Supporting Materials

Supporting Tables

Table S1 Gill metabolites extract changes after various concentrations of brevotoxin treatment. FDR was calculated based on 36 metabolites. The FDR was only for the confidence of single metabolites not for the combinational effects.

		0.1 ppb		0.5 ppb		1 ppb		2 ppb		5 ppb		10 ppb	
Metabolites	ppm	p values	FDR	p values	FDR	p values	FDR	p values	FDR	p values	FDR	p values	FDR
2-Oxoisocaproate	2.6	7.90E-01	8.36E-01	9.95E-01	9.95E-01	6.30E-01	7.82E-01	5.97E-01	6.51E-01	8.95E-01	9.76E-01	9.63E-01	9.63E-01
3-Hydroxyisovalerate	1.25	4.57E-01	6.86E-01	7.94E-01	1.06E+00	5.49E-01	8.24E-01	3.72E-01	3.72E-01	9.44E-01	9.44E-01	5.92E-01	7.35E-01
4-hydroxyphenylacetate	7.19	2.61E-01	5.53E-01	8.86E-01	1.03E+00	1.30E-01	2.75E-01	1.50E-01	1.86E-01	5.98E-01	7.42E-01	1.55E-02	1.12E-01
Acetate	1.92	3.04E-01	6.08E-01	6.59E-01	9.89E-01	2.86E-01	1.29E+00	4.36E-02	7.47E-02	1.82E-01	3.12E-01	2.30E-02	1.04E-01
Alanine	1.5	3.55E-01	6.39E-01	5.27E-01	9.99E-01	9.36E-01	1.02E+00	7.92E-01	8.15E-01	9.43E-01	9.70E-01	2.59E-01	4.44E-01
Asparagine	2.93	7.52E-02	3.38E-01	1.07E-01	4.82E-01	1.37E-01	2.47E-01	2.42E-01	5.81E-01	1.08E-01	2.59E-01	3.35E-01	5.48E-01
Aspartate	2.72	2.00E-01	5.14E-01	4.44E-01	9.99E-01	4.06E-01	5.41E-01	5.07E-01	9.13E-01	1.79E-01	3.22E-01	6.84E-01	7.94E-01
Betaine	3.27	8.23E-02	2.96E-01	2.51E-01	7.53E-01	3.79E-01	2.27E+00	3.24E-02	6.48E-02	1.30E-01	2.60E-01	5.20E-01	7.80E-01
Biotin	4.46	6.43E-04	2.31E-02	3.43E-02	3.09E-01	6.71E-03	2.68E-02	4.81E-02	2.89E-01	4.05E-02	2.43E-01	1.26E-01	2.84E-01
Butyrate	0.9	1.12E-01	3.36E-01	5.42E-02	3.90E-01	1.35E-01	1.94E-01	3.90E-01	8.78E-01	1.08E-01	2.43E-01	4.53E-01	7.09E-01
Choline	3.2	2.89E-02	2.08E-01	8.78E-02	5.27E-01	5.79E-02	1.60E-01	8.42E-02	3.03E-01	8.56E-02	3.08E-01	1.62E-02	9.72E-02
Formate	8.47	3.21E-01	6.08E-01	2.67E-01	7.39E-01	1.98E-01	5.09E-01	8.96E-02	2.93E-01	8.68E-02	2.84E-01	1.15E-01	2.76E-01
Fumarate	6.5	4.77E-	6.87E-	3.09E	7.42E-	2.91E	8.73E-	7.47E	2.07E-	9.79E	2.71E-	1.32E-	2.80E-

	3	01	01	-01	01	-01	01	-02	01	-02	01	01	01
Glucose	5.23	3.56E-01	6.10E-01	1.04E-01	5.35E-01	1.44E-01	2.73E-01	2.39E-01	2.15E+00	9.35E-03	8.42E-02	9.77E-02	2.71E-01
Glutamate	2.35	7.37E-01	8.56E-01	8.07E-01	1.00E+00	2.33E-01	2.47E-01	8.79E-01	9.89E-01	8.89E-01	1.00E+00	8.03E-01	8.76E-01
Glutamine	2.43	6.32E-01	7.58E-01	6.26E-01	1.07E+00	9.30E-01	1.12E+00	6.99E-01	1.09E+00	2.35E-01	3.68E-01	5.61E-01	7.21E-01
Glycine	3.56	7.02E-02	3.61E-01	2.97E-01	7.64E-01	1.32E-01	6.79E-01	3.98E-02	1.79E-01	4.37E-02	1.97E-01	6.74E-02	2.21E-01
Hypotaurine	2.65	2.02E-03	2.42E-02	9.40E-03	1.13E-01	8.60E-03	3.10E-01	1.23E-03	4.43E-02	3.00E-03	1.08E-01	5.20E-03	9.36E-02
Inosine	8.24	9.40E-01	9.40E-01	7.72E-01	1.07E+00	9.71E-01	1.66E+00	2.51E-01	7.53E-01	8.99E-02	2.70E-01	7.74E-01	8.71E-01
Isoleucine	1.02	7.41E-01	8.34E-01	6.33E-01	1.04E+00	1.08E-01	2.59E-01	1.00E-01	2.12E-01	1.17E-01	2.48E-01	4.35E-02	1.74E-01
Lactate	4.12	8.36E-04	1.50E-02	5.60E-03	1.01E-01	3.64E-04	4.37E-03	7.43E-03	1.34E-01	4.92E-03	8.86E-02	1.51E-02	1.36E-01
Leucine	0.97	5.61E-01	6.96E-01	5.68E-01	1.02E+00	2.06E-01	6.74E-01	7.47E-02	1.92E-01	1.01E-01	2.60E-01	4.18E-03	1.50E-01
Lysine	3	7.48E-01	8.16E-01	9.63E-01	1.02E+00	1.31E-01	2.05E-01	3.33E-01	4.00E-01	6.98E-01	8.38E-01	1.76E-01	3.17E-01
Maltose	5.42	8.83E-01	9.08E-01	2.13E-01	6.97E-01	1.86E-01	2.16E-01	7.38E-01	1.21E+00	2.05E-01	3.35E-01	1.45E-01	2.90E-01
Phenylalanine	7.41	5.53E-01	7.11E-01	8.35E-01	1.00E+00	2.79E-01	4.57E-01	3.02E-01	4.03E-01	4.84E-01	6.45E-01	4.85E-02	1.75E-01
Propylene glycol	1.12	7.88E-02	3.15E-01	1.42E-01	5.68E-01	2.31E-02	1.66E-01	2.53E-02	1.82E-01	1.44E-02	1.04E-01	1.58E-01	2.99E-01
Serine	3.98	1.04E-01	3.40E-01	9.00E-01	1.01E+00	7.53E-01	1.69E+00	1.05E-01	1.35E-01	5.87E-01	7.55E-01	8.43E-01	8.67E-01
sn-glycero-3-phosphocholine	3.23	5.00E-01	6.92E-01	6.86E-01	9.88E-01	4.98E-01	4.98E-01	9.43E-01	1.36E+00	4.64E-01	6.68E-01	5.46E-01	7.56E-01
Succinate	2.41	3.93E-01	6.43E-01	8.05E-01	1.04E+00	4.18E-01	4.70E-01	7.50E-01	1.04E+00	4.79E-01	6.63E-01	5.52E-01	7.36E-01
Tartrate	4.38	5.24E-01	6.99E-01	9.19E-01	1.00E+00	2.84E-01	3.65E-01	5.12E-01	7.68E-01	4.10E-01	6.15E-01	8.20E-01	8.68E-01

Taurine	3.29	3.74E-02	2.24E-01	3.81E-03	1.37E-01	2.30E-02	8.28E-02	7.09E-02	8.51E-01	6.62E-03	7.94E-02	1.05E-01	2.70E-01
Threonine	4.26	2.37E-02	2.13E-01	1.90E-01	6.84E-01	2.37E-02	2.13E-01	2.18E-02	1.12E-01	4.11E-02	2.11E-01	2.00E-02	1.03E-01
Trigonelline	4.45	2.20E-01	5.28E-01	5.23E-01	1.05E+00	9.71E-01	9.99E-01	8.84E-01	1.03E+00	7.02E-01	8.15E-01	6.15E-01	7.38E-01
Tyrosine	6.92	4.56E-01	7.14E-01	9.68E-01	9.96E-01	3.50E-01	4.85E-01	4.06E-01	4.30E-01	8.98E-01	9.51E-01	8.93E-02	2.68E-01
Valine	0.99	1.41E-01	3.90E-01	6.44E-01	1.01E+00	8.56E-02	1.54E+00	4.69E-03	1.88E-02	7.15E-02	2.86E-01	7.05E-03	8.46E-02
β-Alanine	2.56	2.32E-01	5.22E-01	4.48E-01	9.49E-01	4.69E-01	9.38E-01	2.35E-01	4.45E-01	1.67E-01	3.16E-01	5.37E-01	7.73E-01

Table S2 Digestive glands metabolites extract changes after various concentrations of brevotoxin treatment. FDR was calculated based on 35 metabolites. The FDR was only for the confidence of single metabolites not for the combinational effects.

		0.1 ppb		0.5 ppb		1 ppb		2 ppb		5 ppb		10 ppb	
Metabolites	ppm	p values	FC	p values	FC	p values	FC	p values	FC	p values	FC	p values	FC
2,3-Dihydroxyvaleric acid	1.53	9.87E-02	1.15E+00	3.76E-01	4.39E+00	8.16E-01	1.24E+00	6.88E-01	8.92E-01	5.22E-01	1.66E+00	4.38E-01	8.07E-01
2-Octenedioic acid	2.27	2.46E-01	6.62E-01	7.72E-01	1.69E+00	7.54E-01	1.47E+00	9.70E-01	9.99E-01	6.49E-01	1.51E+00	7.39E-01	8.92E-01
Alanine	1.5	2.32E-01	6.77E-01	8.39E-01	1.22E+00	2.76E-01	1.93E+00	7.42E-02	2.60E+00	5.04E-01	1.96E+00	2.17E-01	2.53E+00
Aspartate	2.7	2.87E-01	5.29E-01	8.83E-01	1.14E+00	7.81E-01	1.44E+00	7.80E-01	9.41E-01	8.09E-01	1.01E+00	3.50E-01	1.53E+00
ATP	8.54	2.28E-01	7.98E-01	5.73E-01	2.51E+00	8.91E-01	1.16E+00	5.05E-01	8.42E-01	9.07E-01	9.34E-01	4.47E-01	7.45E-01
Betaine	3.27	2.03E-01	1.02E+00	6.23E-01	2.18E+00	9.15E-01	1.03E+00	8.68E-01	9.49E-01	3.47E-01	1.74E+00	5.20E-01	7.28E-01
Choline	3.21	6.98E	7.63E-	9.04E	1.09E+	7.38E	1.61E+	7.65E	9.56E-	2.84E	2.49E+	3.81E	1.03E+

		-01	01	-01	00	-01	00	-01	01	-01	00	-01	00
D-Arginine	3.78	4.52E-01	6.33E-01	7.07E-01	2.25E+00	9.88E-01	9.88E-01	6.35E-01	8.89E-01	3.34E-01	2.34E+00	3.87E-01	9.03E-01
dCMP	8.04	2.69E-01	6.28E-01	8.38E-01	1.28E+00	9.04E-01	1.05E+00	2.22E-01	8.63E-01	7.57E-01	1.26E+00	3.69E-01	1.08E+00
Dimethylglycine	2.93	4.00E-01	5.83E-01	9.07E-01	1.06E+00	9.27E-01	1.01E+00	2.85E-01	7.67E-01	7.40E-01	1.30E+00	4.93E-01	7.84E-01
Fumarate	6.53	1.83E-01	1.28E+00	7.91E-01	1.46E+00	7.84E-01	1.37E+00	1.23E-01	2.15E+00	5.93E-01	1.60E+00	3.47E-01	1.74E+00
Glucose	5.23	7.58E-01	8.04E-01	8.53E-01	1.19E+00	9.00E-01	1.13E+00	2.28E-01	7.98E-01	2.41E-01	2.81E+00	8.20E-02	1.44E+00
Glutamate	2.35	2.36E-02	8.26E-01	5.41E-01	2.71E+00	2.94E-01	1.72E+00	6.29E-01	9.17E-01	8.75E-01	9.28E-01	8.43E-01	9.84E-01
Glutamine	2.43	2.98E-01	4.97E-01	9.95E-01	9.95E-01	9.71E-01	1.00E+00	3.04E-01	7.60E-01	7.87E-01	1.10E+00	3.55E-01	1.24E+00
Glycine	3.56	9.47E-01	9.47E-01	6.23E-02	2.18E+00	3.14E-01	1.57E+00	5.92E-01	9.01E-01	5.05E-01	1.77E+00	7.90E-02	2.77E+00
Hypotaurine	3.34	7.80E-01	8.03E-01	4.71E-01	3.30E+00	1.51E-01	2.64E+00	2.80E-01	8.17E-01	4.46E-01	1.95E+00	8.54E-01	9.64E-01
Inosine	8.34	2.80E-01	5.44E-01	7.95E-01	1.39E+00	8.25E-01	1.20E+00	1.77E-01	1.24E+00	7.75E-01	1.23E+00	3.56E-01	1.13E+00
Isoleucine	1.02	4.78E-01	6.43E-01	9.16E-01	1.03E+00	5.73E-01	2.01E+00	2.69E-01	8.56E-01	7.99E-01	1.04E+00	6.62E-01	8.28E-01
Lactate	4.12	2.13E-01	9.32E-01	9.67E-01	9.95E-01	1.18E-01	4.13E+00	3.64E-01	7.96E-01	7.38E-01	1.36E+00	3.98E-01	8.19E-01
Leucine	1.71	3.94E-01	6.00E-01	9.58E-01	1.05E+00	7.89E-01	1.32E+00	8.06E-01	9.40E-01	7.37E-01	1.43E+00	9.16E-01	9.43E-01
Lysine	3.05	4.80E-01	6.22E-01	9.62E-01	1.02E+00	9.59E-01	1.02E+00	4.32E-01	8.89E-01	6.70E-01	1.47E+00	4.98E-01	7.58E-01
Malate	4.29	3.83E-02	6.70E-01	4.01E-01	3.51E+00	6.03E-01	1.92E+00	4.63E-01	8.53E-01	3.36E-01	1.96E+00	2.27E-01	1.99E+00
Methionine	2.65	2.66E-01	6.65E-01	8.31E-01	1.32E+00	1.58E-01	1.84E+00	3.13E-01	7.30E-01	9.62E-01	9.62E-01	9.02E-01	9.57E-01
Neopterin	8.63	1.98E-01	1.16E+00	5.06E-01	2.95E+00	8.46E-01	1.18E+00	4.84E-01	8.47E-01	8.16E-01	9.85E-01	3.89E-01	8.51E-01

Phenylalanine	7.42	2.87E-01	5.02E-01	8.80E-01	1.18E+00	7.41E-01	1.53E+00	1.82E-01	1.06E+00	7.83E-01	1.14E+00	3.54E-01	1.38E+00
Serine	3.98	2.72E-01	5.60E-01	8.22E-01	1.37E+00	6.53E-01	1.90E+00	6.66E-01	8.97E-01	5.80E-01	1.69E+00	5.15E-01	7.51E-01
sn-Glycero-3-phosphocholine	3.23	4.94E-01	6.18E-01	7.26E-01	1.82E+00	9.04E-01	1.09E+00	8.34E-01	9.42E-01	1.82E-01	3.19E+00	2.61E-01	1.52E+00
Succinate	2.41	4.97E-01	6.00E-01	1.73E-01	3.03E+00	2.63E-01	2.30E+00	9.97E-01	9.97E-01	1.46E-01	5.11E+00	8.95E-01	9.79E-01
Tartrate	4.37	2.29E-01	7.29E-01	7.16E-01	2.09E+00	6.68E-01	1.67E+00	1.43E-01	1.67E+00	8.26E-01	9.33E-01	9.69E-01	9.69E-01
Taurine	3.44	5.29E-01	6.17E-01	7.41E-01	1.73E+00	5.12E-01	1.99E+00	9.10E-01	9.65E-01	8.22E-01	9.59E-01	4.03E-01	7.84E-01
Threonine	4.26	1.09E-01	9.54E-01	5.78E-01	2.25E+00	4.80E-01	2.10E+00	5.48E-01	8.72E-01	7.89E-01	1.06E+00	6.29E-01	8.15E-01
Tyrosine	6.89	2.71E-01	5.93E-01	7.77E-01	1.60E+00	8.78E-01	1.18E+00	2.15E-01	9.41E-01	7.76E-01	1.18E+00	3.85E-01	9.63E-01
Uracil	7.55	2.14E-01	8.32E-01	7.86E-01	1.53E+00	8.01E-01	1.27E+00	1.87E-01	9.35E-01	7.13E-01	1.47E+00	2.42E-01	1.69E+00
Valine	1.06	5.52E-01	6.23E-01	7.16E-01	1.93E+00	6.60E-01	1.78E+00	1.54E-01	1.35E+00	6.13E-01	1.53E+00	4.41E-01	7.72E-01
β-Alanine	2.56	3.42E-01	5.44E-01	8.88E-01	1.11E+00	6.95E-01	1.62E+00	4.52E-01	8.79E-01	8.27E-01	9.05E-01	6.06E-01	8.16E-01

Supporting Figures

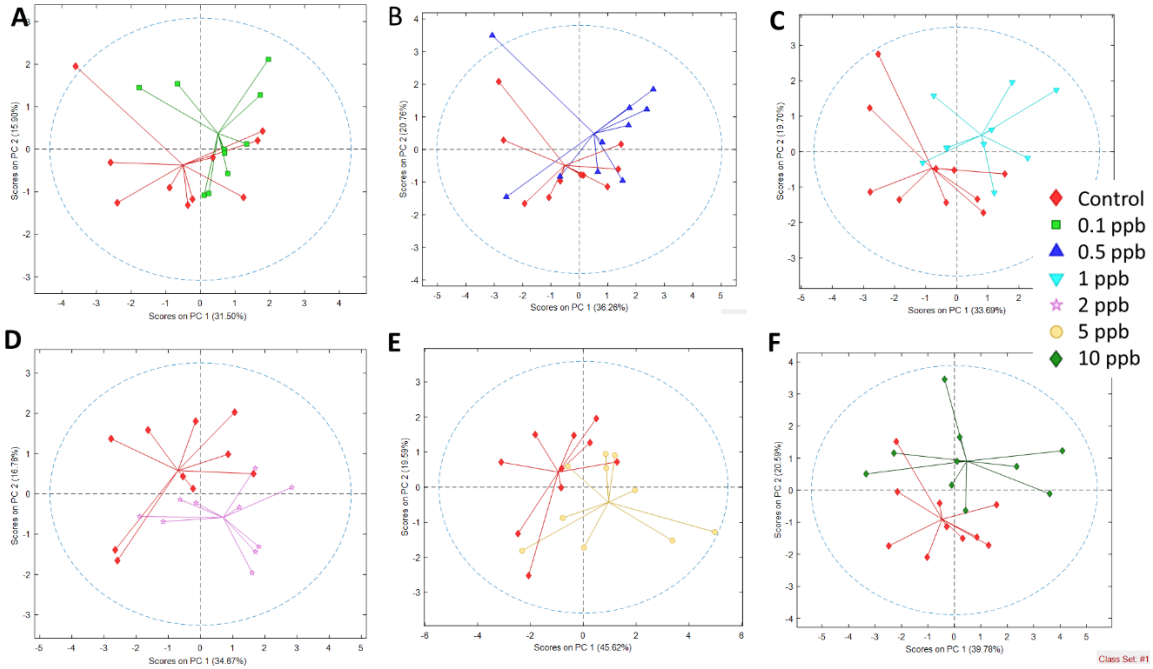

Figure S1 The PCA score plot of metabolites in the gill extracts. **A**, Control vs 0.1 ppb; **B**, Control vs 0.5 ppb; **C**, Control vs 1 ppb; **D**, Control vs 2 ppb; **E**, Control vs 5 ppb; **F**, Control vs 10ppb

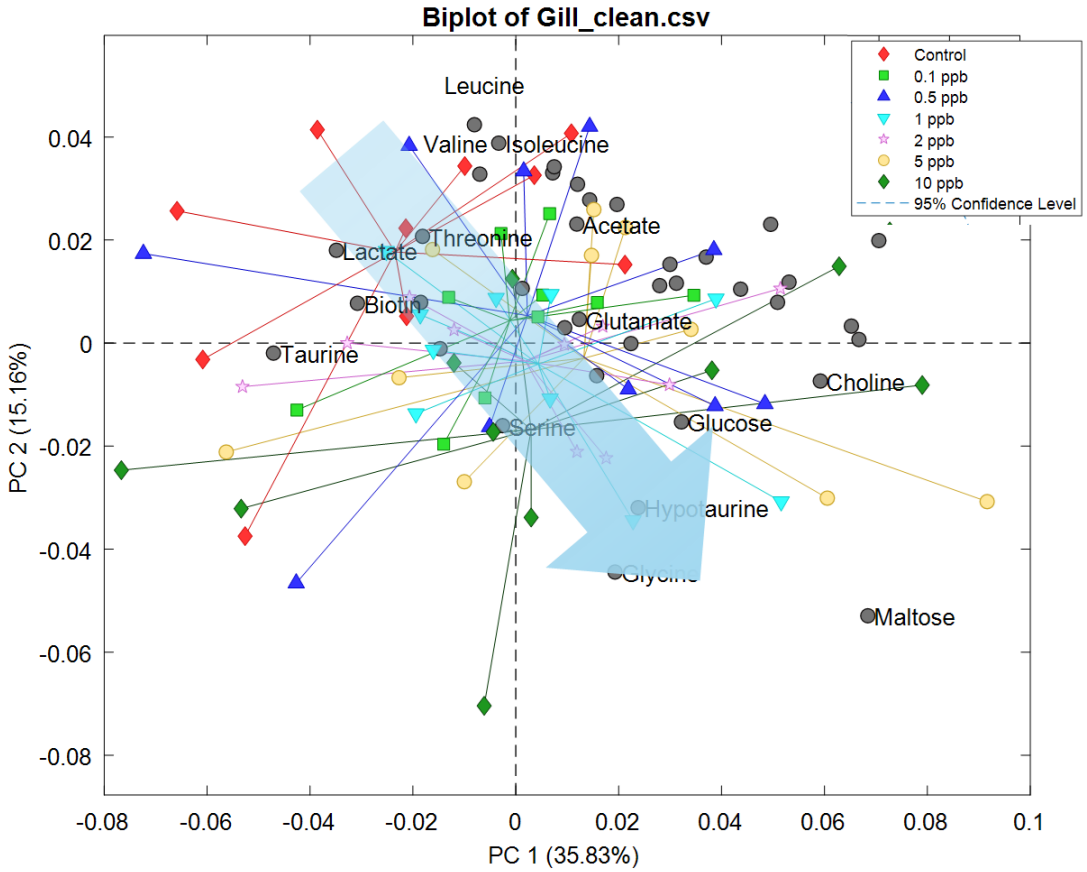

Figure S2 The PCA score and loading biplot for all the treatment groups in gill extracts which can show the trend of the concentration influence.

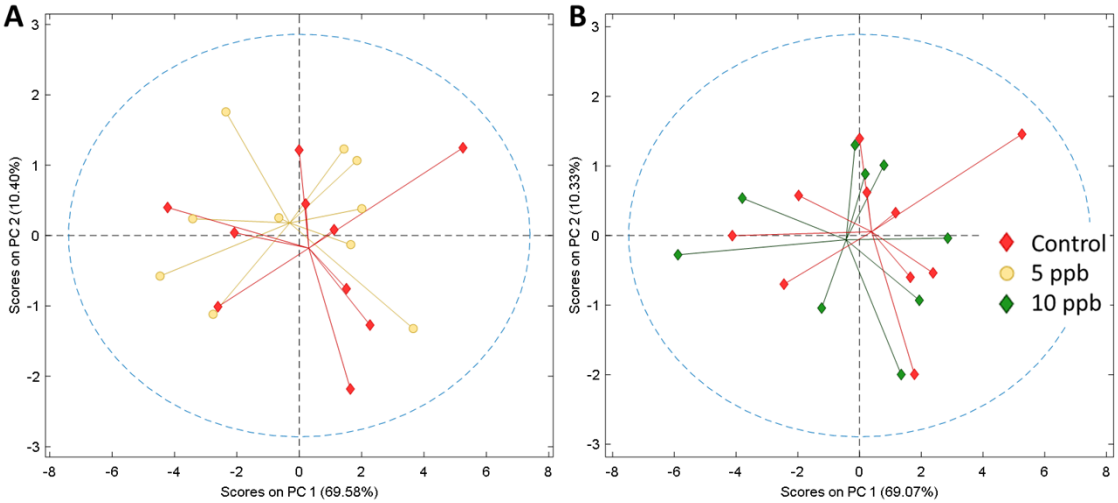

Figure S3 The PCA changing patterns after treatment for 5 and 10 ppb in the digestive glands extracts.

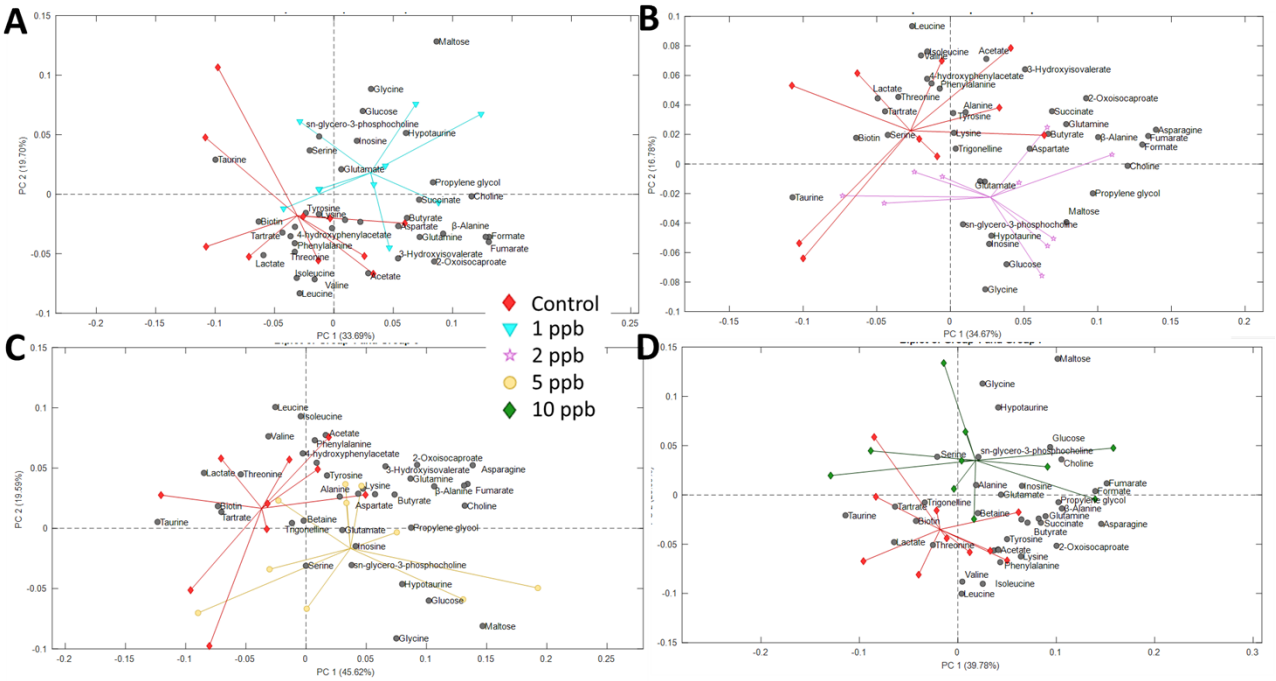

Figure S4 The PCA of gill metabolites extracts for brevetoxins treatment from 1 ppb to 10 ppb concentrations.

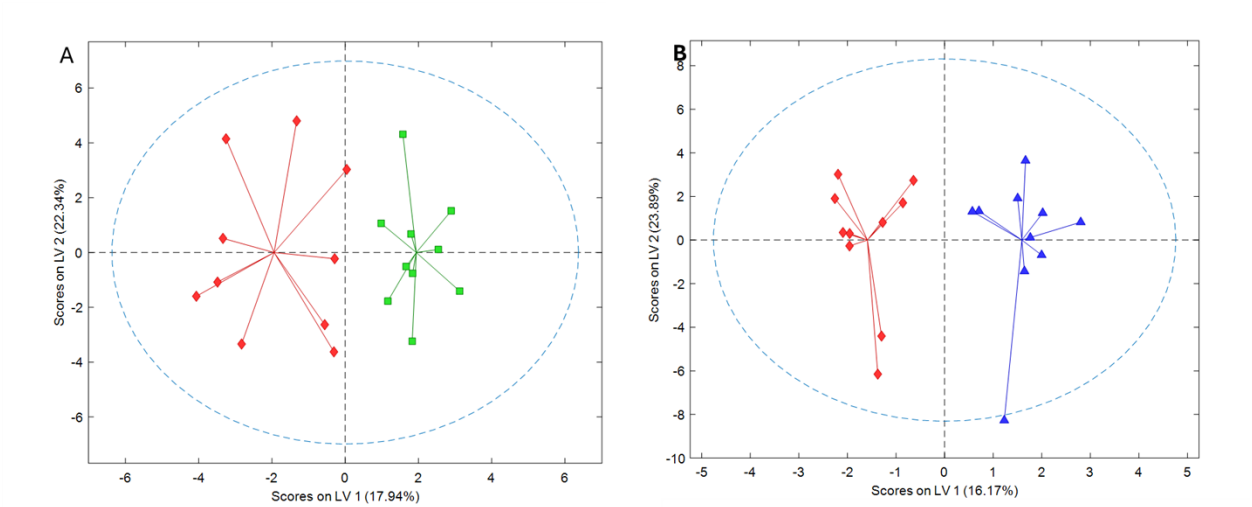

Figure S5. The OPLS-DA score plot for lower concentration treatment (0.1 and 0.5 ppb) for gill samples. PLS-DA Error rate (average of false positive rate and false negative rate for class) in cross validation is **A**, 0.35 and **B**, 0.35 A, R2 0.88 Q2 0.15 B, R2 0.60 Q2 0.31. The permutation test with 1000 trials showed Cross-Validated Wilcoxon P values A 0.143 and B 0.344.

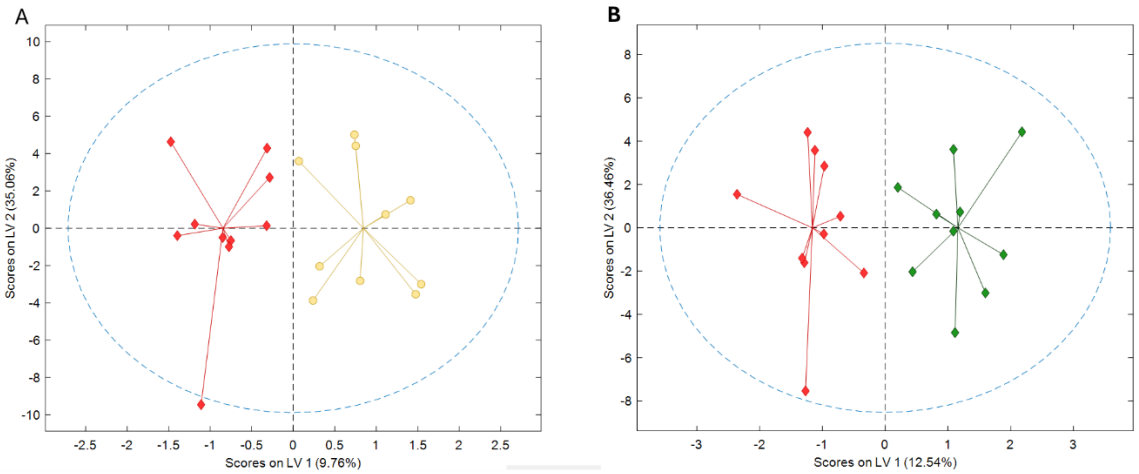

Figure S6. The digestive glands study. The OPLS-DA score plot of the control vs 5 and 10 ppb treatment, cross validation error rates for **A** and **B** are 0.4 (5 LVs) and 0.35 (3 LVs). A, R2 0.58 Q2 0.14 B, R2 0.67 Q2 0.03. The permutation test with 1000 trials showed the cross-validated Wilcoxon p values A 0.424 and B 0.288.

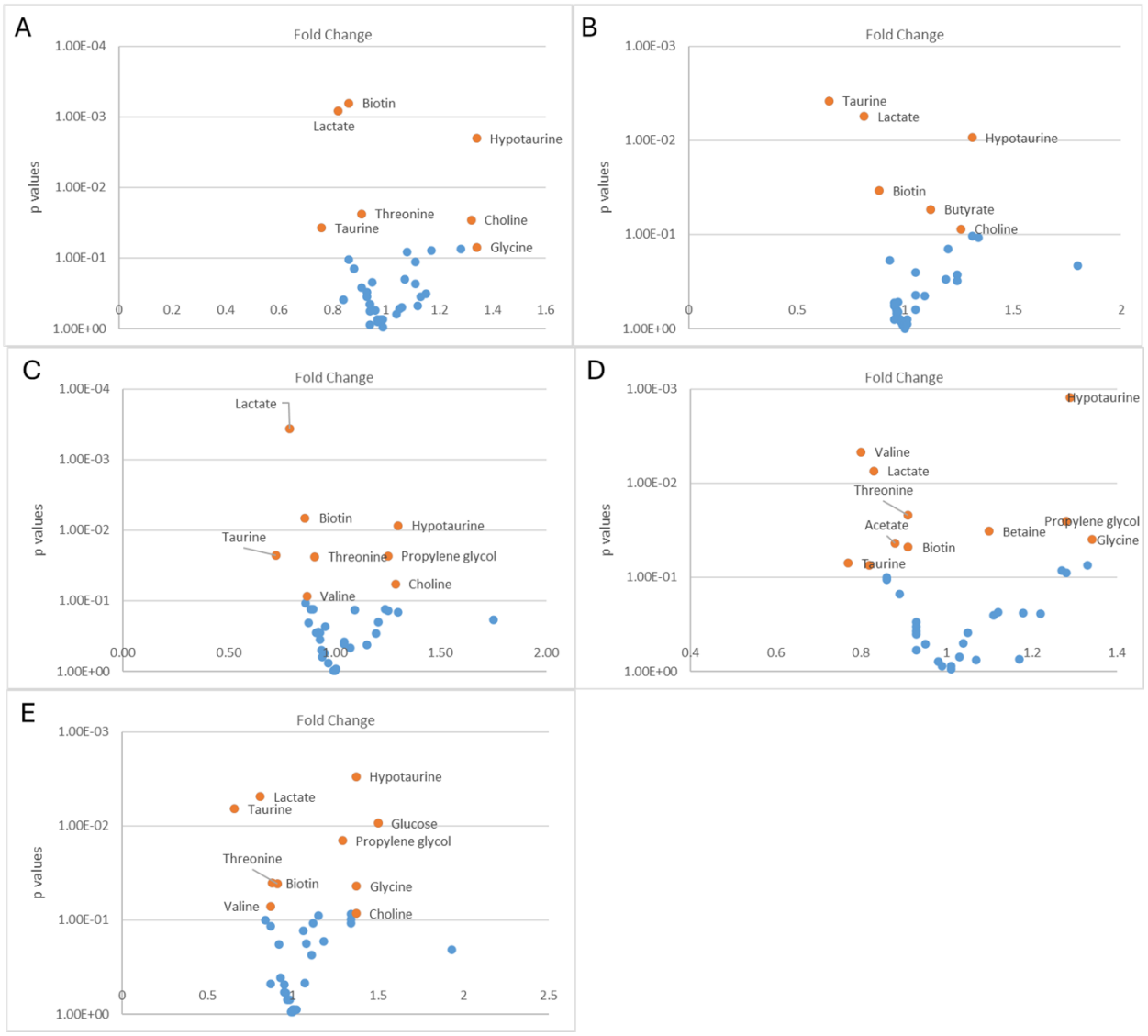

Figure S7 The Volcano plot of gill extracts with selected metabolites highlighted. A 0.1 ppb, B 0.5 ppb, C 1 ppb, D 5 ppb, E 10 ppb

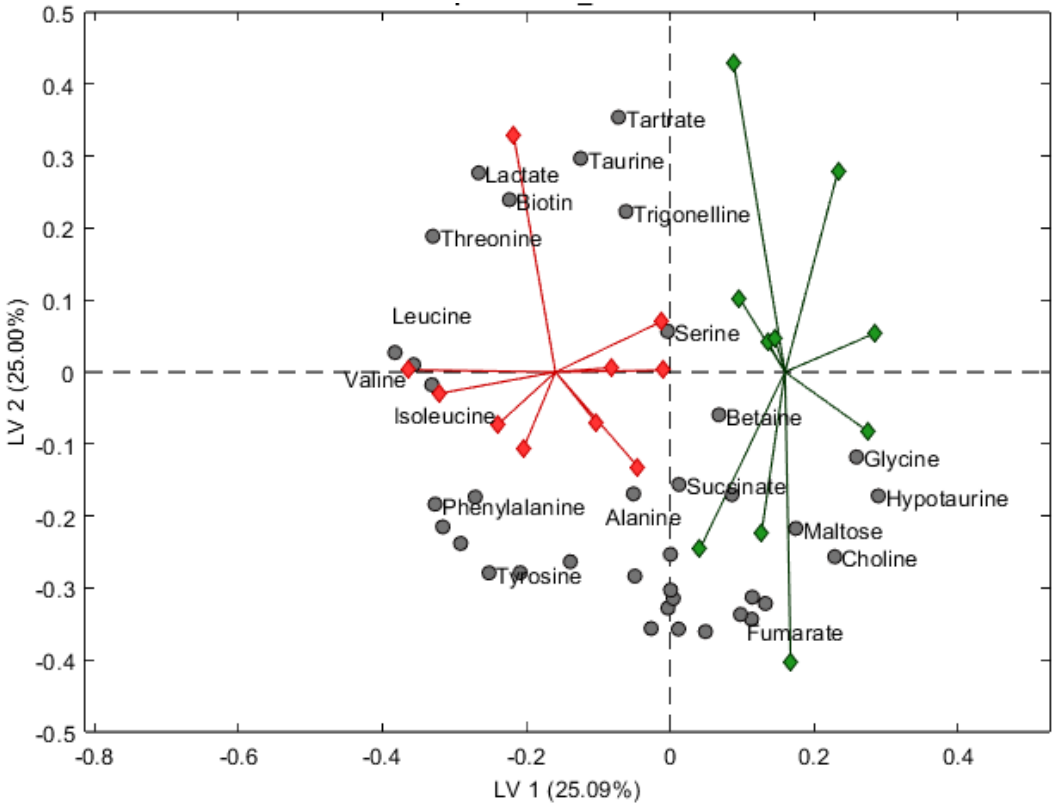

Figure S8 The OPLS-DA biplot for 10 ppb concentration treatment (the highest concentration in the study) for gill samples. PLS-DA Error rate (average of false positive rate and false negative rate for class) in cross-validation is 0.25, and Q2 is 0.34. The permutation test with 1000 trials showed the cross-validated Wilcoxon p-value is 0.083.