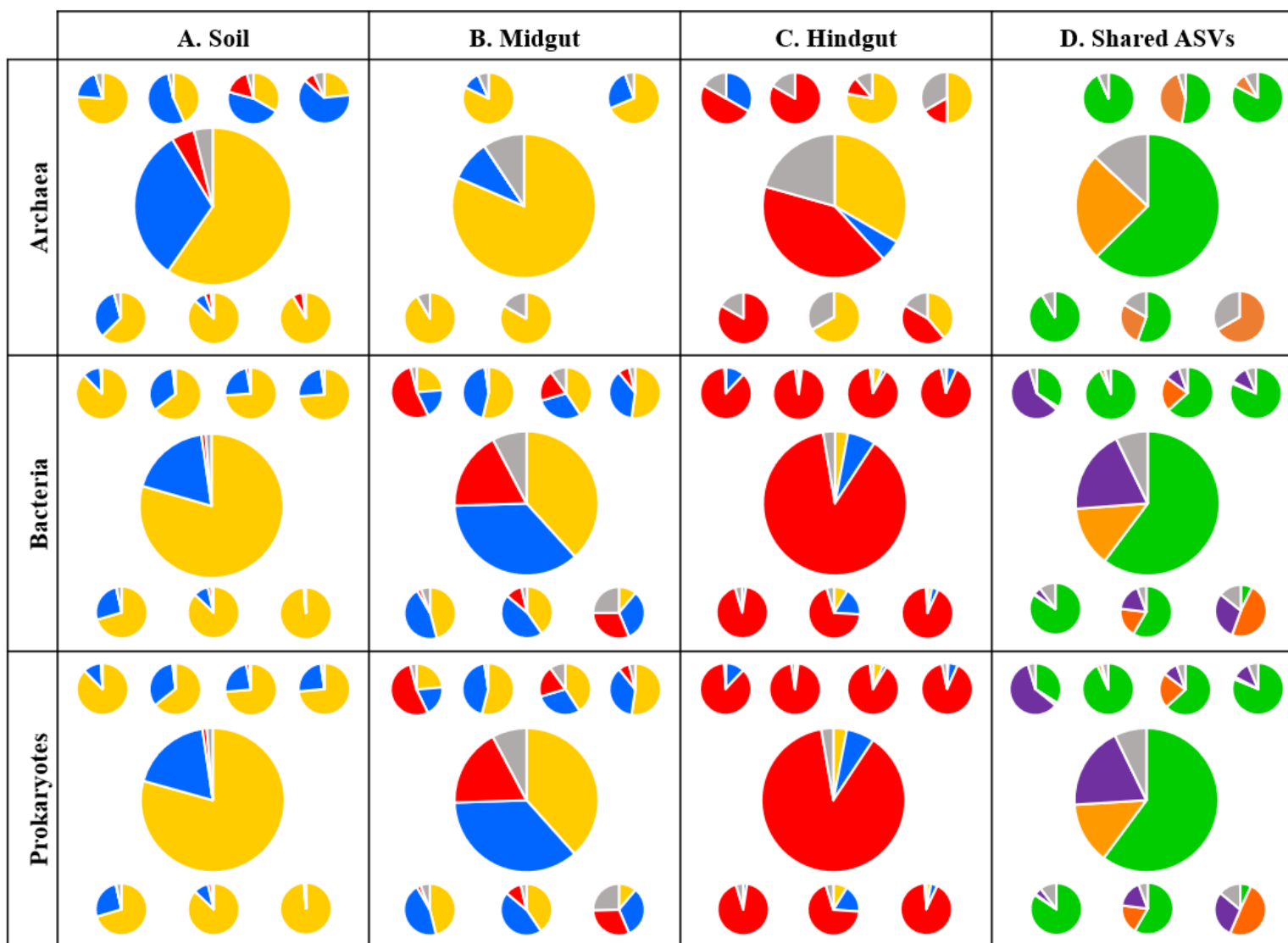
(B)



Supplementary Figure 1. Dissected alimentary tract from a neonate (A) and (B) a third instar Japanese beetle (*Popillia japonica* Newman) larvae. Alimentary tract from neonates were used entirely while alimentary tract from third instar larvae were divided into midgut (between the first set of gastric caeca after the head and the pyloric sphincter where the Malpighian tubules emerge) and hindgut (including ileum, colon, and rectum) sections.



Supplementary Figure 2. Rarefaction curves for α -diversity metrics of prokaryotic communities in guts from third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil, at a depth of 11,781 sequences. (A) Observed amplicon sequence variants (ASVs), (B) Shannon diversity, (C) Faith's phylogenetic diversity. Line color represents sample type (gut region or soil). Individual samples are represented by thin lines while average per sample type are represented by thick lines.



ASVs within compartment (A, B, and C):

■ Soil
 ■ Midgut
 ■ Hindgut
 ■ All

Shared ASVs across compartments (D):

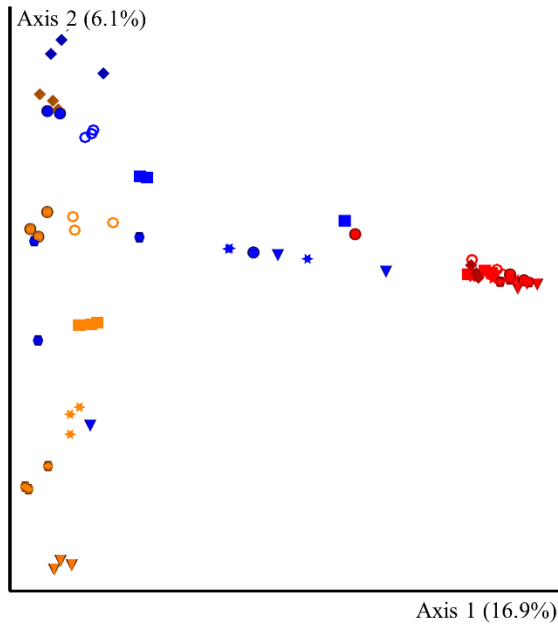
■ Soil & Midgut
 ■ Soil & Hindgut
 ■ Midgut & Hindgut
 ■ All

Supplementary Figure 3. Distribution (percentage) of unique and shared observed amplicon sequence variants (ASVs) of the archaeal, bacterial and combined prokaryotic community within compartment: soil (A), and midgut (B) and hindgut (C) of third instar Japanese beetle (*Popillia japonica* Newman) larvae across locations. Shared ASVs (D) represents an overview of the distribution (percentage) of ASVs that are shared between two or three (all) compartments. Central larger chart represents the average ASVs across all locations. Smaller charts represent average ASVs per location as follows (left to right), in top row: Blackhawk, Culver, Janesville1, and Janesville2, and in bottom row: Nursery, Purdy, and TPAC. The cases where a smaller chart is not presented means that ASVs were not detected in that compartment/comparison at the location.

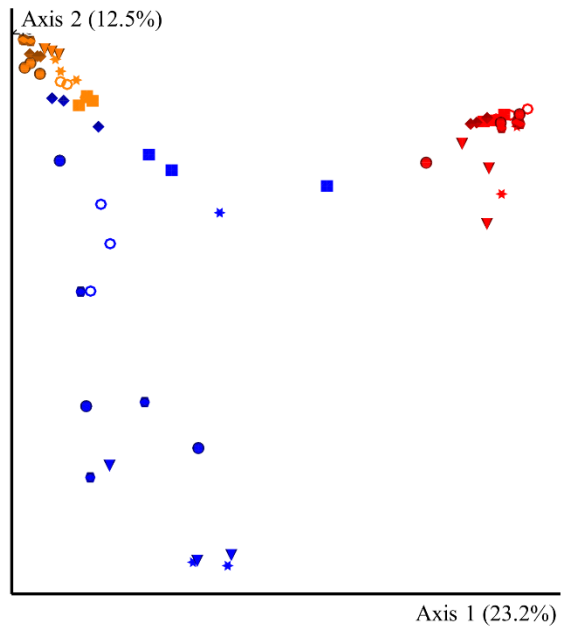


Supplementary Figure 4. Prokaryotic community based on V4 16S rRNA gene sequences at the order rank in gut (i.e., midgut, hindgut) from third instar larvae of the Japanese beetle (*Popillia japonica* Newman) and associated soil, at seven locations across Indiana and Wisconsin, USA. All taxa belong to *Bacteria* except to *Nitrososphaerales* and *Methanobacteriales* that belong to *Archaea*. Taxa with ASVs $\geq 0.016\%$ in abundance were considered. The dendrogram is based on Euclidean dissimilarities between taxa. Average is presented, $n=3$ for each compartment at each location. For taxonomic affiliation at order rank refer to **Supplementary Table 3.**

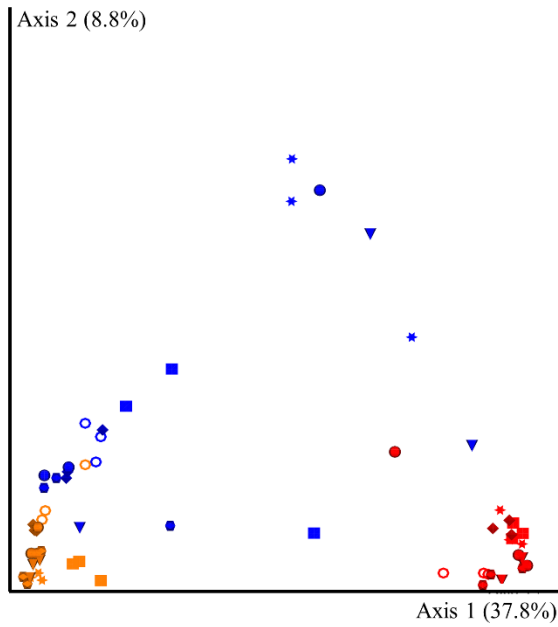
(A) Jaccard



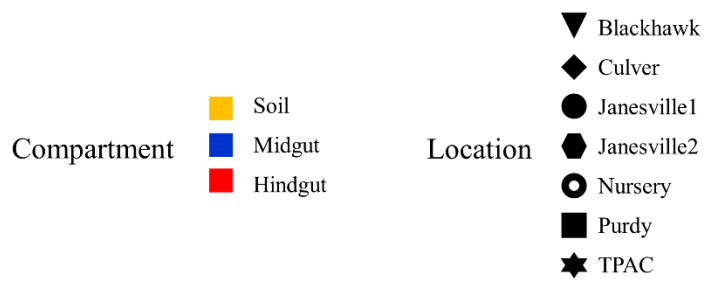
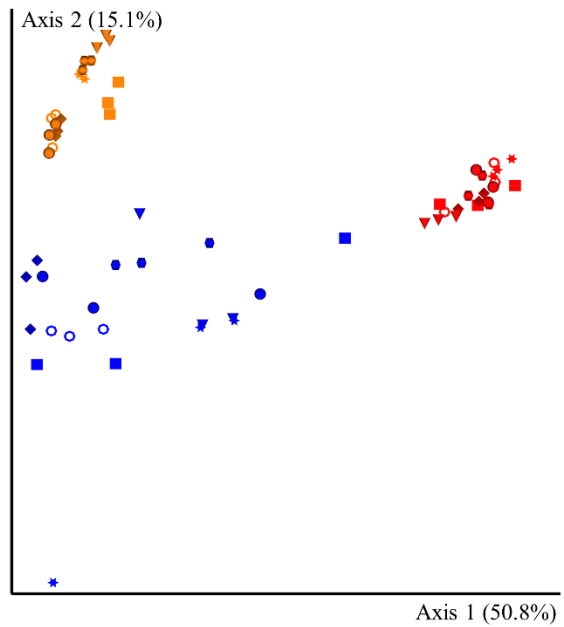
(B) Bray-Curtis



(C) Unweighted UniFrac



(D) Weighted UniFrac



Supplementary Figure 5. Microbial compositional profiles of prokaryotic communities in midgut and hindgut of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil visualized using the principal coordinate analysis (PCoA) based on (A) Jaccard-, (B) Bray-Curtis-, (C) Unweighted UniFrac-, and (D) Weighted UniFrac dissimilarity matrices. Data points represent individual samples where symbol color denotes compartment (i.e., midgut, hindgut, or soil) while symbol shape denotes location.

Supplementary Table 1. 16S rRNA gene reads recovered from Illumina MiSeq sequencing in the whole gut of first instar hatched under sterile conditions, and the midgut and hindgut regions of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil. For each compartment at each location, average \pm SD are presented, n=3, except for first instar whole guts, where n=5.

	Raw sequences		Filtered and denoised (DADA2)		Merged		Non-chimeric		Filtering based on >2, >p, nmnc*	
First instar	129,020	\pm 60,928	123,366	\pm 58,408	119,623	\pm 55,525	114,977	\pm 52,326	106,348	\pm 49,771
Janesville	157,560	\pm 45,011	150,458	\pm 43,240	144,671	\pm 39,708	138,876	\pm 38,325	122,641	\pm 41,543
TPAC	100,480	\pm 65,499	96,275	\pm 63,018	94,576	\pm 61,577	91,078	\pm 57,129	90,056	\pm 56,429
Total	1,290,197		1,233,663		1,196,234		1,149,772		1,063,481	
Soil	1,392,495	\pm 35,222	1,340,202	\pm 33,953	1,044,949	\pm 28,311	1,034,937	\pm 28,016	973,411	\pm 25,544
Blackhawk	134,338	\pm 12,416	129,145	\pm 11,925	100,949	\pm 10,563	100,446	\pm 10,463	96,187	\pm 9,561
Culver	136,652	\pm 7,996	131,393	\pm 7,727	97,518	\pm 5,804	96,471	\pm 5,758	92,394	\pm 5,446
Janesville1	151,081	\pm 17,118	144,982	\pm 16,425	109,459	\pm 13,143	108,075	\pm 12,937	103,631	\pm 12,148
Janesville2	170,905	\pm 28,866	164,409	\pm 27,642	125,351	\pm 22,537	124,344	\pm 22,265	119,028	\pm 20,731
Nursery	139,828	\pm 27,826	134,643	\pm 26,754	101,971	\pm 21,310	101,003	\pm 21,054	94,461	\pm 19,203
Purdy	337,849	\pm 40,889	326,078	\pm 39,372	263,046	\pm 33,750	260,442	\pm 33,439	244,827	\pm 30,424
TPAC	321,842	\pm 21,079	309,552	\pm 20,328	246,655	\pm 17,036	244,156	\pm 16,950	222,883	\pm 14,828
Midgut	1,967,400	\pm 33,350	1,897,148	\pm 32,216	1,735,308	\pm 28,012	1,712,549	\pm 27,576	1,524,972	\pm 27,507
Blackhawk	306,525	\pm 18,011	294,899	\pm 17,219	280,535	\pm 15,610	277,163	\pm 15,982	262,166	\pm 18,313
Culver	307,283	\pm 32,489	296,168	\pm 31,796	262,339	\pm 28,234	258,648	\pm 27,905	208,132	\pm 23,098
Janesville1	270,936	\pm 40,640	261,758	\pm 39,021	238,114	\pm 32,697	234,161	\pm 32,408	223,857	\pm 29,879
Janesville2	362,008	\pm 29,827	349,464	\pm 29,229	313,901	\pm 26,535	309,146	\pm 25,917	298,798	\pm 25,653
Nursery	325,813	\pm 7,981	313,611	\pm 7,561	286,940	\pm 5,376	282,613	\pm 4,912	249,585	\pm 2,991
Purdy	268,530	\pm 33,353	259,521	\pm 32,332	233,973	\pm 26,976	232,081	\pm 26,802	168,835	\pm 31,325
TPAC	126,305	\pm 18,477	121,727	\pm 17,677	119,506	\pm 16,961	118,737	\pm 16,633	113,599	\pm 18,325
Hindgut	2,030,261	\pm 33,947	1,952,179	\pm 32,718	1,771,213	\pm 30,454	1,760,927	\pm 30,214	1,711,363	\pm 29,260
Blackhawk	381,377	\pm 19,597	366,796	\pm 19,047	335,318	\pm 18,482	333,820	\pm 18,224	327,428	\pm 17,838
Culver	210,721	\pm 36,657	203,900	\pm 36,047	186,581	\pm 34,215	185,455	\pm 33,969	178,363	\pm 33,031
Janesville1	308,466	\pm 21,861	296,541	\pm 21,520	271,187	\pm 21,845	269,520	\pm 21,308	260,446	\pm 18,445
Janesville2	333,289	\pm 23,887	319,211	\pm 23,051	284,944	\pm 21,846	284,307	\pm 21,824	276,631	\pm 19,942
Nursery	370,986	\pm 23,993	356,022	\pm 23,098	321,129	\pm 21,526	318,670	\pm 21,554	307,611	\pm 20,159
Purdy	151,478	\pm 11,652	145,404	\pm 11,107	128,729	\pm 11,177	128,387	\pm 11,202	124,472	\pm 10,948
TPAC	273,944	\pm 29,896	264,305	\pm 29,090	243,325	\pm 27,691	240,768	\pm 27,110	236,412	\pm 26,879
Total	5,390,156		5,189,529		4,551,470		4,508,413		4,209,746	

*: >2: present in at least 2 samples; >p: assigned at least to the phylum rank of taxonomy classification; nmnc: were not classified as mitochondrial or chloroplast.

Supplementary Table 2. Number of observed amplicon sequence variants (ASVs) including taxonomic affiliation at different ranks, present in the prokaryotic community from midgut and hindgut of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil. Taxa for unrarefied and rarefied libraries are presented.

			Phylum		Order		Species		ASVs	
			<i>Archaea</i>	<i>Bacteria</i>	<i>Archaea</i>	<i>Bacteria</i>	<i>Archaea</i>	<i>Bacteria</i>	<i>Archaea</i>	<i>Bacteria</i>
No rarefaction	Total	ALL	3	34	4	355	15	1,885	37	8,381
		Soil	3	34	4	338	13	1,616	34	5,385
		Midgut	2	27	2	248	9	1,349	26	4,274
		Hindgut	2	17	2	99	6	437	11	2,762
	Unique	Soil	1	7	2	105	5	471	10	2,530
		Midgut	0	0	0	11	1	104	1	653
		Hindgut	0	0	0	1	1	53	1	1,513
	Shared	ALL	2	17	2	92	5	260	9	355
		Soil & Midgut	0	10	0	140	3	873	15	2,436
		Soil & Hindgut	0	0	0	1	0	12	0	64
		Midgut & Hindgut	0	0	0	5	0	112	1	830
	Rarefaction at 11,781	Total	ALL	3	34	4	355	15	1,877	37
Soil			3	33	4	334	13	1,589	33	5,294
Midgut			2	24	2	236	9	1,293	25	4,026
Hindgut			2	15	2	87	5	400	9	2,676
Unique		Soil	1	10	2	117	5	516	11	2,678
		Midgut	0	0	0	14	1	118	2	716
		Hindgut	0	0	0	2	1	58	1	1,580
Shared		ALL	2	14	2	80	4	220	7	304
		Soil & Midgut	0	9	0	137	4	843	15	2,263
		Soil & Hindgut	0	0	0	0	0	10	0	49
		Midgut & Hindgut	0	1	0	5	0	112	1	743
Rarefaction coverage (%)		ALL	100.0	100.0	100.0	100.0	100.0	99.6	100.0	99.4
	Soil	100.0	97.1	100.0	98.8	100.0	98.3	97.1	98.3	
	Midgut	100.0	88.9	100.0	95.2	100.0	95.8	96.2	94.2	
	Hindgut	100.0	88.2	100.0	87.9	83.3	91.5	81.8	96.9	

Supplementary Table 3. Taxonomic affiliation (SILVA v132) of prokaryotic community (up to order rank when available) in gut (i.e., midgut, hindgut) from third instar larvae of the Japanese beetle (*Popillia japonica* Newman) and associated soil.

Label in heatmap	Kingdom	Phylum	Class	Order
11-24	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	11-24
Acetobacterales	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales
Acidobacteria	Bacteria	Actinobacteria		
Acidobacteriales	Bacteria	Acidobacteria	Acidobacteriia	Acidobacteriales
Actinomarinales	Bacteria	Actinobacteria	Acidimicrobiia	Actinomarinales
Alphaproteobacteria	Bacteria	Proteobacteria	Alphaproteobacteria	uncultured
Anaeromyxobacter	Bacteria	Acidobacteria	Subgroup 6	uncultured Anaeromyxobacter sp.
Azospirillales	Bacteria	Proteobacteria	Alphaproteobacteria	Azospirillales
Bacillales	Bacteria	Firmicutes	Bacilli	Bacillales
Bacteroidales	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales
Bacteroidia	Bacteria	Bacteroidetes	Bacteroidia	
Betaproteobacteriales	Bacteria	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales
Blastocatellales	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	Blastocatellales
BRC1__	Bacteria	BRC1	uncultured Acidobacteria bacterium	uncultured Acidobacteria bacterium
BRH-c20a	Bacteria	Firmicutes	BRH-c20a	uncultured bacterium
Caulobacterales	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales
Cellvibrionales	Bacteria	Proteobacteria	Gammaproteobacteria	Cellvibrionales
Chitinophagales	Bacteria	Bacteroidetes	Bacteroidia	Chitinophagales
Chthoniobacteriales	Bacteria	Verrucomicrobia	Verrucomicrobiae	Chthoniobacteriales
Clostridia	Bacteria	Firmicutes	Clostridia	
Clostridiales	Bacteria	Firmicutes	Clostridia	Clostridiales
Corynebacteriales	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales
Cytophagales	Bacteria	Bacteroidetes	Bacteroidia	Cytophagales
Deferribacterales	Bacteria	Deferribacteres	Deferribacteres	Deferribacterales
Deltaproteobacteria	Bacteria	Proteobacteria	Deltaproteobacteria	
Desulfarculales	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfarculales
Desulfovibrionales	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales
Desulfuromonadales	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales
Dongiales	Bacteria	Proteobacteria	Alphaproteobacteria	Dongiales
DS-100	Bacteria	Acidobacteria	Blastocatellia (Subgroup 4)	DS-100
Enterobacteriales	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales
Erysipelotrichales	Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales
Fibrobacterales	Bacteria	Fibrobacteres	Fibrobacteria	Fibrobacterales
Firmicutes	Bacteria	Firmicutes		
Flavobacteriales	Bacteria	Bacteroidetes	Bacteroidia	Flavobacteriales
Frankiales	Bacteria	Actinobacteria	Actinobacteria	Frankiales
Gaiellales	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales
Gammaproteobacteria_	Bacteria	Proteobacteria	Gammaproteobacteria	Gammaproteobacteria Incertae Sedis
Gastranaerophilales	Bacteria	Cyanobacteria	Melainabacteria	Gastranaerophilales
Gemmatales	Bacteria	Planctomycetes	Planctomycetacia	Gemmatales
Gemmatimonadales	Bacteria	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales
IMCC26256	Bacteria	Actinobacteria	Acidimicrobiia	IMCC26256

Supplementary Table 3. *Continued*

Label in heatmap	Kingdom	Phylum	Class	Order
<i>KD4-96_a</i>	<i>Bacteria</i>	<i>Chloroflexi</i>	<i>KD4-96</i>	
<i>KD4-96_b</i>	<i>Bacteria</i>	<i>Chloroflexi</i>	<i>KD4-96</i>	<i>uncultured Chloroflexi bacterium</i>
<i>KD4-96_c</i>	<i>Bacteria</i>	<i>Chloroflexi</i>	<i>KD4-96</i>	<i>uncultured bacterium</i>
<i>KI89A</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>KI89A clade</i>
<i>Kineosporiales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Kineosporiales</i>
<i>Ktedonobacteriales</i>	<i>Bacteria</i>	<i>Chloroflexi</i>	<i>Ktedonobacteria</i>	<i>Ktedonobacteriales</i>
<i>Latescibacteriales</i>	<i>Bacteria</i>	<i>Latescibacteria</i>	<i>Latescibacteria</i>	<i>Latescibacteriales</i>
<i>Latescibacteria</i>	<i>Bacteria</i>	<i>Latescibacteria</i>	<i>metagenome</i>	<i>metagenome</i>
<i>MB-A2-108</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>MB-A2-108</i>	<i>metagenome</i>
<i>MBNT15</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>MBNT15</i>
<i>Methanobacteriales</i>	<i>Archaea</i>	<i>Euryarchaeota</i>	<i>Methanobacteria</i>	<i>Methanobacteriales</i>
<i>Micrococcales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Micrococcales</i>
<i>Micromonosporales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Micromonosporales</i>
<i>Micropepsales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Micropepsales</i>
<i>Microtrichales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Acidimicrobiia</i>	<i>Microtrichales</i>
<i>Mollicutes RF39</i>	<i>Bacteria</i>	<i>Tenericutes</i>	<i>Mollicutes</i>	<i>Mollicutes RF39</i>
<i>Myxococcales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Myxococcales</i>
<i>Nitrososphaerales</i>	<i>Archaea</i>	<i>Thaumarchaeota</i>	<i>Nitrososphaeria</i>	<i>Nitrososphaerales</i>
<i>Nitrospirales</i>	<i>Bacteria</i>	<i>Nitrospirae</i>	<i>Nitrospira</i>	<i>Nitrospirales</i>
<i>Opitutales</i>	<i>Bacteria</i>	<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	<i>Opitutales</i>
<i>Pedosphaerales</i>	<i>Bacteria</i>	<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	<i>Pedosphaerales</i>
<i>Pirellulales</i>	<i>Bacteria</i>	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Pirellulales</i>
<i>Planctomycetales</i>	<i>Bacteria</i>	<i>Planctomycetes</i>	<i>Planctomycetacia</i>	<i>Planctomycetales</i>
<i>PLTA13</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>PLTA13</i>
<i>Propionibacteriales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Propionibacteriales</i>
<i>Proteobacteria</i>	<i>Bacteria</i>	<i>Proteobacteria</i>		
<i>Pseudomonadales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>
<i>Pseudonocardiales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Pseudonocardiales</i>
<i>Pyrinomonadales</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Blastocatellia (Subgroup 4)</i>	<i>Pyrinomonadales</i>
<i>R7C24</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>R7C24</i>
<i>RCP2-54</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>RCP2-54</i>
<i>Reyranelles</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Reyranelles</i>
<i>Rhizobiales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhizobiales</i>
<i>Rhodospirillales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>
<i>Rokubacteriales</i>	<i>Bacteria</i>	<i>Rokubacteria</i>	<i>NC10</i>	<i>Rokubacteriales</i>
<i>Rubrobacteriales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Rubrobacteria</i>	<i>Rubrobacteriales</i>
<i>SBR1031</i>	<i>Bacteria</i>	<i>Chloroflexi</i>	<i>Anaerolineae</i>	<i>SBR1031</i>
<i>Selenomonadales</i>	<i>Bacteria</i>	<i>Firmicutes</i>	<i>Negativicutes</i>	<i>Selenomonadales</i>
<i>Solibacteriales</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Acidobacteriia</i>	<i>Solibacteriales</i>
<i>Solirubrobacteriales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Thermoleophilia</i>	<i>Solirubrobacteriales</i>
<i>Sphingobacteriales</i>	<i>Bacteria</i>	<i>Bacteroidetes</i>	<i>Bacteroidia</i>	<i>Sphingobacteriales</i>
<i>Sphingomonadales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Sphingomonadales</i>
<i>Spirochaetales</i>	<i>Bacteria</i>	<i>Spirochaetes</i>	<i>Spirochaetia</i>	<i>Spirochaetales</i>
<i>Steroidobacteriales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Steroidobacteriales</i>
<i>Streptomycetales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Streptomycetales</i>
<i>Streptosporangiales</i>	<i>Bacteria</i>	<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Streptosporangiales</i>

Supplementary Table 3. Continued

Label in heatmap	Kingdom	Phylum	Class	Order
<i>Subgroup 11</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 11</i>	<i>uncultured bacterium</i>
<i>Subgroup 17_a</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 17</i>	
<i>Subgroup 17_b</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 17</i>	<i>uncultured Acidobacteriales bacterium</i>
<i>Subgroup 17_c</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 17</i>	<i>uncultured Acidobacteria bacterium</i>
<i>Subgroup 17_d</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 17</i>	<i>uncultured bacterium</i>
<i>Subgroup 2</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Acidobacteriia</i>	<i>Subgroup 2</i>
<i>Subgroup 22</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 22</i>	<i>uncultured Holophaga sp.</i>
<i>Subgroup 25</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 25</i>	<i>uncultured soil bacterium</i>
<i>Subgroup 5</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 5</i>	
<i>Subgroup 6_a</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 6</i>	<i>Unknown Order</i>
<i>Subgroup 6_b</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 6</i>	
<i>Subgroup 6_c</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Subgroup 6</i>	<i>uncultured Acidobacterium sp.</i>
<i>Subgroup 7</i>	<i>Bacteria</i>	<i>Acidobacteria</i>	<i>Holophagae</i>	<i>Subgroup 7</i>
<i>Syntrophobacterales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Deltaproteobacteria</i>	<i>Syntrophobacterales</i>
<i>Tepidisphaerales</i>	<i>Bacteria</i>	<i>Planctomycetes</i>	<i>Phycisphaerae</i>	<i>Tepidisphaerales</i>
<i>Tistrellales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Tistrellales</i>
<i>TK10</i>	<i>Bacteria</i>	<i>Chloroflexi</i>	<i>TK10</i>	
<i>Xanthomonadales</i>	<i>Bacteria</i>	<i>Proteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Xanthomonadales</i>

Supplementary Table 4. Influence of compartment (i.e., midgut, hindgut, or soil), location, and their interaction on variation in α -diversity of prokaryotic communities in the alimentary tract of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil. F-statistics and p -values for Aligned Rank Transform (ART) nonparametric factorial ANOVA.

Factor	df^1	Observed ASVs ²		Evenness		Shannon Diversity		Faith's PD ³	
		F	p -value	F	p -value	F	p -value	F	p -value
Compartment	2	37.9	< 0.001	106.7	< 0.001	111.0	< 0.001	38.6	< 0.001
Location	6	0.9	0.505	7.4	< 0.001	8.1	< 0.001	2.2	0.061
Compartment \times Location	12	4.8	< 0.001	5.7	< 0.001	7.7	< 0.001	5.2	< 0.001
Error	42								

¹ df = degrees of freedom; ²ASVs= Amplicon sequence variants (Richness); ³Faith's PD= Faith's phylogenetic distance

Supplementary Table 5. Pairwise comparisons between compartments on prokaryotic α -diversity in the alimentary tract of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil. Across-location comparisons emphasize relationships between compartments by comparing the slopes between compartments across locations using contrasts for the Aligned Rank Transform (ART) nonparametric ANOVA to generate differences of differences (entries with different letters are significantly different at $\alpha=0.05$ using Tukey-corrected for multiple comparisons p -values). Within-location comparisons emphasize relationships between compartments (soil *versus* midgut, soil *versus* hindgut, and midgut *versus* hindgut) at each location independently by comparing mean α -diversity values using Kruskal-Wallis nonparametric ANOVA (> and < indicate statistical significance and the relative trajectory of those differences at $\alpha=0.05$). $n=3$ for each compartment at each location.

	Location	Observed ASVs ¹		Evenness		Shannon Diversity		Faith's PD ²	
		Across locations	Within location	Across locations	Within location	Across locations	Within location	Across locations	Within location
Soil vs. Midgut	Blackhawk	ab	Soil > Midgut	a	Soil > Midgut	a	Soil > Midgut	ab	Soil > Midgut
	Culver	a	ND	b	Soil > Midgut	b	Soil > Midgut	c	ND
	Janesville1	ab	ND	c	Soil > Midgut	c	Soil > Midgut	abc	ND
	Janesville2	a	ND	c	Soil > Midgut	c	Soil > Midgut	ac	Soil > Midgut
	Nursery	a	ND	bc	Soil > Midgut	bc	Soil > Midgut	c	ND
	Purdy	b	Soil > Midgut	bc	Soil > Midgut	c	Soil > Midgut	b	Soil > Midgut
	TPAC	c	Soil > Midgut	a	Soil > Midgut	a	Soil > Midgut	d	Soil > Midgut
Soil vs. Hindgut	Blackhawk	a	ND	a	Soil > Hindgut	ab	ND	ab	Soil > Hindgut
	Culver	a	Soil > Hindgut	a	Soil > Hindgut	ab	Soil > Hindgut	ac	Soil > Hindgut
	Janesville1	a	ND	a	Soil > Hindgut	ab	Soil > Hindgut	ac	Soil > Hindgut
	Janesville2	a	ND	a	Soil > Hindgut	a	Soil > Hindgut	a	Soil > Hindgut
	Nursery	a	ND	a	Soil > Hindgut	ab	ND	b	ND
	Purdy	b	Soil > Hindgut	a	Soil > Hindgut	ab	Soil > Hindgut	d	Soil > Hindgut
	TPAC	b	Soil > Hindgut	a	Soil > Hindgut	b	Soil > Hindgut	cd	Soil > Hindgut
Midgut vs. Hindgut	Blackhawk	a	Midgut < Hindgut	a	Midgut < Hindgut	a	Midgut < Hindgut	ab	ND
	Culver	b	ND	b	Midgut > Hindgut	b	Midgut > Hindgut	c	Midgut > Hindgut
	Janesville1	ab	ND	bcd	ND	c	ND	acd	ND
	Janesville2	ab	ND	ce	Midgut < Hindgut	c	Midgut < Hindgut	acd	Midgut > Hindgut
	Nursery	ab	Midgut < Hindgut	bd	ND	bc	Midgut < Hindgut	abd	ND
	Purdy	b	ND	cd	ND	bc	ND	cd	ND
	TPAC	a	Midgut < Hindgut	ae	Midgut < Hindgut	a	Midgut < Hindgut	b	Midgut < Hindgut

¹ASVs= Amplicon sequence variants (Richness); ²Faith's PD=Faith's phylogenetic distance; ND= no significant difference between samples.

Supplementary Table 6. Pairwise comparisons within compartment on prokaryotic α -diversity in the alimentary tract of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil. Comparisons emphasize differences within a single compartment (soil, midgut, or hindgut) across locations by comparing mean values using a one-way ANOVA and Tukey's-HSD post hoc (entries with different letters are statistically significant at $\alpha=0.05$). $n=3$ for each compartment at each location.

	Location	Observed ASVs ¹	Evenness	Shannon Diversity	Faith's PD ²
Soil	ANOVA: $F_{6,14}$, p -value	5.030, 0.006	18.46, <0.001	5.795, 0.003	5.613, 0.004
	Blackhawk	bc	bc	bc	abc
	Culver	bc	a	abc	bc
	Janesville1	abc	a	abc	abc
	Janesville2	abc	c	bc	abc
	Nursery	c	ab	c	c
	Purdy	ab	a	ab	ab
	TPAC	a	a	a	a
Midgut	ANOVA: $F_{6,14}$, p -value	2.592, 0.067	4.882, 0.007	5.722, 0.003	2.552, 0.070
	Blackhawk	a	b	bc	a
	Culver	a	a	a	a
	Janesville1	a	ab	abc	a
	Janesville2	a	ab	abc	a
	Nursery	a	ab	ab	a
	Purdy	a	ab	abc	a
	TPAC	a	b	c	a
Hindgut	ANOVA: $F_{6,14}$, p -value	6.955, 0.001	3.245, 0.033	3.644, 0.022	11.470, <0.001
	Blackhawk	a	a	a	abc
	Culver	c	a	ab	d
	Janesville1	abc	a	ab	bcd
	Janesville2	ab	a	ab	ab
	Nursery	ab	a	ab	a
	Purdy	c	a	ab	d
	TPAC	bc	a	b	cd

¹ASVs= Amplicon sequence variants (Richness); ²Faith's PD=Faith's phylogenetic distance

Supplementary Table 7. Influence of compartment (midgut, hindgut, soil), location, and their interaction on variation in β -diversity of prokaryotic communities in the alimentary tract of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil. Pseudo-F statistics, R^2 and p -values for factorial permutational analysis of variance (PERMANOVA, Adonis) model used 999 permutations.

Term	df [†]	Jaccard			Bray-Curtis			Unweighted UniFrac			Weighted UniFrac			DEICODE		
		pseudo-F	p-value	R ²	pseudo-F	p-value	R ²	pseudo-F	p-value	R ²	pseudo-F	p-value	R ²	pseudo-F	p-value	R ²
Compartment	2	10.9	0.001	0.191	27.1	0.001	0.313	30.2	0.001	0.389	76.1	0.001	0.599	174.2	0.001	0.654
Location	6	3.6	0.001	0.187	5.5	0.001	0.189	3.7	0.001	0.144	4.2	0.001	0.099	9.0	0.001	0.101
Compartment × Location	12	2.4	0.001	0.254	3.7	0.001	0.257	2.5	0.001	0.196	2.9	0.001	0.138	7.4	0.001	0.166
Residuals	42			0.368			0.242			0.271			0.165			0.079

[†]df=degrees of freedom

Supplementary Table 8. Homogeneity of dispersion among compartments (midgut, hindgut, soil) and location of prokaryotic communities in the alimentary tract of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil. Notice that the test of dispersions was run separately and independently for the two factors. F-statistics and p -values for permutational analysis of multivariate dispersion (PERMDISP) models used 999 permutations.

Term	n	Groups	Jaccard		Bray-Curtis		Unweighted UniFrac		Weighted UniFrac		DEICODE	
			F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value
Compartment	63	3	30.0	0.001	12.0	0.001	36.1	0.001	22.8	0.001	8.3	0.001
Soil vs. Midgut	42	2	11.4	0.007	2.6	0.073	20.8	0.001	22.3	0.002	0.2	0.596
Soil vs. Hindgut	42	2	25.7	0.002	18.4	0.002	26.4	0.001	1.4	0.003	14.3	0.002
Midgut vs. Hindgut	42	2	47.5	0.002	18.8	0.002	53.5	0.001	26.5	0.002	13.9	0.002
Location	63	7	0.4	0.627	0.4	0.501	0.5	0.366	0.6	0.403	0.1	0.977

Supplementary Table 9. Selection of key prokaryotic taxa for pairwise analysis of log-ratios across compartments: midgut and hindgut from third instar larvae of the Japanese beetle (*Popillia japonica* Newman), and associated soil. Taxa are presented in order of criteria for consideration, including most prevalent and/or top taxa driving most of the difference among compartments. Number of amplicon sequence variants (ASVs) assigned to each taxon, as well as their prevalence in samples (21 samples= 100%) and locations (7 locations= 100%) per compartment are presented.

Taxa	Criteria for consideration*	# ASVs	Soil		Midgut		Hindgut		Key taxa for log ratio analysis**
			Sample prevalence (%)	Location prevalence (%)	Sample prevalence (%)	Location prevalence (%)	Sample prevalence (%)	Location prevalence (%)	
<i>Rhizobiales</i>	<i>i. s, m; ii.</i>	234	100	100	76	100	100	100	Yes
<i>Chthoniobacterales</i>	<i>i. s; ii.</i>	142	100	100	95	100	100	100	Yes
<i>Betaproteobacterales</i>	<i>i. s.</i>	222	100	100	90	100	100	100	Yes
<i>Enterobacterales</i>	<i>i. m; ii.</i>	10	95	100	100	100	100	100	Yes
<i>Bacillales</i>	<i>i. m; ii.</i>	61	100	100	100	100	100	100	Yes
<i>Clostridiales</i>	<i>i. h; ii.</i>	2,106	95	100	95	100	100	100	Yes
<i>Bacteroidales</i>	<i>i. h; ii.</i>	47	81	86	95	100	100	100	Yes
<i>Desulfovibrionales</i>	<i>i. h; ii.</i>	22	43	71	71	100	100	100	Yes
<i>Chitinophagales</i>	<i>ii</i>	263	95	100	81	100	14	29	No
<i>Erysipelotrichales</i>	<i>ii</i>	24	29	29	57	100	100	100	No
<i>Steroidobacterales</i>	<i>ii</i>	23	100	100	76	86	29	71	No

***Criteria for consideration:**

i. The three most prevalent orders in the prokaryotic core microbiota of each compartment (**Figure 3**), s=soil, m=midgut, h=hindgut;

ii. Orders of top 20 ASVs driving most of the variation among compartments in the ordination space (**Figure 4**);

****Criteria for selection:** Sample and location prevalence are both $\geq 33\%$ in all compartments (the log-ratio method is sensitive to sparsity).

Supplementary Table 10. Pairwise analysis of prokaryotic taxa across compartments: midgut and hindgut from third instar larvae of the Japanese beetle (*Popillia japonica* Newman), and associated soil. The natural log-ratio of taxa in each sample was calculated using *qurro*⁶⁸. Taxa were chosen according to their prevalence among compartments (**Figure 3**) and their influence on variation along the first axis of the ordination space, Robust Aitchison PCA (**Figure 4**). Statistical significance (Brown-Forsythe Test) is indicated with one star (*) or two stars (**) for $p < 0.05$ or $p < 0.001$, respectively.

Comparison	Sample prevalence (%)			Location prevalence (%)			Brown-Forsythe Test		<i>p</i> -value for Posthoc Brown-Forsythe Test with Bonferroni correction						
	Numerator/Denominator	Soil	Midgut	Hindgut	Soil	Midgut	Hindgut	Statistic	<i>p</i> -value	Soil vs. Midgut	Soil vs. Hindgut	Midgut vs. Hindgut	Soil vs. Midgut	Soil vs. Hindgut	Midgut vs. Hindgut
<i>Bacillales/Bacteroidales</i>	81	95	100	86	100	100	126.1	0.000	**	0.278		0.000	**	0.000	**
<i>Bacillales/Betaproteobacteriales</i>	100	90	100	100	100	100	39.5	0.000	**	0.000	**	0.357		0.000	**
<i>Bacillales/Clostridiales</i>	95	95	100	100	100	100	146.6	0.000	**	0.754		0.000	**	0.000	**
<i>Bacillales/Desulfovibrionales</i>	43	71	100	71	100	100	166.5	0.000	**	0.187		0.000	**	0.000	**
<i>Bacteroidales/Betaproteobacteriales</i>	81	86	100	86	100	100	63.4	0.000	**	0.007	*	0.000	**	0.000	**
<i>Bacteroidales/Desulfovibrionales</i>	43	67	100	71	100	100	1.7	0.197		1.000		0.273		0.482	
<i>Chthoniobacterales/Bacillales</i>	100	95	100	100	100	100	50.6	0.000	**	0.000	**	0.000	**	1.000	
<i>Chthoniobacterales/Bacteroidales</i>	81	90	100	86	100	100	96.9	0.000	**	0.002	*	0.000	**	0.000	**
<i>Chthoniobacterales/Betaproteobacteriales</i>	100	86	100	100	100	100	68.8	0.000	**	0.048	*	0.000	**	0.000	**
<i>Chthoniobacterales/Clostridiales</i>	95	95	100	100	100	100	139.3	0.000	**	0.000	**	0.000	**	0.000	**
<i>Chthoniobacterales/Desulfovibrionales</i>	43	71	100	71	100	100	82.4	0.000	**	0.019	*	0.000	**	0.000	**
<i>Chthoniobacterales/Enterobacteriales</i>	95	95	100	100	100	100	33.4	0.000	**	0.000	**	0.000	**	0.711	
<i>Clostridiales/Bacteroidales</i>	81	90	100	86	100	100	0.9	0.400		1.000		0.122		1.000	
<i>Clostridiales/Betaproteobacteriales</i>	95	86	100	100	100	100	88.6	0.000	**	0.003	*	0.000	**	0.000	**
<i>Clostridiales/Desulfovibrionales</i>	43	71	100	71	100	100	0.5	0.586		0.974		0.608		1.000	
<i>Desulfovibrionales/Betaproteobacteriales</i>	43	67	100	71	100	100	75.8	0.000	**	0.026	*	0.000	**	0.000	**
<i>Enterobacteriales/Bacillales</i>	95	100	100	100	100	100	3.8	0.031	*	0.055		0.031	*	1.000	
<i>Enterobacteriales/Bacteroidales</i>	81	95	100	86	100	100	75.4	0.000	**	0.002	*	0.000	**	0.000	**

Supplementary Table 10. *Continued.*

Comparison	Sample prevalence (%)			Location prevalence (%)			Brown-Forsythe Test			<i>p</i> -value for Posthoc Brown-Forsythe Test with Bonferroni correction					
	Numerator/Denominator	Soil	Midgut	Hindgut	Soil	Midgut	Hindgut	Statistic	<i>p</i> -value	Soil vs. Midgut	Soil vs. Hindgut	Midgut vs. Hindgut			
<i>Enterobacteriales/Betaproteobacteriales</i>	95	90	100	100	100	100	21.5	0.000	**	0.000	**	0.021	*	0.002	*
<i>Enterobacteriales/Clostridiales</i>	90	95	100	100	100	100	72.1	0.000	**	0.017	*	0.000	**	0.000	**
<i>Enterobacteriales/Desulfovibrionales</i>	43	71	100	71	100	100	90.0	0.000	**	0.003	*	0.000	**	0.000	**
<i>Rhizobiales/Bacillales</i>	100	76	100	100	100	100	21.9	0.000	**	0.012	*	0.000	**	0.204	
<i>Rhizobiales/Bacteroidales</i>	81	71	100	86	100	100	89.5	0.000	**	1.000		0.000	**	0.000	**
<i>Rhizobiales/Betaproteobacteriales</i>	100	76	100	100	100	100	86.4	0.000	**	0.006	*	0.000	**	0.000	**
<i>Rhizobiales/Chthoniobacterales</i>	100	76	100	100	100	100	12.9	0.000	**	0.000	**	0.006	*	0.063	
<i>Rhizobiales/Clostridiales</i>	95	76	100	100	100	100	124.9	0.000	**	0.780		0.000	**	0.000	**
<i>Rhizobiales/Desulfovibrionales</i>	43	57	100	71	100	100	114.1	0.000	**	1.000		0.000	**	0.000	**
<i>Rhizobiales/Enterobacteriales</i>	95	76	100	100	100	100	15.0	0.000	**	0.018	*	0.000	**	0.646	

Supplementary Table 11. Influence of location (all locations), soil management history (TPAC *versus* naturally infested locations), and manipulated larval treatment (Purdy *versus* Nursery) on variation in β -diversity of prokaryotic communities per compartment in the alimentary tract of third instar Japanese beetle (*Popillia japonica* Newman) larvae and associated soil. Permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersion (PERMDISP) methods were used to test differences between locations and homogeneity of dispersion among locations within each compartment. Test statistic (pseudo-F and F-value for PERMANOVA and PERMDISP, respectively) and *p*-values for both models using 999 permutations.

	Compartment	Method	Jaccard		Bray-Curtis		Unweighted UniFrac		Weighted UniFrac		DEICODE	
			Test statistic	<i>p</i> -value	Test statistic	<i>p</i> -value	Test statistic	<i>p</i> -value	Test statistic	<i>p</i> -value	Test statistic	<i>p</i> -value
All locations Sample size=21 Groups=7	Soil	PERMANOVA	7.0	0.001	16.3	0.001	6.7	0.001	25.6	0.001	91.2	0.001
		PERMDISP	0.4	0.323	0.5	0.249	0.4	0.350	0.6	0.203	0.3	0.700
	Midgut	PERMANOVA	2.1	0.001	3.4	0.001	2.2	0.001	2.3	0.008	8.9	0.001
		PERMDISP	0.8	0.210	0.5	0.413	0.7	0.202	0.7	0.316	0.6	0.251
	Hindgut	PERMANOVA	1.6	0.001	1.8	0.001	2.0	0.001	2.6	0.005	4.9	0.001
		PERMDISP	1.2	0.212	2.7	0.007	0.6	0.587	0.3	0.670	0.5	0.667
Soil management history Sample size=18 Groups=2	Soil	PERMANOVA	3.1	0.003	3.6	0.006	3.0	0.007	2.3	0.083	11.8	0.005
		PERMDISP	150.5	0.009	53.9	0.006	75.6	0.006	25.9	0.008	2.4	0.184
	Midgut	PERMANOVA	1.8	0.010	1.5	0.124	2.9	0.012	2.1	0.078	1.1	0.398
		PERMDISP	4.1	0.653	2.3	0.184	1.0	0.457	0.0	0.909	4.5	0.082
	Hindgut	PERMANOVA	1.6	0.007	1.9	0.006	1.5	0.043	4.5	0.004	1.7	0.175
		PERMDISP	13.5	0.086	44.2	0.009	6.6	0.061	3.3	0.124	2.1	0.183
Manipulated larval treatment Sample size=6 Groups=2	Soil	PERMANOVA	6.4	0.099	13.5	0.100	6.9	0.114	21.9	0.104	3.9	0.102
		PERMDISP	0.5	0.102	0.3	0.109	0.7	0.103	0.3	0.046	1.9	0.186
	Midgut	PERMANOVA	2.6	0.112	2.8	0.101	2.5	0.112	1.3	0.202	3.5	0.171
		PERMDISP	5.7	0.107	26.4	0.043	2.9	0.086	10.6	0.121	0.1	0.808
	Hindgut	PERMANOVA	1.3	0.106	1.3	0.211	2.4	0.104	0.8	0.387	1.9	0.098
		PERMDISP	6.2	0.087	1.9	0.104	0.8	0.045	0.0	0.897	11.1	0.372