

Supplementary materials for:

## The gut microbiome of two wild bumblebee species native of South America: *Bombus pauloensis* and *Bombus bellicosus*

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**Supplementary table S1:** The table describes qPCR results on the gut pathogen *N. ceranae* and Eubacteria expressed in Log, and the amount of DNA extracted per sample expressed in ng/μl.

Species	Sampling Site	DNA Quantification (ng/μl)	<i>Nosema ceranae</i> units (Log)	Eubacteria (Log)
<i>B. pauloensis</i>	NRP	5.78	0.00	7.385
<i>B. pauloensis</i>	NRP	1.84	0.00	7.613
<i>B. pauloensis</i>	NRP	3.19	0.00	8.520
<i>B. pauloensis</i>	NRP	12.40	4.89	8.449
<i>B. pauloensis</i>	NRP	72.00	0.00	9.726
<i>B. pauloensis</i>	NRP	11.60	0.00	9.299
<i>B. pauloensis</i>	NRP	75.3	4.82	8.187
<i>B. pauloensis</i>	NRP	48.7	8.24	10.207
<i>B. pauloensis</i>	NRP	7.67	5.14	9.576
<i>B. pauloensis</i>	NRP	47.50	4.89	8.436
<i>B. pauloensis</i>	SP	163.00	6.29	8.998
<i>B. pauloensis</i>	SP	30.80	5.26	8.260
<i>B. pauloensis</i>	SP	217.00	7.17	8.067
<i>B. pauloensis</i>	SP	8.87	4.35	9.446
<i>B. pauloensis</i>	SP	31.30	5.42	8.790
<i>B. pauloensis</i>	SP	86.70	5.26	8.600
<i>B. pauloensis</i>	SP	22.50	6.15	8.325
<i>B. pauloensis</i>	SP	15.80	5.17	9.138
<i>B. pauloensis</i>	SP	221.00	8.52	9.394
<i>B. pauloensis</i>	SP	15.7	8.21	8.743
<i>B. pauloensis</i>	VA	9.47	7.44	8.363
<i>B. pauloensis</i>	VA	7.13	5.21	9.490
<i>B. pauloensis</i>	VA	27.20	4.86	8.063
<i>B. pauloensis</i>	VA	27.10	7.19	9.713
<i>B. pauloensis</i>	VA	3.44	4.9	7.385
<i>B. pauloensis</i>	VA	3.00	4.55	7.613
<i>B. pauloensis</i>	VA	9.47	5.13	8.520
<i>B. pauloensis</i>	VA	2.01	6.59	8.449
<i>B. pauloensis</i>	VA	14.00	7.45	9.726
<i>B. pauloensis</i>	VA	109.00	4.25	9.299
<i>B. belicosus</i>	NRP	14.90	4.84	8.169
<i>B. belicosus</i>	NRP	3.03	4.47	7.727
<i>B. belicosus</i>	NRP	1.68	5.01	8.064
<i>B. belicosus</i>	NRP	1.88	4.67	7.689
<i>B. belicosus</i>	NRP	1.15	0.00	9.507
<i>B. belicosus</i>	NRP	14.30	4.72	9.213
<i>B. belicosus</i>	NRP	15.00	4.93	7.974
<i>B. belicosus</i>	NRP	1.35	4.87	9.792
<i>B. belicosus</i>	NRP	10.30	0.00	8.169
<i>B. belicosus</i>	NRP	15.20	6.06	7.727

**Supplementary table S2:** Table with complete list of primers used for PCR and qPCR analysis

TARGET	PRIMER NAME	SEQUENCE	AMPLICON MELTING TEMPERATURE	ANNEALING TEMPERATURE	AMPLICON SIZE	References
<i>Lotmaria passim</i>	qCrFw1 153	5'- TCCACTCTGCAAACGATGAC -3'	87.0	56	153	<a href="#">Arismendi et al., 2016</a>
	qCrRev1 153	5'- GGGCCGAATGGAAAAGATAC -3'				
<i>Ascospaera spp.</i>	AscoAll1	5'- GCACTCCCACCCTTGTCTA -3'	89.8	58	550	<a href="#">James &amp; Skinner 2005</a>
	AscoAll2	5'- GAACACGACGCCGTCCTACT -3'				
<i>Acarapis woody</i>	AcarFor	5'- CGGGCCCGAGCTTATTTTACTGCTG -3'	78.5	57	162	<a href="#">Garrido-Bailón et al., 2012</a>
	AcarRev	5'- GCGCCTGTCAATCCACCTACAGAA -3'				
<i>Nosema apis</i>	321 apis Fw	5'- GGGGGCATGTCTTTGACGTACTATGTA -3'	84.3	60	321	<a href="#">Martín-Hernández et al., 2007</a>
	321 apis Rev	5'- GGGGGGCGTTTAAAAATGTGAAACAACCTATG -3'				
<i>Nosema cerenae</i>	218mitoc Fw	5'- CGGCGACGATGTGATATGAAAATATTA -3'	77.0	60	218	<a href="#">Martín-Hernández et al., 2007</a>
	218mitoc Rev	5'- CCCGGTCATTCTCAAACAACAAAACCG -3'				
<i>Apicystis bombi</i>	ApBF1293 Fw	5'- CGTACTGCCCTGAATACTCCAG -3'	81.7	58	293	<a href="#">Meeus et al, 2018</a>
	ApBR1293 Rv	5'- TGAAAGCGGCGTATACATGA -3'				
<i>Crithidia bombi</i>	Crithidia119fw	5'- CCAACGGTGAGCCGCATTCAGT -3'	83.7	58	119	<a href="#">Huang et al., 2015</a>
	Crithidia119rv	5'- CGCGTGTGCGCCAGAACATTGA -3'				
<i>Nosema bombi</i>	NBom323 fw	5'- CCATGCATGTTTTTGAAGATTATTAT -3'	78.1	56	323	<a href="#">Klee et al., 2006</a>
	NBom 323 rv	5'- CATATATTTTTTAAAATATGAAACAATAA -3'				
AmFV	AmFV 551 -F	5'- CAGAGAATTCGGTTTTTGTGAGTG -3'	84.7	52	551	<a href="#">Hartmann et al., 2015</a>
	AmFV 551 -R	5'- CATGGTGCCCAAGTCTTGCT -3'				
Eubacteria	Eub338-F	5'- ACTCCTACGGGAGGCAGCAG-3'	-	60	200	<a href="#">Favaro et al., 2023</a>
	Eub518-R	5'- ATTACCGCGGCTGCTGG -3'				
16S rRNA region V7-V8 with adapters	799F	5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACMGGATTAGATACCCG -3			470 and 720	
	1139R	5'- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGTCATCCCCACCTTC -3				

**Supplementary table S3:** Relative abundance at Phylum level. The sampling sites reported: Natural Reserve Paititi (NRP); Santa Paula's Farm (SP); Vivero Antoniucci (VA)

Sample			Phylum				
Code	Sampling site	Bumblebee Specie	Proteobacteria	Firmicutes	Bacteroidota	Actinobacteriota	Others
BB71	NRP	<i>B. bellicosus</i>	0.9573	0.0208	0.0008	0.0082	0.0129
BB72	NRP	<i>B. bellicosus</i>	0.9346	0.0346	0.0017	0.0229	0.0063
BB90	NRP	<i>B. bellicosus</i>	0.9385	0.0336	0.0007	0.0173	0.0099
BB91	NRP	<i>B. bellicosus</i>	0.8773	0.0612	0.0016	0.0424	0.0176
BB92	NRP	<i>B. bellicosus</i>	0.7529	0.0164	0.0010	0.2250	0.0047
BB93	NRP	<i>B. bellicosus</i>	0.7626	0.0947	0.0001	0.1302	0.0124
BB94	NRP	<i>B. bellicosus</i>	0.9318	0.0347	0.0000	0.0179	0.0157
BB96	NRP	<i>B. bellicosus</i>	0.5242	0.0412	0.4267	0.0069	0.0010
BP02	NRP	<i>B. pauloensis</i>	0.6551	0.1022	0.0449	0.1955	0.0023
BP04	NRP	<i>B. pauloensis</i>	0.7568	0.1800	0.0038	0.0525	0.0069
BP06	NRP	<i>B. pauloensis</i>	0.9106	0.0751	0.0001	0.0143	0.0000
BP07	NRP	<i>B. pauloensis</i>	0.5060	0.2953	0.0018	0.1960	0.0009
BP08	NRP	<i>B. pauloensis</i>	0.5721	0.3259	0.0024	0.0988	0.0009
BP09	NRP	<i>B. pauloensis</i>	0.2460	0.6098	0.1105	0.0336	0.0001
BP11	SP	<i>B. pauloensis</i>	0.9217	0.0405	0.0004	0.0253	0.0120
BP13	SP	<i>B. pauloensis</i>	0.6517	0.2444	0.0000	0.1029	0.0010
BP15	SP	<i>B. pauloensis</i>	0.8879	0.0445	0.0003	0.0673	0.0000
BP16	SP	<i>B. pauloensis</i>	0.5903	0.3432	0.0000	0.0665	0.0000
BP17	SP	<i>B. pauloensis</i>	0.3713	0.6017	0.0025	0.0240	0.0005
BP18	SP	<i>B. pauloensis</i>	0.8913	0.0847	0.0006	0.0234	0.0000
BP19	SP	<i>B. pauloensis</i>	0.7091	0.2211	0.0004	0.0695	0.0000
BP20	VA	<i>B. pauloensis</i>	0.6511	0.3296	0.0000	0.0193	0.0000
BP21	VA	<i>B. pauloensis</i>	0.5903	0.3571	0.0258	0.0262	0.0007
BP22	VA	<i>B. pauloensis</i>	0.5556	0.0991	0.3313	0.0136	0.0004
BP23	VA	<i>B. pauloensis</i>	0.5103	0.4864	0.0003	0.0026	0.0004
BP24	VA	<i>B. pauloensis</i>	0.7719	0.1985	0.0002	0.0292	0.0004
BP25	VA	<i>B. pauloensis</i>	0.6510	0.0766	0.2555	0.0168	0.0000
BP26	VA	<i>B. pauloensis</i>	0.8704	0.0089	0.0000	0.1197	0.0010
BP27	VA	<i>B. pauloensis</i>	0.8560	0.1258	0.0000	0.0182	0.0000
BP28	VA	<i>B. pauloensis</i>	0.9910	0.0048	0.0010	0.0015	0.0016
BP03	NRP	<i>B. pauloensis</i>	0.7293	0.2142	0.0454	0.0086	0.0024
BP30	VA	<i>B. pauloensis</i>	0.9120	0.0617	0.0047	0.0148	0.0069

**Supplementary table S4:** Relative abundance at Family level. The sampling sites reported: Natural Reserve Paititi (NRP); Santa Paula’s Farm (SP); Vivero Antoniucci (VA)

Sample			Family										
Code	Sampling site	Bumblebee Specie	Neisseriaceae	Pseudomonadaceae	Morganellaceae	Enterobacterales	Lactobacillaceae	Orbaceae	Weeksellaceae	Bifidobacteriaceae	Anaplasmataceae	Pectobacteriaceae	Erwiniaceae
BB71	NRP	<i>B. bellicosus</i>	0.348	0.517	0.000	0.025	0.014	0.037	0.001	0.002	0.004	0.002	0.000
BB72	NRP	<i>B. bellicosus</i>	0.665	0.112	0.000	0.021	0.024	0.110	0.000	0.017	0.005	0.002	0.000
BB90	NRP	<i>B. bellicosus</i>	0.675	0.094	0.013	0.018	0.031	0.107	0.001	0.013	0.005	0.002	0.001
BB91	NRP	<i>B. bellicosus</i>	0.523	0.133	0.005	0.037	0.055	0.104	0.001	0.011	0.006	0.002	0.000
BB92	NRP	<i>B. bellicosus</i>	0.006	0.079	0.042	0.016	0.016	0.011	0.001	0.220	0.010	0.004	0.571
BB93	NRP	<i>B. bellicosus</i>	0.495	0.088	0.015	0.009	0.093	0.137	0.000	0.124	0.001	0.000	0.000
BB94	NRP	<i>B. bellicosus</i>	0.491	0.145	0.006	0.053	0.013	0.092	0.000	0.000	0.003	0.000	0.091
BB96	NRP	<i>B. bellicosus</i>	0.478	0.010	0.001	0.002	0.038	0.028	0.427	0.007	0.001	0.001	0.003
BP02	NRP	<i>B. pauloensis</i>	0.248	0.044	0.001	0.030	0.101	0.095	0.044	0.190	0.026	0.013	0.000
BP03	NRP	<i>B. pauloensis</i>	0.043	0.226	0.004	0.145	0.206	0.026	0.045	0.001	0.156	0.065	0.001
BP04	NRP	<i>B. pauloensis</i>	0.267	0.143	0.006	0.122	0.171	0.048	0.003	0.039	0.077	0.048	0.001
BP06	NRP	<i>B. pauloensis</i>	0.309	0.001	0.510	0.002	0.075	0.087	0.000	0.014	0.001	0.000	0.000
BP07	NRP	<i>B. pauloensis</i>	0.375	0.003	0.064	0.004	0.294	0.057	0.002	0.196	0.002	0.000	0.000
BP08	NRP	<i>B. pauloensis</i>	0.269	0.110	0.022	0.051	0.324	0.045	0.002	0.095	0.043	0.019	0.000
BP09	NRP	<i>B. pauloensis</i>	0.091	0.003	0.034	0.002	0.610	0.112	0.111	0.034	0.001	0.000	0.000
BP11	SP	<i>B. pauloensis</i>	0.889	0.019	0.001	0.010	0.041	0.003	0.000	0.026	0.006	0.004	0.000
BP13	SP	<i>B. pauloensis</i>	0.579	0.020	0.010	0.013	0.245	0.017	0.000	0.100	0.007	0.000	0.000
BP15	SP	<i>B. pauloensis</i>	0.860	0.004	0.006	0.001	0.044	0.011	0.000	0.067	0.003	0.000	0.001
BP16	SP	<i>B. pauloensis</i>	0.572	0.003	0.007	0.002	0.341	0.003	0.000	0.067	0.002	0.000	0.000
BP17	SP	<i>B. pauloensis</i>	0.189	0.014	0.004	0.009	0.603	0.142	0.002	0.015	0.004	0.000	0.000
BP18	SP	<i>B. pauloensis</i>	0.762	0.007	0.007	0.004	0.085	0.106	0.000	0.021	0.002	0.000	0.000
BP19	SP	<i>B. pauloensis</i>	0.581	0.018	0.080	0.008	0.218	0.005	0.000	0.068	0.016	0.000	0.000
BP20	VA	<i>B. pauloensis</i>	0.603	0.007	0.002	0.003	0.328	0.032	0.000	0.018	0.003	0.000	0.000
BP21	VA	<i>B. pauloensis</i>	0.451	0.005	0.005	0.004	0.357	0.116	0.026	0.021	0.004	0.002	0.001
BP22	VA	<i>B. pauloensis</i>	0.375	0.036	0.000	0.020	0.098	0.084	0.331	0.010	0.026	0.007	0.000
BP23	VA	<i>B. pauloensis</i>	0.001	0.012	0.002	0.012	0.417	0.002	0.000	0.000	0.012	0.004	0.001
BP24	VA	<i>B. pauloensis</i>	0.742	0.001	0.000	0.002	0.197	0.026	0.000	0.029	0.001	0.000	0.000
BP25	VA	<i>B. pauloensis</i>	0.533	0.007	0.000	0.000	0.076	0.109	0.256	0.017	0.000	0.000	0.000
BP26	VA	<i>B. pauloensis</i>	0.669	0.168	0.001	0.004	0.009	0.010	0.000	0.119	0.001	0.000	0.006
BP27	VA	<i>B. pauloensis</i>	0.759	0.004	0.000	0.000	0.126	0.090	0.000	0.018	0.000	0.000	0.000
BP28	VA	<i>B. pauloensis</i>	0.000	0.520	0.000	0.315	0.004	0.000	0.001	0.000	0.019	0.129	0.000
BP30	VA	<i>B. pauloensis</i>	0.003	0.339	0.003	0.047	0.042	0.459	0.001	0.000	0.025	0.013	0.001

**Supplementary table S5:** Relative abundance at genus level. The sampling sites reported: Natural Reserve Paititi (NRP); Santa Paula´s Farm (SP); Vivero Antoniucci (VA)

Sample			Genera												
Code	Sampling site	Bumblebee Specie	Apibacter	Arsenophonus	Bifidobacterium	Gilliamella	Kluyvera	Lactobacillus	Pantoea	Pseudomonas	Rosenbergiella	Snodgrassella	Sodalis	Wolbachia	Others
BB71	NRP	<i>B. bellicosus</i>	0.03	0.00	0.21	3.32	0.00	1.43	0.00	51.65	0.00	34.72	0.23	0.38	8.05
BB72	NRP	<i>B. bellicosus</i>	0.00	0.00	1.55	5.00	0.00	2.43	0.00	11.22	0.00	66.50	0.25	0.48	12.56
BB90	NRP	<i>B. bellicosus</i>	0.03	1.27	1.23	8.03	0.00	3.13	0.00	9.35	0.00	67.22	0.21	0.49	9.04
BB91	NRP	<i>B. bellicosus</i>	0.00	0.50	1.09	8.79	0.00	5.49	0.05	13.31	0.00	52.30	0.25	0.58	17.66
BB92	NRP	<i>B. bellicosus</i>	0.00	4.22	21.94	0.28	0.00	1.59	26.56	7.90	13.21	0.54	0.36	1.00	22.39
BB93	NRP	<i>B. bellicosus</i>	0.00	1.51	12.31	12.5	0.00	9.26	0.00	8.74	0.00	49.18	0.00	0.12	6.30
BB94	NRP	<i>B. bellicosus</i>	0.00	0.65	0.03	2.19	0.00	1.33	3.54	14.44	5.06	49.03	0.00	0.35	23.39
BB96	NRP	<i>B. bellicosus</i>	42.67	0.05	0.65	2.57	0.00	3.84	0.00	1.04	0.06	47.57	0.06	0.07	1.41
BP02	NRP	<i>B. pauloensis</i>	0.00	0.14	2.52	0.00	0.00	4.01	0.00	1.83	0.00	87.85	0.36	0.59	2.67
BP03	NRP	<i>B. pauloensis</i>	0.00	0.98	9.94	1.41	0.00	24.35	0.00	2.05	0.00	57.82	0.00	0.71	2.74
BP04	NRP	<i>B. pauloensis</i>	0.00	0.64	6.73	0.10	0.00	4.38	0.00	0.43	0.00	86.00	0.00	0.25	1.46
BP06	NRP	<i>B. pauloensis</i>	0.00	0.71	6.65	0.32	0.00	34.14	0.00	0.34	0.00	57.24	0.00	0.19	0.40
BP07	NRP	<i>B. pauloensis</i>	0.00	0.44	1.49	3.49	0.00	60.17	0.00	1.43	0.00	18.86	0.00	0.44	13.68
BP08	NRP	<i>B. pauloensis</i>	0.00	0.74	2.13	10.50	0.00	8.45	0.00	0.66	0.00	76.25	0.00	0.25	1.03
BP09	NRP	<i>B. pauloensis</i>	0.00	7.98	6.76	0.39	0.00	21.77	0.00	1.75	0.00	58.06	0.00	1.65	1.64
BP11	SP	<i>B. pauloensis</i>	4.02	0.12	18.92	6.44	0.00	10.08	0.00	4.36	0.00	24.81	1.30	2.63	27.32
BP13	SP	<i>B. pauloensis</i>	0.00	0.19	1.78	2.25	0.00	32.82	0.00	0.71	0.00	60.23	0.00	0.34	1.68
BP15	SP	<i>B. pauloensis</i>	2.42	0.49	2.11	11.10	0.00	35.64	0.00	0.52	0.12	45.09	0.23	0.39	1.88
BP16	SP	<i>B. pauloensis</i>	33.09	0.03	0.99	1.78	0.00	9.81	0.00	3.56	0.00	37.48	0.74	2.56	9.94
BP17	SP	<i>B. pauloensis</i>	0.00	0.22	0.00	0.04	19.27	41.64	0.00	1.23	0.11	0.12	0.40	1.25	35.72
BP18	SP	<i>B. pauloensis</i>	0.00	0.04	2.86	2.50	0.00	19.73	0.00	0.13	0.00	74.17	0.00	0.08	0.50
BP19	SP	<i>B. pauloensis</i>	25.55	0.03	1.66	4.40	0.00	7.65	0.00	0.72	0.00	53.28	0.00	0.03	6.68
BP20	VA	<i>B. pauloensis</i>	0.00	0.13	11.90	0.99	0.00	0.89	0.00	16.82	0.61	66.78	0.00	0.07	1.81
BP21	VA	<i>B. pauloensis</i>	0.00	0.00	1.81	6.76	0.00	12.58	0.00	0.36	0.00	75.85	0.00	0.00	2.63
BP22	VA	<i>B. pauloensis</i>	0.00	0.04	0.00	0.00	0.00	0.39	0.00	51.97	0.00	0.00	12.89	1.90	32.81
BP23	VA	<i>B. pauloensis</i>	4.03	0.35	0.05	1.50	0.00	20.59	0.08	22.57	0.00	4.24	6.49	15.5	24.54
BP24	VA	<i>B. pauloensis</i>	0.00	0.32	0.00	0.00	0.00	4.22	0.05	33.65	0.05	0.25	1.26	2.46	57.74
BP25	VA	<i>B. pauloensis</i>	0.00	0.62	3.91	2.69	0.00	17.00	0.09	14.23	0.00	26.56	4.82	7.68	22.41
BP26	VA	<i>B. pauloensis</i>	0.00	51.04	1.40	7.35	0.00	7.49	0.00	0.11	0.00	30.88	0.00	0.14	1.59
BP27	VA	<i>B. pauloensis</i>	0.02	6.35	19.32	5.58	0.00	29.41	0.00	0.30	0.00	37.49	0.00	0.22	1.30
BP28	VA	<i>B. pauloensis</i>	0.00	2.15	9.49	4.05	0.00	32.38	0.00	11.04	0.00	26.85	1.93	4.27	7.83

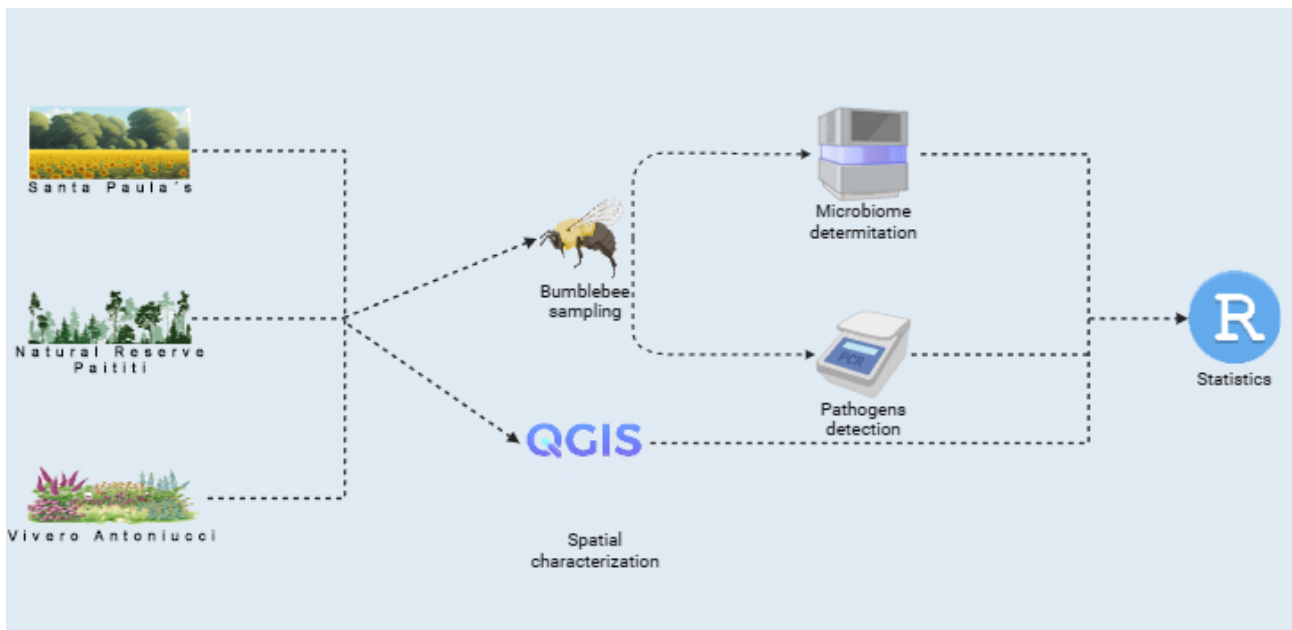
**Supplementary table S6: Generalized Unifrac beta diversity, pairwise comparison of groups.** The sampling sites reported: Natural Reserve Paititi (NRP); Santa Paula's Farm (SP); Vivero Antoniucci (VA). p-values: \* p<0.05; \*\*\* p<0.01.

<b>Group_1</b>	<b>Group_2</b>	<b>Sample size</b>	<b>Permutations</b>	<b>pseudo-F</b>	<b>p-value</b>	<b>q-value</b>
	<i>B. paulensis</i> RPN	15	999	2.161524	0.291	0.292
<i>B. bellicosus</i> RPN	<i>B. paulensis</i> SP	16	999	3.672724	0.031	0.093
	<i>B. paulensis</i> VA	17	999	2.625976	0.292	0.292
<i>B. paulensis</i> RPN	<i>B. paulensis</i> SP	15	999	11.745693	0.004 *	0.024
	<i>B. paulensis</i> VA	16	999	3.024549	0.207	0.292
<i>B. paulensis</i> SP	<i>B. paulensis</i> VA	17	999	4.755364	0.063	0.126

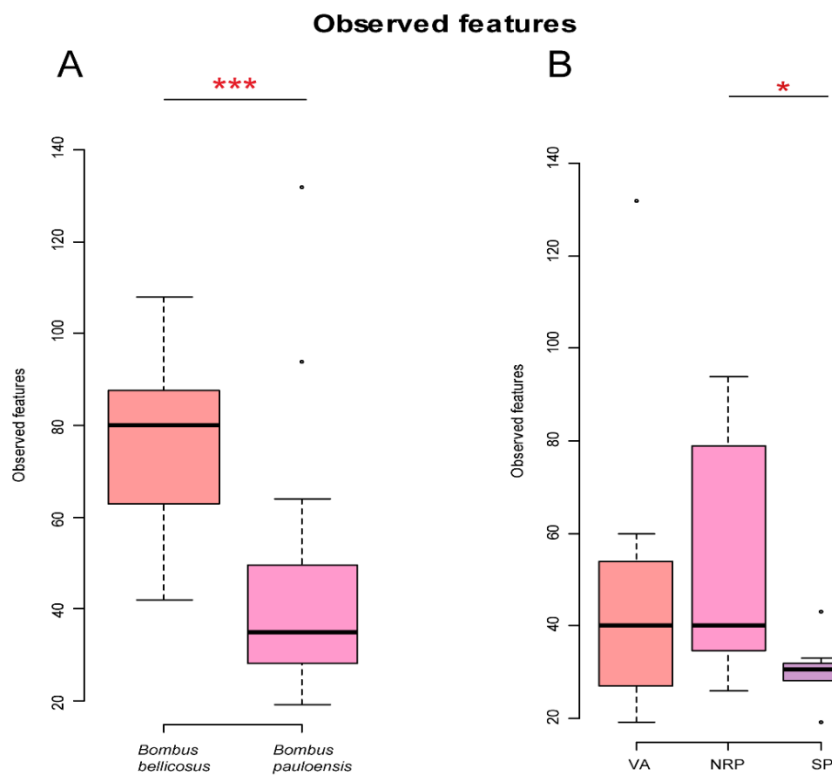
**Supplementary table S7:** Table summarizing the sample identification code and the NCBI Sequence Read Archive (SRA) accession number

Sample Code	Sampling site	Bumblebee Specie	NCBI accession number
BB71	NRP	<i>B. bellicosus</i>	<a href="#">SRR17715434</a>
BB72	NRP	<i>B. bellicosus</i>	<a href="#">SRR17715423</a>
BB90	NRP	<i>B. bellicosus</i>	<a href="#">SRR17715412</a>
BB91	NRP	<i>B. bellicosus</i>	<a href="#">SRR17715401</a>
BB92	NRP	<i>B. bellicosus</i>	<a href="#">SRR17715481</a>
BB93	NRP	<i>B. bellicosus</i>	<a href="#">SRR17715470</a>
BB94	NRP	<i>B. bellicosus</i>	<a href="#">SRR17715459</a>
BB96	NRP	<i>B. bellicosus</i>	<a href="#">SRR17715448</a>
BP02	NRP	<i>B. pauloensis</i>	<a href="#">SRR17715433</a>
BP04	NRP	<i>B. pauloensis</i>	<a href="#">SRR17715431</a>
BP06	NRP	<i>B. pauloensis</i>	<a href="#">SRR17715430</a>
BP07	NRP	<i>B. pauloensis</i>	<a href="#">SRR17715429</a>
BP08	NRP	<i>B. pauloensis</i>	<a href="#">SRR17715428</a>
BP09	NRP	<i>B. pauloensis</i>	<a href="#">SRR17715427</a>
BP11	SP	<i>B. pauloensis</i>	<a href="#">SRR17715425</a>
BP13	SP	<i>B. pauloensis</i>	<a href="#">SRR17715422</a>
BP15	SP	<i>B. pauloensis</i>	<a href="#">SRR17715421</a>
BP16	SP	<i>B. pauloensis</i>	<a href="#">SRR17715420</a>
BP17	SP	<i>B. pauloensis</i>	<a href="#">SRR17715419</a>
BP18	SP	<i>B. pauloensis</i>	<a href="#">SRR17715418</a>
BP19	SP	<i>B. pauloensis</i>	<a href="#">SRR17715417</a>
BP20	VA	<i>B. pauloensis</i>	<a href="#">SRR17715416</a>
BP21	VA	<i>B. pauloensis</i>	<a href="#">SRR17715415</a>
BP22	VA	<i>B. pauloensis</i>	<a href="#">SRR17715414</a>
BP23	VA	<i>B. pauloensis</i>	<a href="#">SRR17715413</a>
BP24	VA	<i>B. pauloensis</i>	<a href="#">SRR17715411</a>
BP25	VA	<i>B. pauloensis</i>	<a href="#">SRR17715410</a>
BP26	VA	<i>B. pauloensis</i>	<a href="#">SRR17715409</a>
BP27	VA	<i>B. pauloensis</i>	<a href="#">SRR17715408</a>
BP28	VA	<i>B. pauloensis</i>	<a href="#">SRR17715407</a>
BP03	NRP	<i>B. pauloensis</i>	<a href="#">SRR17715432</a>
BP30	VA	<i>B. pauloensis</i>	<a href="#">SRR17715406</a>

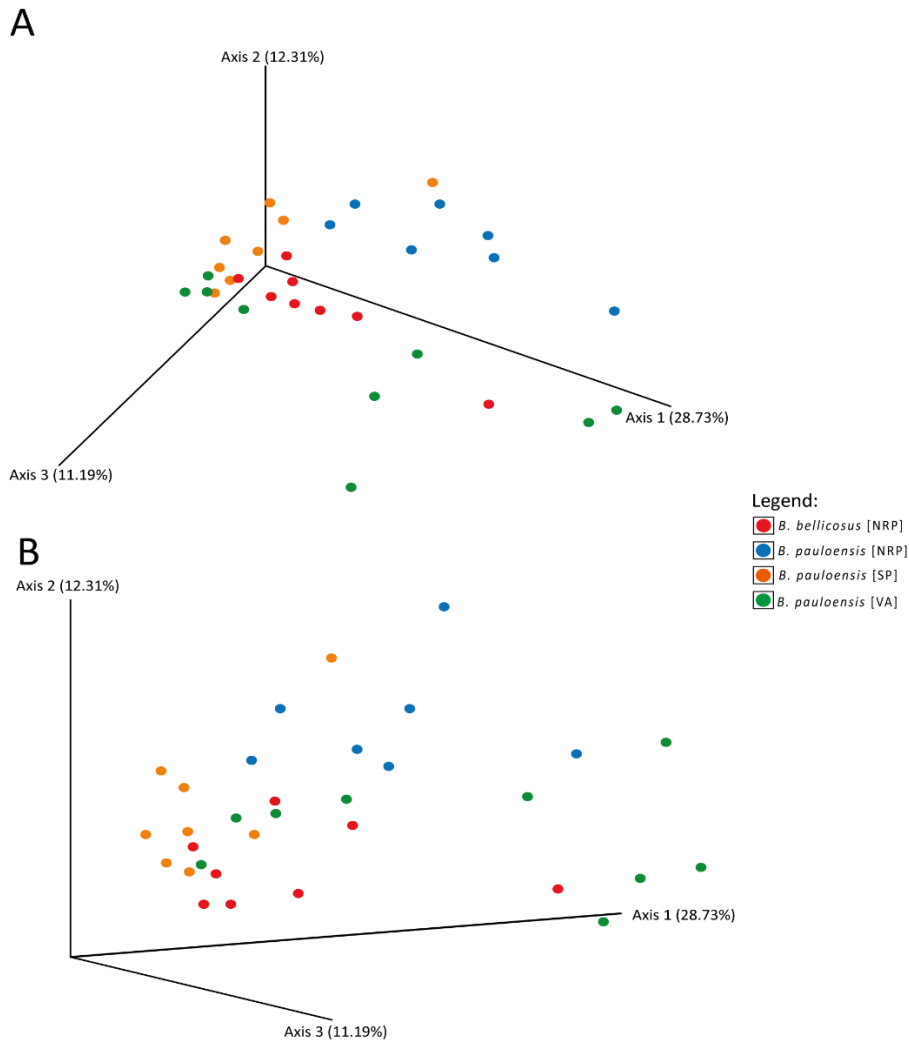




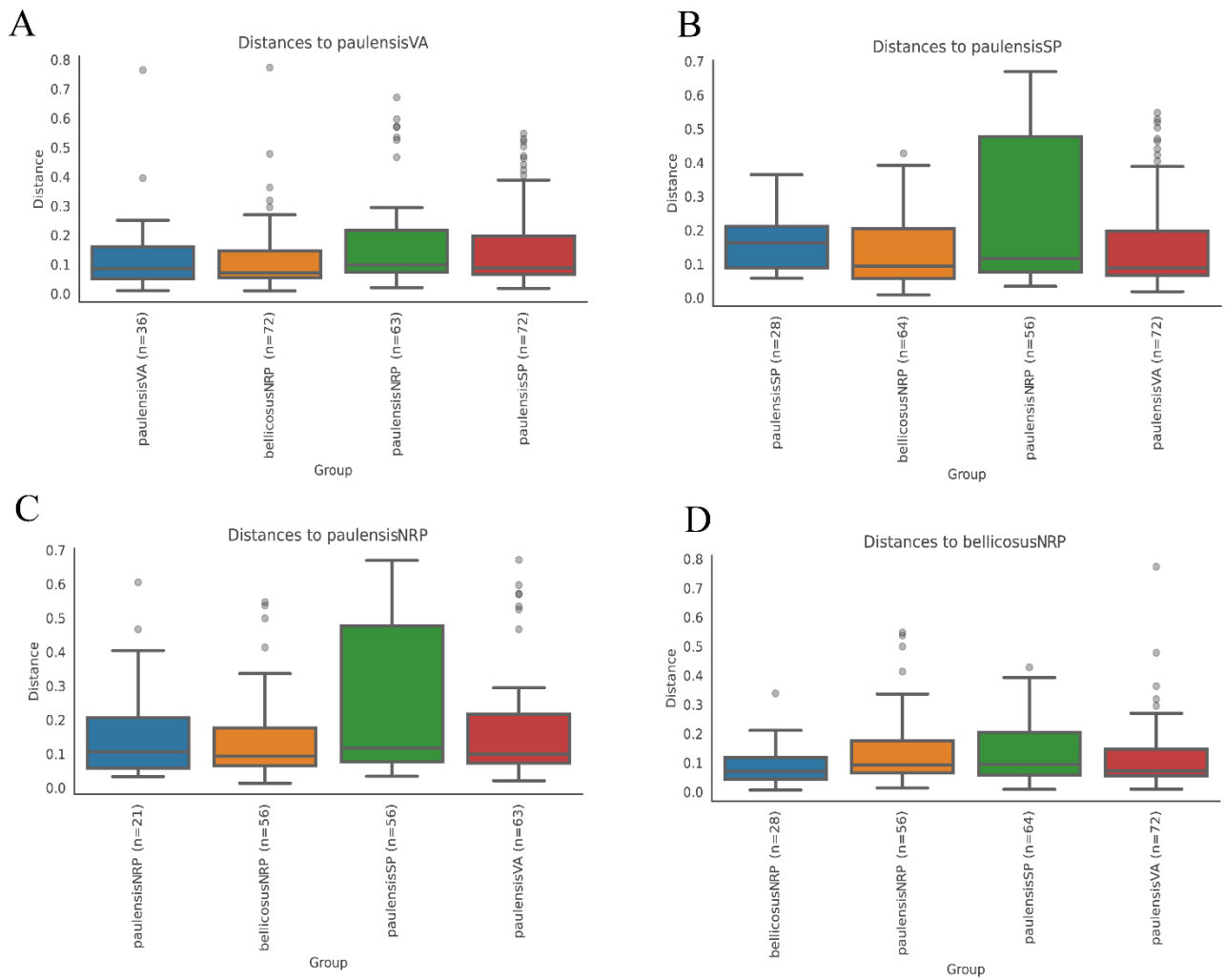
**Supplementary figure S1:** Workflow of the presented research illustrating the sampling sites and the analysis carried out.



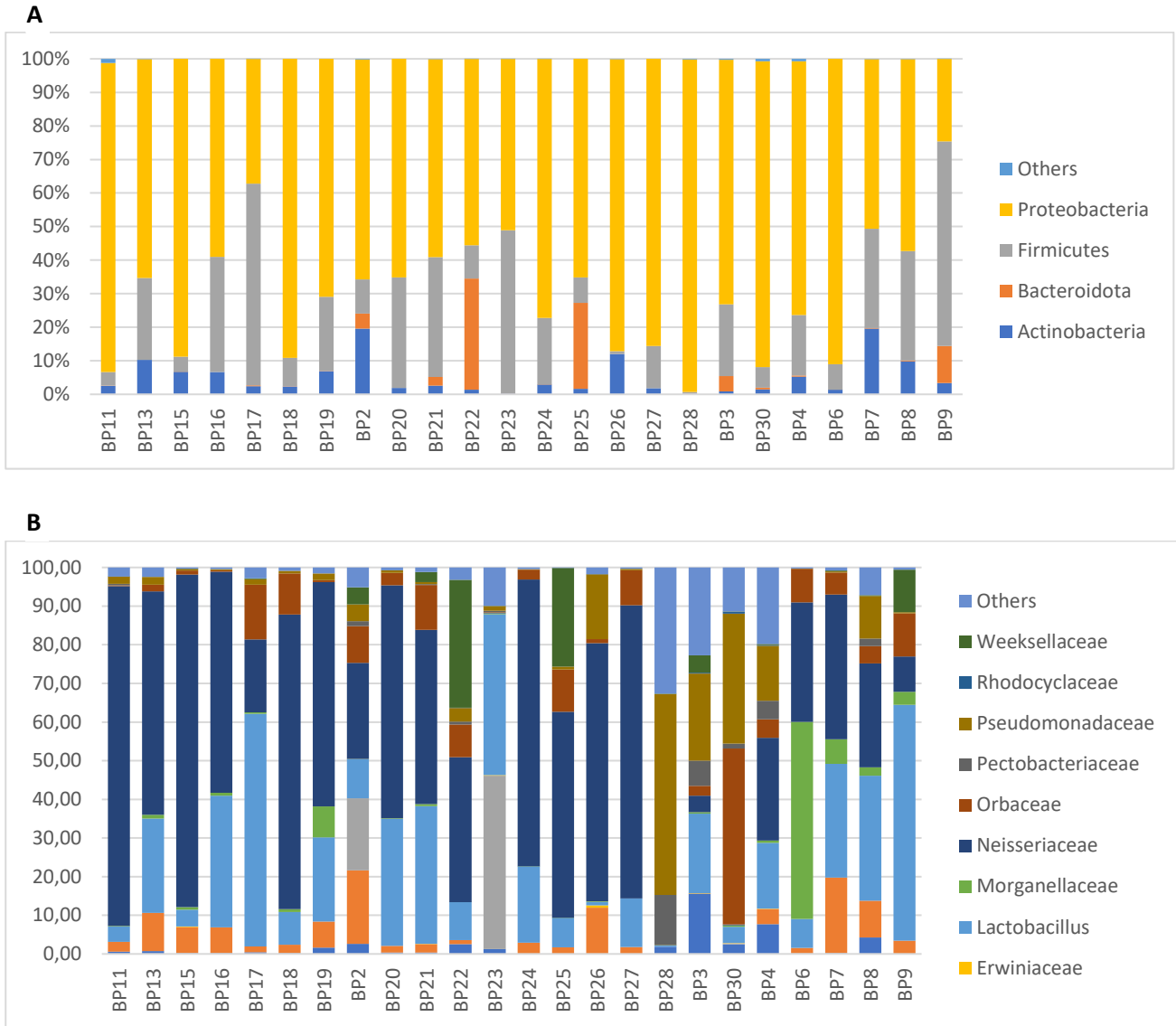
**Supplementary figure S2:  $\alpha$ -diversity indexes.** A) Comparing *B. pauloensis* (n= 24) and *B. bellicosus* (n=8) with Observed features. B) Comparing the different sampling sites Vivero Antoniucci [VA] vs Reserva Natural Paititi [NRP] vs Santa Paula [SP] Observed features. p-values: \* p<0.05; \*\*\* p<0.01.



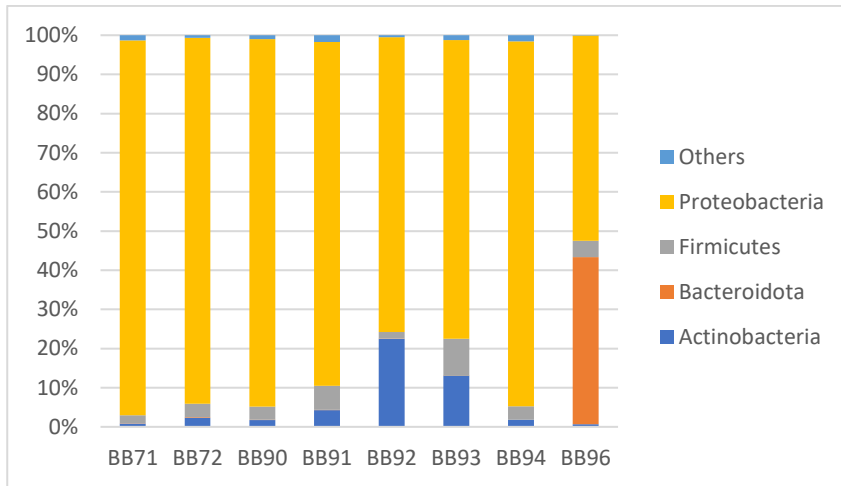
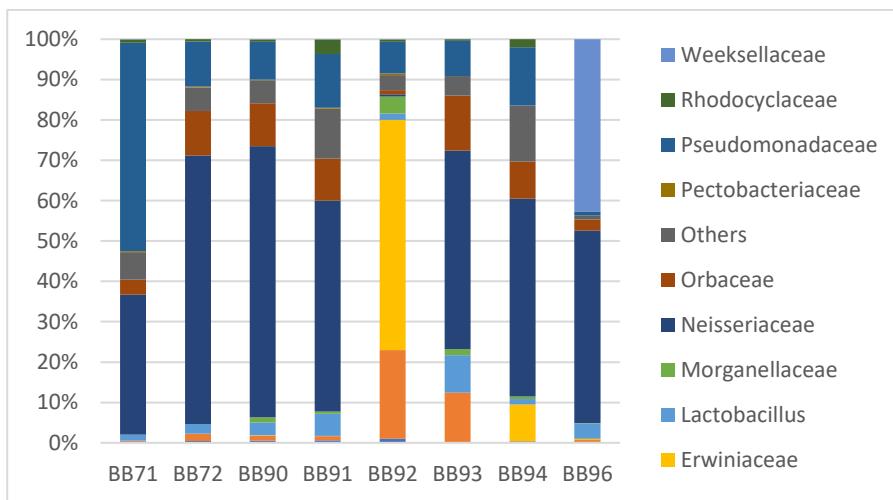
**Supplementary figure S3: PCoA illustrating the Bray-Curtis  $\beta$ -Diversity.** PCoA reports Bray-Curtis index for *B. pauloensis* sampled in Vivero Antoniucci [VA] (n= 9), Reserva Natural Paititi [NRP] (n=7) and Santa Paula [SP] (n=8), and for *B. bellicosus* sampled in [NRP]. Figure A) shows the samples distribution on axes 1-3, whereas, B) shows the same samples distribution on axes 1-2



**Supplementary figure S4: Generalized Unifrac.** A) *B. paulensis* VA vs all sites and species. B) *B. paulensis* SP vs all sites and species.

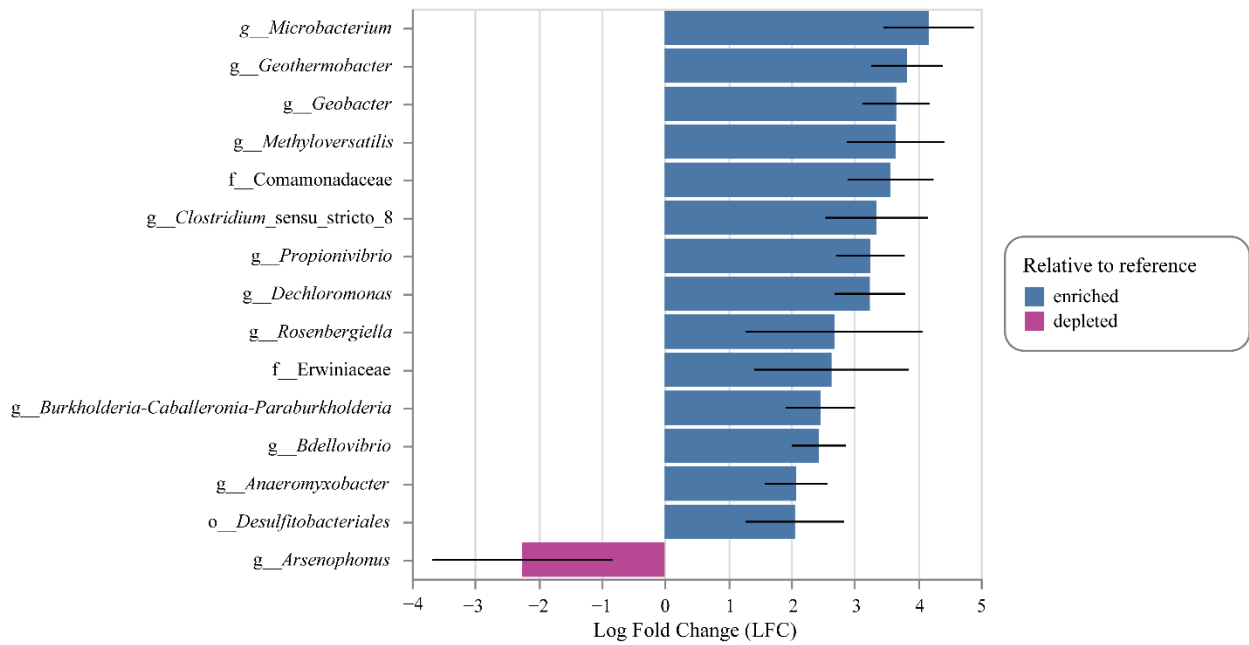


**Supplementary figure S5: Diversity of the gut microbiota of *Bombus pauloensis*.** Overlaid bar chart representing in percentage the composition of the microbiota (Y-axis) of each analyzed individual of *B. pauloensis* species (X-axis), for the different taxonomic levels studied, Phylum (A) and Family (B) level

**A****B**

**Supplementary figure S6: Diversity of the gut microbiota of *Bombus bellicosus*.** Overlaid bar chart representing in percentage the composition of the microbiota (Y-axis) of each analyzed individual of *B. bellicosus* species (X-axis), for the different taxonomic levels studied, Phylum (A) and Family (B) level

Differential abundance analysis *Bombus bellicosus* VS *Bombus pauloensis* (NRP)



**Supplementary figure S7:** Differential abundance analysis (DAA) *Bombus bellicosus* compared to *Bombus pauloensis* in NRP sampling site. Only the differential values above Log Fold Change (LFC) of 2 are shown.



**Supplementary figure S8:** *Bombus pauloensis* foraging on a Leguminosae (Reserva Natural Paititi – Buenos Aires province December 2023)



**Supplementary figure S9:** *Bombus bellicosus* foraging on a Leguminosae (Reserva Natural Paititi – Buenos Aires province, December 2023)

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