iScience, Volume *26*

Supplemental information

Integrated FT-ICR MS and metabolome reveals diatom-derived organic matter by bacterial transformation under warming and acidification

Yang Liu, Chao Ma, and Jun Sun

Journal: *iScience*

Supplementary Information for

Integrated FT-ICR MS and metabolome analysis reveals diatom-derived organic matter by bacterial transformation under warming and acidification

Yang Liu[1, 2, 3], Chao Ma[4], Jun Sun[1, 2]*

[1] Institute for Advance Marine Research, China University of Geosciences, Guangzhou 511462, China.

[2] State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, China.

[3] Institute of Marine Science and Technology, Shandong University, Qingdao 266237, China.

[4] Institute of Surface-Earth System Science, School of Earth System Science, Tianjin University, Tianjin 300072, China.

* Corresponding author: Jun Sun (phytoplankton@163.com)

Contents of this file:

- **Figures: 13**
- **Tables: 6**

Figure S1. The phylogenetic tree of the isolated bacteria (*Roseobacter* sp. SD-R1) based on NJ analysis of the 16S rRNA gene.

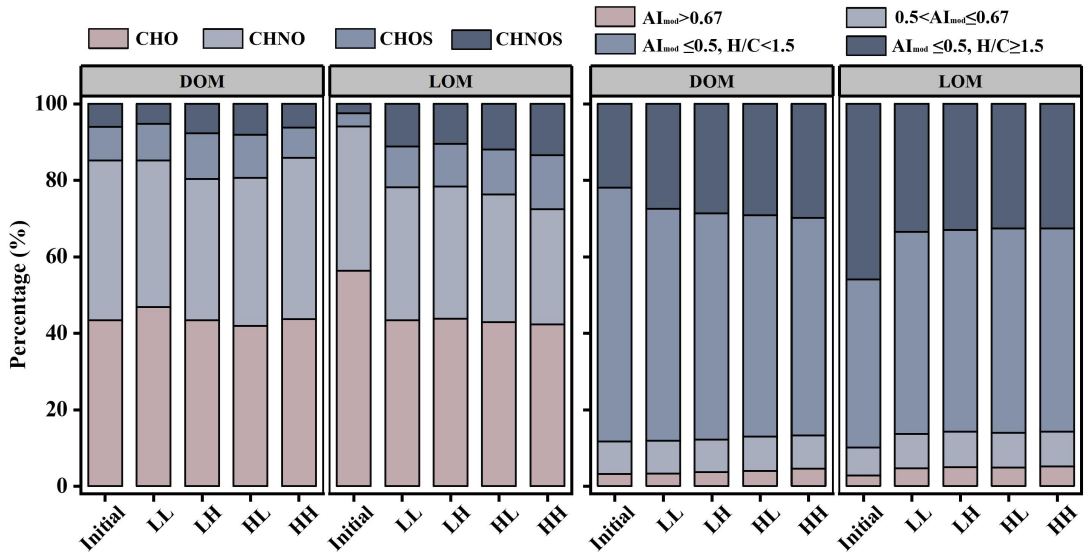

Figure S2. Properties of the assigned features in diatom-derived DOM and LOM under warming and acidification: Compound classes (including CHO, CHNO, CHOS, and CHNOS). Proportional distributions of polycyclic aromatic compounds (AI_{mod}>0.67), aromatic compounds (0.5<AI_{mod}≤0.67), highly unsaturated and phenolic compounds (AI_{mod}≤0.5 and H/C<1.5), and aliphatic compounds (AI_{mod}≤0.5 and H/C≥1.5).

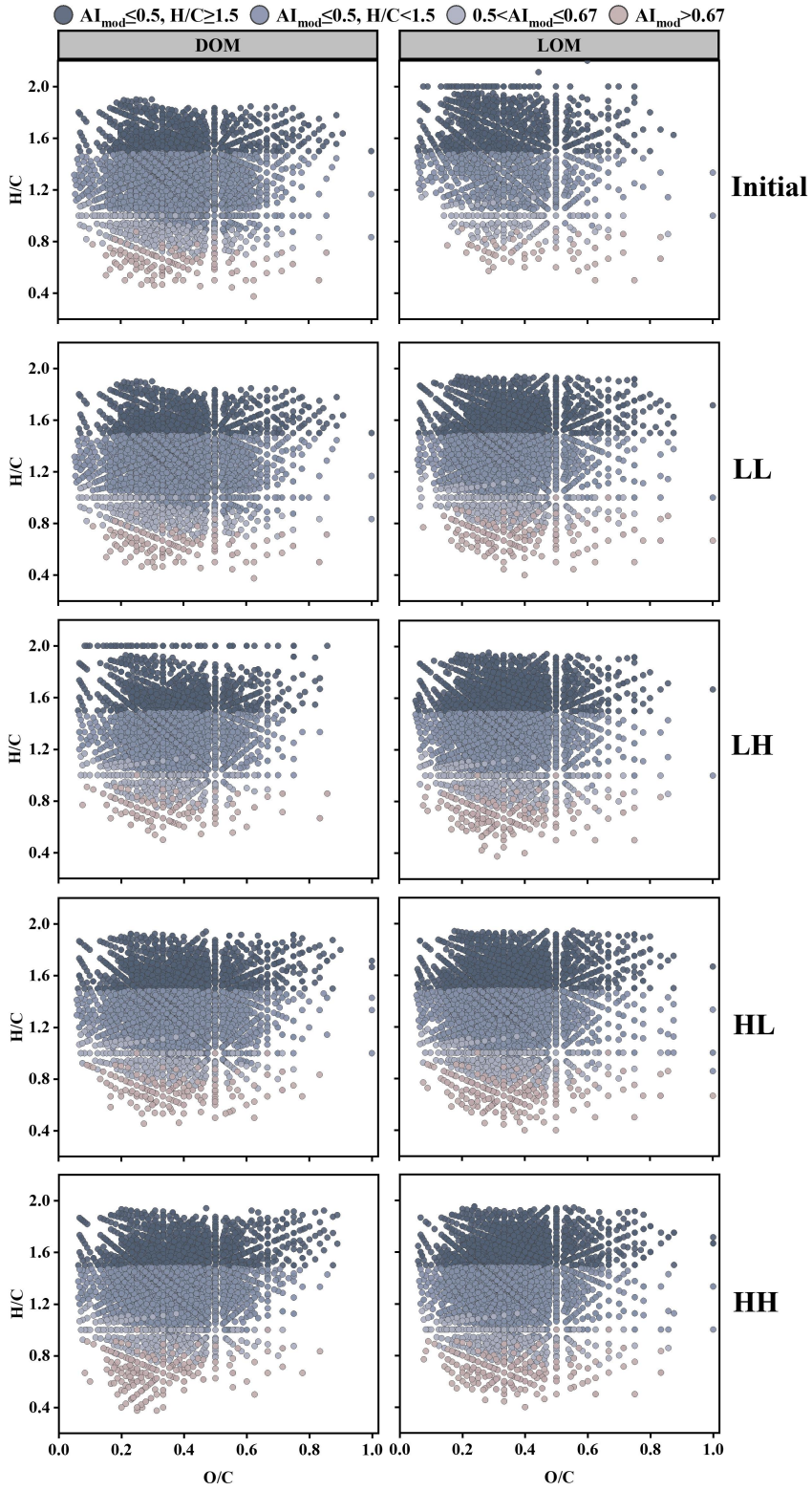

Figure S3. Classification of OM molecular functional groups by modified aromaticity index. Highly unsaturated and phenolic compounds (AI_{mod}≤0.5 and H/C<1.5), aliphatic compounds (AI_{mod}≤0.5 and H/C≥1.5), aromatic compounds (0.5<AI_{mod}≤0.67), and polycyclic aromatic compounds (AI_{mod}>0.67).

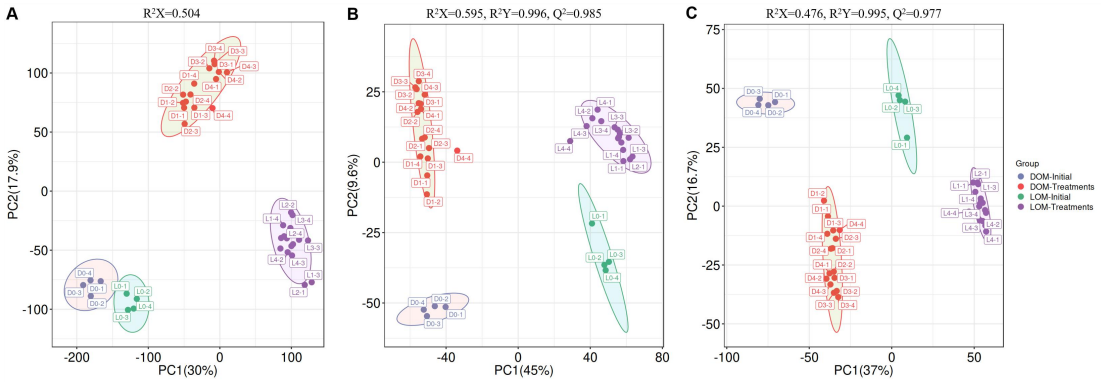

Figure S4. The score plots of principal component analysis (A), partial least squares discriminate analysis (B), and orthogonal projections to latent structures discriminant analysis (C) from the metabolite profiles. Different colors represent different groups and each point represents a sample. Comparing the DOM treatments and LOM treatments, including D1 and L1 (LL), D2 and L2 (LH), D3 and L3 (HL), and D4 and L4 (HH) treatments. Four biological replicates per group.

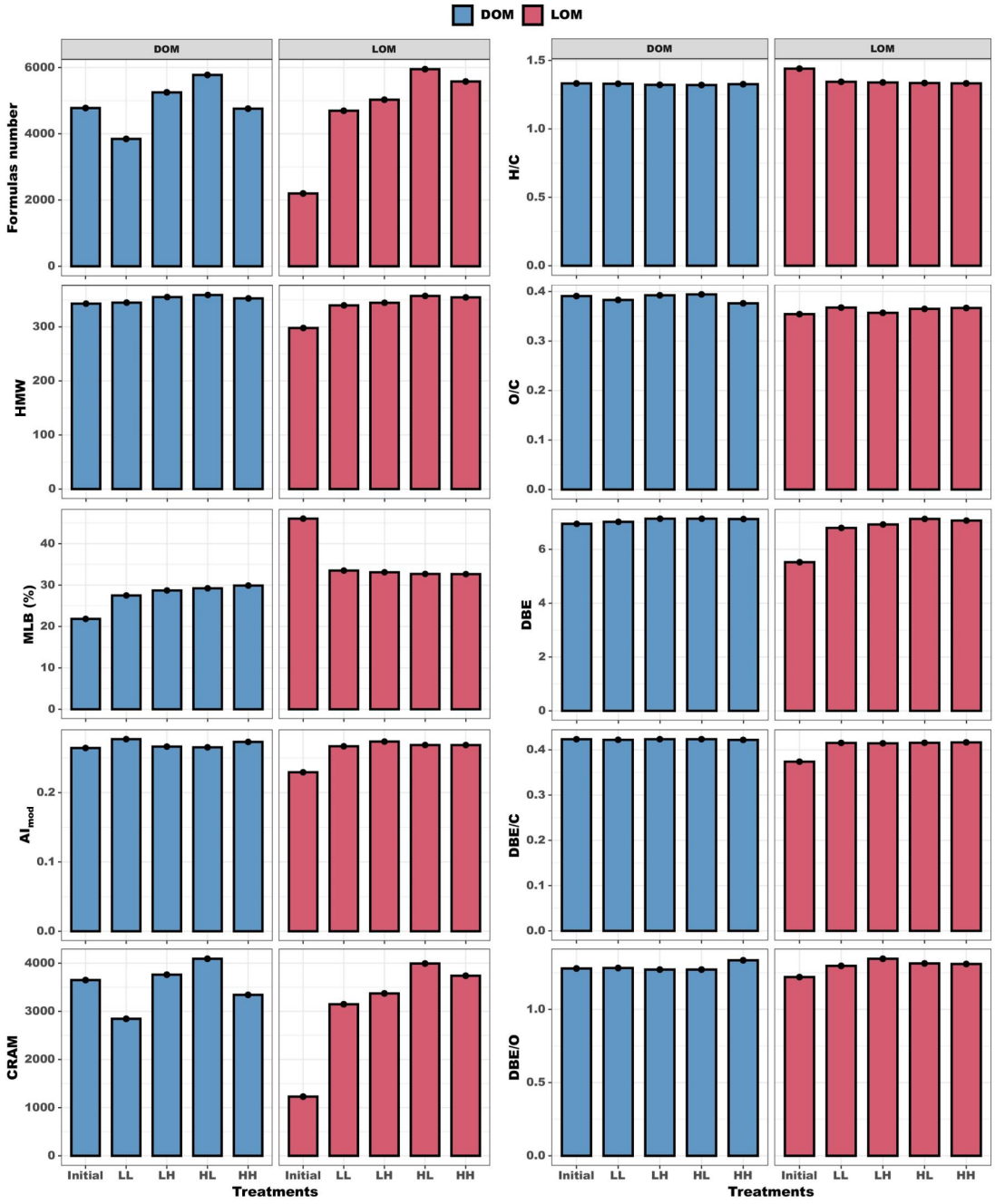

Figure S5. Properties of the assigned features in diatom-derived DOM and LOM under warming and acidification: number of formulas; high molecular weight (HMW); MLB (%); AI_{mod}; CRAM; H/C; O/C; DBE; DBE/C; DBE/O.

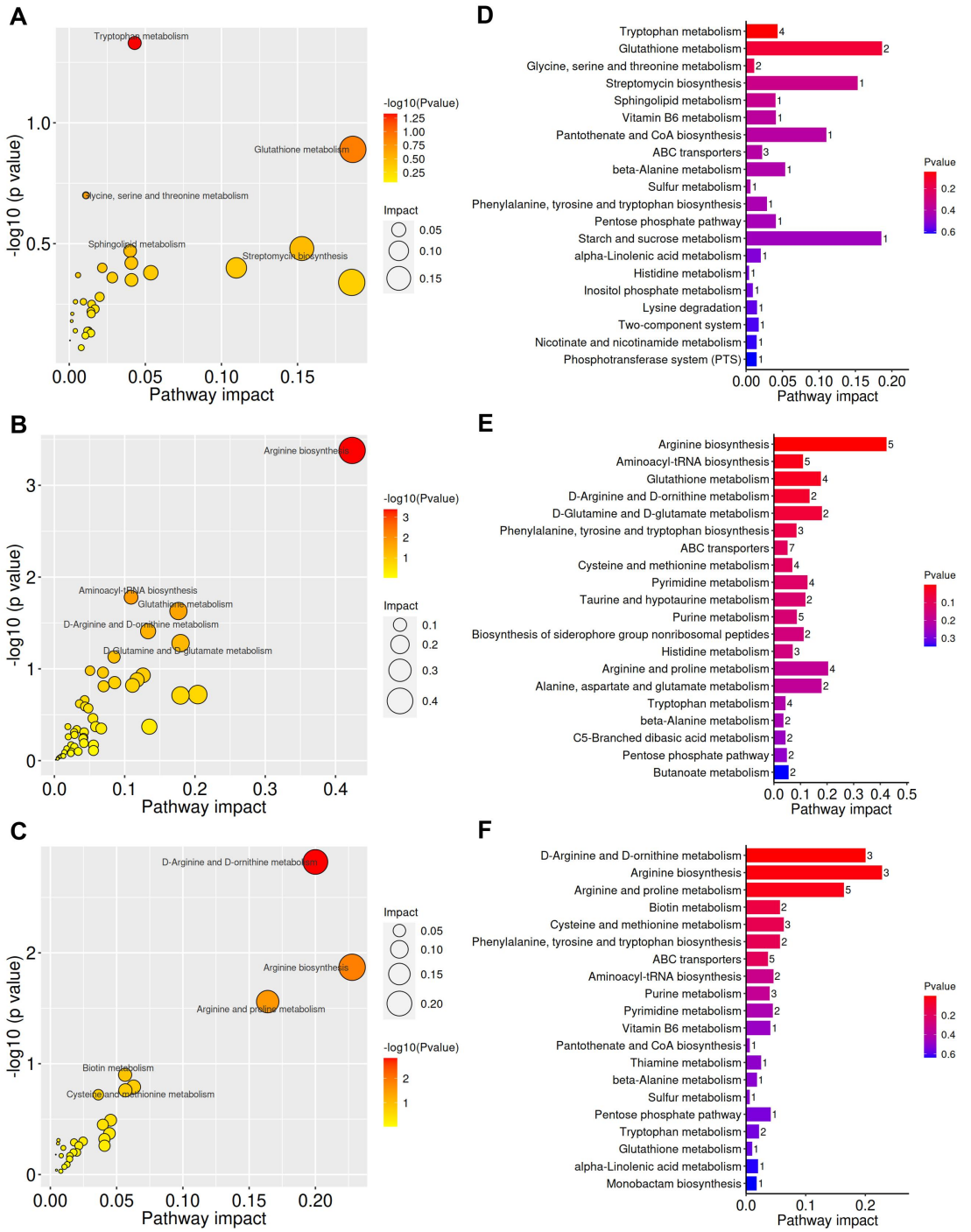

Figure S6. Statistics and analysis of differential metabolites in the different treatments in DOM groups. Bubble diagram (A–C) and bar graph (D–F) of metabolic pathways. A and D belong to the "LH vs. LL" groups; B and E belong to the "HL vs. LL" groups; C and F belong to the "HH vs. LL" groups. LL, 26ºC and 400 ppm; LH, 26ºC and 1000 ppm; HL, 30ºC and 400 ppm; HH, 30ºC and 1000 ppm.

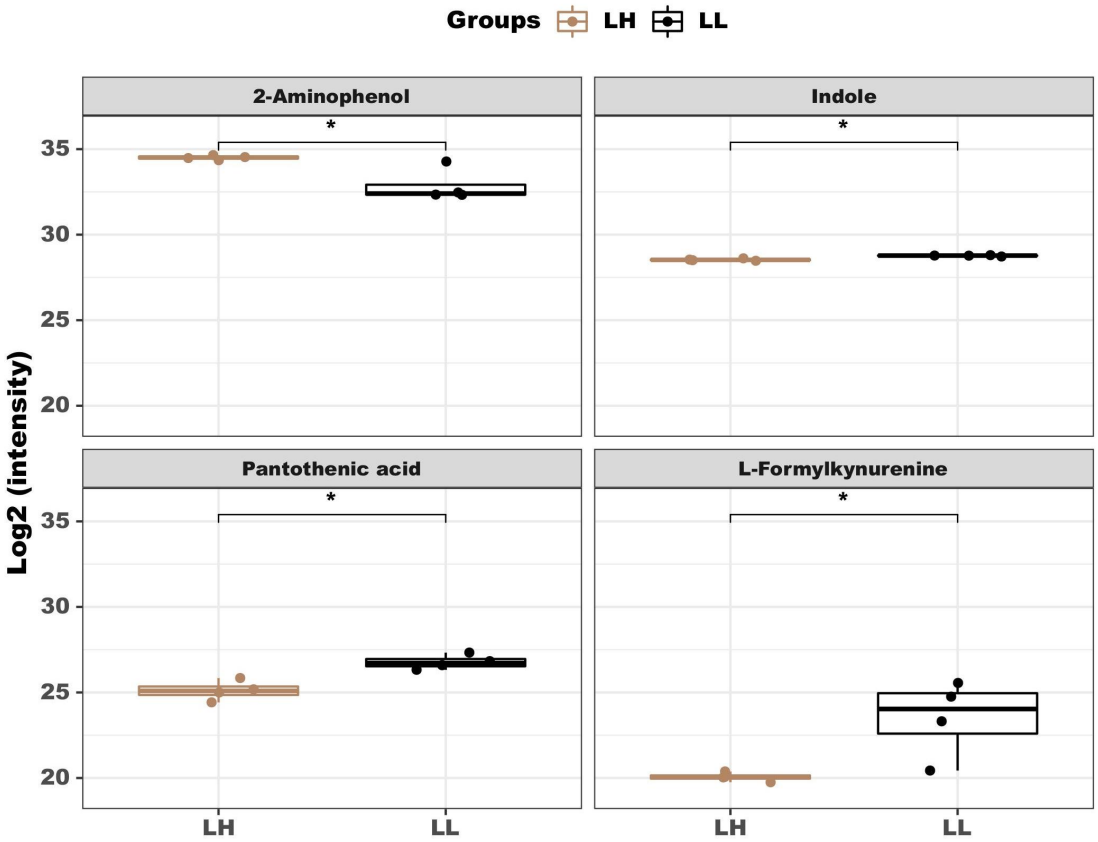

Figure S7. Box plots of differential metabolites in DOM treatments: 2-Aminophenol, indole, picolinic acid, and L-formylkynurenine. The Wilcoxon rank sum tests were performed using the 'wilcox.test' function in R. Asterisks represent significance as follows: *, $p < 0.05$.

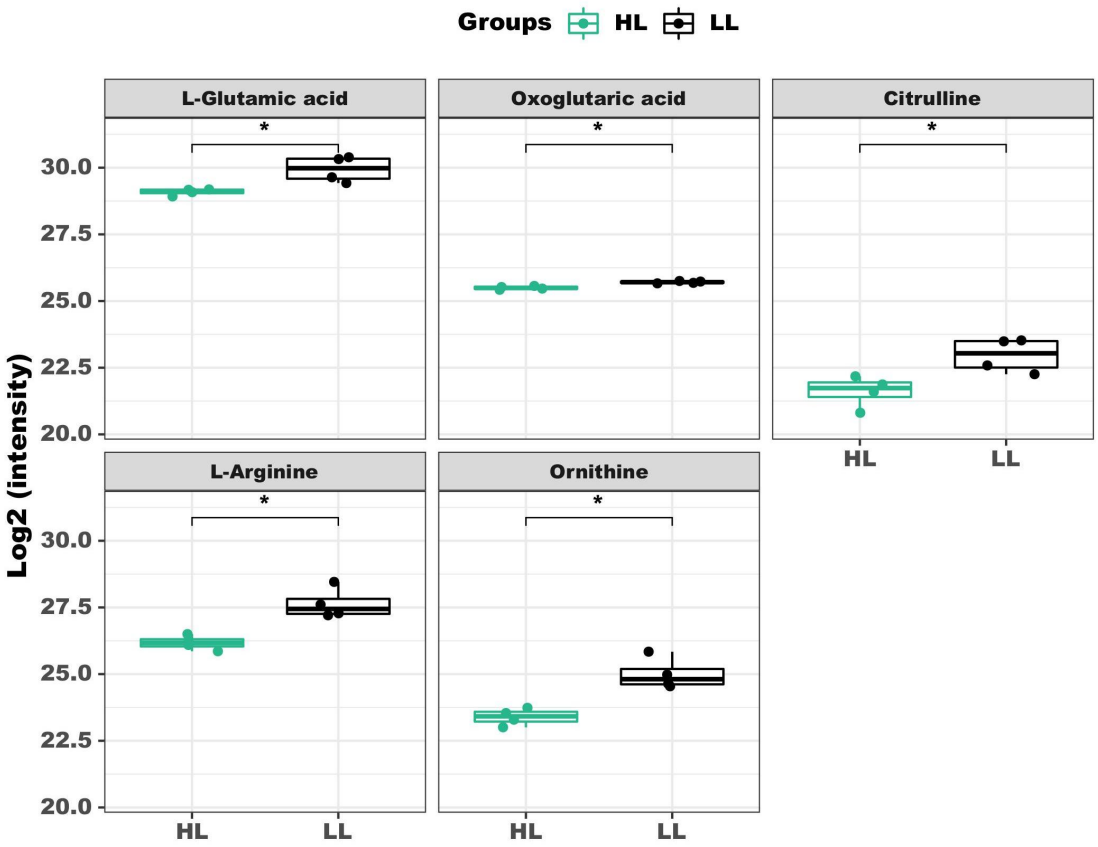

Figure S8. Box plots of differential metabolites in DOM treatments: L-Glutamic acid, oxoglutaric acid, citrulline, L-arginine, and ornithine. The Wilcoxon rank sum tests were performed using 'wilcox.test' function in R. Asterisks represent significance as follows: *, $p < 0.05$.

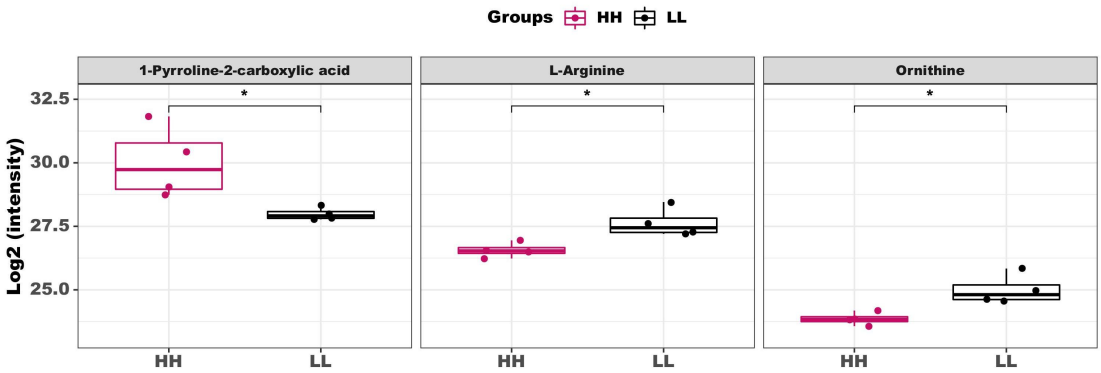

Figure S9. Box plots of differential metabolites in DOM treatments: 1-Pyrroline-2-carboxylic acid, L-arginine, and ornithine. The Wilcoxon rank sum tests were performed using 'wilcox.test' function in R. Asterisks represent significance as follows: *, $p < 0.05$.

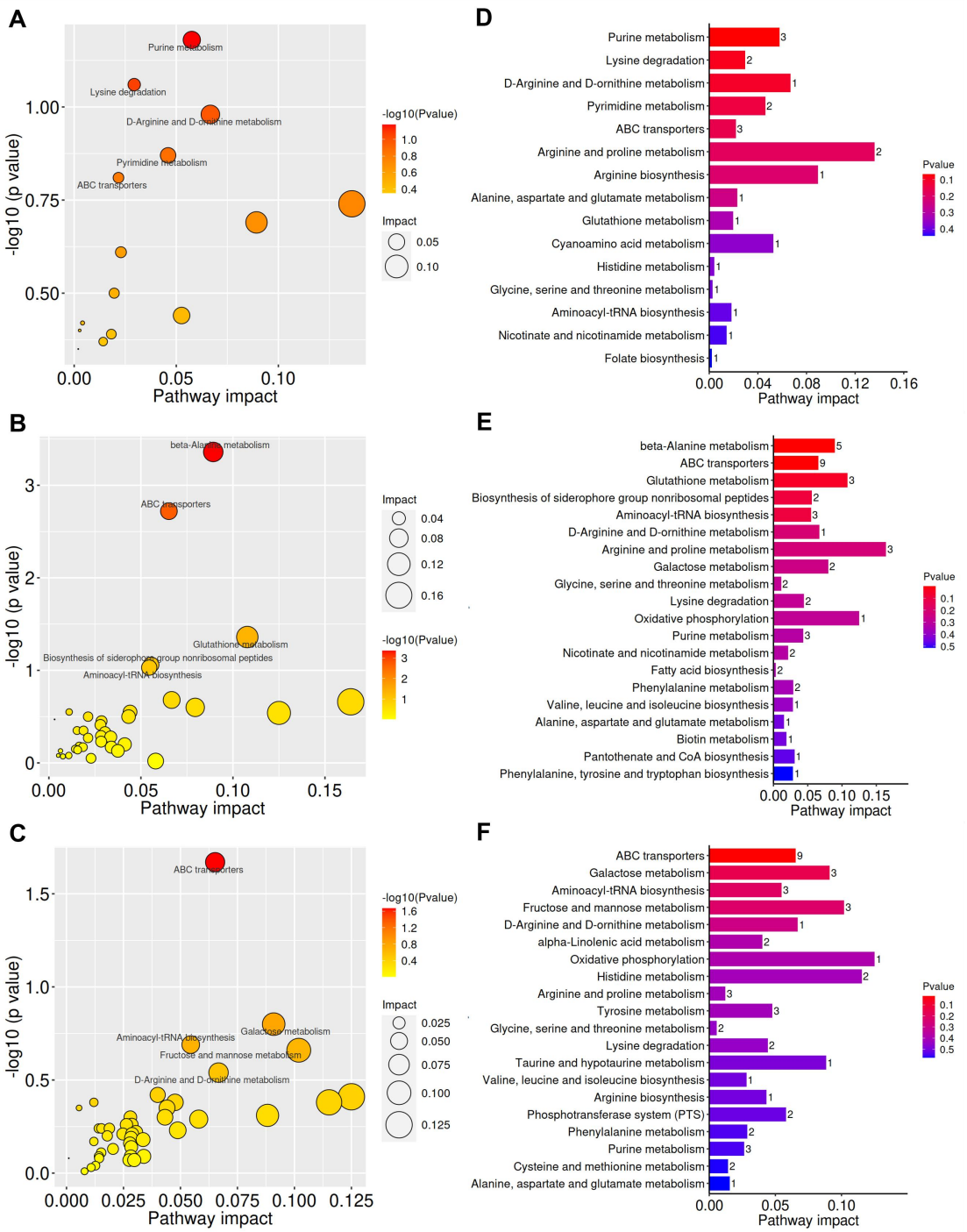

Figure S10. Statistics and analysis of differential metabolites in the different treatments in LOM groups. Bubble diagram (A–C) and bar graph (D–F) of metabolic pathways. A and D belong to the "LH vs. LL" groups; B and E belong to the "HL vs. LL" groups; C and F belong to the "HH vs. LL" groups. LL, 26ºC and 400 ppm; LH, 26ºC and 1000 ppm; HL, 30ºC and 400 ppm; HH, 30ºC and 1000 ppm.

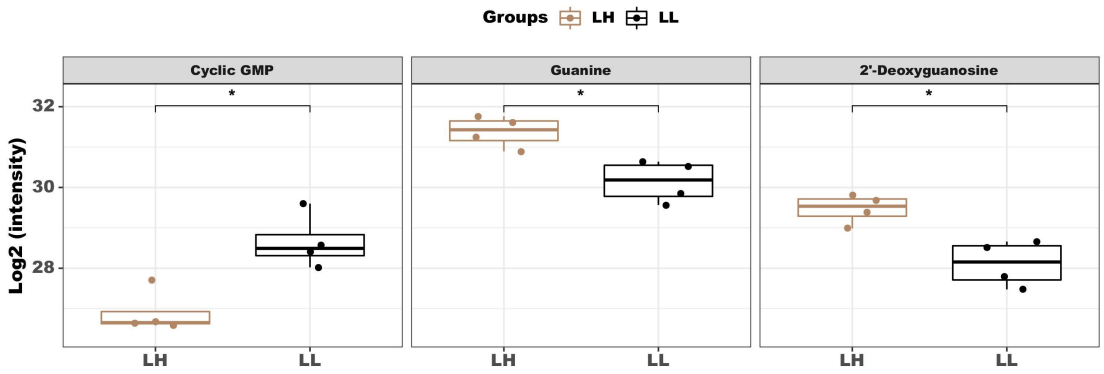

Figure S11. Box plots of differential metabolites in LOM treatments: Cyclic GMP, guanine, and 2'-deoxyguanosine. The Wilcoxon rank sum tests were performed using 'wilcox.test' function in R. Asterisks represent significance as follows: *, $p < 0.05$.

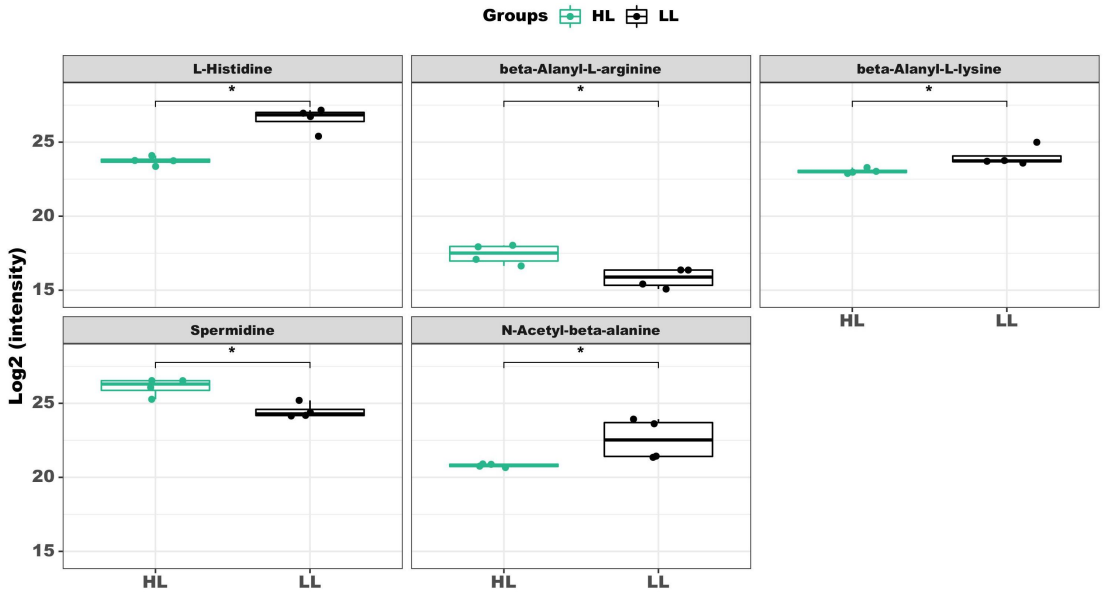

Figure S12. Box plots of differential metabolites in LOM treatments: L-Histidine; beta-alanyl-L-arginine, beta-alanyl-L-lysine, spermidine, and N-acetyl-beta-alanine. The Wilcoxon rank sum tests were performed using 'wilcox.test' function in R. Asterisks represent significance as follows: *, $p < 0.05$.

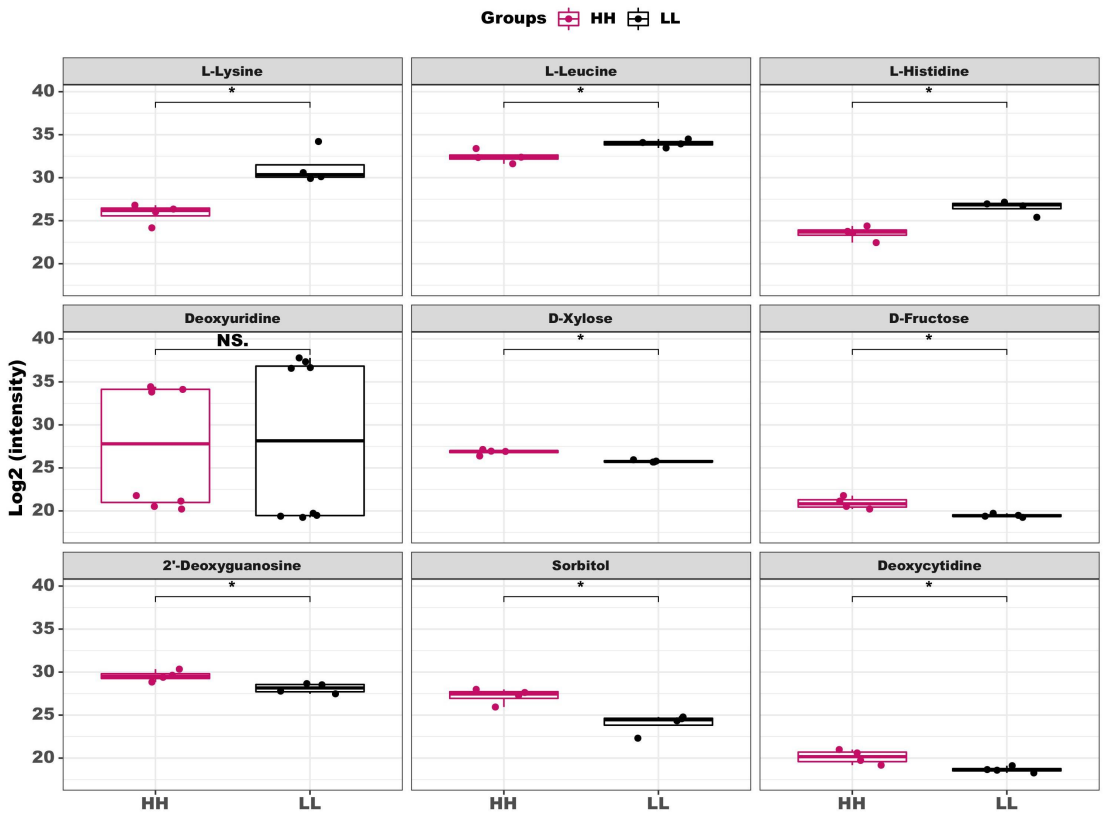

Figure S13. Box plots of differential metabolites in LOM treatments: L-Lysine, L-leucine, L-histidine, deoxyuridine, D-xylose, D-fructose, 2'-deoxyguanosine, sorbitol, and deoxycytidine. The Wilcoxon rank sum tests were performed using 'wilcox.test' function in R. Asterisks represent significance as follows: *, $p < 0.05$. NS stands for non-significant.

Table S1. The results of differential metabolites in the LH vs LL group of DOM.

KEGG	Metabolites	VIP	Regulation	P value
C14691	Triethylamine	1.61	↑	<0.05
C01987	2-Aminophenol	1.59	↑	<0.05
C00463	Indole	1.88	↑	<0.05
C00719	Betaine	1.87	↑	<0.05
C11004	2,6-Dimethylaniline	1.82	↑	<0.05
C00153	Niacinamide	1.02	↑	<0.05
C10164	Picolinic acid	1.43	↑	<0.05
C00436	N-Carbamoylputrescine	1.64	↑	<0.05
C02486	Beta-Leucine	1.62	↑	<0.05
C05650	Tetrahydropteridine	1.81	↑	<0.05
C06231	Ectoine	1.96	↑	<0.05
C05572	4-Oxoglutaramate	1.62	↑	<0.05
C00314	Pyridoxine	1.54	↑	<0.05
C01551	Allantoin	1.71	↑	<0.05
C03153	N5-Methyl-L-glutamine	1.85	↑	<0.05
C00318	L-Carnitine	1.88	↑	<0.05
C05343	(R)-4-Hydroxymandelate	1.93	↑	<0.05
C02710	N-Acetylleucine	1.71	↑	<0.05
C01047	L-Theanine	1.79	↑	<0.05
C02727	N6-Acetyl-L-lysine	1.68	↑	<0.05
C12989	N-Alpha-acetyllysine	1.74	↑	<0.05
C00355	L-Dopa	1.53	↑	<0.05
C06841	Baclofen	1.73	↑	<0.05
C03017	Propionylcarnitine	1.80	↑	<0.05
C03740	gamma-Glutamylalanine	1.60	↑	<0.05
C00864	Pantothenic acid	1.74	↑	<0.05
C16765	Citrinin	1.84	↑	<0.05
C07375	Procaine	1.57	↑	<0.05
C02700	L-Formylkynurenine	1.53	↑	<0.05
C03137	N-Acetyl-D-tryptophan	1.64	↑	<0.05
C05926	Neopterin	1.56	↑	<0.05
C09827	(S)-Pinocembrin	1.69	↑	<0.05
C13454	Xanthoxic acid	1.79	↑	<0.05
C05282	(5-L-Glutamyl)-L-glutamate	1.78	↑	<0.05
C07239	Ritodrine	1.63	↑	<0.05
C16324	9,10-EOT	1.63	↑	<0.05
C07319	Sumatriptan	1.47	↑	<0.05
C20674	N7-Methylguanosine	1.81	↑	<0.05
C00836	Sphinganine	1.03	↑	<0.05
C08019	Oxymorphone	1.50	↑	<0.05
C14749	19(S)-HETE	1.66	↑	<0.05
C00051	Glutathione	1.65	↑	<0.05

C00575	Cyclic AMP	1.59	↑	<0.05
C03557	Ciliatine	1.85	↓	<0.05
C16357	3-Methylxanthine	1.91	↓	<0.05
C00345	6-Phosphogluconic acid	1.86	↓	<0.05
C00092	Glucose 6-phosphate	1.74	↓	<0.05
C10098	Kaempferide	2.28	↓	<0.05
C15519	25-Hydroxycholesterol	2.17	↓	<0.05
C11142	Dimethyl sulfone	1.72	↓	<0.05

VIP: variable importance for the projection; ↑, up-regulation; ↓, down-regulation.

Table S2. The results of differential metabolites in the HL vs LL group of DOM.

KEGG	Metabolites	VIP	Regulation	P value
C12455	5-Aminopentanal	1.27	↑	<0.05
C05942	Pyrrole-2-carboxylic acid	1.67	↑	<0.05
C00463	Indole	1.69	↑	<0.05
C02115	2-Methylserine	1.42	↑	<0.05
C11004	2,6-Dimethylaniline	1.71	↑	<0.05
C00153	Niacinamide	1.06	↑	<0.05
C01108	1,2,3-Trihydroxybenzene	1.29	↑	<0.05
C00178	Thymine	1.26	↑	<0.05
C00026	Oxoglutaric acid	1.64	↑	<0.05
C00123	L-Leucine	1.46	↑	<0.05
C01015	cis-4-Hydroxy-L-proline	1.18	↑	<0.05
C17883	2-Methoxy-4-vinylphenol	1.68	↑	<0.05
C16630	5,6-Dihydro-5-fluorouracil	1.62	↑	<0.05
C02505	2-Phenylacetamide	1.40	↑	<0.05
C00262	Hypoxanthine	1.39	↑	<0.05
C00483	Tyramine	1.28	↑	<0.05
C00568	p-Aminobenzoic acid	1.41	↑	<0.05
C06231	Ectoine	1.76	↑	<0.05
C00955	Tryptophanol	1.47	↑	<0.05
C05572	4-Oxoglutaramate	1.56	↑	<0.05
C00025	L-Glutamic acid	1.46	↑	<0.05
C00073	L-Methionine	1.64	↑	<0.05
C00196	2-Pyrocatechuic acid	1.76	↑	<0.05
C19463	1,5-Naphthalenediamine	1.44	↑	<0.05
C01551	Allantoin	1.62	↑	<0.05
C03153	N5-Methyl-L-glutamine	1.69	↑	<0.05
C00318	L-Carnitine	1.55	↑	<0.05
C06180	Anabasine	1.54	↑	<0.05
C10447	3,4-Dihydroxyphenylpropanoate	1.41	↑	<0.05
C02989	L-Methionine S-oxide	1.47	↑	<0.05
C05343	(R)-4-Hydroxymandelate	1.47	↑	<0.05
C02637	3-Dehydroshikimate	1.43	↑	<0.05

C02710	N-Acetylleucine	1.75	↑	<0.05
C01047	L-Theanine	1.69	↑	<0.05
C00327	Citrulline	1.40	↑	<0.05
C01092	8-Amino-7-oxononanoate	1.65	↑	<0.05
C12989	N-Alpha-acetyllysine	1.52	↑	<0.05
C02155	Glycylleucine	1.48	↑	<0.05
C03626	Asymmetric dimethylarginine	1.50	↑	<0.05
C03955	N6-Acetyl-N6-hydroxy-L-lysine	1.65	↑	<0.05
C03519	N-Acetyl-L-phenylalanine	1.30	↑	<0.05
C06841	Baclofen	1.38	↑	<0.05
C05341	beta-Alanyl-L-lysine	1.43	↑	<0.05
C03740	gamma-Glutamylalanine	1.53	↑	<0.05
C00299	Uridine	1.73	↑	<0.05
C04337	N(omega)-Nitro-L-arginine methyl ester	1.36	↑	<0.05
C02700	L-Formylkynurenine	1.41	↑	<0.05
C03137	N-Acetyl-D-tryptophan	1.73	↑	<0.05
C00647	Pyridoxamine 5'-phosphate	1.56	↑	<0.05
C06960	Diphenhydramine	1.66	↑	<0.05
C00774	Phloretin	1.52	↑	<0.05
C09980	Yangonin	1.48	↑	<0.05
C00670	Glycerophosphocholine	1.55	↑	<0.05
C11500	5'-Dehydroadenosine	1.48	↑	<0.05
C20579	Cyclopeptine	1.42	↑	<0.05
C01595	Linoleic acid	1.44	↑	<0.05
C02494	1-Methyladenosine	1.37	↑	<0.05
C00387	Guanosine	1.48	↑	<0.05
C07239	Ritodrine	1.37	↑	<0.05
C21016	Ophthalmate	1.16	↑	<0.05
C07881	Dyclonine	1.61	↑	<0.05
C07319	Sumatriptan	1.58	↑	<0.05
C20674	N7-Methylguanosine	1.40	↑	<0.05
C00170	5'-Methylthioadenosine	1.71	↑	<0.05
C08019	Oxymorphone	1.33	↑	<0.05
C06967	Dobutamine	1.75	↑	<0.05
C05324	Nicotianamine	1.52	↑	<0.05
C14749	19(S)-HETE	1.38	↑	<0.05
C00239	dCMP	1.58	↑	<0.05
C00575	Cyclic AMP	1.25	↑	<0.05
C00360	dAMP	1.49	↑	<0.05
C06176	Senecionine	1.59	↑	<0.05
C07119	Medroxyprogesterone	1.74	↑	<0.05
C21160	L-Alanyl-gamma-D-glutamyl-L-lysine	1.50	↑	<0.05
C00908	Gentamicin C1a	1.43	↑	<0.05
C01746	Piperidine	1.38	↑	<0.05

C01073	N-Acetyl-beta-alanine	1.80	↓	<0.05
C00077	Ornithine	1.97	↓	<0.05
C00181	D-Xylose	1.43	↓	<0.05
C00062	L-Arginine	1.96	↓	<0.05
C00977	Tryptophanamide	1.81	↓	<0.05
C00078	L-Tryptophan	1.81	↓	<0.05
C00620	Ribose 1-phosphate	1.63	↓	<0.05
C00669	gamma-Glutamylcysteine	1.84	↓	<0.05
C00345	6-Phosphogluconic acid	1.85	↓	<0.05
C00389	Quercetin	1.88	↓	<0.05
C00364	dTMP	1.84	↓	<0.05
C16417	Xanthohumol	2.02	↓	<0.05

VIP: variable importance for the projection; ↑, up-regulation; ↓, down-regulation.

Table S3. The results of differential metabolites in the HH vs LL group of DOM.

KEGG	Metabolites	VIP	Regulation	P value
C11118	N-Methyl-2-pyrrolidinone	1.57	↑	<0.05
C00388	Histamine	1.60	↑	<0.05
C05942	Pyrrole-2-carboxylic acid	1.54	↑	<0.05
C03564	1-Pyrroline-2-carboxylic acid	1.38	↑	<0.05
C00463	Indole	1.66	↑	<0.05
C00719	Betaine	1.76	↑	<0.05
C11004	2,6-Dimethylaniline	1.83	↑	<0.05
C10164	Picolinic acid	1.40	↑	<0.05
C02183	Phloroglucinol	1.63	↑	<0.05
C05938	L-4-Hydroxyglutamate semialdehyde	1.15	↑	<0.05
C01015	cis-4-Hydroxy-L-proline	1.56	↑	<0.05
C02486	Beta-Leucine	1.66	↑	<0.05
C05650	Tetrahydropteridine	1.45	↑	<0.05
C10761	p-Anisaldehyde	1.21	↑	<0.05
C04294	5-(2-Hydroxyethyl)-4-methylthiazole	1.52	↑	<0.05
C00073	L-Methionine	1.57	↑	<0.05
C01551	Allantoin	1.49	↑	<0.05
C03153	N5-Methyl-L-glutamine	1.74	↑	<0.05
C05343	(R)-4-Hydroxymandelate	1.71	↑	<0.05
C02710	N-Acetylleucine	1.52	↑	<0.05
C01047	L-Theanine	1.77	↑	<0.05
C00327	Citrulline	1.55	↑	<0.05
C01092	8-Amino-7-oxononanoate	1.72	↑	<0.05
C02727	N6-Acetyl-L-lysine	1.52	↑	<0.05
C12989	N-Alpha-acetyllysine	1.59	↑	<0.05
C02155	Glycylleucine	1.55	↑	<0.05
C07577	3,4-Methylenedioxyamphetamine	1.66	↑	<0.05
C05944	Pantothenol	1.32	↑	<0.05

C06841	Baclofen	1.63	↑	<0.05
C01909	Dethiobiotin	1.25	↑	<0.05
C05341	beta-Alanyl-L-lysine	1.60	↑	<0.05
C03017	Propionylcarnitine	1.73	↑	<0.05
C09627	Capsidiol	1.58	↑	<0.05
C08322	Myristoleic acid	1.73	↑	<0.05
C00526	Deoxyuridine	1.58	↑	<0.05
C09376	Confertifolin	1.40	↑	<0.05
C03137	N-Acetyl-D-tryptophan	1.72	↑	<0.05
C00647	Pyridoxamine 5'-phosphate	1.50	↑	<0.05
C13456	Abscisic alcohol	1.28	↑	<0.05
C00774	Phloretin	1.69	↑	<0.05
C09980	Yangonin	1.52	↑	<0.05
C11500	5'-Dehydroadenosine	1.54	↑	<0.05
C02713	N-Acetylmuramate	1.46	↑	<0.05
C21730	epsilon-(gamma-L-Glutamyl)-L-lysine	1.78	↑	<0.05
C17748	1-(3,4-Dihydroxyphenyl)-5-hydroxy-3-decanone	1.58	↑	<0.05
C00387	Guanosine	1.58	↑	<0.05
C21016	Ophthalmate	1.74	↑	<0.05
C16324	9,10-EOT	1.51	↑	<0.05
C00170	5'-Methylthioadenosine	1.79	↑	<0.05
C08019	Oxymorphone	1.41	↑	<0.05
C06967	Dobutamine	1.77	↑	<0.05
C05324	Nicotianamine	1.66	↑	<0.05
C14749	19(S)-HETE	1.47	↑	<0.05
C00239	dCMP	1.51	↑	<0.05
C00575	Cyclic AMP	1.34	↑	<0.05
C00360	dAMP	1.62	↑	<0.05
C06176	Senecionine	1.71	↑	<0.05
C08380	2-Heptanone	1.77	↓	<0.05
C00077	Ornithine	1.79	↓	<0.05
C00493	Shikimic acid	1.67	↓	<0.05
C00062	L-Arginine	1.75	↓	<0.05
C00977	Tryptophanamide	1.78	↓	<0.05
C00345	6-Phosphogluconic acid	1.77	↓	<0.05
C10098	Kaempferide	1.95	↓	<0.05
C14828	9,10-DHOME	1.73	↓	<0.05
C16417	Xanthohumol	1.80	↓	<0.05
C11142	Dimethyl sulfone	1.70	↓	<0.05

VIP: variable importance for the projection; ↑, up-regulation; ↓, down-regulation.

Table S4. The results of differential metabolites in the LH vs LL group of LOM.

KEGG	Metabolites	VIP	Regulation	P value

C14691	Triethylamine	1.69	↑	<0.05
C00178	Thymine	2.07	↑	<0.05
C00408	Pipecolic acid	2.14	↑	<0.05
C03440	cis-4-Hydroxy-D-proline	1.66	↑	<0.05
C06231	Ectoine	2.24	↑	<0.05
C05572	4-Oxoglutaramate	2.01	↑	<0.05
C12115	3-Amino-4-hydroxybenzoate	2.19	↑	<0.05
C19719	D-Alanyl-D-serine	1.56	↑	<0.05
C01551	Allantoin	1.90	↑	<0.05
C02710	N-Acetylleucine	1.77	↑	<0.05
C00327	Citrulline	2.04	↑	<0.05
C02727	N6-Acetyl-L-lysine	1.84	↑	<0.05
C12989	N-Alpha-acetyllysine	2.23	↑	<0.05
C03017	Propionylcarnitine	2.20	↑	<0.05
C02587	Pyrimidodiazepine	1.48	↑	<0.05
C00526	Deoxyuridine	2.07	↑	<0.05
C09317	Xanthyletin	1.62	↑	<0.05
C03137	N-Acetyl-D-tryptophan	2.39	↑	<0.05
C05841	Nicotinate D-ribonucleoside	1.63	↑	<0.05
C00774	Phloretin	2.08	↑	<0.05
C11500	5'-Dehydroadenosine	2.25	↑	<0.05
C19530	Sulfamethazine	1.85	↑	<0.05
C11501	9-Riburonosyladenine	2.12	↑	<0.05
C05324	Nicotianamine	2.25	↑	<0.05
C00942	Cyclic GMP	2.04	↑	<0.05
C00134	Putrescine	1.41	↑	<0.05
C08380	2-Heptanone	1.84	↓	<0.05
C00152	L-Asparagine	1.68	↓	<0.05
C00330	2'-Deoxyguanosine	2.10	↓	<0.05
C00242	Guanine	2.07	↓	<0.05

VIP: variable importance for the projection; ↑, up-regulation; ↓, down-regulation.

Table S5. The results of differential metabolites in the HL vs LL group of LOM.

KEGG	Metabolites	VIP	Regulation	P value
C14691	Triethylamine	1.71	↑	<0.05
C00463	Indole	1.83	↑	<0.05
C00719	Betaine	1.69	↑	<0.05
C01108	1,2,3-Trihydroxybenzene	1.47	↑	<0.05
C11918	Maltol	1.44	↑	<0.05
C00408	Pipecolic acid	1.88	↑	<0.05
C00123	L-Leucine	1.72	↑	<0.05
C03440	cis-4-Hydroxy-D-proline	1.88	↑	<0.05
C00805	Salicylic acid	1.50	↑	<0.05
C06231	Ectoine	1.80	↑	<0.05

C01046	N-Methyl-L-glutamic acid	1.73	↑	<0.05
C00940	2-Keto-glutaramic acid	1.70	↑	<0.05
C00966	2-Dehydropantoate	1.53	↑	<0.05
C00047	L-Lysine	1.65	↑	<0.05
C00135	L-Histidine	1.85	↑	<0.05
C03153	N5-Methyl-L-glutamine	1.57	↑	<0.05
C06180	Anabasine	1.83	↑	<0.05
C02989	L-Methionine S-oxide	1.61	↑	<0.05
C05343	(R)-4-Hydroxymandelate	1.65	↑	<0.05
C11016	Diphenylamine	1.67	↑	<0.05
C02710	N-Acetylleucine	1.76	↑	<0.05
C10454	Methyleugenol	1.73	↑	<0.05
C00984	D-Galactose	1.79	↑	<0.05
C00794	Sorbitol	1.42	↑	<0.05
C03626	Asymmetric dimethylarginine	1.84	↑	<0.05
C05341	beta-Alanyl-L-lysine	1.48	↑	<0.05
C03740	gamma-Glutamylalanine	1.48	↑	<0.05
C00931	Porphobilinogen	1.37	↑	<0.05
C00526	Deoxyuridine	1.88	↑	<0.05
C04337	N(omega)-Nitro-L-arginine methyl ester	1.34	↑	<0.05
C05340	beta-Alanyl-L-arginine	1.56	↑	<0.05
C00399	Ubiquinone-1	1.80	↑	<0.05
D05229	OPEO	1.39	↑	<0.05
C05841	Nicotinate D-ribonucleoside	1.55	↑	<0.05
C00249	Palmitic acid	1.64	↑	<0.05
C09980	Yangonin	1.81	↑	<0.05
C00858	Formononetin	1.55	↑	<0.05
C19530	Sulfamethazine	1.80	↑	<0.05
C00387	Guanosine	1.53	↑	<0.05
C01530	Stearic acid	1.92	↑	<0.05
C07881	Dyclonine	1.84	↑	<0.05
C05324	Nicotianamine	1.69	↑	<0.05
C14827	9(S)-HPODE	1.86	↑	<0.05
C05484	Pregnanediol	1.48	↑	<0.05
C02105	(S)-Reticuline	1.73	↑	<0.05
C08316	Erucic acid	1.01	↑	<0.05
C21160	L-Alanyl-gamma-D-glutamyl-L-lysine	1.60	↑	<0.05
C00942	Cyclic GMP	1.82	↑	<0.05
C00362	dGMP	1.80	↑	<0.05
C11171	Sodium deoxycholate	1.29	↑	<0.05
C07837	Fomepizole	1.21	↑	<0.05
C01746	Piperidine	1.70	↑	<0.05
C00134	Putrescine	1.88	↑	<0.05
C05332	Phenylethylamine	1.62	↓	<0.05

C06813	Nitrobenzene	1.58	↓	<0.05
C01073	N-Acetyl-beta-alanine	1.48	↓	<0.05
C01020	6-Hydroxynicotinate	1.59	↓	<0.05
C00977	Tryptophanamide	1.66	↓	<0.05
C16537	Pentadecanoic acid	1.67	↓	<0.05
C07635	Epiandrosterone	1.95	↓	<0.05
C07461	Chlorothiazide	1.13	↓	<0.05
C16417	Xanthohumol	1.85	↓	<0.05
C00315	Spermidine	1.68	↓	<0.05

VIP: variable importance for the projection; ↑, up-regulation; ↓, down-regulation.

Table S6. The results of differential metabolites in the HH vs LL group of LOM.

KEGG	Metabolites	VIP	Regulation	P value
C03564	1-Pyrroline-2-carboxylic acid	1.30	↑	<0.05
C00463	Indole	1.50	↑	<0.05
C00860	L-Histidinol	1.66	↑	<0.05
C11918	Maltol	1.46	↑	<0.05
C00408	Pipecolic acid	1.65	↑	<0.05
C17714	Heptanoic acid	1.22	↑	<0.05
C00300	Creatine	1.39	↑	<0.05
C00123	L-Leucine	1.38	↑	<0.05
C03440	cis-4-Hydroxy-D-proline	1.51	↑	<0.05
C03758	Dopamine	1.34	↑	<0.05
C10761	p-Anisaldehyde	1.59	↑	<0.05
C00483	Tyramine	1.41	↑	<0.05
C00227	Acetylphosphate	1.51	↑	<0.05
C06231	Ectoine	1.33	↑	<0.05
C04294	5-(2-Hydroxyethyl)-4-methylthiazole	1.54	↑	<0.05
C00940	2-Keto-glutaramic acid	1.53	↑	<0.05
C00966	2-Dehydropantoate	1.12	↑	<0.05
C00047	L-Lysine	1.46	↑	<0.05
C05527	3-Sulfinylpyruvic acid	1.48	↑	<0.05
C12115	3-Amino-4-hydroxybenzoate	1.50	↑	<0.05
C00135	L-Histidine	1.52	↑	<0.05
C03030	Uracil 5-carboxylate	1.43	↑	<0.05
C03153	N5-Methyl-L-glutamine	1.65	↑	<0.05
C00318	L-Carnitine	1.18	↑	<0.05
C01127	4-Hydroxy-2-oxoglutaric acid	1.55	↑	<0.05
C06180	Anabasine	1.61	↑	<0.05
C00507	L-Fucose	1.48	↑	<0.05
C02989	L-Methionine S-oxide	1.33	↑	<0.05
C11016	Diphenylamine	1.56	↑	<0.05
C05576	3,4-Dihydroxyphenylglycol	1.58	↑	<0.05
C17366	(2S,5S)-trans-Carboxymethylproline	1.52	↑	<0.05

C02710	N-Acetylleucine	1.52	↑	<0.05
C01047	L-Theanine	1.57	↑	<0.05
C10454	Methyleugenol	1.66	↑	<0.05
C00095	D-Fructose	1.44	↑	<0.05
C00984	D-Galactose	1.60	↑	<0.05
C00794	Sorbitol	1.46	↑	<0.05
C12989	N-Alpha-acetyllysine	1.55	↑	<0.05
C07577	3,4-Methylenedioxyamphetamine	1.44	↑	<0.05
C03626	Asymmetric dimethylarginine	1.60	↑	<0.05
C18828	Ethirimol	1.50	↑	<0.05
C03017	Propionylcarnitine	1.35	↑	<0.05
C08322	Myristoleic acid	1.09	↑	<0.05
C00881	Deoxycytidine	1.36	↑	<0.05
C00526	Deoxyuridine	1.63	↑	<0.05
C04337	N(omega)-Nitro-L-arginine methyl ester	1.48	↑	<0.05
C09376	Confertifolin	1.62	↑	<0.05
C03137	N-Acetyl-D-tryptophan	1.54	↑	<0.05
C09537	Quadrone	1.44	↑	<0.05
C00399	Ubiquinone-1	1.55	↑	<0.05
D05229	OPEO	1.33	↑	<0.05
C05841	Nicotinate D-ribonucleoside	1.36	↑	<0.05
C09980	Yangonin	1.62	↑	<0.05
C04148	Phenylacetylglutamine	1.54	↑	<0.05
C13454	Xanthoxic acid	1.28	↑	<0.05
C00330	2'-Deoxyguanosine	1.38	↑	<0.05
C00858	Formononetin	1.46	↑	<0.05
C19530	Sulfamethazine	1.58	↑	<0.05
C11993	10-Deoxymethynolide	1.58	↑	<0.05
C01595	Linoleic acid	1.64	↑	<0.05
C11501	9-Riburonosyladenine	1.23	↑	<0.05
C02494	1-Methyladenosine	1.64	↑	<0.05
C01530	Stearic acid	1.30	↑	<0.05
C00376	Retinal	1.42	↑	<0.05
C07881	Dyclonine	1.57	↑	<0.05
C16324	9,10-EOT	1.50	↑	<0.05
C16321	9(S)-HPOT	1.56	↑	<0.05
C11045	Aspartame	1.25	↑	<0.05
C11285	Tridemorph	1.42	↑	<0.05
C05324	Nicotianamine	1.58	↑	<0.05
C14827	9(S)-HPODE	1.62	↑	<0.05
C02165	12-Keto-tetrahydro-leukotriene B4	1.39	↑	<0.05
C14750	alpha-Zearalenol	1.31	↑	<0.05
C05484	Pregnanediol	1.59	↑	<0.05
C06429	Docosahexaenoic acid	1.46	↑	<0.05

C02105	(S)-Reticuline	1.29	↑	<0.05
C06176	Senecionine	1.38	↑	<0.05
C08316	Erucic acid	1.01	↑	<0.05
C00942	Cyclic GMP	1.61	↑	<0.05
C00362	dGMP	1.36	↑	<0.05
C14151	Delta-Tocopherol	1.55	↑	<0.05
C11962	5-Oxoavermectin "1b" aglycone	1.24	↑	<0.05
C05332	Phenylethylamine	1.65	↓	<0.05
C00181	D-Xylose	1.83	↓	<0.05
C00624	N-Acetylglutamic acid	1.29	↓	<0.05
C02678	Dodecanedioic acid	1.64	↓	<0.05
C16537	Pentadecanoic acid	1.73	↓	<0.05
C07635	Epiandrosterone	1.75	↓	<0.05
C07461	Chlorothiazide	1.17	↓	<0.05
C16527	Adrenic acid	1.56	↓	<0.05
C16417	Xanthohumol	1.72	↓	<0.05

VIP: variable importance for the projection; ↑, up-regulation; ↓, down-regulation.