

Supporting Information

Short-term effects of salt stress on the amino acids of *Phragmites australis* root exudates in constructed wetlands

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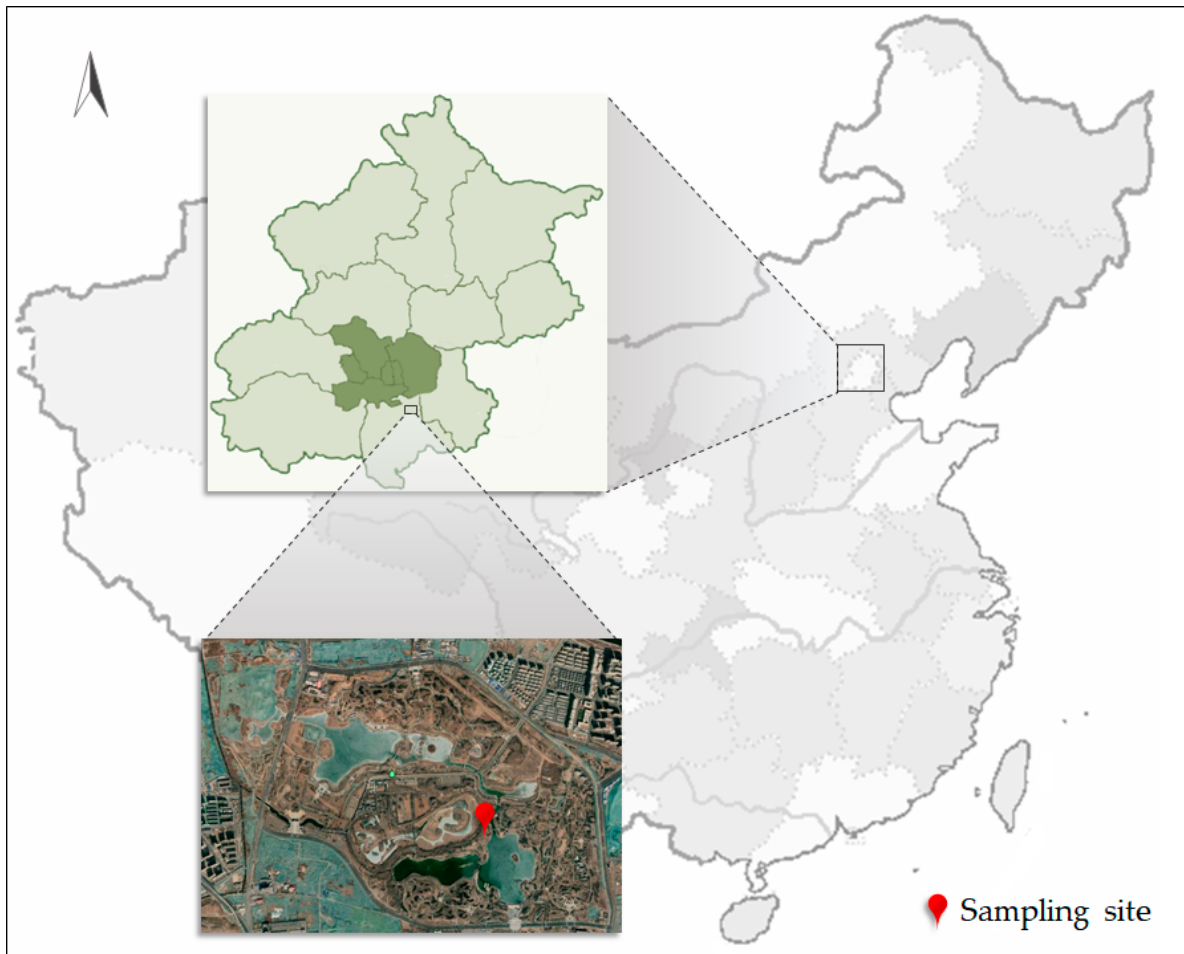


Figure S1 Spring buds of *P. australis* Sampling site in wetland of Daxing District, Beijing

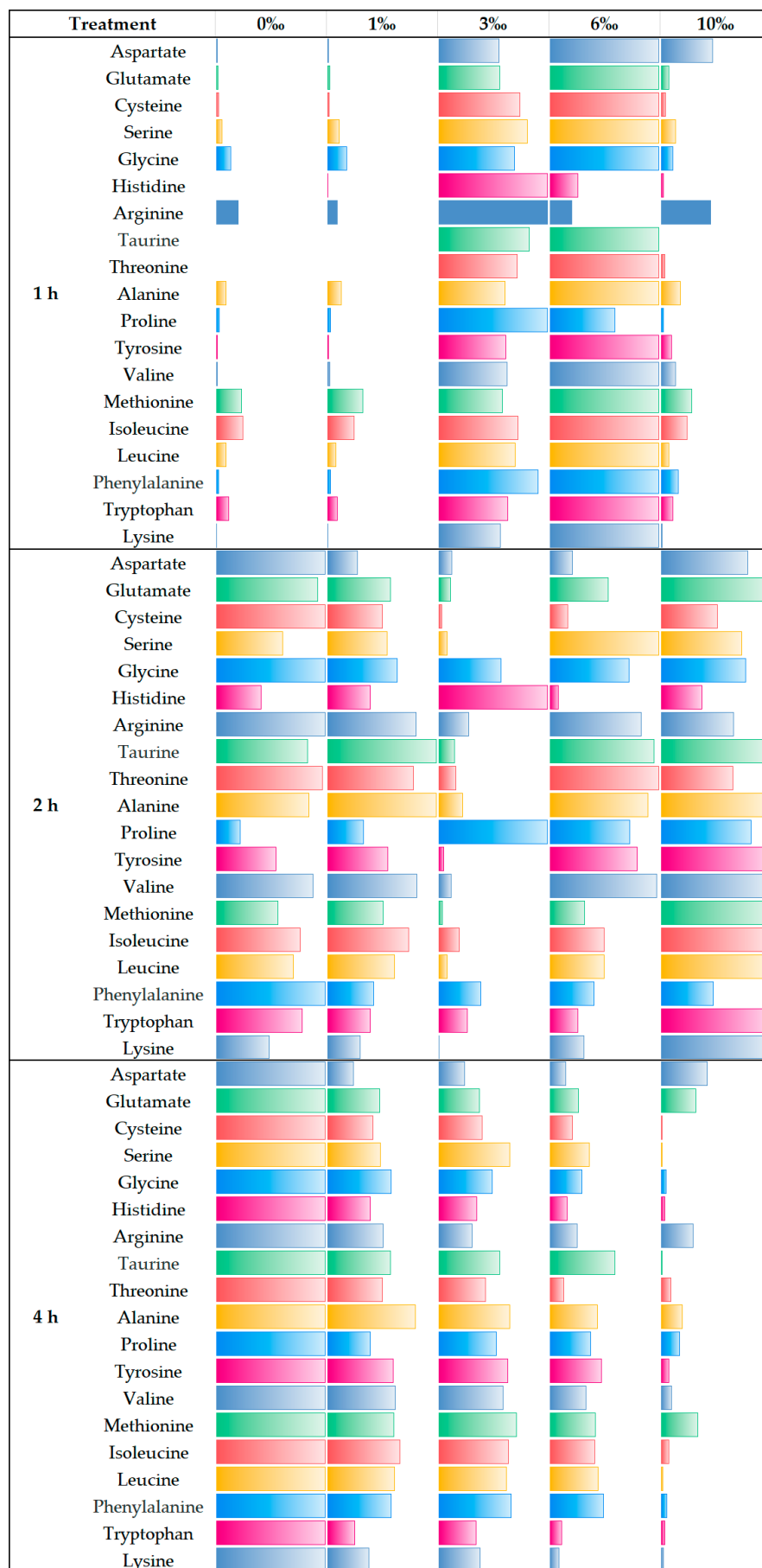


Figure S2 Changes of amino acids in different treatments in each period. Defined the highest in each row as 100%, the rest of each row is converted to percentage by relative value

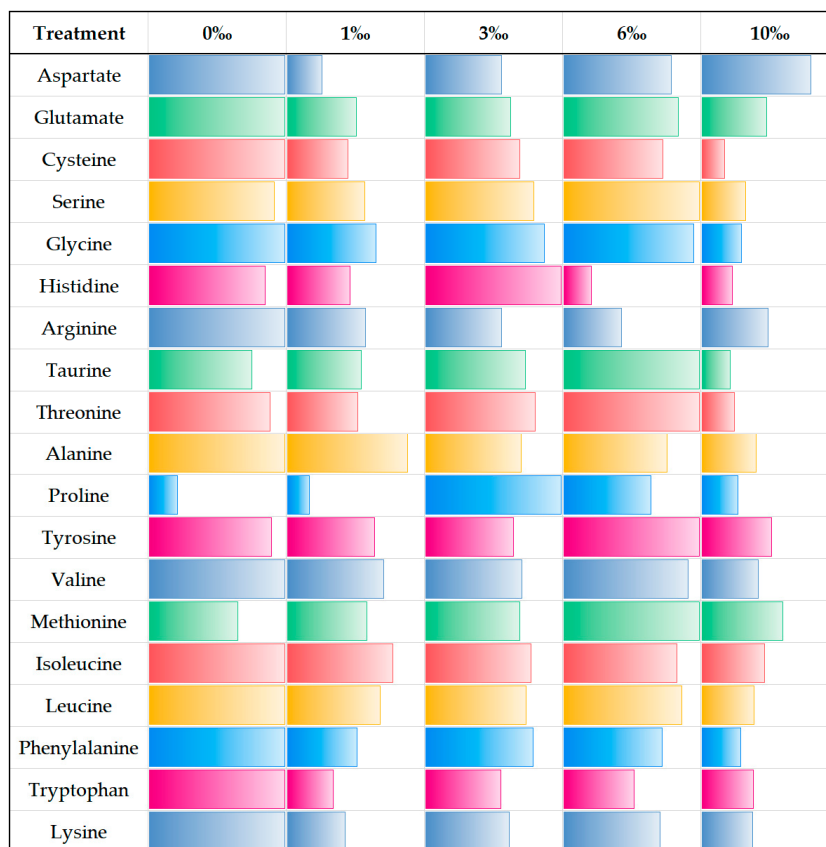


Figure S3 Changes of amino acids in different treatments throughout all the experimental period. Defined the highest in each row as 100%, the rest of each row is converted to percentage by relative value

Table S1 The recovery rate of NaCl remove procedure

Amino acids	Recovery (%)	Amino acids	Recovery (%)
Aspartate	90.97±1.04	Proline	84.09±3.86
Glutamate	97.19±0.01	Tyrosine	88.09±0.06
Cysteine	90.00±9.67	Valine	98.80±1.09
Serine	106.62±2.20	Methionine	112.66±4.94
Glycine	97.34±4.87	Isoleucine	97.98±1.02
Histidine	116.35±8.57	Leucine	101.31±3.82
Arginine	104.06±2.81	Phenylalanine	105.47±11.72
Taurine	98.12±1.07	Tryptophan	87.88±10.27
Threonine	97.94±1.57	Lysine	106.06±5.17
Alanine	87.61±0.60		

All the data are mean ± standard deviation (SD) from three duplicates

Table S2 The gradient elution process of amino acid mobile phase A and B

Grades	Time(min)	A	B
		Sodium acetate: Acetonitrile (93:7, V/V)(%)	acetonitrile: water (80:20,V/V)(%)
1	0	100	0
2	11	93	7
3	14	85	15
4	29	66	34
5	32	30	70
6	35	0	100
7	42	0	100
8	45	100	0
9	55	100	0

Table S3 The HPLC elution time for amino acids

Amino acids	Elution time (min)	Amino acids	Elution time (min)
Aspartate	5.71	Proline	19.67
Glutamate	6.44	Tyrosine	23.07
Cysteine	12.47	Valine	24.28
Serine	13.45	Methionine	25.38
Glycine	15.05	Isoleucine	28.00
Histidine	16.98	Leucine	28.37
Arginine	17.38	Phenylalanine	30.55
Taurine	17.95	Tryptophan	32.41
Threonine	18.24	Lysine	22.08
Alanine	18.76		

Table S4 The calibration curve for each kind of amino acid and relative standard deviation (RSD)

Amino acids	calibration curve	R ²	RSD (%)
Aspartate	$y = 0.0752x - 0.0822$	0.9990	3.30
Glutamate	$y = 0.0544x - 0.0264$	0.9994	2.30
Cysteine	$y = 0.0597x - 0.0625$	0.9995	2.67
Serine	$y = 0.1413x - 0.0425$	0.9994	2.33
Glycine	$y = 0.0812x - 0.0805$	0.9995	2.49
Histidine	$y = 0.0673x - 0.0054$	0.9990	2.35
Arginine	$y = 0.0530x + 0.0203$	0.9989	2.38
Taurine	$y = 0.0690x + 0.0703$	0.9987	2.17
Threonine	$y = 0.0639x + 0.0473$	0.9985	2.84
Alanine	$y = 0.2503x + 0.0674$	0.9995	1.94
Proline	$y = 0.0310x - 0.1162$	0.9949	6.13
Tyrosine	$y = 0.1119x + 0.1377$	0.9972	4.20
Valine	$y = 0.0825x - 0.1077$	0.9962	4.99
Methionine	$y = 0.0604x + 0.0080$	0.9962	2.97
Isoleucine	$y = 0.0830x + 0.0776$	0.9973	3.23
Leucine	$y = 0.0751x + 0.0818$	0.9977	3.08
Phenylalanine	$y = 0.0856x + 0.0563$	0.9979	2.99
Tryptophan	$y = 0.0159x + 0.0095$	0.9962	3.17
Lysine	$y = 0.0976x + 0.1093$	0.9910	5.43

Table S5 Content of total free amino acids (TotAA) and single compounds (absolute content) of root exudates of *Phragmites australis* clones (stress time 1, 2, 4h) compares for five NaCl-salinity levels

Time	Amino acid	NaCl-Salinity (‰)									
		0‰		1‰		3‰		6‰		10‰	
		Concentration ($\mu\text{M}\cdot\text{g}^{-1}\text{DW}$)	Diversity	Concentration ($\mu\text{M}\cdot\text{g}^{-1}\text{DW}$)	Diversity	Concentration ($\mu\text{M}\cdot\text{g}^{-1}\text{DW}$)	Diversity	Concentration ($\mu\text{M}\cdot\text{g}^{-1}\text{DW}$)	Diversity	Concentration ($\mu\text{M}\cdot\text{g}^{-1}\text{DW}$)	Diversity
1 h	TotAA	45.97±1.44	d	48.64±0.18	d	1067.91±2.09	b	1098.79±1.77	a	106.32±0.67	c
	Aspartate	0.8±0.00	d	1.0±0.30	d	40.2±0.56	b	72.5±0.90	a	34.3±0.06	c
	Glutamate	3.10±0.09	d	3.32±0.01	d	82.28±0.59	b	146.81±0.84	a	10.64±0.10	c
	Cysteine	1.01±0.23	c	0.99±0.13	c	34.24±0.21	b	45.88±1.78	a	2.02±0.11	c
	Serine	1.00±0.08	d	2.04±0.01	c	14.58±0.76	b	17.88±0.07	a	2.38±0.01	c
	Glycine	2.70±0.18	c	3.61±0.37	c	14.13±0.71	b	20.28±3.32	a	2.20±0.02	c
	Histidine	0.10±0.08	c	0.30±0.12	c	34.35±1.90	a	8.81±0.71	b	0.95±0.21	c
	Arginine	2.16±0.00	c	0.97±0.11	d	10.70±0.89	a	2.20±0.25	c	4.89±0.11	b
	Taurine	0.08±0.04	c	0.01±0.01	c	82.58±3.13	b	98.95±4.28	a	0.03±0.01	c
	Threonine	0.35±0.21	c	0.27±0.01	c	108.71±5.69	b	151.03±0.28	a	5.40±0.49	c
	Alanine	1.34±0.28	d	1.94±0.16	cd	8.95±0.69	b	14.67±0.55	a	2.64±0.15	c
	Proline	13.59±0.43	cd	13.90±0.08	c	484.80±0.64	a	290.96±1.56	b	11.46±0.78	d
	Tyrosine	0.18±0.04	d	0.20±0.05	d	11.78±0.76	b	19.15±0.16a		1.92±0.11	c

	Valine	0.42±0.01	e	1.05±0.06	d	26.02±0.21	b	41.33±0.24	a	5.65±0.01	c
	Methionine	3.85±0.37	d	5.36±0.28	c	9.57±0.22	b	16.26±0.34	a	4.62±0.23	c
	Isoleucine	5.00±0.25	c	5.04±0.02	c	14.95±0.56	b	20.49±0.66	a	4.95±0.13	c
	Leucine	3.16±0.19	c	2.64±0.18	c	23.79±1.27	b	33.79±0.86	a	2.55±0.23	c
	Phenylalanine	0.54±0.42	d	0.59±0.09	d	18.69±0.46	b	20.51±0.40	a	3.24±0.30	c
	Tryptophan	6.44±0.15	c	5.26±0.88	c	35.35±0.64	b	55.65±1.74	a	6.18±0.18	c
	Lysine	0.15±0.04	c	0.19±0.06	c	12.24±2.22	b	21.64±0.74	a	0.29±0.06	c
2 h	TotAA	457.00±1.04	b	378.47±4.53	d	350.48±0.30	e	436.35±6.76	c	582.53±2.06	a
	Aspartate	25.84±0.83	a	7.24±0.42	c	3.22±0.03	e	5.46±0.01	d	20.55±0.56	b
	Glutamate	45.27±0.34	b	28.09±0.39	c	5.39±0.12	e	25.88±0.99	d	48.42±0.43	a
	Cysteine	23.02±0.04	a	11.67±0.14	b	0.66±0.01	d	3.82±0.07	c	11.89±0.18	b
	Serine	9.66±0.24	c	8.67±0.16	d	1.26±0.02	e	15.82±0.50	a	11.69±0.00	b
	Glycine	9.15±0.35	a	5.89±0.02	cd	5.25±0.13	d	6.65±0.15	bc	7.10±0.64	b
	Histidine	30.69±0.01	b	29.49±0.44	c	74.20±0.32	a	6.18±0.07	e	27.96±0.22	d
	Arginine	8.47±0.57	a	6.91±0.14	b	2.33±0.13	d	7.12±0.12	b	5.65±0.01	c
	Taurine	38.60±1.65	b	45.83±2.04	a	6.71±0.08	c	43.84±2.76	a	44.57±1.36	a
	Threonine	52.19±0.45	a	42.11±0.12	b	8.47±0.01	d	53.41±0.40	a	35.30±0.98	c

	Alanine	11.61±0.05	c	13.61±0.08	a	3.03±0.00	d	12.29±0.16	b	13.51±0.11	a
	Proline	45.74±0.51	e	68.30±0.81	d	203.45±0.06	a	149.90±5.65	c	168.89±1.07	b
	Tyrosine	13.42±0.04	d	13.58±0.01	c	1.28±0.03	e	19.69±0.02	b	24.52±0.03	a
	Valine	27.97±0.31	b	25.88±0.22	c	3.75±0.05	d	30.96±0.76	a	31.55±0.19	a
	Methionine	3.16±0.05	b	2.87±0.04	c	0.22±0.08	e	1.78±0.00	d	5.58±0.02	a
	Isoleucine	12.28±0.55	b	11.90±0.09	b	3.02±0.01	d	8.00±0.18	c	15.97±0.04	a
	Leucine	21.72±0.44	b	18.87±0.45	c	2.36±0.12	e	15.33±0.61	d	30.54±0.71	a
	Phenylalanine	32.69±0.52	a	13.90±0.14	c	12.66±0.61	d	13.29±0.00	cd	15.77±0.62	b
	Tryptophan	38.57±0.11	b	19.36±0.41	c	13.10±0.21	d	12.58±0.01	d	48.94±1.47	a
	Lysine	6.93±0.03	b	4.30±0.28	c	0.13±0.05	d	4.42±0.00	c	14.14±0.47	a
4 h	TotAA	1379.37±13.15	a	667.67±6.32	b	639.68±6.18	c	407.55±2.09	d	191.00±1.63	e
	Aspartate	88.63±1.01	a	21.40±0.07	c	21.11±0.88	c	13.34±0.03	d	37.68±0.11	b
	Glutamate	228.03±1.32	a	109.09±0.93	b	85.46±0.85	c	61.15±1.41	e	72.86±3.68	d
	Cysteine	61.56±1.03	a	25.84±0.11	b	24.61±0.12	b	13.07±0.48	c	0.64±0.04	d
	Serine	30.69±1.41	a	15.06±0.87	c	20.17±0.18	b	11.24±0.58	d	0.41±0.10	e
	Glycine	23.62±0.64	a	13.80±0.25	b	11.69±0.23	c	7.03±0.11	d	1.11±0.17	e
	Histidine	88.66±0.83	a	35.25±1.13	b	31.33±1.72	c	14.20±0.43	d	3.03±0.10	e

Arginine	27.65±0.40	a	14.18±0.82	b	8.55±0.06	c	7.00±0.00	d	8.22±0.05	c
Taurine	126.91±3.85	a	73.86±3.10	b	71.51±11.10	b	76.06±0.22	b	1.74±0.07	c
Threonine	146.68±3.05	a	74.27±5.92	b	63.32±4.62	c	18.89±2.78	d	13.78±0.01	d
Alanine	53.36±0.16	a	43.22±0.30	b	34.98±0.00	c	23.50±0.33	d	10.54±0.15	e
Proline	97.73±0.98	a	38.80±1.88	c	52.00±0.94	b	36.77±0.93	c	16.86±0.59	d
Tyrosine	37.47±0.01	a	22.77±0.13	c	23.84±0.51	b	17.89±0.33	d	2.86±0.00	e
Valine	79.57±0.18	a	49.66±0.34	b	47.08±0.42	c	26.65±0.19	d	7.99±0.13	e
Methionine	6.63±0.11	a	4.06±0.06	c	4.76±0.08	b	2.80±0.02	d	2.24±0.01	e
Isoleucine	33.24±0.38	a	22.26±0.21	b	21.29±1.23	b	13.81±0.11	c	2.42±0.05	d
Leucine	64.12±0.06	a	39.45±0.87	b	39.81±1.47	b	28.46±0.81	c	1.19±0.37	d
Phenylalanine	41.08±0.20	a	23.97±0.56	c	27.48±0.18	b	20.33±0.27	d	2.26±0.07	e
Tryptophan	110.06±6.19	a	27.81±0.28	c	37.81±4.04	b	12.38±0.26	d	4.33±0.49	d
Lysine	33.69±0.17	a	12.93±0.45	b	12.90±0.45	b	2.97±0.06	c	0.86±0.13	d

Data points are means ± S.D. (n = 2). Different letters indicate significant differences between the treatments according to ANOVA, $p < 0.05$.