

Article

# Effect of Salinity and Nitrogen Form in Irrigation Water on Growth, Antioxidants and Fatty Acids Profiles in Halophytes *Salsola australis*, *Suaeda maritima*, and *Enchylaena tomentosa* for a Perspective of Biosaline Agriculture

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## Artworks - Supporting Tables

**Table S1.** Mean proportions of all fatty acids and fatty acid classes (%TFA) identified in leaves of halophytes *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa*, after one-month irrigation water with different salinities (control, moderate, high) and N-ratios (NO<sub>3</sub><sup>-</sup>-N:NH<sub>4</sub><sup>+</sup>-N ratio). Data are mean ± SD on all combined treatments (n=45 for each species).

	<i>Salsola australis</i>	<i>Suaeda maritima</i>	<i>Enchylaena tomentosa</i>
14:0	1.32±0.30	1.46±0.22	3.16±2.02
15:0	0.03±0.02	0.02±0.01	0.03±0.02
16:0	14.76±1.89	14.56±0.71	16.99±1.45
16:1n-13t	1.54±0.31	1.02±0.11	1.16±0.17
16:1n-7	0.15±0.06	0.17±0.04	0.50±0.24
16:1n-9	0.39±0.09	0.36±0.10	0.28±0.05
16:2n-6	0.03±0.01	0.02±0.01	0.05±0.02
16:3n-3	0.06±0.04	0.05±0.02	0.08±0.05
16:4n-1	0.01±0.01	0.04±0.09	0.05±0.07
16:4n-3	0.31±0.07	0.38±0.06	0.26±0.06
17:0	0.18±0.07	0.20±0.05	0.30±0.08
18:0	2.78±0.48	4.19±0.65	3.03±0.33
18:1n-7	0.85±0.26	0.46±0.10	0.67±0.27
18:1n-9	11.02±5.54	4.80±1.05	9.36±0.94
18:2n-6	26.03±3.13	21.24±2.27	18.31±1.39
18:3n-3	34.95±7.77	45.96±2.81	37.33±4.46
20:0	1.01±0.17	0.72±0.09	1.10±0.20
20:1n-9	0.12±0.06	0.10±0.03	0.26±0.07
20:3n-3	0.02±0.01	0.12±0.02	0.10±0.12
21:0	0.08±0.03	0.11±0.02	0.10±0.06
22:0	0.97±0.21	0.74±0.12	1.46±0.36
22:1n-7	0.02±0.01	0.02±0.03	0.03±0.03
22:1n-9	0.05±0.04	0.02±0.01	0.03±0.11
24:0	0.87±0.17	0.88±0.14	1.71±0.43
Unknow 1	0.31±0.07	0.37±0.04	0.25±0.06
Unknow 2	0.07±0.02	0.18±0.22	0.05±0.02
Unknow 3	0.05±0.02	0.10±0.05	0.07±0.07
Unknow 4	0.33±0.10	0.38±0.10	0.26±0.06
Unknow 5	0.03±0.01	0.04±0.06	0.04±0.01
Unknow 6	0.16±0.09	0.05±0.09	0.05±0.05
Unknow 7	0.10±0.07	0.34±0.20	0.84±0.30
Unknow 8	0.08±0.04	0.07±0.06	0.16±0.05
Unknow 9	0.98±0.58	0.24±0.14	0.46±0.24
Unknow 10	0.12±0.07	0.43±0.28	0.98±0.36
Unknow 11	0.02±0.01	0.08±0.02	0.08±0.01
Unknow 12	0.17±0.08	0.10±0.02	0.39±0.06
Total SFA	22.01±2.65	22.88±0.94	27.89±3.83
Total MUFA	14.14±5.59	6.95±1.05	12.30±0.87
Total PUFA	61.42±4.99	67.81±1.34	56.19±4.43
Total Unknow	2.43±0.83	2.37±0.59	3.63±0.77

Data are mean±SD (n=3).

**Table S2.** Two-way analysis of variance on rank investigating significant variations of relative growth rate (RGR) ( $\text{mg g day}^{-1}$ ), water content (WC), ( $\%_{\text{FW}}$ ), antioxidant activity index (AAI), total phenolic compound (TPC) content ( $\text{mg}_{\text{GAE}} \text{g}_{\text{DW}}^{-1}$ ), total fatty acid (TFA) content ( $\text{mg g}_{\text{DW}}^{-1}$ ), proportions ( $\%_{\text{TFA}}$ ) of major fatty acids (palmitic acid 16:0, stearic acid 18:0, oleic acid 18:1n-9, linoleic acid 18:2n-6 and  $\alpha$ -linolenic acid 18:3n-3), ratio of polyunsaturated on saturated fatty acids (PUFA:SFA) and ratio of omega 6 on omega 3 fatty acids ( $n - 6:n - 3$ ) in *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa* according to salinity (control, moderate, high), N-ratio ( $\text{NO}_3\text{-N}:\text{NH}_4\text{-N}$  ratio) and Salinity:N-ratio interaction.

		<i>Salsola australis</i>		<i>Suaeda maritima</i>		<i>Enchylaena tomentosa</i>	
		H	p-value	H	p-value	H	p-value
Relative growth rate (RGR)	Salinity	18.5300	0.0001	10.9109	0.0043	0.0048	0.9976
	N-form	6.1665	0.1870	10.1060	0.0387	16.5031	0.0024
	Interaction	8.3778	0.3975	2.4208	0.9653	14.0042	0.0817
Water content	Salinity	11.3985	0.0033	9.17932	0.0102	0.8912	0.6404
	N-form	7.9282	0.0942	4.5130	0.3410	10.1668	0.0377
	Interaction	3.8328	0.8719	7.4274	0.4913	6.2328	0.6212
Antioxidant activity index (AAI)	Salinity	3.5026	0.1735	16.0988	0.0003	7.2381	0.0268
	N-form	7.3686	0.0251	1.6332	0.4419	14.0670	0.0009
	Interaction	10.7478	0.0295	1.9506	0.7448	1.4039	0.8435
Total phenolic compounds	Salinity	5.2310	0.0731	2.6490	0.2660	2.8078	0.2456
	N-form	0.8360	0.6584	6.9876	0.0304	10.6808	0.0048
	Interaction	13.1711	0.0105	6.4374	0.1688	4.0247	0.4027
Total fatty acid (TFA) content	Salinity	12.7050	0.0017	1.6286	0.4429	26.8545	0.0000
	N-form	15.4860	0.0038	21.6953	0.0002	1.4969	0.8272
	Interaction	4.2690	0.8321	4.2200	0.8367	2.7867	0.9470
palmitic acid (16:0) %	Salinity	0.7335	0.6930	5.0790	0.0789	13.8110	0.0010
	N-form	12.3517	0.0149	5.0229	0.2850	8.4522	0.0763
	Interaction	12.7003	0.1226	10.6559	0.2220	9.0296	0.3398
stearic acid (18:0) %	Salinity	7.9034	0.0192	9.3519	0.0093	2.6875	0.2609
	N-form	6.7800	0.1480	3.6032	0.4624	12.7704	0.0124
	Interaction	5.7687	0.6731	6.3608	0.6069	11.7575	0.1623
oleic acid (18:1n-9) %	Salinity	4.0912	0.1293	2.7260	0.2559	7.1196	0.0284
	N-form	5.3630	0.2520	11.0058	0.0265	7.8003	0.0992
	Interaction	11.0328	0.1998	7.7030	0.4630	15.1709	0.0559
linoleic acid (18:2n-6) %	Salinity	20.0510	0.0000	0.0703	0.9654	4.7693	0.0921
	N-form	0.3710	0.9848	12.8953	0.0118	11.4821	0.0216
	Interaction	4.2388	0.8349	5.5309	0.6996	4.6153	0.7978
$\alpha$ -linolenic acid (18:3n-3) %	Salinity	14.8784	0.0006	0.6330	0.7287	20.0263	0.0000
	N-form	0.7330	0.9472	11.7385	0.0194	4.1649	0.3841
	Interaction	8.3306	0.4019	5.4024	0.7138	7.7354	0.4597
PUFA:SFA	Salinity	19.2858	0.0001	5.5845	0.0613	13.5722	0.0011
	N-form	4.4341	0.3504	3.5298	0.4734	5.7417	0.2193
	Interaction	3.3873	0.9078	3.5852	0.8925	6.9238	0.5449
$n - 6:n - 3$	Salinity	18.6597	0.0001	0.0649	0.9681	15.4118	0.0005
	N-form	0.4006	0.9824	13.4232	0.0094	3.9923	0.4071
	Interaction	4.9049	0.7677	4.8433	0.7742	9.0674	0.3366

Data are mean $\pm$ SD (n=3).

**Table S3.** Multiple comparisons of relative growth rate (mg g day<sup>-1</sup>), water content (%<sub>FW</sub>), antioxidant activity index (AAI), total phenolic compound (TPC) content (mg<sub>GAE</sub> g<sub>DW</sub><sup>-1</sup>), total fatty acid (TFA) content (mg g<sub>DW</sub><sup>-1</sup>), proportions (%TFA) of major fatty acids (palmitic acid 16:0, stearic acid 18:0, oleic acid 18:1n-9, linoleic acid 18:2n-6 and α-linolenic acid 18:3n-3), ratio of polyunsaturated on saturated fatty acids (PUFA:SFA) and ratio of omega 6 on omega 3 fatty acids (n - 6:n - 3) in *Salsola australis* between combined treatments of salinity and N-ratio in irrigation water.

<i>Salsola australis</i>	100:0		100:0		75:25		75:25		50:50		50:50		25:75		25:75		0:100		0:100	
	control	moderate	high	moderate	high	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high	
<b>RGR</b>	26.0±3.9	23.5±5.1	3.4±1.6	31.4±5.1	13.8±6.7	13.5±5.1	29.3±3.3	25.6±4.0	11.1±1.8	36.0±5.5	17.9±2.1	8.1±1.1	32.7±4.1	28.3±3.5	21.9±1.6					
<b>Water content</b>	77.6±1.4	70.7±7.6	56.1±2.7	64.4±6.2	64.9±9.8	42.0±3.7	59.4±8.2	62.5±12.9	63.2±5.3	77.4±0.5	68.2±6.2	61.1±4.6	76.1±7.3	77.6±2.0	63.1±9.2					
<b>AAI</b>	0.20±0.01	0.24±0.07	0.35±0.07	N.D	N.D	N.D	0.21±0.04	0.14±0.02	0.11±0.01	N.D	N.D	N.D	0.16±0.04	0.15±0.05	0.50±0.12					
<b>TPC content</b>	30.8±6.9	47.2±2.6	37.9±7.3	N.D	N.D	N.D	40.3±2.5	44.5±9.3	42.6±10.9	N.D	N.D	N.D	32.5±6.9	27.0±2.1	66.7±5.3					
<b>TFA content</b>	15.9±2.4	15.3±0.9	14.4±1.0	15.5±2.7	14.2±0.6	10.9±1.2	14.5±0.5	14.8±0.9	11.0±0.3	13.3±0.9	11.5±4.4	9.9±2.1	11.0±0.7	12.7±0.4	10.4±0.6					
<b>Palmitic acid 16:0</b>	14.3±0.9	14.4±0.2	12.4±0.3	14.4±2	13.9±0.9	15.3±0.4	14.5±0.5	13.7±0.2	14.5±0.4	13.9±0.8	16.2±2.1	15.1±2.3	15.5±0.7	14.8±0.7	18.4±4.7					
<b>Stearic acid 18:0</b>	2.6±0.3	2.4±0.1	2.8±0.1	2.6±0	2.7±0.1	2.6±0.1	2.6±0.1	2.7±0.1	2.9±0	2.7±0.2	2.8±0.8	3±0.3	2.8±0.3	2.7±0.3	3.9±1.2					
<b>Oleic acid 18:1n-9</b>	10.8±5.2	6.8±2.2	23.0±0.3	9.3±10.2	13.5±3.8	9.4±1.2	10.9±3.6	11.8±1.0	12.7±2.9	13.3±5.5	7.4±1.8	12.7±8.5	5.8±1.1	7.6±4.1	10.2±4.7					
<b>Linoleic acid 18:2n-6</b>	23.7±1.6	23.6±1.5	30.1±0.2	24.3±5.1	26.0±2.2	26.2±0.7	24.2±1.8	24.6±1.3	29.2±1.0	24.7±2.1	27.2±6.6	27.7±2.2	24.5±0.4	24.1±1.7	30.4±0.9					
<b>α-linolenic acid 18:3n-3</b>	38.9±4.8	43.0±3.6	22.4±0.6	39.5±12.6	33.6±4.7	35.7±1.5	38.1±4.9	37.2±2.4	30.5±3.0	34.7±7.1	34.9±12.6	30.1±7.1	41.0±0.4	40.0±4.6	24.6±7.5					
<b>n - 6:n - 3</b>	0.61±0.06	0.55±0.05	1.33±0.02	0.70±0.24	0.78±0.10	0.71±0.02	0.64±0.07	0.66±0.05	0.96±0.07	0.73±0.11	0.92±0.37	0.96±0.20	0.59±0.01	0.60±0.06	1.06±0.13					
<b>PUFA:SFA</b>	3.00±0.18	3.20±0.18	2.74±0.09	3.00±0.19	2.87±0.03	2.80±0.12	2.94±0.09	3.03±0.08	2.74±0.07	2.83±0.19	2.68±0.60	2.56±0.23	2.89±0.12	2.95±0.18	2.43±0.09					

Data are mean±SD (n=3). N.D = no data

**Table S4.** Multiple comparisons of relative growth rate ( $\text{mg g day}^{-1}$ ), water content ( $\%_{\text{ofW}}$ ), antioxidant activity index (AAI), total phenolic compound (TPC) content ( $\text{mg}_{\text{GAE}} \text{g}_{\text{DW}}^{-1}$ ), total fatty acid (TFA) content ( $\text{mg g}_{\text{DW}}^{-1}$ ), proportions ( $\%_{\text{TFA}}$ ) of major fatty acids (palmitic acid 16:0, stearic acid 18:0, oleic acid 18:1n-9, linoleic acid 18:2n-6 and  $\alpha$ -linolenic acid 18:3n-3), ratio of polyunsaturated on saturated fatty acids (PUFA:SFA) and ratio of omega 3 fatty acids ( $n - 6:n - 3$ ) in *Suaeda maritima* between combined treatments of salinity and N-ratio in irrigation water.

<i>Suaeda maritima</i>	100:0		100:0		75:25		75:25		50:50		50:50		25:75		25:75		0:100		0:100	
	control	moderate	high	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high
<b>RGR</b>	22.4±5.7	20.1±2.2	17.4±4.8	25.9±2.9	20.1±3.3	20.0±2.5	33.4±6.4	23.6±2.0	20.9±0.8	30.2±7.9	26.7±4.1	19.1±3.1	9.2±1.8	10.2±3.3	13.3±2.0					
<b>Water content</b>	64.5±21.6	74.0±3.6	67.1±6.0	69.3±14.5	73.0±8.1	73.0±5.0	75.2±1.8	66.8±7.4	71.4±0.4	79.2±6.0	74.5±2.3	73.1±2.6	80.3±1.6	77.7±1.7	67.0±7.0					
<b>AAI</b>	0.21±0.03	0.70±0.36	0.77±0.18	N.D	N.D	N.D	0.46±0.21	0.60±0.05	0.90±0.10	N.D	N.D	N.D	0.33±0.18	0.83±0.28	1.11±0.16					
<b>TPC content</b>	53.8±4.4	69.2±2.8	65.3±5.5	N.D	N.D	N.D	56.4±8.2	46.2±3.0	54.7±4.1	N.D	N.D	N.D	52.8±4.7	55.9±12.3	65.8±9.5					
<b>TFA content</b>	13.5±0.9	14.5±2.2	12.6±0.9	16.6±1.2	14.2±2.4	13.7±1.8	17.9±0.6	17.5±1.9	18.4±1.0	18.2±1.3	19.0±2.5	15.8±2.6	16.8±2.9	15.8±0.6	16.1±2.2					
<b>Palmitic acid 16:0</b>	15.0±0.6	14.2±0.5	15.0±0.3	14.1±0.6	14.3±0.3	14.3±0.7	15.1±0.8	14.5±1.5	15.1±0.6	13.9±0.5	14.3±0.6	15.5±0.1	14.8±0.7	14.1±0.6	14.1±0.3					
<b>Stearic acid 18:0</b>	3.4±0.3	4.1±0.9	4.8±0.1	4.2±0.3	3.9±0.6	4.3±0.6	3.7±0.4	4.0±0.5	4.4±1.0	4.3±0.9	3.7±0.7	4.6±0.7	4.1±0.7	4.6±0.2	4.9±0.3					
<b>Oleic acid 18:1n-9</b>	6.0±0.6	5.5±1.1	4.7±0.4	5.7±1.8	4.9±0.5	5.2±0.1	4.0±0.8	3.8±0.5	4.7±0.7	5.7±1.8	4.7±0.6	3.9±0.7	5.1±1.0	4.1±0.1	4.0±1.3					
<b>Linoleic acid 18:2n-6</b>	20.5±1.1	21.6±0.4	20.7±0.8	20.6±1.3	19.8±1.4	22.9±4.2	19.2±1.0	20.6±1.4	19.4±0.9	21.2±2.1	20.8±1.9	20.3±0.3	25.3±2.7	23.1±3.0	22.7±2.4					
<b><math>\alpha</math>-linolenic acid 18:3n-3</b>	44.9±1.9	45.2±0.8	46.0±0.7	46.6±2.2	48.1±1.5	44.7±4.7	48.8±1.6	47.2±3.0	47.8±2.8	46.1±3.1	47.6±2.3	45.6±2.9	41.2±1.9	44.7±2.0	45.0±4.1					
<b>n - 6:n - 3</b>	0.45±0.04	0.47±0.01	0.44±0.02	0.44±0.05	0.41±0.04	0.52±0.15	0.39±0.03	0.43±0.05	0.40±0.04	0.46±0.07	0.43±0.06	0.44±0.03	0.61±0.09	0.51±0.09	0.50±0.09					
<b>PUFA:SFA</b>	2.89±0.18	2.99±0.09	2.80±0.05	3.00±0.04	3.04±0.08	3.05±0.17	3.01±0.23	3.02±0.33	2.91±0.24	3.02±0.12	3.09±0.07	2.81±0.18	2.99±0.23	3.00±0.11	2.93±0.12					

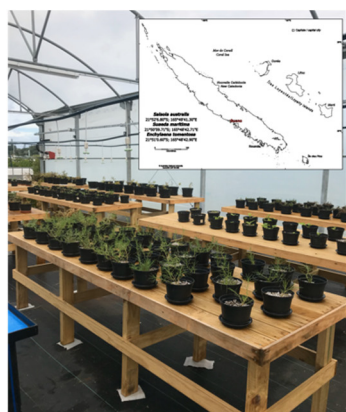
Data are mean±SD (n=3). N.D = no data

**Table S5.** Multiple comparisons of relative growth rate (mg g day<sup>-1</sup>), water content (%FW), antioxidant activity index (AAI), total phenolic compound (TPC) content (mg<sub>GAE</sub> g<sub>DW</sub><sup>-1</sup>), total fatty acid (TFA) content (mg g<sub>ow</sub><sup>-1</sup>), proportions (%TFA) of major fatty acids (palmitic acid 16:0, stearic acid 18:0, oleic acid 18:1n-9, linoleic acid 18:2n-6 and α-linolenic acid 18:3n-3), ratio of polyunsaturated on saturated fatty acids (PUFA:SFA) and ratio of omega 3 fatty acids (n - 6:n - 3) in *Enchylaena tomentosa* between combined treatments of salinity and N-ratio in irrigation water

<i>Enchylaena tomentosa</i>	100:0		100:0		75:25		75:25		50:50		50:50		25:75		25:75		0:100		0:100			
	control	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high	control	moderate	high	
<b>RGR</b>	9.2±1.6	6.1±1.7	5.6±2.7	8.9±1.0	7.0±4.2	9.0±1.2	13.6±4.2	12.7±2.0	8.5±1.9	9.1±1.4	10.7±1.5	18.3±2.9	7.5±2.3	14.3±0.7	12.7±3.2							
<b>Water content</b>	46.5±11.5	41.6±25.0	46.6±13.3	39.6±9.6	22.3±18.5	18.0±11.4	50.4±2.1	40.0±14.3	43.9±6.1	41.8±3.8	39.6±15.7	45.9±4.2	43.6±3.1	46.7±2.0	45.2±1.4							
<b>AAI</b>	0.28±0.11	0.49±0.02	0.24±0.04	N.D	N.D	N.D	0.64±0.07	0.81±0.15	0.48±0.02	N.D	N.D	N.D	0.76±0.22	1.36±0.06	0.53±0.11							
<b>TPC content</b>	40.8±6.1	70.7±15.1	47.5±6.2	N.D	N.D	N.D	74.8±3.7	68.7±21.1	57.1±5.3	N.D	N.D	N.D	87.4±4.4	103.6±23.1	72.5±24.3							
<b>TFA content</b>	10.4±1.6	8.3±0.8	7.1±0.4	11.5±2.7	9.8±1.0	6.6±0.5	7.5±0.4	9.2±1.0	5.7±0.3	11.1±1.3	8.0±3.8	7.4±0.3	9.9±1.2	9.4±0.8	6.5±0.2							
<b>Palmitic acid 16:0</b>	16.0±1.1	16.3±1.3	18.3±0.5	17.9±1.0	15.7±0.5	19.0±1.2	17.6±0.5	16.7±0.3	19.4±0.1	14.9±0.5	16.7±1.8	16.9±0.3	15.8±0.8	16.6±0.7	17.6±0.9							
<b>Stearic acid 18:0</b>	2.9±0.3	3.4±0.1	3.1±0.3	3.2±0.3	3.0±0.2	3.2±0.0	3.4±0.2	2.8±0.1	3.5±0.0	2.8±0.2	3.0±0.6	3.0±0.0	2.6±0.2	2.7±0.2	2.9±0.0							
<b>Oleic acid 18:1n-9</b>	8.8±0.3	9.3±0.3	9.0±0.8	9.6±0.1	8.5±0.3	8.4±0.5	8.4±0.4	9.1±0.1	9.6±0.1	9.7±0.4	8.8±0.0	10.8±0.8	9.4±0.4	9.0±0.1	11.7±0.8							
<b>Linoleic acid 18:2n-6</b>	17.5±0.8	18.1±1.6	18.0±1.2	16.8±1.5	18.3±0.4	18.2±0.9	18.3±0.4	18.2±0.3	18.5±0.3	17.0±0.1	17.9±1.2	18.2±1.0	18.4±1.4	19.6±0.8	21.7±0.9							
<b>α-linolenic acid 18:3n-3</b>	40.7±2.5	36.8±2.0	33.4±2.1	34.9±3.4	40.6±1.9	32.9±1.9	35.4±0.5	39.9±1.3	30.2±0.2	43.0±2.1	39.8±6.2	37.3±0.9	42.8±2.8	39.3±2.5	32.3±2.0							
<b>n - 6:n - 3</b>	0.43±0.01	0.49±0.02	0.53±0.01	0.48±0.04	0.45±0.03	0.55±0.01	0.51±0.01	0.45±0.02	0.60±0.01	0.40±0.03	0.45±0.04	0.49±0.04	0.43±0.06	0.50±0.05	0.67±0.06							
<b>PUFA:SFA</b>	2.36±0.43	1.99±0.38	1.64±0.23	1.67±0.39	2.32±0.14	1.64±0.24	1.80±0.08	2.31±0.08	1.50±0.02	2.45±0.28	2.25±0.69	2.05±0.11	2.63±0.25	2.28±0.24	2.02±0.17							

Data are mean±SD (n=3). N.D = no data

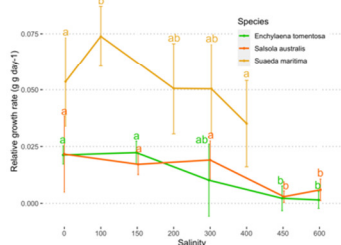
### Artworks - Supporting Figures



Collect and cultivation of mother plants

June 2018 - today

- Freshwater nebulization 3 time / day
- Saline irrigation 2 time / week
- NPK fertilization 2 time / month



Preliminary test on mother plants

July-August 2018

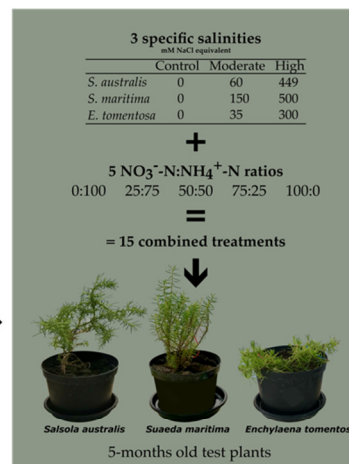
- Irrigation according to salt gradients (1 month)
- Relative growth rate measurements
- selection of "moderate" or "high" thresholds of saline application for future tests



Multiplication by germination

February 2019

- 4 months of cultivation under same design as mother plants
- Followed by 1 month of standardization of conditions (exclusive freshwater) for the 45 test seedlings / species



Experiment

July-August 2019 (1 month)

**Figure S1.** Experimental design of combined salinity and nitrogen ratio experiment on halophytes *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa*.