

Effect of Alkaline and Mechanical Pretreatment of Wheat Straw on Enrichment Cultures from *Pachnoda marginata* Larva Gut

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Supplementary Figures:

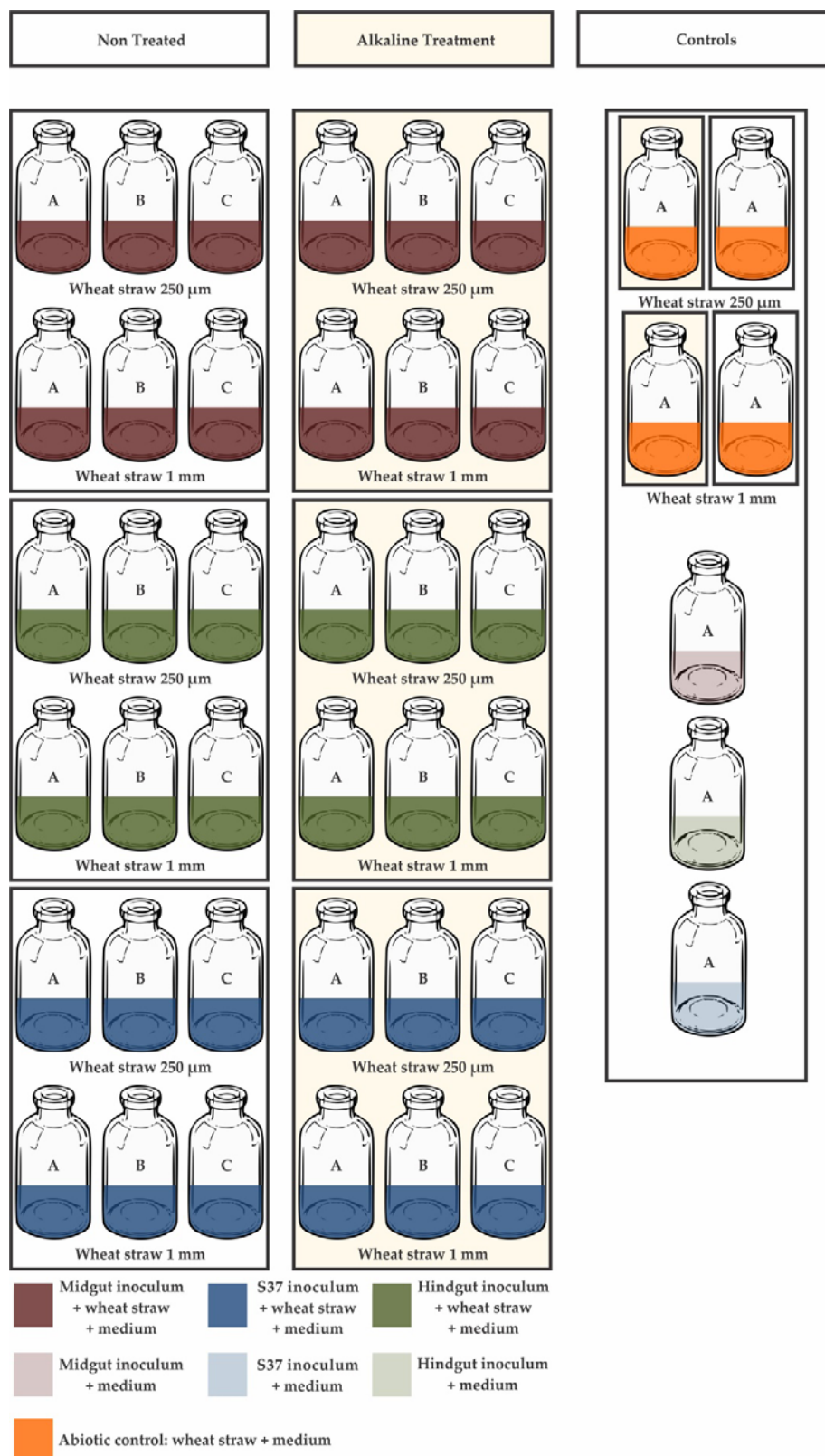


Figure S1: Experimental design.

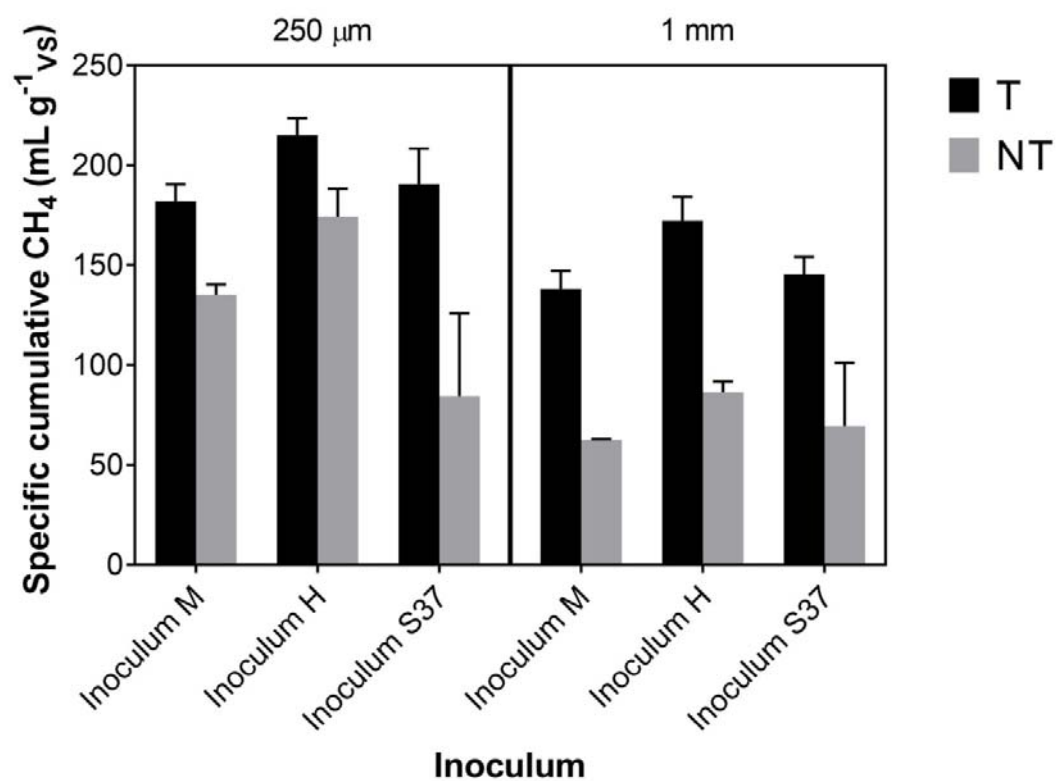


Figure S2: Specific cumulative methane at 30 days of cultivation.

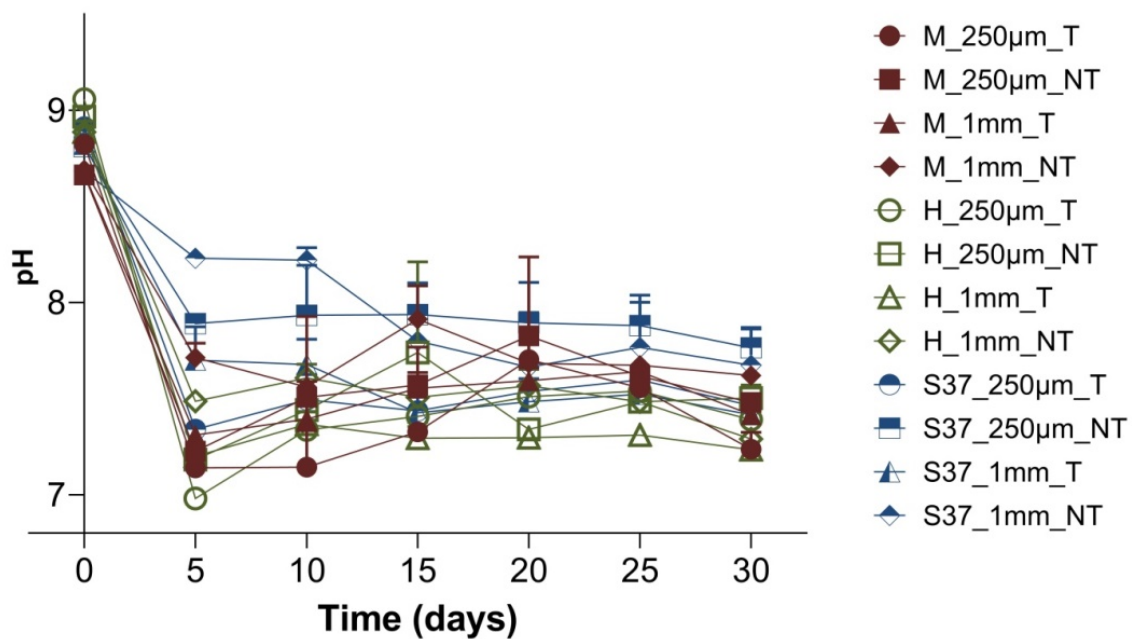


Figure S3: pH over 30 days of cultivation in the midgut (**M**), hindgut (**H**) larva cultures and Szarvas soda lake (**S37**) culture. The error bars represent the standard deviation of the mean of $n = 3$ (invisible error bars are smaller than the symbol).

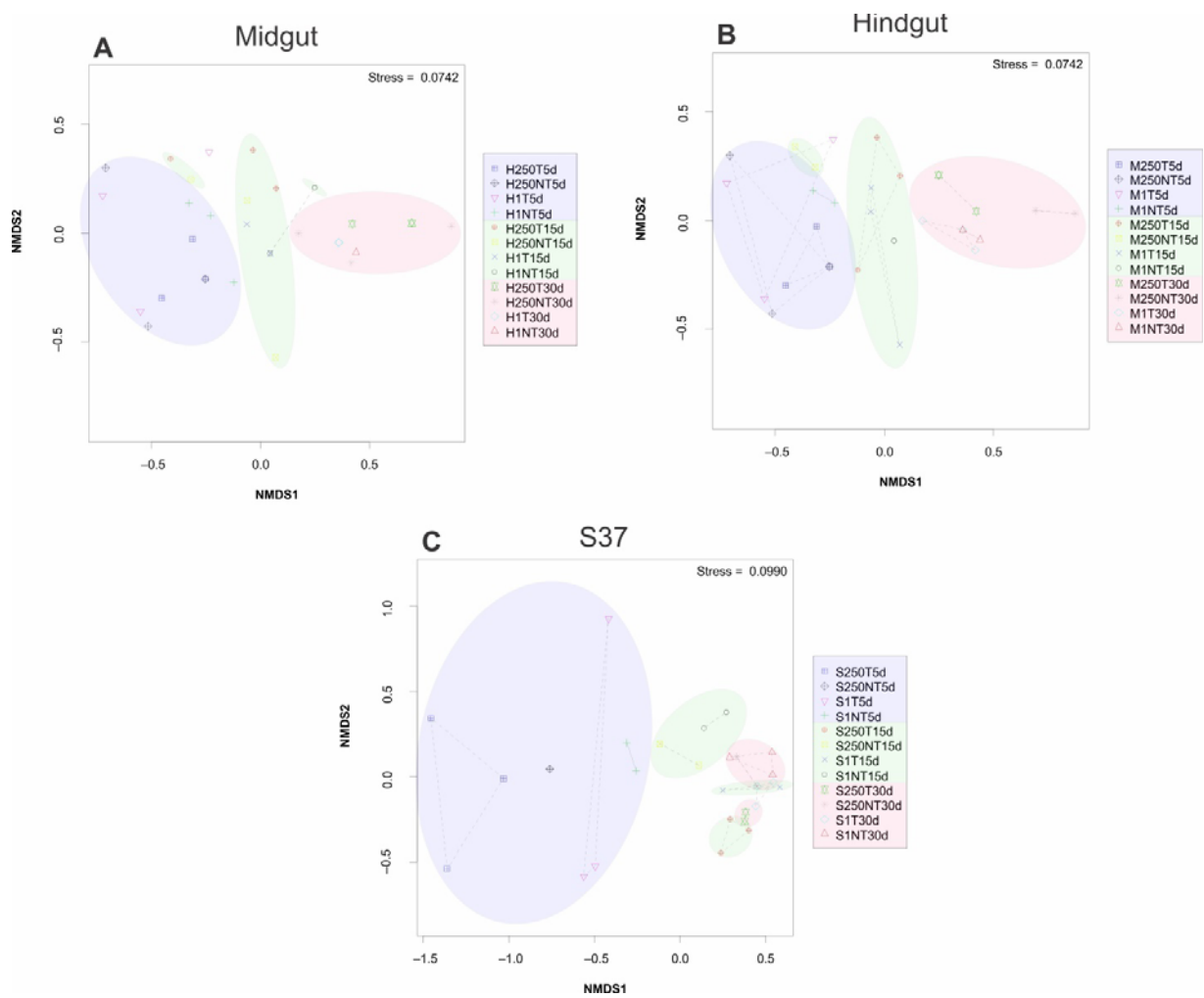


Figure S4: Bacterial community dynamics at 5, 15 and 30 days of cultivation. The non-metric multidimensional scaling (NMDS) plot of T-RFLP profiles of bacterial 16S rRNA gene amplicons obtained by *RSa I* restriction enzyme. Plot based on Bray-Curtis dissimilarity index showing the similarity relationships of the (A) midgut cultures, (B) hindgut cultures and (C) Szarvas soda lake cultures.

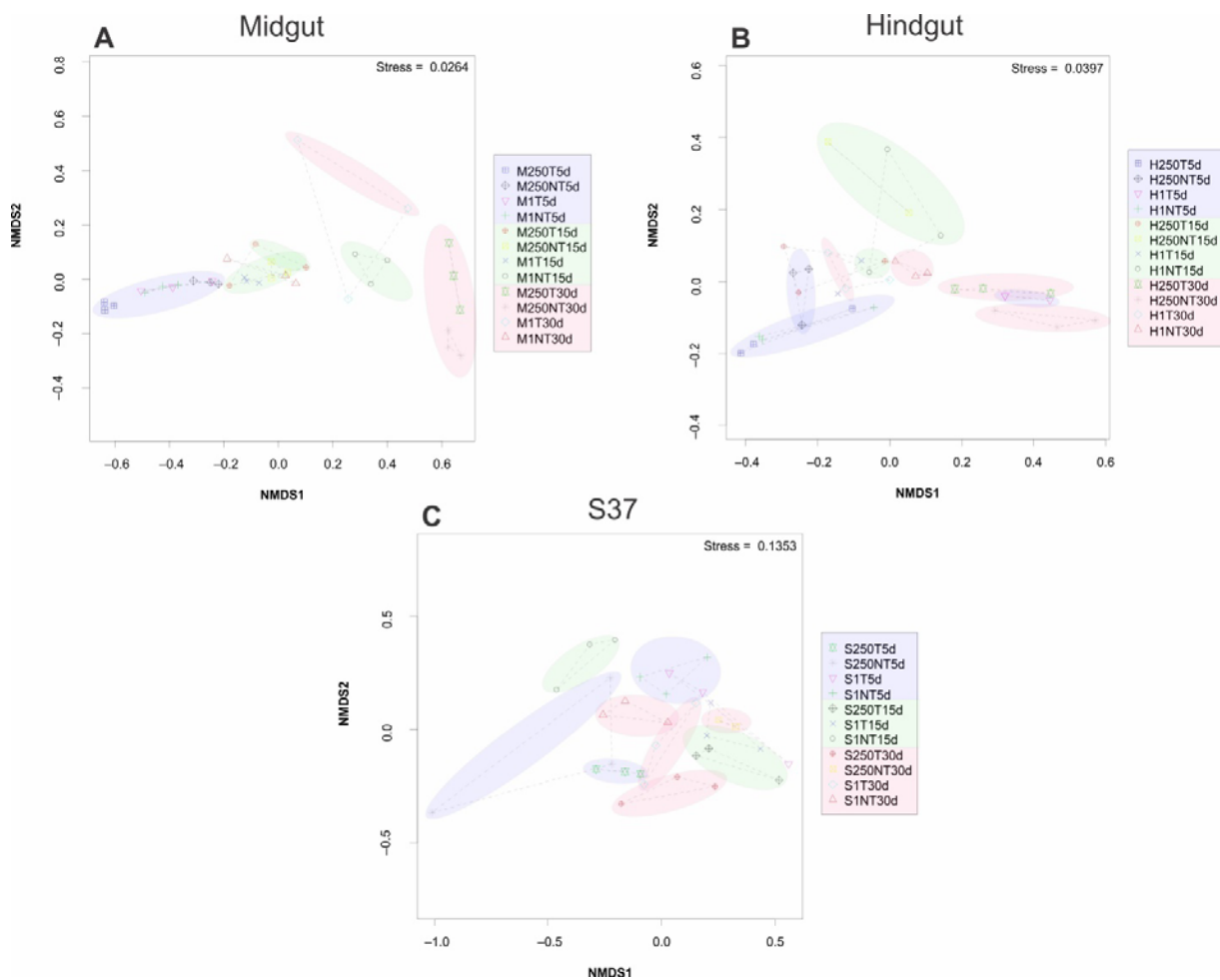


Figure S5: Methanogenic community dynamics at 5, 15 and 30 days of cultivation. The non-metric multidimensional scaling (NMDS) plot of the T-RFLP data obtained by *Bst*NI restriction enzyme. Plot based on Bray–Curtis dissimilarity index showing the similarity relationships of the (A) midgut cultures, (B) hindgut cultures and (C) Szarvas soda lake cultures.

Table S1: Specific cumulative methane at 30 days of cultivation data.

	A			B			C			D		
	250 μ m			250 μ m			1 mm			1 mm		
	T			NT			T			NT		
Inoculum M	172.306	184.8334	188.8305	139.69	136.44	129.48	127.84	144.01	142.7	62.99	61.92	62.37
Inoculum H	206.78	223.5951	215.0911	158.05	183.84	180.76	159.4374	174.7111	182.8481	88.22303	80.59464	90.5458
Inoculum S37	191.1593	208.096	172.807	66.01834	131.8814	55.58448	153.8788	136.5968	146.0129	87.30989	88.13875	32.99552

Table S2: The three-way ANOVA analysis of specific cumulative methane at 30 days of cultivation. P value lower than 0.05 is considered significant.

Table Analyzed		Specific methane 30 days		
Three-way ANOVA		Ordinary		
Alpha		0.05		
Source of Variation	% of total variation	P value	P value summary	Significant?
Row factor		11.26 <0.0001 ****		Yes
(AB vs CD)		24.9 <0.0001 ****		Yes
(AC vs BD)		49.08 <0.0001 ****		Yes
Row factor x (AB vs CD)		2.195 0.0516 ns		No
Row factor x (AC vs BD)		1.751 0.0888 ns		No
(AB vs CD) x (AC vs BD)		0.504 0.2261 ns		No
Row factor x (AB vs CD) x (AC vs BD)		2.476 0.037 *		Yes
ANOVA table	SS	DF	MS	F (DFn, DFd) P value
Row factor		10680	2	5340 F (2, 24) = 17.24 P<0.0001
(AB vs CD)		23620	1	23620 F (1, 24) = 76.27 P<0.0001
(AC vs BD)		46563	1	46563 F (1, 24) = 150.4 P<0.0001
Row factor x (AB vs CD)		2082	2	1041 F (2, 24) = 3.362 P=0.0516
Row factor x (AC vs BD)		1662	2	830.8 F (2, 24) = 2.683 P=0.0888
(AB vs CD) x (AC vs BD)		478.1	1	478.1 F (1, 24) = 1.544 P=0.2261
Row factor x (AB vs CD) x (AC vs BD)		2349	2	1175 F (2, 24) = 3.793 P=0.0370
Residual		7432	24	309.7

Table S3: Tukey's multiple comparisons test of specific cumulative methane at 30 days of cultivation. P value lower than 0.05 is considered significant.

Compare each cell mean with every other cell mean					
Number of families	1				
Number of comparisons per family	66				
Alpha	0.05				
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Inoculum M:Group A vs. Inoculum M:Group B	46.79 -5.021 to 98.59		No	ns	0.1034
Inoculum M:Group A vs. Inoculum M:Group C	43.81 -8.001 to 95.61		No	ns	0.1544
Inoculum M:Group A vs. Inoculum M:Group D	119.667.76 to 171.4		Yes	****	<0.0001
Inoculum M:Group A vs. Inoculum H:Group A	-33.17 -84.97 to 18.64		No	ns	0.4971
Inoculum M:Group A vs. Inoculum H:Group B	7.773 -44.03 to 59.58		No	ns	>0.9999
Inoculum M:Group A vs. Inoculum H:Group C	9.658 -42.15 to 61.47		No	ns	0.9999
Inoculum M:Group A vs. Inoculum H:Group D	95.5443.73 to 147.3		Yes	****	<0.0001
Inoculum M:Group A vs. Inoculum S37:Group A	-8.697 -60.51 to 43.11		No	ns	>0.9999
Inoculum M:Group A vs. Inoculum S37:Group B	97.545.69 to 149.3		Yes	****	<0.0001
Inoculum M:Group A vs. Inoculum S37:Group C	36.49 -15.31 to 88.30		No	ns	0.3633
Inoculum M:Group A vs. Inoculum S37:Group D	112.560.70 to 164.3		Yes	****	<0.0001
Inoculum M:Group B vs. Inoculum M:Group C	-2.98 -54.79 to 48.83		No	ns	>0.9999
Inoculum M:Group B vs. Inoculum M:Group D	72.7820.97 to 124.6		Yes	**	0.0017
Inoculum M:Group B vs. Inoculum H:Group A	-79.95 -131.8 to -28.14		Yes	***	0.0005
Inoculum M:Group B vs. Inoculum H:Group B	-39.01 -90.82 to 12.79		No	ns	0.277
Inoculum M:Group B vs. Inoculum H:Group C	-37.13 -88.94 to 14.68		No	ns	0.3402
Inoculum M:Group B vs. Inoculum H:Group D	48.75 -3.059 to 100.6		No	ns	0.0783
Inoculum M:Group B vs. Inoculum S37:Group A	-55.48 -107.3 to -3.676		Yes	*	0.0285
Inoculum M:Group B vs. Inoculum S37:Group B	50.71 -1.099 to 102.5		No	ns	0.0589
Inoculum M:Group B vs. Inoculum S37:Group C	-10.29 -62.10 to 41.52		No	ns	0.9998
Inoculum M:Group B vs. Inoculum S37:Group D	65.7213.91 to 117.5		Yes	**	0.0055
Inoculum M:Group C vs. Inoculum M:Group D	75.7623.95 to 127.6		Yes	**	0.001
Inoculum M:Group C vs. Inoculum H:Group A	-76.97 -128.8 to -25.16		Yes	***	0.0008
Inoculum M:Group C vs. Inoculum H:Group B	-36.03 -87.84 to 15.77		No	ns	0.3807
Inoculum M:Group C vs. Inoculum H:Group C	-34.15 -85.96 to 17.66		No	ns	0.4557
Inoculum M:Group C vs. Inoculum H:Group D	51.73 -0.07902 to 103.5		No	ns	0.0506
Inoculum M:Group C vs. Inoculum S37:Group A	-52.5 -104.3 to -0.6962		Yes	*	0.045
Inoculum M:Group C vs. Inoculum S37:Group B	53.691.881 to 105.5		Yes	*	0.0376
Inoculum M:Group C vs. Inoculum S37:Group C	-7.313 -59.12 to 44.50		No	ns	>0.9999
Inoculum M:Group C vs. Inoculum S37:Group D	68.716.89 to 120.5		Yes	**	0.0033
Inoculum M:Group D vs. Inoculum H:Group A	-152.7 -204.5 to -100.9		Yes	****	<0.0001
Inoculum M:Group D vs. Inoculum H:Group B	-111.8 -163.6 to -59.98		Yes	****	<0.0001
Inoculum M:Group D vs. Inoculum H:Group C	-109.9 -161.7 to -58.10		Yes	****	<0.0001
Inoculum M:Group D vs. Inoculum H:Group D	-24.03 -75.84 to 27.78		No	ns	0.8637
Inoculum M:Group D vs. Inoculum S37:Group A	-128.3 -180.1 to -76.45		Yes	****	<0.0001
Inoculum M:Group D vs. Inoculum S37:Group B	-22.07 -73.88 to 29.74		No	ns	0.9153
Inoculum M:Group D vs. Inoculum S37:Group C	-83.07 -134.9 to -31.26		Yes	***	0.0003
Inoculum M:Group D vs. Inoculum S37:Group D	-7.055 -58.86 to 44.75		No	ns	>0.9999
Inoculum H:Group A vs. Inoculum H:Group B	40.94 -10.87 to 92.75		No	ns	0.2212
Inoculum H:Group A vs. Inoculum H:Group C	42.82 -8.985 to 94.63		No	ns	0.1752
Inoculum H:Group A vs. Inoculum H:Group D	128.776.89 to 180.5		Yes	****	<0.0001
Inoculum H:Group A vs. Inoculum S37:Group A	24.47 -27.34 to 76.28		No	ns	0.8502
Inoculum H:Group A vs. Inoculum S37:Group B	130.778.85 to 182.5		Yes	****	<0.0001
Inoculum H:Group A vs. Inoculum S37:Group C	69.6617.85 to 121.5		Yes	**	0.0028
Inoculum H:Group A vs. Inoculum S37:Group D	145.793.87 to 197.5		Yes	****	<0.0001
Inoculum H:Group B vs. Inoculum H:Group C	1.884 -49.92 to 53.69		No	ns	>0.9999
Inoculum H:Group B vs. Inoculum H:Group D	87.7635.95 to 139.6		Yes	***	0.0001
Inoculum H:Group B vs. Inoculum S37:Group A	-16.47 -68.28 to 35.34		No	ns	0.9886
Inoculum H:Group B vs. Inoculum S37:Group B	89.7237.91 to 141.5		Yes	****	<0.0001
Inoculum H:Group B vs. Inoculum S37:Group C	28.72 -23.09 to 80.53		No	ns	0.69
Inoculum H:Group B vs. Inoculum S37:Group D	104.752.93 to 156.5		Yes	****	<0.0001
Inoculum H:Group C vs. Inoculum H:Group D	85.8834.07 to 137.7		Yes	***	0.0002
Inoculum H:Group C vs. Inoculum S37:Group A	-18.36 -70.16 to 33.45		No	ns	0.9745
Inoculum H:Group C vs. Inoculum S37:Group B	87.8436.03 to 139.6		Yes	***	0.0001
Inoculum H:Group C vs. Inoculum S37:Group C	26.84 -24.97 to 78.64		No	ns	0.7666
Inoculum H:Group C vs. Inoculum S37:Group D	102.951.04 to 154.7		Yes	****	<0.0001
Inoculum H:Group D vs. Inoculum S37:Group A	-104.2 -156.0 to -52.43		Yes	****	<0.0001
Inoculum H:Group D vs. Inoculum S37:Group B	1.96 -49.85 to 53.77		No	ns	>0.9999
Inoculum H:Group D vs. Inoculum S37:Group C	-59.04 -110.8 to -7.234		Yes	*	0.0162
Inoculum H:Group D vs. Inoculum S37:Group D	16.97 -34.83 to 68.78		No	ns	0.9856
Inoculum S37:Group A vs. Inoculum S37:Group B	106.254.38 to 158.0		Yes	****	<0.0001
Inoculum S37:Group A vs. Inoculum S37:Group C	45.19 -6.617 to 97.00		No	ns	0.1285
Inoculum S37:Group A vs. Inoculum S37:Group D	121.269.40 to 173.0		Yes	****	<0.0001
Inoculum S37:Group B vs. Inoculum S37:Group C	-61 -112.8 to -9.194		Yes	*	0.0118
Inoculum S37:Group B vs. Inoculum S37:Group D	15.01 -36.79 to 66.82		No	ns	0.9945
Inoculum S37:Group C vs. Inoculum S37:Group D	76.0124.21 to 127.8		Yes	***	0.001