Supporting Information

Metabolomics Reveals Cryptic Interactive Effects of Species Interactions and Environmental Stress on Nitrogen and Sulfur Metabolism in Seagrass

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S1 Experimental setup and specimen collection Sandy sediment was collected from unvegetated sea bottom adjacent to seagrass meadows at Svenstrup Strand, Denmark (+55°28'70", +9°45'20"), sieved through a 1 mm sieve and placed in a total of 24 pots (20 cm i.d., 17 cm height) filled with 3.5 L sediment per pot. Ten days later, apical eelgrass shoots from Svenstrup Strand were collected randomly by harvesting turfs with intact ramets. Senescent leaves, rhizome parts older than 5 internodes and epiphytes were gently removed. Twenty-eight shoots were transplanted into each pot yielding a shoot density of 891 shoots m^{-2} and placed into mesocosms filled with sea water from Svenstrup Strand. Ten shoots of each replicate were marked to estimate leaf growth, as described by Sand-Jensen [1]. Salinity and temperature were kept constant at 14 and 15°C respectively and the water was constantly aerated. Illumination (Phillips SONT-T Agro 400W) was set to a diurnal cycle of 12:12h at a photonflux density of 550 µmol photons (PAR) m^{-2} s^{-1} at canopy level for the high-light treatment and to 100 µmol photons (PAR) m^{-2} s^{-1} at canopy level for the low light treatment respectively. Blue mussels were gathered from the Marine Biological Research Centre and twenty-eight blue mussels were added to half of the mesocosms (= 891 mussels m^{-2}; 28 mussels per mesocosm in 12 mesocosm), creating 100% mussel cover. To support nutrition of the blue mussels 2 g dry d^{-1} resuspended dried Spirulina sp. were continuously added to the mesocosms.

After 21 days of exposure the seagrasses in the 24 pots were randomly harvested. Macroscopic epiphytes were removed by hand, the plants were rinsed with deionized water separated into leaves, rhizome and roots (yielding 72 samples), followed by immediate transfer to perforated aluminum bags and snap freezing in liquid nitrogen. The handling was fast (<20 sec) to preserve metabolites. The 72 samples were lyophilized for 48h and homogenized in a ball mill before further processing and analysis for metabolites.

Metabolite extraction All solvents and tubes were pre cooled to -20 °C. Ten mg of lyophilized and homogenized plant material (leaf, rhizome and root) were extracted for 8 min on ice in 1mL methanol/water (5:1 [v/v]) spiked with 1 µg of $^{13}C_6$-Sorbitol and 1 µg or Reserpine per sample as internal standard, followed by centrifugation at 14,000 g for 4 min. The supernatants (900 µL) were transferred to a test tube and split in a ration of 1:7:1 for quality control (QC), LC-MS and GC-MS respectively, the QC aliquots of all samples were pooled, aliquoted in 700 µL portions LC-MS and 100 µL for GC-MS respectively. All extracts were dried overnight in a speed-vac. For LC-MS analysis the dried samples were resuspended in 100 µL LC-solvent A (0.1% formic acid in water) and for GC-MS analysis derivatised as described below.

Metabolite profiling Here we present data collected by five different analytical conditions for roots, rhizomes and leaves of Z. marina (1) RP-LC-MS ESI+; (2) RP-LC-MS ESI−; (3) HILIC-MS ESI+; (4) HILIC-MS ESI−; and (5) GC-MS (RP=Reverse phase, LC= Liquid chromatography, MS= Mass spectroscopy, ESI-/+= negative/positive ionization, HILIC=Hydrophobic interaction liquid chromatography, GC=Gas chroatography).

LC-MS Q-TOF analysis A 1290 quaternary UHPLC system (Agilent Technologies, Santa Clara, CA, USA) (equipped with a degaser, autosampler, a temperature controlled column module) and an Agilent 6530 quadrople-time of flight (Q-TOF) mass spectrometer (MS) with an ESI source (Agilent Technologies, Santa Clara, CA, USA) was used for metabolomics analysis. Separation of apolar metabolites was achieved by injecting 5 µl sample on reversed phase column (Agilent Zorbax EclipsePlus C18; 150x2.1 mm, 1.8µm) maintained at 40 °C. The mobile phase was a mixture of solvent A (0.1%

formic acid in water and solvent B (0.1% formic acid in acetonitrile). The gradient elution program at a flow rate of 0.4 ml/min was: held 97% A (0-1 min), 97-2% A (1-15 min), held at 2% A (15-19 min) and returned to 97% A (19-20 min) followed by an equilibration time of 5 min at 97% A. Polar metabolites were separated on a HILIC column (Merck SeQuant ZIC-pHILIC column; 150x2.1mm, 5 µm) maintained at 40°C by injection of 3 µL of sample. QC samples were injected every 8 samples to control for RT drift and ionization patterns. The mobile phase was a mixture of solvent A (0.1% formic acid in water and solvent B (0.1% formic acid in acetonitrile). The gradient elution program at a flow rate of 0.3 ml/min was: held at 20% A (0-1) min, 20-80% A (1-15 min), held at 80% A (15-20 min) and returned to 20% A (20-21.5 min) followed by an equilibration time of 5 min at 20% A. MS-data was collected in full scan modus at 3 scans/s and a mass range of 50 – 1200 m/z in extended dynamic range mode.

ESI source settings were: VCap 3500 V, skimmer 65 V, fragmentor 165 V, nebulizer 40 psig, nitrogen gas flow 8 L/min at 250 °C. MS-data was collected at 3 scans/s and a mass range of 50 – 1200 m/z in the extended dynamic range mode (2GHz) and in positive and negative ionization. Initial tuning and auto-calibration (to two reference masses 121.050873 and 922.009798) was achieved to meet an mass accuracy of < 1 ppm before analysis.

Data processing of LC-MS data Data were collected and examined using Agilent MassHunter (MH) B7.02 (Agilent Technologies, Santa Clara, CA, USA) and deconvoluted, aligned, cleaned for back ground noise and unrelated ions in Agilent Profinder B6.0 (Agilent Technologies, Santa Clara, CA, USA) by batch recursive feature extraction an untargeted data-analysis algorithm. Agilent Mass Profiler Professional 13 (MPP) (Agilent Technologies, Santa Clara, CA, USA) was used for statistical analysis and metabolite annotation. The metabolites were annotated as MZ@RT on MSI level 4[2].

GC-MS Q-TOF analysis The dried samples (100 µL) were derivatised by adding 20 µL of methoxyamine hydrochloride (40 mg mL^{-1}) at 30 °C for 90 min followed by 30 min at 37°C in 80 µL MSTFA (spiked with 5 µl myristic-d_{27} acid (0.75 mg ml^{-1}) and 5 µL FAME mix (0.75 mg mL^{-1}) into 1 mL MSTFA for retention time locking respectively indexing). The metabolites were separated on Agilent 7890B gas chromatograph equipped with a DB5-MS Ultra-inert column (30 m, 0.25 mm, 0.25 µm) (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 7200 GC-QTOF-MS (Agilent Technologies, Santa Clara, CA, USA) after injecting 1 µL in split 1:10 mode. The GC temperature gradient was at 60 °C for 1 min, followed by a ramp of 10 °C per min to 325 °C held for 10 min. The TOF acquisition rate was set to 10 spectra/s in the extended dynamic range mode (2GHz). QC samples were injected every 8 samples to control for RT drift and ionization patterns.

Data processing of GC-MS data Data were collected using MassHunter Data Acquisition B7.02, examined in MassHunter qual B7.02. The deconvolution was achieved in Masshunter Unknown analysis B7.0 (UA) (Agilent Technologies, Santa Clara, CA, USA). The peak alignment, normalization and metabolite annotation was done in MPP 13. Analytes were considered as putatively annotated (MSI level 2, after Sumner et al. 2007) by matching the deconvoluted and aligned mass spectra against an in house library as well as the Fiehn-lib[3] (match factor >80) and annotation was further supported by manual comparison of retention indices.

General data analysis Peak areas were standardized for sample weight and to the internal standard, and later log2(x+1) transformed and baselined by unit scaling (mean-centered and divided by standard deviation of each variable) and log2(x+1) transformed. To exclude false positive hits only entities that sowed a coefficient of variation (CV) of <35% and were present in at least 80% of the QC samples were

used for analysis. These stringent filters kept the number of false positive entities low, but might also have led to an increase in the number of false negatives.

The effects of light availability and species interaction were compared in MPP using univariate and multivariate methods. A cut-off value of $p<0.05$ was considered as significant in the two-way ANOVA (tukey post-hoc test) applying a Benjamini Hochberg FDR procedure , regarding a adjusted p value <0.05 as significant. To identify the most influential metabolites in separating the treatment groups along the principal components (PC) the CC-plots (Covariance vs. Correlation) of all 3 components were inspected[4]. The CC-plot combines the covariance and correlation loading profiles resulting from the PCA in a scatter plot. In this plot both magnitude (covariance) and reliability (correlation) are visualized. The P-Cov axis describes the magnitude of each variable in the data matrix X. The P-Cor axis represents the reliability of each variable in X. P-Cor axis always spans between ±1 as the correlation has a theoretical minimum of -1 and a maximum of +1[4]. An alpha value of 0.05 was applied consistently.

Ceramide and SP1 measurements Ceramide was assessed as ceramide (validated as d18:1/12:0 with a mass of 481.4495)[5] and sphigosine-1-phosphate with a mass of 379.2488 by RP-LC-MS as described above. We confirmed the annotation of ceramide and S1P by comparison with standards purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA).

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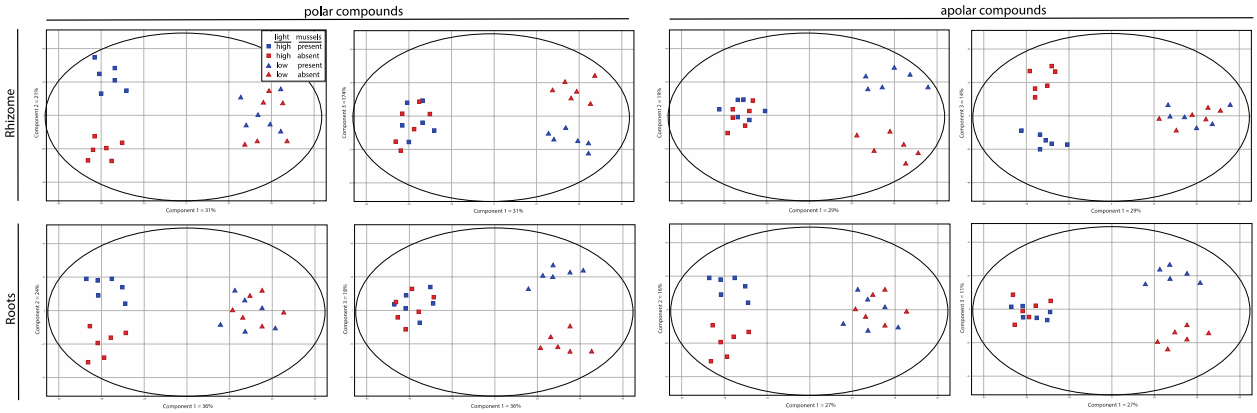

Figure S1. PCA plots of polar (two left panels) and apolar (two right panels) compounds in *Zostera marina* rhizomes (upper row) and roots (lower row), exposed to varying light and mussels. The first and third column indicates PC1 vs PC2 and the second column indicate P1 vs PC3. Squares indicate samples under high light intensities and triangles samples under low light intensities; blue colored samples indicate mussel presence and red colored samples indicate mussel absence. Only metabolites that passed the quality control filters are included.

Table S1: List of all putatively annotated metabolites (MSI level 2, after Sumner et al. 2007) reproducibly detected in all tissues and conditions by GC-QTOF-MS. Metabolites indicated as MZ@RT were not annotated, but kept for analysis. The significant results ($p<0.05$) of the 2-way ANOVA with a Bonferroni correction for multiple comparisons are indicated in bold. Pearson correlations between each metabolites and elemental sulfur (S^0) correlations <0.6 are shown in red for negative correlations and green for positive correlations >0.6 respectively. The up or down regulation of a specific metabolites between each treatment is indicated by up or down. H, high light; L, low light; +, mussel presence; -, mussel absence;

	ANOVA p-value			Spearman R	Regulation					
Source of variation	Light	Mussel	L x M	S^0	H- vs H+	H- vs L-	H- vs L+	H+ vs L-	H+ vs L+	H- vs L+
Metabolite										
75.03@6.66	0.228	0.875	0.836	-0.57	down	down	down	down	down	up
140.0315@6.68	0.895	0.718	0.999	-0.07	down	down	down	up	down	down
167.0359@6.88	0.726	0.875	0.999	-0.18	down	down	down	up	down	down
207.0325@7.1	0.646	0.875	0.897	0.64	down	down	down	up	up	up
hydroxypyridine	0.640	0.875	0.849	0.84	down	down	down	up	up	down
207.0334@7.32	0.246	0.756	0.649	0.82	down	down	up	up	up	up
lactic acid	0.560	0.305	0.405	0.87	up	down	up	down	up	up
234.1041@7.48	0.426	0.875	0.999	-0.13	down	down	down	down	down	down
89.0327@7.54	0.014	0.531	0.897	0.55	up	down	down	down	down	up
73.0472@7.63	0.796	0.664	0.914	-0.14	down	down	down	up	up	down
L-alanine	<.001	0.973	0.999	0.89	up	down	down	down	down	up
acetohydroxamic acid	0.383	0.875	0.649	0.76	down	down	up	up	up	up
oxalic acid	0.335	0.973	0.999	0.45	up	down	down	down	down	up
281.0515@8.83	0.446	0.875	0.897	0.59	up	down	down	down	down	down
97.1011@9.23	0.646	0.875	0.836	0.84	down	down	up	up	up	up
147.0661@9.30	0.495	0.745	0.649	0.45	up	up	down	up	down	down
L-valine	<.001	0.875	0.649	0.88	up	down	down	down	down	down
147.0661@9.77	0.243	0.875	0.913	0.18	up	down	down	down	down	down
urea	0.009	0.902	0.836	0.86	up	down	down	down	down	down
299.0739@10.18	0.495	0.910	0.999	-0.22	down	down	down	down	down	down
ethanolamine	0.030	0.875	0.656	0.81	down	up	up	up	up	up
phosphoric acid	0.139	0.875	0.999	0.79	down	up	up	up	up	down
147.0664@10.38	0.020	0.864	0.999	-0.42	down	up	up	up	up	down

73.0472@10.42	0.446	0.875	0.999	0.8	down	down	down	up	down	down
DL-isoleucine	<.001	0.745	0.649	0.91	up	down	down	down	down	down
142.1054@10.71	0.396	0.984	0.999	0.27	up	down	down	down	down	down
L-proline	<.001	0.539	0.649	-0.99	up	up	up	up	up	up
glycine	0.009	0.973	0.999	0.75	down	up	up	up	up	down
succinic acid	0.230	0.875	0.938	0.13	up	down	down	down	down	up
glyceric acid	0.979	0.875	0.649	-0.52	up	up	down	up	down	down
glyoxylic acid	0.484	0.892	0.649	0.77	down	down	down	down	up	up
fumaric acid	0.085	0.539	0.296	0.8	up	up	up	up	down	down
L-serine 2	0.075	0.718	0.683	0.72	down	up	up	up	up	down
156.1205@11.70	<.001	0.125	0.175	0.82	down	down	down	down	down	down
L-threonine	0.414	0.875	0.897	0.02	down	down	down	down	down	down
NA sulfamate	0.016	0.944	0.999	0.76	up	up	up	up	up	down
228.066@12.38	0.321	0.875	0.961	0.79	down	down	down	down	down	down
147.0661@12.77	0.076	0.951	0.999	0.09	up	down	down	down	down	up
155.1003@13.05	0.224	0.875	0.649	0.84	up	up	down	down	down	down
D-malic acid	0.454	0.973	0.999	-0.04	down	down	down	down	down	up
73.0472@13.2	0.230	0.539	0.999	0.85	up	up	up	down	up	up
100.0573@13.44	0.228	0.059	0.982	0.29	down	down	down	up	down	down
156.0846@13.45	0.530	0.875	0.897	0.42	up	down	down	down	down	up
232.1195@13.55	0.102	0.951	0.649	-0.99	up	down	down	down	down	down
aspartic acid	0.230	0.913	0.836	0.86	up	down	down	down	down	down
L-glutamic acid	0.414	0.539	0.982	-0.8	up	down	up	down	down	up
L-pyroglutamic acid	0.351	0.539	0.405	0.86	down	down	up	down	up	up
gamma-aminobutyric acid (GABA)	<.001	0.875	0.649	0.88	up	down	down	down	down	down
butantriol	0.875	0.910	0.999	0.86	up	up	up	down	up	up
threonic acid	0.129	0.875	0.740	0.87	up	down	down	down	down	down
2-isopropylmalic acid	0.626	0.539	0.296	-0.09	down	down	up	down	up	up
100.0578@14.32	0.559	0.801	0.786	0.68	down	up	down	up	down	down
146.0821@14.77	<.001	0.875	0.897	0.82	down	down	down	down	down	down
246.1346@14.82	0.560	0.875	0.897	0.43	up	down	up	down	down	up
L-phenylalanine	0.521	0.875	0.897	0.57	up	down	down	down	down	up

147.0661@15.07	0.646	0.745	0.740	0.35	down	up	down	up	down	down
73.0472@15.32	0.230	0.913	0.649	0.82	down	down	down	down	down	up
L-asparagine	0.171	0.875	0.897	0.72	up	down	down	down	down	down
xylose	<.001	0.875	0.897	0.79	down	up	up	up	up	down
243.1272@15.59	0.230	0.875	0.740	0.84	down	down	down	down	down	up
xylitol	0.007	0.973	0.999	0.83	up	down	down	down	down	down
73.0472@15.71	0.036	0.875	0.740	0.87	down	down	down	down	down	up
156.0849@15.80	0.169	0.127	0.897	0.65	down	down	down	up	down	down
97.1011@15.89	0.646	0.875	0.961	0.84	up	down	down	down	down	up
217.1072@15.91	0.406	0.913	0.999	0.01	down	down	down	down	down	down
ribitol	<.001	0.973	0.999	0.81	up	up	up	up	up	down
Glutamine	0.084	0.973	0.649	0.85	up	down	down	down	down	down
73.047@16.18	0.448	0.992	0.999	0.24	up	down	down	down	down	down
179.0529@16.26	<.001	<.001	<.001	1	down	down	down	down	down	down
glycerol 1-phosphate	0.206	0.875	0.999	0.78	up	up	up	up	up	up
73.047@16.47	0.171	0.875	0.849	-0.1	up	down	down	down	down	down
156.0846@16.57	0.394	0.913	0.999	0.03	down	down	down	down	down	down
L-glutamine	0.307	0.929	0.836	0.88	up	down	down	down	down	down
205.1083@16.64	0.335	0.586	0.649	0.75	down	up	down	up	down	down
204.1@16.89	0.462	0.539	0.836	-0.13	down	up	down	up	down	down
citric acid	0.127	0.180	0.296	0.31	up	down	up	down	up	up
225.0935@17.12	0.979	0.875	0.649	-0.32	down	down	up	down	up	up
Hexadecanal	0.285	0.756	0.649	0.81	down	down	up	up	up	up
myristic acid	0.089	0.973	0.836	0.88	down	down	down	down	down	up
tagatose 1	<.001	0.003	0.011	0.73	up	up	up	up	up	up
149.0236@17.61	0.016	0.973	0.999	-0.64	down	down	down	down	down	down
adenine	<.001	0.539	0.961	0.79	up	up	up	up	up	up
73.047@17.71	0.081	0.875	0.836	0.08	down	up	up	up	up	up
allantoin	<.001	0.944	0.897	0.85	down	down	down	down	down	up
D-glucose	<.001	0.226	0.649	0.81	up	up	up	up	up	up
hydroxyphenyl lactic acid	0.094	0.557	0.999	0.76	up	up	up	up	up	up
D-sorbitol	0.426	0.875	0.897	0.45	up	down	down	down	down	down

D (+) galactose	0.005	0.531	0.782	0.77	up	up	up	up	up	up
D-mannitol	0.168	0.539	0.649	0.02	up	down	down	down	down	up
D-sorbitol	0.029	0.973	0.999	-0.09	up	up	up	up	up	up
galactitol	0.285	0.973	0.999	-0.01	up	down	down	down	down	down
hydroxycinnamic acid	0.011	0.875	0.908	0.76	down	up	up	up	up	down
NA Inositol	0.002	0.942	0.740	0.5	up	down	down	down	down	down
gluconic acid	0.009	0.875	0.897	-0.8	up	down	down	down	down	down
Mannose	0.707	0.910	0.649	-0.09	up	up	up	up	down	down
73.047@18.81	0.524	0.989	0.649	0.8	up	up	down	down	down	down
mucic acid	0.009	0.875	0.897	0.87	up	down	down	down	down	up
NA Inositol	0.377	0.875	0.897	0.62	up	down	down	down	down	up
Chizo-Inositol	0.560	0.892	0.897	-0.13	up	down	down	down	down	down
313.3062@19.37	0.446	0.531	0.740	0.59	down	up	down	up	down	down
palmitic acid	0.524	0.539	0.999	0.82	up	down	up	down	down	up
dihydroxyphenylalanine (DOPA)	0.407	0.875	0.405	0.3	up	up	down	down	down	down
355.0705@19.56	0.322	0.875	0.897	-0.08	down	down	down	down	down	down
myo-inositol	0.079	0.951	0.999	0.84	up	up	up	up	up	up
galactose oxime hexakis	0.313	0.875	0.649	0.25	up	up	up	up	down	down
caffeic acid	0.167	0.610	0.897	0.88	up	down	down	down	down	up
phytol	0.886	0.233	0.982	0.82	up	down	up	down	up	up
linoleic acid	0.063	0.875	0.649	0.84	up	down	down	down	down	down
octadecatrienoic acid	0.097	0.745	0.849	0.84	up	up	up	up	up	up
stearic acid	0.640	0.718	0.897	0.86	up	up	up	down	up	up
73.047@21.39	0.006	0.973	0.897	0.83	up	down	down	down	down	down
204.1003@21.69	0.020	0.875	0.836	0.84	down	down	down	down	down	up
73.0472@21.87	0.446	0.539	0.897	0.8	up	up	up	down	up	up
73.0472@21.91	0.600	0.943	0.649	-0.05	up	up	down	down	down	down
73.047@22.58	0.020	0.992	0.999	-0.19	up	up	up	up	up	down
73.0472@22.73	0.859	0.967	0.649	0.26	up	up	up	up	down	down
430.177@22.83	0.089	0.305	0.999	-0.58	up	up	up	down	up	up
73.047@22.86	0.024	0.875	0.836	0.01	up	up	up	up	up	up
73.0472@23.14	0.109	0.875	0.961	-0.29	down	down	down	down	down	down

lactobionic acid	<.001	0.875	0.740	0.72	up	up	up	up	up	down
hexadecanoic acid	0.462	0.875	0.897	-0.16	down	down	down	down	down	down
eicosapentaenoic acid	0.168	0.875	0.999	-1	down	down	down	down	down	down
kestose	0.626	0.539	0.897	-0.89	up	up	up	down	up	up
NA Disacaride	0.009	0.875	0.649	0.22	up	down	down	down	down	down
sucrose	<.001	0.125	0.175	-0.94	up	up	up	up	up	up
eicosapentaenoic acid	0.322	0.875	0.836	0.65	up	down	down	down	down	down
73.047@25.32	0.061	0.875	0.897	0.11	up	up	up	up	up	up
73.047@25.80	0.224	0.875	0.897	0.32	up	down	down	down	down	down
tetracosanoic acid	0.232	0.557	0.649	0.86	up	down	up	down	down	up
204.1007@26.81	0.892	0.729	0.740	0.08	up	down	up	down	up	up
alpha tocophereol	0.600	0.875	0.740	0.83	up	up	up	down	down	down
73.0472@28.06	0.859	0.875	0.358	0.79	up	up	up	down	down	down
caffeic acid	<.001	0.059	0.649	0.77	up	up	up	up	up	up
443.135@28.59	0.006	0.875	0.662	-0.27	up	up	up	up	up	down
501.1581@28.68	<.001	0.875	0.897	0.56	down	up	up	up	up	down
501.158@28.93	<.001	0.875	0.739	-0.37	up	up	up	up	up	down
559.182@28.96	<.001	0.875	0.897	0.54	down	up	up	up	up	down
83.0852@29.08	<.001	0.875	0.897	0.53	up	down	down	down	down	down
beta-sitosterol	0.079	0.875	0.649	0.8	up	down	down	down	down	down
296.25@29.72	<.001	0.875	0.999	0.64	up	up	up	up	up	up
rosmarinic acid	0.035	0.875	0.836	-0.43	up	up	up	up	up	up
647.4216@31.80	0.610	0.352	0.999	0.12	up	up	up	down	up	up

NA indicates metabolites that belong to a certain compound class but could not be annotated as a specific metabolite.

Table S2: Elemental sulfur (S^0) and pore water ammonium levels for Z. marina as a function of light and mussel treatment.

Light treatment	High light		Low light	
Mussel treatment	Absent	Present	Absent	Present
Response variable				
Root S^0 (µmol S gDW^{-1})	$0^{B,b}$	$3.54\pm1.34^{B,a}$	$6.80\pm2.28^{A,b}$	$17.95\pm6.16^{A,a}$
Pore water nutrients				
Ammonium (µmol l^{-1})	632.4 ± 71.2^{2}	1279.7 ± 51.9^{1}	1218.2 ± 107.4^{1}	1320.2 ± 163.1^{1}

Data are represented as mean (±SEM). Levels not connected by the same letter indicate significant differences (ANOVA-Tukey Post Hoc <0.05). Capital letters indicate light dependent differences, lower case letters indicate mussel dependent differences; uppercase numbers indicate interaction effects; data from Castorani et al.[6]

Table S3: Results of 2-way ANOVA testing the effects of light and mussels on seagrass performance, nutrients, sediment biogeochemical and production and respiration.

Source of variation	Light			Mussels			Light x mussels		
Response variable	df	F	p	df	F	p	df	F	p
Seagrass survival, growth, and energy stores									
Total shoot density	1,20	139.401	<0.001	1,20	1.024	0.324	1,20	0	1
Terminal shoot density	1,20	99.011	<0.001	1,20	0.309	0.584	1,20	1.104	0.306
Lateral shoot density	1,20	198.416	<0.001	1,20	0.218	0.646	1,20	1.422	0.247
Leaf growth rate	1,20	211.888	<0.001	1,20	2.235	0.151	1,20	0.553	0.466
Rhizome soluble sugars	1,20	218.559	<0.001	1,20	0.059	0.81	1,20	0.383	0.543
Rhizome starches	1,20	0.391	0.539	1,20	0.018	0.895	1,20	0.001	0.999
Root elemental sulfur	1,20	18.853	<0.001	1,20	6.444	0.02	1,20	0.598	0.448
Seagrass nutrient condition									
Leaf percent carbon	1,20	7.693	0.012	1,20	1.196	0.287	1,20	0.574	0.458
Leaf percent nitrogen	1,20	119.71	<0.001	1,20	1.513	0.233	1,20	0.757	0.395
Leaf carbon:nitrogen	1,20	201.834	<0.001	1,20	1.21	0.284	1,20	0.597	0.449
Sediment biochemical conditions									
Porewater ammonium	1,20	8.542	0.008	1,20	12.231	0.002	1,20	6.48	0.019
Porewater dissolved organic carbon	1,19	2.469	0.133	1,19	0.822	0.376	1,19	0.096	0.761
Sediment acid-volatile sulfides	1,19	1.097	0.308	1,19	10.234	0.005	1,19	1.419	0.248

P values <0.05 are shown in bold; data from Castorani et al.