

## 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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**Abstract:** Cultivation of salt-tolerant crops help to face to irreversible global salinization of freshwater and soils. In New-Caledonia, three halophytes are candidates for saline crops, *Salsola australis* R.Br., *Suaeda maritima* (L.) Dumort and *Enchylaena tomentosa* R.Br. Their success and quality depend yet on availabilities of salinity and essential nutrients in agrosystems. So, we investigated effects of three salinities, i.e., control moderate and high, and five nitrogen ratios, i.e., 100:0, 75:25, 50:50, 25:75 or 0:100 NO<sub>3</sub><sup>-</sup>-N:NH<sub>4</sub><sup>+</sup>-N ratio on their growth and functional value for fatty acids and antioxidants. Results show that the leaf fatty acid and antioxidant profiles of species, emphasize their good potential to become functional crop products, based on comparison with other functional plants, dietetic recommendation, or functional indices. However, their total phenolic compounds (TPC) content can be influenced by N-ratio (*Suaeda maritima* and *Enchylaena tomentosa*) and their antioxidant activity index (AAI) can be influenced by salinity (*Suaeda maritima*), N-ratio (*Salsola australis*) or both (*Enchylaena tomentosa*). Their quantitative and/or qualitative fatty acid profiles can also be influenced by salinity (*Enchylaena tomentosa*), N-ratio (*Suaeda maritima*), or both (*Salsola australis*). Regarding these variations, involving salt tolerance and nitrogen nutrition mechanisms, we recommend suitable treatments to maintain or optimize the growth and the functional quality of leaves in the three species.

**Keywords:** halophyte; *Salsola australis*; *Suaeda maritima*; *Enchylaena tomentosa*; salt tolerance; nitrogen form; fatty acid; phenolic compounds; antioxidant activity

## 1. Introduction

The salinization of land is a serious environmental problem limiting productivity and sustainability of crops worldwide [1,2]. Halophytes are cosmopolitan plants capable of ensuring their complete life cycle under salinity of 200 mM NaCl or more. To overcome salt and take advantage of it, they use complex and multigene salt tolerance process [1,3].

Thus, promoting biosaline agriculture by cultivating halophytes appears as a judicious opportunity to valorize salted soils and sustainably diversify food production.

Based on intra-genera potentials, three halophytes from *Amaranthaceae* family drew attention to test their saline crop potential in New Caledonia: *Salsola australis* R.Br. (APNI), *Suaeda maritima* (L.) Dumort and *Enchylaena tomentosa* R.Br. (APNI). In fact, the leaf extracts of *Salsola* and *Suaeda* sp. are promising source of antioxidant and antimicrobial agents [4–6]. Many *Suaeda* sp., are also promising oilseed crops with high polyunsaturated fatty acids contents [7]. Forage and seed products from *Suaeda* sp. and *Enchylaena tomentosa* have also demonstrated potential to replace traditional ingredients in domestic animal diets [7,8]. Moreover, the salty taste and fleshy texture of species' leaves (for *Salsola* species, this is the case only for the young leaves) adds also food value to them for human consumption. *Salsola soda* species is notably cultivated in Italy as a functional vegetable [9] and young shoots of *Suaeda* species are widely consumed worldwide [10].

The domestication of wild halophytic plant species into viable crop has yet to be undertaken with studies on their biological variations according to biological factors as genotype, organ and ontogeny and environmental factors as salinity, nutrient and water availability, temperature or light intensity [2,6]. The primary reason for changes in biochemical composition of halophytes' tissues is to combat salt stress and result often in a qualitative improvement of functional value [11–13]. As salinity is perceived as oxidative stress in many plants [1,3], halophytes have generally very effective antioxidant systems to counteract this [3,6,14]. So, there is an increasing interest to identify among halophyte species those with high antioxidant content for their use in the agri-food industry and/or pharmaceutical and cosmetic applications. Among natural antioxidants extracted from plants, phenolic compounds, which are widely distributed, exhibit a broad spectrum of antioxidant activity and medicinal properties and are used in many applications [6,15,16]. In addition, it is widely accepted that the increase of fatty acid (FA) unsaturation level and balance between linoleic acid (18:2n – 6) and  $\alpha$ -linolenic acid (18:3n – 3) proportions in cell membranes participate to salt resistance in halophytes [12,13,17]. Such changes interfere with ion uptake and sequestration, participate in the prevention of photosynthetic damage and hinder the loss of water, nutrient or osmolytes. Besides, the use of an appropriate nitrogen form is generally beneficial for halophyte plant growth and functional value [18–21]. In high saline environments, i.e., 300–500 mM NaCl, photosynthate biosynthesis is reduced, thus the type of transportable nitrogen compounds is critical for the achievement of energetic demand in plants [20,22–24].

Given this, we hypothesize that the crop potential of our three candidate species can be also modulated by main cultural parameters as salinity and nitrogen form in irrigation. However, hardly any information is available on the effects of both factors, individual or combine, on the growth or biochemical composition of aerial tissues belonging to the three genera. We know that *Salsola* species are drought shrubs with optimum growth at low or moderate salinities (70–150 mM NaCl) [25,26], adopting frequently C4 photosynthesis [27], which we suppose be an adaptation to reduce water loss in dry or saline lands. Salinity is further known to influence growth and water relation and induce antioxidant activity in *Salsola crassa* [28]. Regarding *Suaeda* species, we know that they are coastal halophyte shrubs, whose succulent leaves accumulate large amounts of ions, showing typical Cl<sup>-</sup> excluders or salt-includers [29]. Their growths are often reported as not affected or stimulated by high salinities (300–500 mM NaCl equivalent) [30] but also influenced by NO<sub>3</sub><sup>-</sup>-N amount in media [31,32]. A significant positive correlation is further known between salinity, total phenolic compound content and capacity of *Suaeda maritima* to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and O<sub>2</sub>•-radicals [14]. In addition, salinity is also known to influence FA profiles of *Suaeda* species [13,17] as in *Suaeda salsa* where saline irrigation at 300 mM NaCl increased plant growth and unsaturation degree in major leaf membrane lipids. Finally, *Enchylaena tomentosa* is a drought-hardy shrub reported to tolerate low to moderate salinities (equivalent 25 to 300 mM NaCl) [33,34].

Face to agronomic aim, it thus appears critical to determine demonstrators of crop potential in the three candidate species as well as their specific relationships with external constraints. So, the aim of the study was first to determine a part of this demonstrator through evaluation of growth and specific functional values of edible tissues, i.e., for fatty acids and antioxidants. Secondly, it was to evaluate the effects of two main irrigation parameters, salinity and nitrogen ratio in irrigation, on these demonstrators. Based on salt tolerance and nitrogen nutrition, this allows recommending experimental treatments to maintain or optimize the growth and functional quality of leaves in the three species.

## 2. Materials and Methods

### 2.1. Reagents

All chemicals used were of analytical grade. Magnesium sulfate, potassium nitrate, ammonium dihydrogen phosphate, calcium nitrate, potassium dihydrogen phosphate, potassium chloride, ammonium chloride, calcium chloride used for the preparation of nutrient saline solutions were purchased from Merck KGaA® (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH•) and standard solutions of gallic acid and ascorbic acid used in DPPH• free radical scavenging activity for evaluation of antioxidant activity were purchased from Merck KGaA®. Standard solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) used in the same essay was purchased from Thermo Fisher Scientific® (Waltham, MA, USA). Solvents used for extraction of hydrophilic antioxidants (ethanol) or fatty acids (chloroform and methanol) as well as sulfuric acid used for esterification of fatty acids were purchased from Merck KGaA®. Sodium carbonate used in the Folin–Ciocalteu assay for the dosage of total phenolic compounds (TPC) was purchased from Merck KGaA®.

### 2.2. Seedling Collect, Plant Cultivation and Multiplication

Mother plants were collected on west coast of New Caledonia in June 2018. *Salsola australis* (S21°52'12.0" E165°49'39.5"; altitude = 9.14 m) was collected in St-Sauveur beach in Ouano. *Suaeda maritima* (S21°51'00.3" E165°48'43.8"; altitude = 3.96 m) and *Enchylaena tomentosa* (S21°51'00.6" E165°48'42.9"; altitude = 4.26 m) were collected on a "tanne" (salt-pan back to the mangrove) in Ouano. They were potted and grown with a substrate containing natural soil, sand, potting soil, vermiculite and perlite 30:25:25:10:10 (v:v:v:v:v). They were kept in a greenhouse equipped with a shade house, with thrice-daily freshwater irrigation completed with bi-weekly saline irrigation, i.e., 200–400 mL of 50% seawater and bi-monthly 1.5% (m:v) Welgro® fertilization, i.e., 17:30:15 N:P:K added of Fe, Mn, Zn, B, Bo microelements. Multiplication by germination was conducted in a seeding cup filled with a 1:1 (v:v) mixture of sand and potting soil. Daughter seedlings were conditioned under the same condition as adult plants for 4 months. For experiments, 45 seedlings were randomly selected from the same generation and irrigated exclusively with freshwater nebulization to standardize conditions, Figure S1.

### 2.3. Combined Salinity and Nitrogen Ratio Experiment

Experiment design. Preliminary tests were conducted on mother plants to determine the best growth rates in response to a salt gradient for the three species. According to these results, specific values (in mM NaCl equivalent) of "moderate" and "high" salinities to apply to each species were chosen, Table 1a. For the experiment, 5-months old seedlings were watered with saline irrigation according to these salinities, i.e., made by dilutions of seawater, and five nitrogen ratios (N-ratio) of nitrate to ammonium  $\text{NO}_3^-$ -N: $\text{NH}_4^+$ -N, i.e., 0:100, 25:75, 50:50, 75:25 and 100:0, Table 1b. Then, fifteen conditions were tested for each species on three individuals, Figure S1. Irrigations were carried out over a month, three times a week with a volume corresponding to 50% (*Salsola australis* and *Enchylaena tomentosa*) or 75% (*Suaeda maritima*) of substrate retention capacity (182.1 mL) [35].

**Table 1.** Salinity and NO<sub>3</sub><sup>-</sup>-N:NH<sub>4</sub><sup>+</sup>-N ratio used in combined irrigations (a) Specific salinities (mM NaCl equivalent) for *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa*. (b) Macronutrient compositions (mM) in the five N-ratios (NO<sub>3</sub><sup>-</sup>-N:NH<sub>4</sub><sup>+</sup>-N).

	(a) Salinity			(b) NO <sub>3</sub> <sup>-</sup> -N:NH <sub>4</sub> <sup>+</sup> -N				
	Control	Moderate	High	0:100	25:75	50:50	75:25	100:0
<i>S. australis</i>	0	60	449					
<i>S. maritima</i>	0	150	500					
<i>E. tomentosa</i>	0	35	300					
Ca(NO <sub>3</sub> ) <sub>2</sub>	0.0	1.8	3.6	2.7	4.3			
KNO <sub>3</sub>	0.0	0.0	0.0	5.4	5.7			
MgSO <sub>4</sub>	2.0	2.0	2.0	2.0	2.0			
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>	1.0	1.0	1.0	1.0	0.0			
KH <sub>2</sub> PO <sub>4</sub>	0.0	0.0	0.0	0.0	1.0			
KCl	7.7	7.7	7.7	2.3	1.0			
NH <sub>4</sub> Cl	13.0	9.7	6.1	2.5	0.0			
CaCl <sub>2</sub>	4.3	2.4	0.7	1.5	0.0			

Experimental measurements. Experiments were conducted between June and July 2019, i.e., during austral winter. Atmospheric parameters were recorded every ten minutes by a weather station (HOBO 0664 H21-USB, ONSET®, Cape Cod, Massachusetts, USA) equipped with two external sensors of temperature and relative humidity (HOBO0338 S-THB-M002) and two external sensors of photosynthetic active radiations (HOBO0042 S-LIA-M00). The mean daily temperature was 18.6 ± 2.1 °C during nighttime and 26.1 ± 5.9 °C during the daytime, the mean relative humidity was 91.3 ± 5.7% and 69.4 ± 18.4% during nighttime and daytime, respectively, and the mean photosynthetic active radiation between 11 am and 1 pm was 639.6 ± 314.2 μmol m<sup>-2</sup> s<sup>-1</sup> day. The salinity of nitrogen solutions was checked by a refractometer (TM Hand Held Refractometer, ATAGO CO.LTD., Tokyo, Japan) and the salinity of the percolation water was evaluated weekly using the refractometer.

Growth evaluation. Five secondary shoots of each individual were harvested (from the node), dried and weighed at the beginning (t1) and at the end (t2) of the experiment. Relative growth rates (RGR) were determined on a dry mass basis (1) [36].

$$\text{Relative growth rate} = \frac{\ln W2 - \ln W1}{t2 - t1} \quad (1)$$

where *W1* and *W2* are means of five shoots dry weight at times *t1* and *t2* respectively.

Harvest and sample preparation of leaves. After experimentation, leaves, i.e., edible tissues were harvested, frozen at -20 °C and lyophilized (lyophilizer alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen®, Osterode am Harz, Deutschland). Leaves were then milled using a rotor laboratory mill for biochemicals analyzes. Plant fresh weights (FW) and dry weights (DW) were determined before and after lyophilization to assess the plant water content. It allows for expressing biochemical parameters on a fresh weight basis.

#### 2.4. Determination of Radical Scavenging Activity and Total Phenolic Compound Contents

Extraction procedure. Hydrophilic antioxidants were extracted from 500 mg of dried leaves according to adapted method from [37] by addition of 6.25mL of water:ethanol mixture (1:1, *v:v*) in a dark recipe (50 mL), taking into account that ethanol and water are food grade and environmentally friendly solvents [38,39] showing molecular affinity and

good mass transfer for phenolic compounds [38,40]. After agitation (1 h, 40 °C) and centrifugation (15 min, 40 °C, 3000× g), the supernatant was filtered using cellulose nitrate filters (Merck KGaA®). Three cycles of pellet exhaustion were carried out in this way. Three filtrates were mixed in a 50 mL dark balloon previously weighed. Then, ethanol was removed in a rotary evaporator (Hei-VAP Core HL, Heidolph Instruments®, Kelheim, Deutschland) at 35 °C, with regular weightings until that mass reached less than 4.5g, i.e., total evaporation of ethanol. The crude aqueous extract obtained was aliquoted in wrapped Eppendorf® (Hambourg, Deutschland) tubes, frozen and lyophilized (lyophilizer alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen®). Powder extracts were weighted to determine extraction yields and were used to evaluate antioxidant activities and phenolic compound content. For each sample, a triplicate of extraction was carried out.

DPPH• free radical scavenging activity. It was evaluated by spectrophotometric measurement of their ability to scavenge DPPH• free radicals [41,42] following a method adapted from [37]. Despite the many modes of action of antioxidants, the determination of free DPPH• radical scavenging activity was often recommended for samples containing compounds with SH-, NH- and OH- chemical groups [42] as phenolic compounds [39]. Our assay was done on 96-well-plates (Greiner bio-one®, Kremsmünster, Austria) from powder extracts solubilized in distilled water to form a 1.2 g L<sup>-1</sup> stock solution. Eight aqueous dilutions of stock solution (9.4–1200 mg L<sup>-1</sup>) were made in triplicate in microplate with a final volume of 100 µL. Eight dilutions (2.1–400 mg L<sup>-1</sup>) of three standard solutions of gallic acid, ascorbic acid and trolox were made in the same way. The 100 µM DPPH• solutions (3.94 mg of DPPH• radical (Merck KGaA®) in 100 mL of ethanol), were made daily as well as the control of their stability and linearity ranges. Tests were performed by addition of 80 µL of the DPPH• solution to the 100 µL of the sample (stock solution, positive standard or aqueous blank), incubation (dark, 60 min, 23 °C) and reading of absorbance at 515 nm (MultiskanMS, Thermo Fisher Scientific®, Waltham, Massachusetts, USA). For each sample, radical scavenging activity was expressed as the percent of inhibition (2).

$$\text{Percent of inhibition} = \left[ \frac{(A_0 - A_t)}{A_0} \right] \times 100 \quad (2)$$

where  $A_0$  and  $A_t$  are the absorbance values in the absence (blank) or presence (extract or standard) of antioxidants, respectively. The antioxidant concentration necessary to decrease initial DPPH• concentration by 50% (IC<sub>50</sub>) was calculated graphically using a calibration curve in the linear range plotting the I% against the sample concentration. Antioxidant activity was expressed as antioxidant activity index (AAI), to overcome methodology variability related to the concentrations of DPPH• and samples used (3) [43].

$$\text{Antioxidant activity index} = \frac{\text{final concentration of DPPH} \cdot (\text{mg L}^{-1})}{\text{IC}_{50} (\text{mg L}^{-1})} \quad (3)$$

Total phenolic compounds (TPC) content. It was determined according to the Folin-Ciocalteu method [44] adapted from [37] using gallic acid as a standard. Five dilutions of standard (10–500 mg L<sup>-1</sup>) were made in triplicate in microplate from 500 mg L<sup>-1</sup> stock solution and with a final volume of 20 µL. Tests were performed by addition of 130 µL of distilled water, 10 µL of Folin-Ciocalteu reagent, and 40 µL of sodium carbonate solution (200 g L<sup>-1</sup>) to 20 µL of the sample (stock solution, positive standard or aqueous blank), incubation (dark, 10 min, 70 °C), cooling (ice) and reading of absorbance at 765 nm (MultiskanMS, Thermo Fisher Scientific®, Waltham, Massachusetts, USA). Total phenolic compound content was determined using a standard curve and expressed in milligram of gallic acid equivalent (GAE) per gram of dry weight thanks to extraction yields. The productivities of the three species in TPC were estimated thanks to the factor of TPC fresh contents (mg<sub>GAE</sub> g<sub>FW</sub><sup>-1</sup>) by relative growth rate (mg g<sup>-1</sup> day<sup>-1</sup>).

## 2.5. Determination of Fatty Acid Profiles

Lipids were extracted according to Folch et al.'s (1957) modified method [45] from 50 mg of dried leaves or leafless shoots, by addition of 6 mL of chloroform:methanol mixture (2:1, *v:v*). After agitation and centrifugation (15 min, ambient T°C, 3000× *g*), 1 mL of supernatant corresponding to total lipid fraction (neutral and polar lipids) was added to a known amount (20 µL) of C23:0 (0.115 µg µL<sup>-1</sup>) used as an internal standard for quantitative determinations. The C:Xn-Y notation was adopted for FA notation where C was the number of carbons, X the number of double bonds and n-Y the position of the first double bond from the terminal methyl group. Total lipids were then vacuum dried under N<sub>2</sub> for 2 h and then directly trans esterified with 800 µL of sulfuric acid:methanol (3.4% *v:v*) for 10 min at 100 °C. After cooling, fatty acid methyl esters were extracted by the addition of 800 µL of hexane. This organic fraction was washed three times with 1.5 mL of hexane saturated water. Then, it was recovered and analyzed in a gas chromatograph (CP 8400, VARIAN®) equipped with a flame ionization detector (GC-FID CP-3800, Agilent Technologies, Inc., Santa Clara, California, USA). Fatty acid methyl esters were analyzed using polar and non-polar capillary columns, temperature-programmed at 220 °C with hydrogen as the carrier gas. A check on outputs of two columns helps to identify co-eluted compounds [46]. Peak integrations and calculations of Fatty acid methyl esters were done with the software program Galaxie 1.9.3.2 (Agilent Technologies, Inc., Santa Clara, California, USA). They were identified by comparison of their retention time with those of standards and they were quantified by comparison of their peak areas to the peak area of internal standard C23:0. Contents were added to determine the total fatty acid (TFA) content.

The intakes of fresh leaves (g day<sup>-1</sup>) necessary to achieve the adequate intake indexes for men and women [47] of  $\alpha$ -linolenic acid were estimated by the conversion of  $\alpha$ -linolenic acid proportion on a quantitative fresh basis (thanks to TFA and water contents) and their ratio with the reference adequate intake indexes. The productivities in TFA and  $\alpha$ -linolenic acid were also estimated thanks to a factor of TFA or  $\alpha$ -linolenic acid fresh contents (mg g<sup>FW-1</sup>) by the relative growth rate (mg g<sup>-1</sup> day<sup>-1</sup>).

## 2.6. Statistical Analysis

All statistical analyzes were performed with R software [48]. Significance levels were set at  $p < 0.05$ . A regression analysis was performed after verification of prerequisites to explore the relation between AAI and TPC content. Then, a correlation analysis by Pearson's method [49] was performed to evaluate the strength of this relation. For comparison between treatments, nonparametric statistics were used due to a low amount of data per condition. First, a two-way ANOVA on rank [50] was performed to investigate significant variations of biological variables according to salinity, N-ratio and the interaction of salinity and N-ratio. The five N-ratios were compared for fatty acid variables and three N-ratios (100:0, 50:50 and 0:100) were compared for antioxidant variables. Next, a one-way Kruskal-Wallis Rank Sum Test [51] and multiple comparisons via post hoc Dunn tests [52], with adjustment of Holm  $p$ -value [53] were performed on individual factors showing significant effects. A multiple comparisons with the same one-way statistical approach was also carried out on the variable corresponding to the combined treatments. We focus description on the effect of salinities versus control for each N-ratio and then on the effects of N-ratio at high salinity.

## 3. Results

### 3.1. Mean Growth and Functional Values for Fatty Acids and Antioxidants

Mean RGR is  $21.7 \pm 10.2$  mg g<sup>-1</sup> day<sup>-1</sup> (ranging from  $3.4 \pm 1.6$  to  $36.0 \pm 5.4$  mg g<sup>-1</sup> day<sup>-1</sup>) for *Salsola australis*,  $21.3 \pm 7.1$  mg g<sup>-1</sup> day<sup>-1</sup> (ranging from  $9.2 \pm 1.8$  to  $33.4 \pm 6.4$  mg g<sup>-1</sup> day<sup>-1</sup>) for *Suaeda maritima* and  $10.1 \pm 3.9$  mg g<sup>-1</sup> day<sup>-1</sup> (ranging from  $5.6 \pm 2.7$  to  $18.3 \pm 2.8$  mg g<sup>-1</sup> day<sup>-1</sup>) for *Enchylaena tomentosa*.

Mean water contents are  $65.4 \pm 13.3\%$  of fresh weight (%FW) in *Salsola australis*,  $72.4 \pm 8.1\%$ FW in *Suaeda maritima* and  $40.6 \pm 13.1\%$ FW in *Enchylaena tomentosa*.

Mean AAI are  $0.23 \pm 0.13$  in *Salsola australis*,  $0.66 \pm 0.32$  in *Suaeda maritima* and  $0.62 \pm 0.34$  in *Enchylaena tomentosa*. All antioxidant activities are lower than for the positive controls that are gallic acid (AAI =  $23.8 \pm 1.1$ ), ascorbic acid (AAI =  $18.5 \pm 0.7$ ) and trolox (AAI =  $16.4 \pm 0.3$ ). Mean TPC contents are  $41.1 \pm 12.6 \text{ mg}_{\text{GAE}} \text{ g}_{\text{DW}}^{-1}$  in *Salsola australis*,  $57.8 \pm 9.1 \text{ mg}_{\text{GAE}} \text{ g}_{\text{DW}}^{-1}$  in *Suaeda maritima* and  $69.0 \pm 22.6 \text{ mg}_{\text{GAE}} \text{ g}_{\text{DW}}^{-1}$  in *Enchylaena tomentosa*. Regression-correlation analysis finds significant linear dependence between AAI and TPC content in *Salsola australis* ( $R^2 = 0.54$ ,  $p < 0.01$ ,  $y = 53.2x + 28.9$ ), *Enchylaena tomentosa* ( $R^2 = 0.72$ ,  $p < 0.001$ ,  $y = 47.7x + 39.5$ ) and in *Suaeda maritima* ( $R^2 = 0.39$ ,  $p < 0.05$ ,  $y = 11x + 50.6$ ).

Up to 36 FA are identified including common wide-spread FA, i.e., with a number of carbon atoms in FA chain  $\leq 18$  and "unusual" FA ( $n = 17$ ) in low proportions representing very long-chain fatty acids, i.e., with a number of carbon atoms in FA chain  $\geq 18$  and a higher retention time of their esters, Table S1. A focus is made on common FA whose sum represent more than 80% of TFA content (%TFA), i.e., 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), Table 2. The main classes of FA are polyunsaturated fatty acids (PUFA) including linoleic acid and  $\alpha$ -linolenic acid, mono unsaturated fatty acids (MUFA) including oleic acid and saturated fatty acids (SFA) including palmitic acid stearic acid. The predominant PUFA class represent  $56.1 \pm 4.4$  to  $67.8 \pm 1.3\%$  of TFA, and contain mainly linoleic acid and  $\alpha$ -linolenic acid. Mean PUFA:SFA ratios are  $2.84 \pm 0.26$  in *Salsola australis*,  $2.97 \pm 0.16$  in *Suaeda maritima* and  $2.07 \pm 0.42$  in *Enchylaena tomentosa* and mean n - 6:n - 3 ratios are  $0.78 \pm 0.29$  in *Salsola australis*,  $0.46 \pm 0.08$  in *Suaeda maritima* and  $0.50 \pm 0.08$  in *Enchylaena tomentosa*.

**Table 2.** Mean total fatty acid (TFA) content ( $\text{mg g}_{\text{DW}}^{-1}$ ) and 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  $\alpha$ -linolenic acid 18:3n - 3) and of fatty acids classes (saturated SFA, monounsaturated MUFA, polyunsaturated PUFA and unknown) in leaves of the three species, on all treatments.

	16:0	18:0	18:1n - 9	18:2n - 6	18:3n - 3	SFA	MUFA	PUFA	Unknown	TFA
<i>S. australis</i>	$14.8 \pm 1.9$	$2.8 \pm 0.5$	$11.0 \pm 5.5$	$26.0 \pm 3.1$	$34.9 \pm 7.8$	$21.8 \pm 2.0$	$14.1 \pm 5.6$	$61.8 \pm 4.6$	$2.4 \pm 0.8$	$13.0 \pm 2.5$
<i>S. maritima</i>	$14.6 \pm 0.7$	$4.2 \pm 0.6$	$4.8 \pm 1.0$	$21.2 \pm 2.3$	$45.9 \pm 2.8$	$22.9 \pm 0.9$	$6.9 \pm 1.0$	$67.8 \pm 1.3$	$2.4 \pm 0.6$	$16.0 \pm 2.5$
<i>E. tomentosa</i>	$17.0 \pm 1.4$	$3.0 \pm 0.3$	$9.4 \pm 0.9$	$18.3 \pm 1.4$	$37.3 \pm 4.4$	$27.9 \pm 3.8$	$12.3 \pm 0.9$	$56.1 \pm 4.4$	$3.6 \pm 0.8$	$8.6 \pm 2.1$

Data are mean  $\pm$  SD,  $n = 45$ .

### 3.2. Influence of Salinity and $\text{NO}_3^-$ -N: $\text{NH}_4^+$ -N Ratio on Growth and Functional Values for Fatty Acids and Antioxydants

Table 3 shows multiple comparisons of biological variables (part of demonstrators of agronomical value), between individual treatments, i.e., salinities (control, moderate or high) or N-ratios ( $\text{NO}_3^-$ -N: $\text{NH}_4^+$ -N).

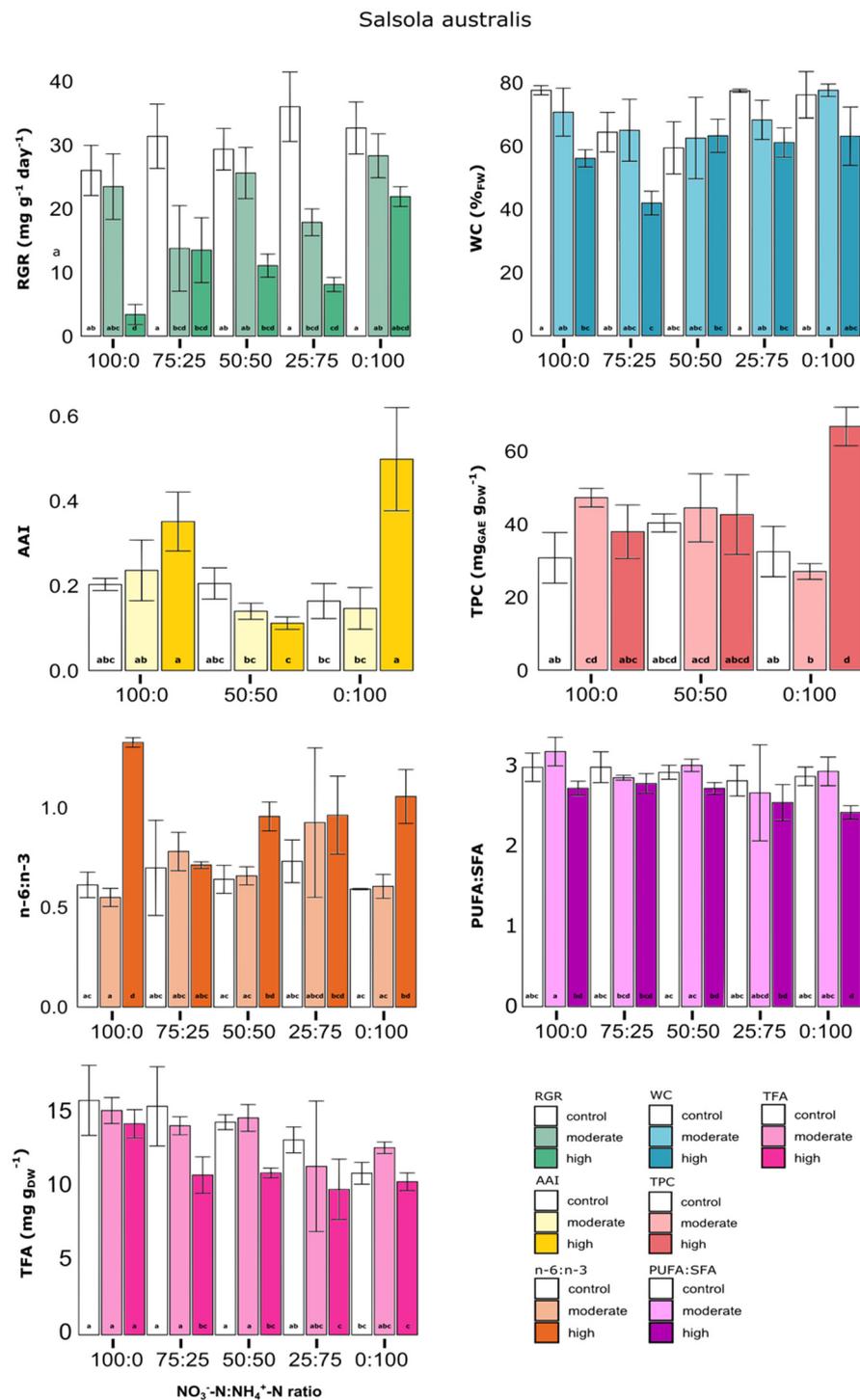
**Table 3.** Multiple comparisons of relative growth rate (RGR) ( $\text{mg g}^{-1} \text{ day}^{-1}$ ), water content (WC) (%FW), antioxidant activity index (AAI), total phenolic compounds (TPC) content ( $\text{mg}_{\text{GAE}} \text{ g}_{\text{DW}}^{-1}$ ), total fatty acid (TFA) content ( $\text{mg g}_{\text{DW}}^{-1}$ ), ratio of polyunsaturated on saturated fatty acids (PUFA:SFA) and ratio of omega 6 on omega 3 fatty acids (n - 6:n - 3) in (a) *Salsola australis*, (b) *Suaeda maritima* and (c) *Enchylaena tomentosa* according to salinities (control, moderate or high) and N-ratios ( $\text{NO}_3^-$ -N: $\text{NH}_4^+$ -N).

	(a) <i>Salsola australis</i>								
	Salinity Factor			N-Ratio Factor					
	Control	Moderate	High	100:0	75:25	50:50	25:75	0:100	
RGR	$31.1 \pm 5.1^a$	$21.8 \pm 6.7^b$	$10.6 \pm 6.4^c$	N.S	N.S	N.S	N.S	N.S	
WC	$70.6 \pm 11.1^a$	$68.8 \pm 13.6^a$	$57.1 \pm 11.6^b$	N.S	N.S	N.S	N.S	N.S	
AAI	N.S	N.S	N.S	$0.26 \pm 0.08^a$	N.D	$0.15 \pm 0.05^b$	N.D	$0.30 \pm 0.18^{a,b}$	
TPC	N.S	N.S	N.S	N.S	N.D	N.S	N.D	N.S	
TFA	$14.0 \pm 2.3^a$	$13.7 \pm 2.3^a$	$11.3 \pm 1.9^b$	$15.2 \pm 1.5^a$	$13.5 \pm 2.6^{a,b}$	$13.4 \pm 1.9^{a,b}$	$11.5 \pm 2.9^{b,c}$	$11.4 \pm 1.2^c$	
PUFA:SFA	$2.93 \pm 0.15^a$	$2.94 \pm 0.31^a$	$2.65 \pm 0.18^b$	N.S	N.S	N.S	N.S	N.S	

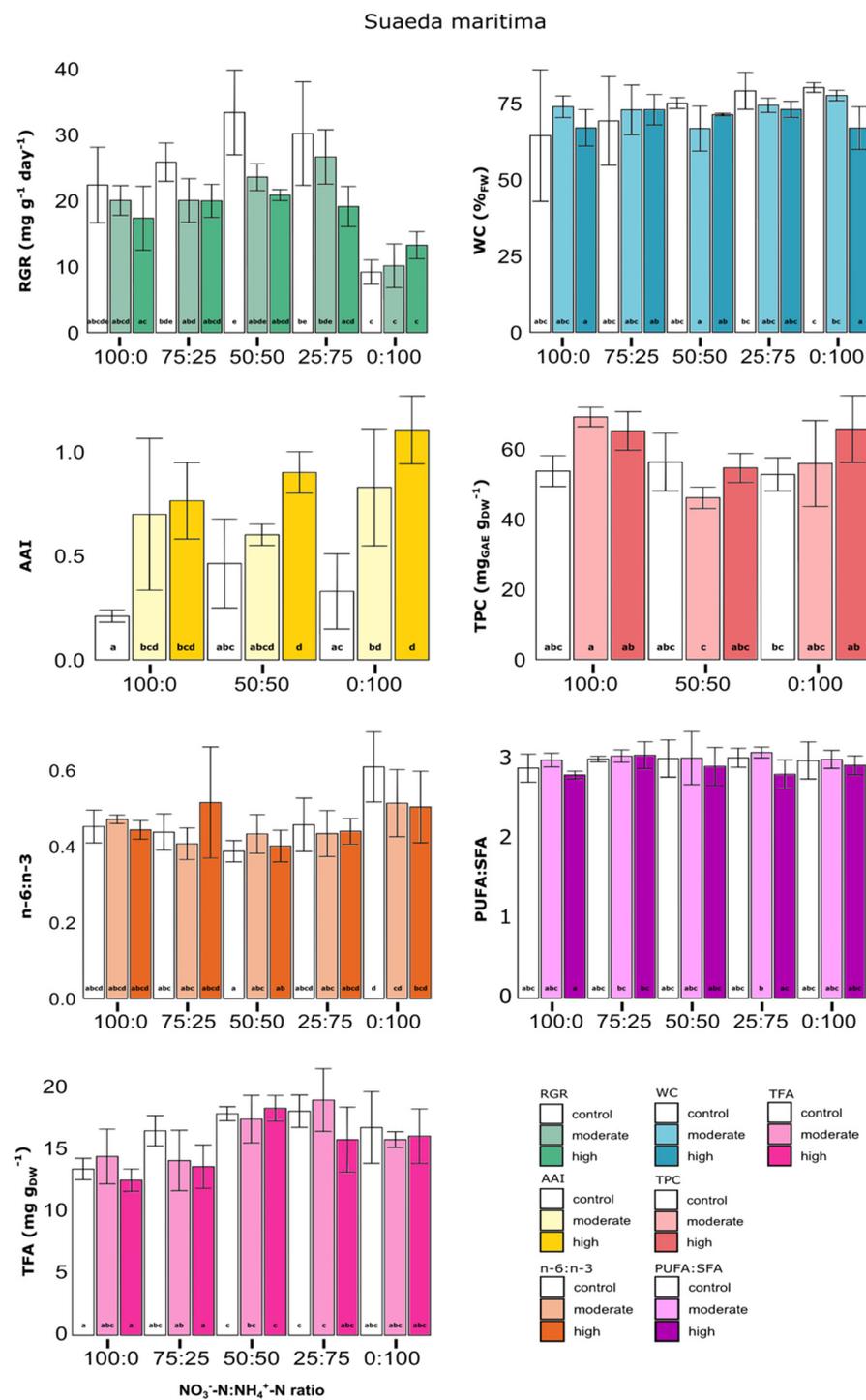
n - 6:n - 3	0.65 ± 0.19 <sup>a</sup>	0.70 ± 0.29 <sup>a</sup>	1.02 ± 0.26 <sup>b</sup>	N.S	N.S	N.S	N.S	N.S
<b>(b) <i>Suaeda maritima</i></b>								
	<b>Salinity Factor</b>			<b>N-Ratio Factor</b>				
	<b>Control</b>	<b>Moderate</b>	<b>High</b>	<b>100:0</b>	<b>75:25</b>	<b>50:50</b>	<b>25:75</b>	<b>0:100</b>
RGR	25.3 ± 9.2 <sup>a</sup>	20.8 ± 5.8 <sup>a,b</sup>	18.1 ± 3.7 <sup>b</sup>	19.9 ± 4.5 <sup>a</sup>	22.0 ± 3.9 <sup>a</sup>	26.0 ± 6.6 <sup>a</sup>	25.3 ± 6.8 <sup>a</sup>	11.2 ± 2.8 <sup>b</sup>
WC	73.7 ± 11.9 <sup>a</sup>	73.2 ± 5.8 <sup>a,b</sup>	70.3 ± 5.0 <sup>b</sup>	N.S	N.S	N.S	N.S	N.S
AAI	0.33 ± 0.18 <sup>a</sup>	0.71 ± 0.25 <sup>b</sup>	0.92 ± 0.2 <sup>b</sup>	N.S	N.D	N.S	N.D	N.S
TPC	N.S	N.S	N.S	62.7 ± 7.9 <sup>a</sup>	N.D	52.4 ± 6.8 <sup>b</sup>	N.D	58.2 ± 10.0 <sup>a,b</sup>
TFA	N.S	N.S	N.S	13.5 ± 1.5 <sup>a</sup>	14.8 ± 2.1 <sup>a,b</sup>	17.9 ± 1.2 <sup>c</sup>	17.7 ± 2.4 <sup>c</sup>	16.3 ± 1.9 <sup>b,c</sup>
PUFA:SFA	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
n - 6:n - 3	N.S	N.S	N.S	0.46 ± 0.03 <sup>a,b</sup>	0.45 ± 0.09 <sup>a,c</sup>	0.41 ± 0.04 <sup>c</sup>	0.44 ± 0.05 <sup>a,c</sup>	0.54 ± 0.09 <sup>b</sup>
<b>(c) <i>Enchylaena tomentosa</i></b>								
	<b>Salinity Factor</b>			<b>N-Ratio Factor</b>				
	<b>Control</b>	<b>Moderate</b>	<b>High</b>	<b>100:0</b>	<b>75:25</b>	<b>50:50</b>	<b>25:75</b>	<b>0:100</b>
RGR	N.S	N.S	N.S	7.0 ± 2.5 <sup>a</sup>	8.3 ± 2.4 <sup>a,b</sup>	11.3 ± 3.2 <sup>b,c</sup>	13.1 ± 4.7 <sup>c</sup>	11.5 ± 3.7 <sup>b,c</sup>
WC	N.S	N.S	N.S	44.9 ± 15.5 <sup>a</sup>	26.6 ± 15.5 <sup>b</sup>	44.0 ± 9.4 <sup>a</sup>	42.4 ± 8.8 <sup>a</sup>	45.1 ± 2.4 <sup>a</sup>
AAI	0.55 ± 0.26 <sup>a,b</sup>	0.89 ± 0.39 <sup>b</sup>	0.42 ± 0.14 <sup>a</sup>	0.34 ± 0.13 <sup>a</sup>	N.D	0.64 ± 0.17 <sup>b</sup>	N.D	0.88 ± 0.39 <sup>b</sup>
TPC	N.S	N.S	N.S	53.0 ± 16.2 <sup>a</sup>	N.D	65.9 ± 14.0 <sup>a</sup>	N.D	87.8 ± 21.6 <sup>b</sup>
TFA	10.3 ± 1.9 <sup>a</sup>	8.9 ± 1.7 <sup>a</sup>	6.6 ± 0.7 <sup>b</sup>	N.S	N.S	N.S	N.S	N.S
PUFA:SFA	2.21 ± 0.48 <sup>a</sup>	2.23 ± 0.34 <sup>a</sup>	1.77 ± 0.27 <sup>b</sup>	N.S	N.S	N.S	N.S	N.S
n - 6:n - 3	0.45 ± 0.05 <sup>a</sup>	0.47 ± 0.04 <sup>a</sup>	0.57 ± 0.07 <sup>b</sup>	N.S	N.S	N.S	N.S	N.S

Data are mean ± SD a.  $n = 15$ , b.  $n = 9$ . For each variable, values with the same letters are not significantly different between treatments ( $p < 0.05$ ). N.S = non-significant effect of factor. N.D = no data.

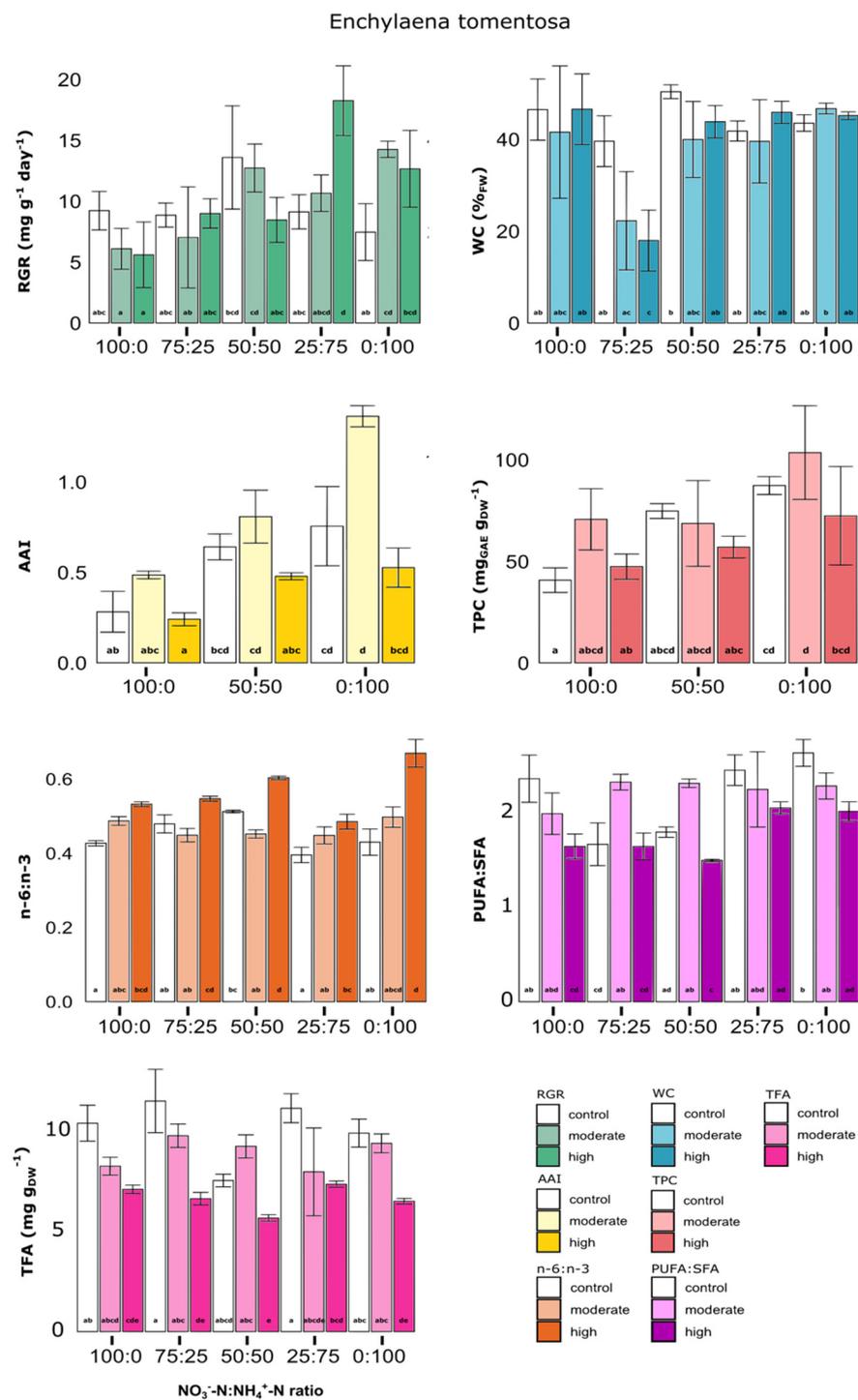
In *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa* respectively, the Figures 1–3 as well as the corresponding Tables S3–S5 specify such individual effects with regard to the combined treatments and allow further to highlight specific effects of some combined treatments.



**Figure 1.** Multiple comparisons of relative growth rate ( $\text{mg g}^{-1} \text{day}^{-1}$ ), water content ( $\%\text{FW}$ ), antioxidant activity index (AAI), total phenolic compound (TPC) content ( $\text{mg}_{\text{GAE}} \text{g}_{\text{DW}}^{-1}$ ), total fatty acid (TFA) content ( $\text{mg g}_{\text{DW}}^{-1}$ ), 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. For each variable, values with the same letters are not significantly different between treatments ( $p < 0.05$ ). Data are mean  $\pm$  SD ( $n = 3$ ).



**Figure 2.** Multiple comparisons of relative growth rate ( $\text{mg g}^{-1} \text{ day}^{-1}$ ), water content ( $\%_{\text{FW}}$ ), antioxidant activity index (AAI), total phenolic compound (TPC) content ( $\text{mg}_{\text{GAE}} \text{g}_{\text{DW}}^{-1}$ ), total fatty acid (TFA) content ( $\text{mg g}_{\text{DW}}^{-1}$ ), ratio of polyunsaturated on saturated fatty acids (PUFA:SFA) and ratio of omega 6 on omega 3 fatty acids ( $n-6:n-3$ ) in *Suaeda maritima*, between combined treatments of salinity and N-ratio in irrigation water. For each variable, values with the same letters are not significantly different between treatments ( $p < 0.05$ ). Data are mean  $\pm$  SD ( $n = 3$ ).



**Figure 3.** Multiple comparisons of relative growth rate ( $\text{mg g}^{-1} \text{ day}^{-1}$ ), water content ( $\%_{\text{FW}}$ ), antioxidant activity index (AAI), total phenolic compound (TPC) content ( $\text{mg}_{\text{GAE}} \text{g}_{\text{DW}}^{-1}$ ), total fatty acid (TFA) content ( $\text{mg g}_{\text{DW}}^{-1}$ ), ratio of polyunsaturated on saturated fatty acids (PUFA:SFA) and ratio of omega 6 on omega 3 fatty acids ( $n - 6:n - 3$ ) in *Enchylaena tomentosa* between combined treatments of salinity and N-ratio in irrigation water. For each variable, values with the same letters are not significantly different between treatments ( $p < 0.05$ ). Data are mean  $\pm$  SD ( $n = 3$ ).

### 3.2.1. *Salsola australis*

The two-way analysis, Table S2, confirms that salinity is a controlling factor for RGR ( $p < 0.001$ ), for water content ( $p < 0.01$ ), for TFA content ( $p < 0.01$ ), for PUFA:SFA ratio ( $p < 0.001$ ) and for  $n - 6:n - 3$  ratio ( $p < 0.001$ ) regardless of the N-ratio. It confirms besides that

the N-ratio is a controlling factor for AAI ( $p < 0.05$ ) and for TFA content ( $p < 0.01$ ) regardless of the salinity. Finally, the interaction is a controlling factor for AAI ( $p < 0.05$ ) and for TPC content ( $p < 0.05$ ).

Moderate or high salinities decrease significantly the RGR versus control and high salinity decreases significantly the RGR versus moderate salinity. High salinity decreases also significantly the water content, versus control or versus moderate salinity. In addition high salinity decreases significantly the TFA content and the PUFA:SFA ratio but increases significantly the n – 6:n – 3 ratio versus control or versus moderate salinity, Table 3a.

Besides, 0:100 N-ratio decreases significantly the TFA content versus 100:0, 75:25 or 50:50 N-ratio as does 25:75 N-ratio versus 100:0 N-ratio. 50:50 N-ratio decreases significantly the AAI versus 100:0 or 0:100 N-ratio, Table 3a.

The Figure 1 and the Table S3 specify that:

- moderate salinity combined with 75:25 or 25:75 N-ratio and high salinity combined with all rich  $\text{NO}_3\text{-N}$  ratios tends to decrease the RGR versus control (ranging from ~57.0% to ~86.9%).
- high salinity combined with 100:0, 75:25 or 25:75 tends to decrease the water content versus control (of ~27.7%, ~34.8% or ~21.1% respectively).
- high salinity combined with 0:100 N-ratio increases significantly the AAI (of ~212.5%;  $p < 0.05$ ) and the TPC content (of ~105.2%;  $p < 0.01$ ) versus control. This combined treatment increases also significantly the AAI versus 50:50 N-ratio (of ~354.0%;  $p < 0.001$ ) and the TPC content versus 100:0 N-ratio (of ~76.0%;  $p < 0.05$ ). Further, high salinity combined with 100:0 N-ratio increases also significantly the AAI versus 50:50 N-ratio (of ~218.2%;  $p < 0.01$ ). In addition, moderate salinity combined with 100:0 N-ratio increases significantly the TPC content versus control (of ~53.2%;  $p < 0.05$ ).
- high salinity combined with 100:0, 50:50 or 0:100 N-ratio tends to increase the n – 6:n – 3 ratio versus 75:25 N-ratio (of ~86.3%, ~34.2% or ~48.2% respectively).
- high salinity combined with 50:50 or 0:100 N-ratio tends to decrease the PUFA:SFA ratio versus control (of 6.9% or ~15.7% respectively).
- high salinity combined with 75:25, 50:50 or 25:75 N-ratio tends to decrease the TFA content versus control (of ~30.1%, ~23.9% or ~25.4% respectively) and when combined with 100:0 N-ratio, it tends to increase the TFA content versus all other ratios (ranging from ~30.3% to ~45.2%).

### 3.2.2. *Suaeda maritima*

The two-way analysis, Table S2, confirms that salinity is a controlling factor for RGR ( $p < 0.05$ ), for water content ( $p < 0.05$ ) and for AAI ( $p < 0.001$ ) regardless of the N-ratio. It confirms besides that the N-ratio is a controlling factor for RGR ( $p < 0.001$ ), for TPC content ( $p < 0.05$ ), for TFA content ( $p < 0.001$ ) and for n – 6:n – 3 ratio ( $p < 0.01$ ) regardless of the salinity.

High salinity decreases significantly the RGR and the water content versus control. Moderate and high salinities significantly increase the AAI versus control, Table 3b.

Besides, 0:100 N-ratio significantly decreases the RGR versus all other N-ratios. 50:50 N-ratio significantly decreases the TPC content versus 100:0 N-ratio. 100:0 N-ratio decreases the TFA content versus 50:50, 25:75 or 0:100 N-ratio as does 75:25 N-ratio versus 50:50 or 25:75 N-ratio. 75:25, 50:50 or 25:75 N-ratio increases the n – 6:n – 3 ratio versus 0:100 N-ratio, Table 3b.

Figure 2 and Table S4 specify that:

- high salinity combined with 50:50 or 25:75 N-ratio tends to decrease the RGR versus control (of ~37.4% and ~36.7% respectively). In addition, control and moderate salinities combined with 0:100 N-ratio tends to decrease the RGR versus 100:0, 75:25, 50:50 or 25:75 N-ratio (ranging from ~58.9% to ~72.4% at control salinity from ~49.4% to ~61.9% at moderate salinity and from ~23.6% to ~36.4% at high salinity).

- high salinity combined with 0:100 N-ratio tends to decrease the water content versus control (of ~16.6%).
- moderate salinity combined with 100:0 or 0:100 N-ratio as well as high salinity combined with all N-ratios tend to increase the AAI versus control (ranging from ~94.4% to ~262.6%).

### 3.2.3. *Enchylaena tomentosa*

The two-way analysis, Table S2, confirms that salinity is a controlling factor for AAI ( $p < 0.05$ ), for TFA content ( $p < 0.001$ ), for PUFA:SFA ratio ( $p < 0.001$ ) and for  $n - 6:n - 3$  ratio ( $p < 0.001$ ) regardless of the N-ratio. It confirms besides that the N-ratio is a controlling factor for RGR ( $p < 0.01$ ), for water content ( $p < 0.05$ ), for AAI ( $p < 0.001$ ) and for TPC content ( $p < 0.01$ ) regardless of the salinity.

No salinities have an effect on the AAI versus control but moderate salinity increases significantly the AAI versus high salinity. High salinity significantly decreases the TFA content, the PUFA:SFA ratio and significantly increases the  $n - 6:n - 3$  ratio versus control or versus moderate salinity, Table 3c.

Besides, 25:75 N-ratio significantly increases the RGR versus 100:0 or 75:25 N-ratio and 50:50 or 0:100 N-ratio significantly increase the RGR versus 100:0 N-ratio. 75:25 N-ratio significantly decreases the water content versus all other N-ratios. 50:50 or 0:100 N-ratio increase significantly the AAI with 50:50 or 0:100 N-ratio versus 100:0 N-ratio. 0:100 N-ratio significantly increase the TPC content versus 50:50 or 100:0 N-ratio, Table 3c.

Figure 3 and Table S5 specify that:

- moderate salinity combined with 0:100 N-ratio tends to increase the RGR of ~91.1% versus control and that high salinity combined with 25:75 N-ratio tends to increase the RGR of ~100.0% versus control. Further, high salinity combined with 25:75 N-ratio tends to increase the RGR versus 100:0 ratio (of ~226.2%), versus 75:25 ratio (of ~102.9%) or versus 50:50 N-ratio (of ~115.7%).
- high salinity combined with 75:25 N-ratio tends to decrease the water content versus control (of ~54.6%) and versus 100:0, 50:50, 25:75 or 0:100 N-ratio (ranging from ~59.0% to ~61.4%).
- all salinities combined with 0:100 N-ratio tend to increase the AAI versus 100:0 N-ratio (ranging from ~118.3% to ~180.6%).
- high salinity combined with all N-ratios tends to increase the  $n - 6:n - 3$  ratio versus control (ranging from ~14.2% to ~55.7%).
- high salinity combined with 100:0, 50:50 or 0:100 N-ratio tends to decrease the PUFA:SFA ratio versus control (ranging from ~16.3% to ~30.4%). Further, moderate salinity combined with 75:25 or 50:50 N-ratio tends to increase the PUFA:SFA ratio versus control (of ~39.5% or ~28.7% respectively)
- high salinity combined with all N-ratios tends to decrease the TFA content versus control (ranging from ~24.8% to ~42.3%).

## 4. Discussion

### 4.1. Mean Growth and Functional Values for Antioxidants and Fatty Acids

Mean RGR of our 5–6 months old *Salsola australis* is lower than the previous report in 1–2 months old *Salsola kali* [54], 2–3 month old *Salsola ikonnikovii* [55] or 2–3 month-old *Salsola crassa* [28] but higher than the previous report in 2–14 month old *Salsola oppositifolia* [56], all potential alternatives crops for the rehabilitation of arid or saline soils, medicine, companion crop, fodder or biofuel [55,57]. Mean RGR of our 5–6 months old *Suaeda maritima* is also lower than the previous report in 2–3 months old *Suaeda salsa* [58], 2–3 months old *Suaeda fruticosa* [59], or 0–4 months old *Suaeda splendens* [60], all three being potential oilseed or forage crops [10]. The difference in developmental stage can explain the differ-

ent growth performances between our species and species of related genera. For *Enchylaena tomentosa*, growth results are in accordance with the highest growths of seedlings (same age) reported in the field [61].

Besides, the antioxidant activity of plant extracts is considered as poor when  $AAI < 0.5$ , moderate when  $0.5 < AAI < 1.0$ , and strong when  $1.0 < AAI < 2.0$  [43]. So, hydroethanolic extracts of leaves indicate moderate to high antioxidant activities for *Suaeda maritima* and *Enchylaena tomentosa* and poor to moderate antioxidant activity for *Salsola australis*. In addition, the three hydroethanolic presented also high [4,37] mean phenolic contents, positively correlated with the AAI. Such correlation has been often reported in other halophytes [6,14]. These results suggest the presence of an interesting pool of natural antioxidants in *Suaeda maritima* and *Enchylaena tomentosa* leaves and require further investigation of specific compounds, such as polyphenols, but also glutathione, terpenoids, vitamins, ureides or polyols as in many other halophytes [4,6,62]. There is increasing interest in identifying plants with high activity or antioxidant content for use in industry [6,15]. Notably, several epidemiological studies in humans demonstrate the protective effects of the consumption of plant polyphenols against oxidative diseases. They are notably known to have anti-carcinogenic, anti-thrombotic, cardio-protective or even antimicrobial and anti-inflammatory effects [63]. However, due to the varied chemical nature of antioxidants and the complexity of oxidation processes, there is no one-size-fits-all method for measuring the antioxidant capacity of a biological sample [39,42]. In addition, each methodology indicates variations at different stages (extraction, quantification, identification) which lead to differences in sensitivity and reproducibility [64].

Besides, the mean TFA contents and FA compositions in the three species are in agreement with those found in edible wild plants [65–67]. In addition, the predominant amounts of dietary essential polyunsaturated acids, i.e., PUFA:SFA ratio  $> 1$ , are above that found in other commonly eaten leafy vegetables like spinach, buttercrunch lettuce, red leaf lettuce, and mustard greens, and slightly below the quantities found in purslane [68]. These predominant amounts suggest a high functional value for species due to the well-known protective role of such fatty acids against cardiac disorders [68,69]. Further, the predominant amounts of omega 3, i.e.,  $n-6:n-3$  ratios  $< 1$ , indicate functional values able to mitigate, through long-term consumption, the persistent imbalance in favor of omega 6 characterizing the diet of many modern societies [68]. An intake of 100 mg of leaves contributes to 8.2(men)–11.9(women)%, 11.6–16.8% and 10.4–15.1% of the dietetic daily recommendation for  $\alpha$ -linolenic acid omega 3 [47] for *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa* respectively. However, beyond global indices, it is also essential to have population-based and food-based dietary recommendations [70].

All these observations suggest that the three species studied have a great potential to become functional plant products. Mean productivities for TFA and  $\alpha$ -linolenic acid, on a fresh weight basis, are estimated at  $97.0 \pm 75.7$  and  $35.9 \pm 30.0$   $\mu\text{g g}^{-1} \text{day}^{-1}$  for *Salsola australis*,  $97.0 \pm 48.1$  and  $45.3 \pm 23.5$   $\mu\text{g g}^{-1} \text{day}^{-1}$  for *Suaeda maritima* and  $49.7 \pm 20.3$  and  $18.8 \pm 8.6$   $\mu\text{g g}^{-1} \text{day}^{-1}$  for *Enchylaena tomentosa*. The mean productivities for TPC, on a fresh weight basis, are estimated at  $309.2 \pm 255.5$   $\mu\text{g g}^{-1} \text{day}^{-1}$  for *Salsola australis*,  $334.0 \pm 166.1$   $\mu\text{g g}^{-1} \text{day}^{-1}$  for *Suaeda maritima* and  $382.6 \pm 219.7$   $\mu\text{g g}^{-1} \text{day}^{-1}$  for *Enchylaena tomentosa*.

#### 4.2. Influence of Salinity and $\text{NO}_3^-$ -N: $\text{NH}_4^+$ -N Ratio on Salt-Tolerance and N-Nutrition

Physiological changes in plants between combined treatments inform on metabolism used by species to face salt under different N-nutritions.

##### 4.2.1. *Salsola australis*

Results show that moderate and high salinities can decrease the RGR regardless of N-ratio. However, they do not show any significant effect of the N-ratio or of a specific combined treatment (interaction) on the RGR. Under saline conditions, plant growth depends firstly on osmotic adjustment [1,3]. Although the interaction is not statistically significant, the lowest growth performances when both salinities are combined with rich-

$\text{NO}_3^-$ -N irrigation waters, suggest yet that  $\text{NO}_3^-$ -N is not used for this purpose. This function could instead be assigned to other ions or to compatible solutes [3,29,71]. In addition, the plants subjected to the exclusive  $\text{NH}_4^+$ -N treatment (0:100 ratio) tend to better maintain the RGR and the water efficiently at moderate and high salinity. This suggests that this species is tolerant to  $\text{NH}_4^+$ -N and efficiently utilizes  $\text{NH}_4^+$ -N uptake products to perform osmoprotective function and/or other salt tolerance processes.

Salt-tolerance and osmoregulation in plants also depend on the ability to increase the FA unsaturation level and the linoleic acid on  $\alpha$ -linolenic acid ratio in cell membranes (the PUFA:SFA ratio and, in our species, the n – 6:n – 3 ratio) what reduces their fluidity and permeability [12,13,17]. Results show such positive variations at high salinity for the n – 6:n – 3 ratio. So, it can be interpreted as the establishment of salt tolerance mechanisms. However, the lower growth performances at high salinity under 100:0, 75:25, 50:50 and 25:75 N-ratios indicate that these FA changes are not sufficient to deal with salt.

Besides, the antioxidant defense is also a well-known salt tolerance mechanism in halophytes [3,6,14]. The results show an increase of the AAI and the TPC content under high salinity when combined with 0:100 N-ratio. This supports the hypothesis of the advantage of  $\text{NH}_4^+$ -N tolerance under high saline conditions. The priming effect of  $\text{NH}_4^+$ -N nutrient on stimulation of salt-tolerance by antioxidant capacity has previously been reported in *Spartina alterniflora* [24]. However, the low mean AAI in *Salsola australis* raises the question of the antioxidant role attributed to the phenolic compounds, i.e., little involved in ROS scavenging or involved in ROS scavenging but with an additional mode of action than that shown by DPPH test.

#### 4.2.2. *Suaeda maritima*

Results show that the RGR depends on the salinity and the N-ratio in water irrigation. However, they do not show any significant effect of a specific combined treatment (interaction) on the RGR. So, lower growth performances are related to a 0:100 N-ratio regardless of salinity, with a more attenuated decrease under high salinity. This suggests that this species prefers  $\text{NO}_3^-$ -N nutrient under all salinities but can better use  $\text{NH}_4^+$ -N nutrient when salinity increase. It was shown for *Suaeda maritima* var. *macrocarpa* that uptake and assimilation of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N (with 66:33 ratio) is affected by salinity and that yields of  $\text{NH}_4^+$ -N nutrient could increase with salinity [72]. However, here, the results also show that plants conserve effectively water relative to control under all saline treatments except when the high salinity is combined with 0:100 N-ratio. This suggests that the increase of  $\text{NH}_4^+$ -N relative to  $\text{NO}_3^-$ -N trends to lower the osmoprotective function at high salinity. Previous reports have shown that the osmotic potential of *Suaeda physophora* at 300 mM NaCl mainly depends on  $\text{NO}_3^-$ -N [73], on proline and sugars [71].

In addition, the results demonstrate that the TFA content and the quality of fatty acids are not affected by moderate or high salinity relative to control regardless of the N-ratio. We suggest that this species does not need to increase the proportion of unsaturated fatty acids or of linoleic acid to tolerate an increase of salinity, unlike other *Suaeda* species [13,17]. However, the plants subject to a 0:100 ratio (with the lowest RGR) tend to have the lowest linoleic acid proportions (the highest n – 6:n – 3 ratio) regardless of salinity, although the interaction is not statistically significant.

Besides, the results do not show any effects of salinity on TPC content, but highlight an increase of AAI with salinity. This supports the hypothesis on the induction of antioxidant processes for salt-tolerance in this species as previously demonstrated for *Suaeda* species [74,75], but with the mobilization of additional antioxidant mechanisms that the activity of phenolic compounds. In fact, the high level of phenolic compounds regardless of salinity may indicate their constitutive antioxidant role [4,37]. In particular, it has been shown for *Suaeda salsa* and for *Suaeda maritima* that the trapping of ROS during salt stress involves the dismutation of superoxide. For *Suaeda salsa*, this could also involve catalase pathway, glutathione peroxidase pathway, peroxiredoxin reductase pathway and ascorbic acid – glutathione cycle.

#### 4.2.3. *Enchylaena tomentosa*

While RGR and the water content depend on N-ratio in water irrigation, the results cannot show any significant effect of salinity or of a specific combined treatment (interaction) on these parameters. Even so, the RGR trends to be favored by moderate or high salinity when combined with the two rich-ammonia ratios, i.e., 25:75 or 0:100 ratios. The level of salt tolerance reported is in agreement with the previous report for this species [33,34]. However, to our better knowledge, nothing is known about nitrogen nutrition for this species. So, our results provide new insights and suggest that this species is  $\text{NH}_4^+$ -N tolerant and can take advantage of this nutrient at moderate or high salinity as many other saline land species [20,22–24]. This advantage was explained in other species by the limited bioavailability of  $\text{NO}_3^-$ -N in Cl-rich-soil [20] and/or by the economic changes of carbon and nitrogen metabolisms [22,76] and/or by the increase in salt tolerance capacity obtained directly by the use of  $\text{NH}_4^+$ -N [23,24]

Besides, the increase of n – 6:n – 3 ratio under high salinity and in particular with 0:100 N-ratio, support the latter hypothesis. This can be interpreted as the establishment of salt tolerance mechanisms involving changes inside polyunsaturated FA. However, the lowest PUFA:SFA ratios at high salinity regardless of the N-ratio suggests that the proportion of polyunsaturated FA is not modulated for this purpose. So, further works will be needed to investigate the possible contribution of structural FA to osmoregulatory processes in saline conditions.

The beneficial effect of  $\text{NH}_4^+$ -N nutrient is also supported by the increase of the AAI and the TPC content in plants when  $\text{NH}_4^+$ -N proportion increases in irrigation water. For the AAI this increase concerns all salinities and for the TPC content, it concerns only control salinity. However, the decrease of the AAI at high salinity relative to moderate salinity could indicate an overtaking of antioxidant capacity face to salt stress. Even so, constant levels of TPC regardless of salinity may further indicate a constitutive role of phenolic compounds. A fundamental role of polyphenols in the protection of the photosynthetic apparatus under salt stress has notably been demonstrated [77], showing photosynthetic tissues as the main sites of flavonoid accumulation. So, further studies have to explore whether the N-modulation of growth and TPC content are correlated with differences in carbon uptake in this species.

## 5. Conclusions

Regarding functional values for antioxidants and fatty acids, our work demonstrates the good potential for biosaline agriculture of *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa*, three halophytes from New Caledonia. However, we report that the quality in terms of antioxidants fatty acids of the leafy product from these species depends on salinity, nitrogen supply or a combination of the two. Based on salt tolerance and nitrogen nutrition, we thus recommend treatments to jointly optimize their growth and functional values for antioxidants and fatty acids. That is to say, the treatments showing a high RGR, a high AAI, a high TPC content, a high TFA content and a high proportion of unsaturated fatty acids (high PUFA:SFA ratio), in particular  $\alpha$ -linolenic acid (low n – 6:n – 3 ratio). For these recommendations, we focus on the highest salinity studied for each species, because such a perspective of irrigation maximizes water savings. For *Salsola australis*, we recommend a combination of a 0:100 N-ratio with high salinity to reach a good compromise between RGR ( $21.9 \pm 1.5 \text{ mg g}^{-1} \text{ day}^{-1}$ ), water content ( $63.1 \pm 16.0\%_{\text{FW}}$ ), functional value for antioxidants (AAI:  $0.49 \pm 0.12$  and TPC content:  $66.7 \pm 5.3 \text{ mg}_{\text{GAE}} \text{ g}_{\text{DW}}^{-1}$ ), and functional value for fatty acids (n – 6:n – 3 ratio:  $1.06 \pm 0.19$ , PUFA:SFA ratio:  $2.43 \pm 0.09$  and TFA content:  $10.4 \pm 0.6 \text{ mg g}_{\text{DW}}^{-1}$ ). For *Suaeda maritima*, we recommend a combination of a 50:50 N-ratio with high salinity to reach a good compromise between RGR ( $20.9 \pm 0.8 \text{ mg g}^{-1} \text{ day}^{-1}$ ), water content ( $71.4 \pm 0.4\%_{\text{FW}}$ ), functional value for antioxidants (AAI:  $0.90 \pm 0.99$  and TPC content:  $54.7 \pm 4.1 \text{ mg}_{\text{GAE}} \text{ g}_{\text{DW}}^{-1}$ ) and functional value for fatty acids (n – 6:n – 3 ratio:  $0.40 \pm 0.04$ , PUFA:SFA ratio:  $2.91 \pm 0.24$  and TFA content:  $18.4 \pm 1.0 \text{ mg g}_{\text{DW}}^{-1}$ ). For

*Enchylaena tomentosa*, we recommend a combination of a 25:75 ratio with high salinity to show a good compromise between RGR ( $18.3 \pm 2.9 \text{ mg g}^{-1} \text{ day}^{-1}$ ), water content ( $45.9 \pm 4.2\%_{\text{FW}}$ ) and functional value for fatty acids (n-6:n-3 ratios:  $0.49 \pm 0.04$ , PUFA:SFA ratio:  $2.05 \pm 0.11$  and TFA content:  $7.4 \pm 0.3 \text{ mg g}_{\text{DW}}^{-1}$ ). We recommend also a combination of a 0:100 ratio with high salinity to reach a good compromise for previous variables, but also the best functional values for antioxidants among the high salinity treatments (AAI:  $0.53 \pm 0.11$  and TPC content:  $72.5 \pm 24.2 \text{ mg}_{\text{GAE}} \text{ g}_{\text{DW}}^{-1}$ ).

Such results have the potential to be used for future field experiments of biosaline agriculture with high salinity in irrigation water. This is particularly important in order to develop solutions for reducing our footprint on freshwaters and lands of territories.

**Supplementary Materials:** The following are available online at [www.mdpi.com/2073-4395/11/3/449/s1](http://www.mdpi.com/2073-4395/11/3/449/s1). Table S1: Mean proportions of all fatty acids and fatty acid classes (%TFA) in halophytes *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa* after one-month irrigation under different salinities and  $\text{NO}_3^-$ -N: $\text{NH}_4^+$ -N ratios. Data are mean  $\pm$  SD on all combined treatments (n = 45), Table S2: Two-way analysis of the variance on ranks studying the significant variations of the RGR, the water content and the profiles of antioxidants and fatty acids of halophytes *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa* as a function of salinity, N-ratio or interaction of salinity and N-ratio, Table S3–S5: Multiple comparisons of relative growth rate ( $\text{mg g day}^{-1}$ ), water content ( $\%_{\text{FW}}$ ), antioxidant activity index (AAI), total phenolic compound (TPC) content ( $\text{mg}_{\text{GAE}} \text{ g}_{\text{DW}}^{-1}$ ), total fatty acid (TFA) content ( $\text{mg g}_{\text{DW}}^{-1}$ ), ratio of polyunsaturated on saturated fatty acids (PUFA:SFA) and ratio of omega 6 sur omega 3 fatty acids (n-6:n-3) in *Salsola australis*, *Suaeda maritima* or *Enchylaena tomentosa* between combined treatments of salinity and N-ratio in irrigation water. Figure S1: Experimental design of combined salinity and nitrogen ratio experiment on halophytes *Salsola australis*, *Suaeda maritima* and *Enchylaena tomentosa*.

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