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Effects of NaCl Salinity on Nitrate Uptake and Partitioning of N and C in *Festuca rubra* L. in Relation to Growth Rate

By

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With 6 figures

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Summary

RUBINIGG M., ELZENGA J. T. M. & STULEN I. 2002. Effects of NaCl salinity on nitrate uptake and partitioning of N and C in *Festuca rubra* L. in relation to growth rate. – *Phyton* (Horn, Austria) 42 (2): 251–267, with 6 figures. – English with German summary.

The effect of salinity on nitrate net uptake rate was studied in the moderately salt tolerant halophyte *Festuca rubra* L., in relation to changes in relative growth rate, root weight ratio and nitrogen and carbon partitioning. Plants were grown for 21 days on nutrient solution containing 50, 100 and 200 mol m⁻³ sodium chloride. Control plants received no additional sodium chloride. Relative growth rate of *Festuca rubra* was reduced by exposure to a sodium chloride concentration as low as 50 mol m⁻³, resulting in a lower nitrogen 'demand' for plant growth and consequently in a lower nitrate net uptake rate. However, the accumulation of reduced nitrogen containing osmotic solutes increased the plant's nitrogen 'demand' slightly. The decreased root weight ratio, caused by the stronger inhibition of root growth as compared to shoot growth upon elevated salinity levels complicated the interpretation of the effects of different salinity treatments on nitrate net uptake rates. Utilization of soluble sugars for osmotic adjustment did not divert significant amounts of carbo-

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hydrates from the carbon pool. To get insight into the physiological background of the interaction between sodium chloride salinity and nitrate uptake, additional complications in the growth analysis and N nutrition must be considered.

Zusammenfassung

RUBINIGG M., ELZENGA J. T. M. & STULEN I. 2002. Auswirkungen von Natriumchlorid-Salinität auf die Nitrataufnahme und die Verteilung von N und C in *Festuca rubra* L. in Relation zur Wachstumsrate. – Phytion (Horn, Austria) 42 (2): 251–267, mit 6 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Auswirkungen von Salzstress auf die Nitrat Nettoaufnahme in dem mäßig salztoleranten Halophyten *Festuca rubra* L., in Relation zu Veränderungen in der relativen Wachstumsrate, im Gewichtsverhältnis zwischen Wurzel und ganzer Pflanze, und in der Verteilung von Stickstoff und Kohlenstoff innerhalb der Pflanze wurden untersucht. Die Pflanzen wurden 21 Tage auf Nährlösung mit einer Natriumchlorid-Konzentration von 50, 100 und 200 mol m⁻³ gezogen. Kontrollpflanzen erhielten kein zusätzliches Natriumchlorid. Die relative Wachstumsrate von *Festuca rubra* nahm bereits bei einer Natriumchlorid-Konzentration von 50 mol m⁻³ signifikant ab, was den Stickstoffbedarf der Pflanze und folglich die Nitrat-Nettoaufnahmerate senkte. Im Gegensatz dazu führte die Akkumulation von reduzierten Stickstoff enthaltenden Osmotika zu einer leichten Erhöhung des pflanzlichen Stickstoffbedarfs. Das niedrigere Gewichtsverhältnis zwischen Wurzel und ganzer Pflanze, verursacht durch eine stärkere Reduktion des Wurzelwachstums im Vergleich zum Sprosswachstum bei erhöhtem Salzgehalt, erschwerte jedoch die Interpretation der Auswirkungen von verschiedenen Salzbehandlungen auf die Nitrat-Nettoaufnahme. Der Gebrauch von löslichen Zuckern als Osmotika beanspruchte keine bedeutenden Kohlenhydrat-Mengen aus dem Kohlenstoff-Pool. Um eine bessere Einsicht in die physiologische Basis der negativen Wechselwirkungen zwischen Natriumchlorid und Nitrataufnahme zu bekommen, müssen zusätzliche Komplikationen bei der Wachstumsanalyse und im Stickstoffhaushalt berücksichtigt werden.

Introduction

Exposure to elevated NaCl concentrations reduces the NO₃⁻ net uptake rate (NUR) in a range of vascular plants, such as *Hordeum vulgare* L. (LUQUE & BINGHAM 1981), *Cucumis sativus* L. (MARTINEZ & CERDÁ 1989), *Gossypium hirsutum* L. and *Phaseolus vulgaris* L. (GOUÏA & al. 1994) or *Ricinus communis* L. (PEUKE & al. 1996). However, the physiological basis of this interaction is not well-established and it is unclear whether this effect is due to a direct interaction between NaCl and processes involved in NO₃⁻ uptake, utilization or translocation within the plant or via a salinity induced reduction of the relative growth rate (RGR), resulting in a lower N 'demand' (GOUÏA & al. 1994, GRATAN & GRIEVE 1999, TOURAINE & al. 1994, ULLRICH 2001). Moreover, it is unclear, whether an increased carbohydrate 'demand' for processes involved in salt exclusion or internal osmotic adjustment may reduce the C availability for NO₃⁻ uptake and reduction.

Soil salinity can affect the RGR of plants and the allocation of biomass between shoot and root (KAFKAFI & BERNSTEIN 1996, MUNNS 1993, MUNNS & TERMAAT 1986). Changes in RGR affect the internal N 'demand' of plants and thereby determine the rate at which NO_3^- is taken up by the root (LAMBERS & POORTER 1992, TOURAINE & al. 1994). In addition, exposure of plants to solutions with a low water potential may induce the accumulation of N containing low molecular weight compounds, referred to as osmotic solutes, thereby increasing the N 'demand' of the plants (STEWART & LEE 1974, STOREY & al. 1977, STOREY & WYN JONES 1977, RHODES & HANSON 1993). Moreover, changes in biomass allocation to the root relative to the whole plant affect the reference value for the expression of $\text{NO}_3^- \text{NUR}$. RGR, root weight ratio (RWR) and N partitioning have therefore to be included in the interpretation of long-term studies on the effects of NaCl on $\text{NO}_3^- \text{NUR}$.

Festuca rubra is a plant species that covers a wide range of contrasting habitats, reaching from acidic and alkaline inland soils to sandy coastal dunes and muddy salt marshes (HANNON & BRADSHAW 1968, RHEBERGEN & NELISSEN 1985, ROZEMA & al. 1978). Salt marsh populations can be characterized as moderately salt tolerant halophytes (ROZEMA & al. 1978, VENABLES & WILKINS 1978, DIERBEN 1996, OLFF & al. 1997). *Festuca rubra*, like many other vascular plants, accumulates proline and methylated quaternary ammonium compounds when exposed to a saline medium (ROZEMA & al. 1985). Besides, as a member of the *Monocotyledoneae* it is assumed to exhibit a lower uptake of salts in general and an efficient exclusion of Na^+ in particular as compared to members of the *Dicotyledoneae*. Instead, these groups use relatively high levels of soluble sugars to maintain an adequate osmotic potential (ALBERT & POPP 1977, 1978).

In the present paper the effects of NaCl on growth, biomass allocation between root and shoot, C and N allocation and partitioning in *Festuca rubra* are described. Possible effects of salinity induced changes in RGR, RWR and N partitioning on the internal N 'demand' and the consequence of changes in the internal N 'demand' on the $\text{NO}_3^- \text{NUR}$ are discussed.

Material and Methods

Plant material

Seeds of *Festuca rubra* L. were collected from a natural population growing on the salt marsh "Oosterkwelder" on the island of Schiermonnikoog, The Netherlands. Plants were germinated for 2 weeks on vermiculite in a climate room with a day/night temperature of 20/20°C, RH of 65%, a photoperiod of 12 h per day and a light intensity of $550 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the plant level (PAR 400–700 nm, measured with a quantum sensor, SKP215, Skye Llandrindod Wells, UK), supplied by fluorescence lamps (F96T12/CW/VHO, 215 W, Sylvana, USA). On day 14 after sowing seedlings which had formed one primary leaf were selected for the experiment. Seedlings were transferred to 0.03 m^3 tanks at a density of 120 plants per tank with aerated nutrient

solution. The nutrient solution was composed as follows (in mol m⁻³): KNO₃, 3.75; CaCl₂, 1.25; MgSO₄, 0.5; KH₂PO₄, 0.2; FeEDTA, 0.0225. Micronutrients were added according to SMAKMAN & HOFSTRA 1982 and pH was adjusted to 5.8 with KOH. Three NaCl treatments were imposed: 50, 100 and 200 mol m⁻³. Control plants, which received no additional NaCl, are referred to as "0 mol m⁻³". NaCl was added in steps of 50 mol m⁻³ day⁻¹ in order to allow osmotic adjustment. Calculations of total plant weight and plant N were made, confirming that NO₃⁻ depletion did not exceed 10% of the initial amount. Solutions were replaced weekly. At harvest plants were divided into shoot and root and each tissue was washed two times for 20 s in double distilled water. Tissue was blotted and fresh weight was determined. Plants for the analysis of insoluble reduced N (IRN), soluble reduced N (SRN), inorganic anion and free amino acid analysis were stored at -80°C, while for all other determinations tissues were dried for 48 h at 70°C and dry weight of the samples was determined.

Chemical analyses

For chemical analyses plants were harvested at day 21 after transfer to the nutrient solution and combined into 3 replicates, the number of plants per replicate was 3-8. Non structural carbohydrates were extracted from dry material in 80% ethanol. Total C and N were determined with an elemental analyzer (model 1106, Carlo Erba Instrumentazione, Milano, Italy). For the determination of free amino acids and NO₃⁻ the plant material was extracted in double distilled water using an Ultra Turrax (T25, Janke & Junkel, Germany). The extract was incubated in a water bath at 100°C for 10 min, filtered and centrifuged at 30000 g (Sorvall RC5C, USA). Anions were measured with HPLC according to STUIVER & al. 1992. For amino acid determination, the supernatant was analyzed according to ROSEN 1957. For this purpose 0.03% (w:v) ninhydrine (Sigma, Switzerland) dissolved in ethyleneglycolmonomethylether, kept at pH 5.4 by a KCN/Na-acetatebuffer was added to the extract and incubated in a water bath at 100°C for 15 min. Isopropanol:H₂O 1:1 (v:v) was added and samples were measured with a spectrometer (Starrcol SC-60-S, R&R Mechatronics, The Netherlands) at 578 nm. Proline was detected at 450 nm. Reduced N (Nr) was calculated from the difference between total N and NO₃⁻.

Insoluble reduced N (IRN) containing mainly proteins and soluble reduced N (SRN) containing mainly amino acids and methylated ammonium compounds was determined according to BAILEY 1967. Fresh material, stored at -80°C, was ground in liquid N₂, incubated in 3% HCl (v:v) for 2 h in order to precipitate the protein present in the IRN fraction and separated from the SRN fraction by filtration through a black ribbon filter (Schleicher & Schüll, Germany). Each fraction was digested in H₂SO₄ at 360°C for 5 h after gradual heating in the presence of K₂SO₄:CuSO₄ 3:1 (w:w). Ammonia was then determined with a spectrometer (Starrcol, SC-60-S, R&R Mechatronics, The Netherlands) at 415 nm using Nessler's reagent A (Merck, Germany) mixed 1:1 with 9 N NaOH (v:v).

The soluble non-structural carbohydrate fraction (SNC) containing mainly mono- and oligosaccharides was separated from the insoluble non-structural carbohydrate fraction (INC) consisting mainly of starch and hemicelluloses by centrifuging the extract at 49500 g (Sorvall RC5C, USA). The pellet containing INC was boiled in 3% HCl (v:v) for 3 h at 127°C. After addition of a 10% Al(OH)₃ (w:v) suspension the extract was centrifuged again. Both fractions were determined according to FALES

1951 with a spectrometer (Starrcol SC-60-S, R&R Mechatronics, The Netherlands) at 620 nm using 0.145% (w:v) Anthrone (Merck, Germany) in ethanol:H₂O:H₂SO₄ 4:15:50 (v:v:v).

Biomass allocation and partitioning, growth and nutrient uptake

RWR was calculated by dividing root fresh weight by plant fresh weight ($\text{mg FW}_{\text{root}} \text{g}^{-1} \text{FW}_{\text{plant}}$). Tissue water content was calculated by dividing the difference between tissue fresh and dry weight by the fresh weight ($\text{mg g}^{-1} \text{FW}$). RGR was obtained from a fit of a linear regression curve to the ln-transformed tissue fresh weights from three consecutive harvests (day 7, 14 and 21 after transfer to the nutrient solution) according to HUNT 1982. Net uptake rates (NUR) of NO₃⁻ were calculated according to WILLIAMS 1948 using the following formula where w_1 and w_2 are root (or plant) fresh weight (g) on t_1 =day 0 and t_2 =day 21, respectively, and N_1 and N_2 are plant N content (μmol) on day 0 and 21, respectively:

$$\text{NUR} = \frac{\ln w_2 - \ln w_1}{t_2 - t_1} \times \frac{N_2 - N_1}{w_2 - w_1}$$

Statistical analyses

Statistical analyses were performed with GraphPad Prism version 3.0 (GraphPad Software Inc., San Diego, USA). For the analysis of three or more groups of means, One Way ANOVA with a Newman-Keuls test has been used. Symbols for levels of significance: n.s., not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Results and Discussion

Festuca rubra is a mainly outbreeding perennial species covering a wide range of contrasting habitats including salt marshes (HANNON & BRADSHAW 1968, RHEBERGEN & NELISSEN 1985, ROZEMA & al. 1978). Populations from different habitats but also individuals in transition zones between different habitats show strong dissimilarities in the response of growth and several other physiological parameters to soil salinity (RHEBERGEN & NELISSEN 1985, ROZEMA & al. 1978). The seed material of *Festuca rubra* used in the present study has been collected on a salt marsh. However, no detailed systematic classification of the material or assignment to a certain ecotype has been made. Conclusions on the species *Festuca rubra* are therefore not possible. The results must be seen as an example for a type of physiological response to soil salinity that is characteristic of many plant species that exhibit a relatively low degree of salt tolerance and accumulate N_r containing osmotic solutes.

Growth parameters and nitrate uptake rate

On day 21 the fresh weights of shoot as well as root tissue (Table 1) of *Festuca rubra* grown on 50, 100 and 200 mol m⁻³ NaCl, respectively, were considerably lower at all NaCl concentrations applied compared to control plants. This was the result of a substantial reduction of the RGR by 24, 30

and 53% at 50, 100 and 200 mol m⁻³ NaCl, respectively as compared to control plants (Table 2). This result does not necessarily disagree with the occurrence of *Festuca rubra* in a saline habitat. Salinity tolerance may depend on the developmental stage of a given plant species. Increased sensitivity towards salinity in the seedling stage as compared to adult plants has been reported for other plant species (MAAS & al. 1983, ZEDLER & al. 1990). Besides, crucial factors for the salinity tolerance such as level and type of soil salinity on a spatial as well as temporal scale in the natural habitat may differ considerably from the conditions in the laboratory. The RGR measured in a highly artificial hydroponic system does therefore not necessarily allow drawing conclusions on the performance of a plant species in its natural habitat.

Table 1.

Biomass production and allocation based on fresh weight in *Festuca rubra* on day 21 after transfer to nutrient solution supplied with 50, 100 and 200 mol m⁻³ NaCl and receiving no additional NaCl. Shoot and root fresh weight (g); water content of shoot and root (mg g⁻¹ FW); RWR, root weight ratio (mg FW_{root} g⁻¹ FW_{plant}). Mean values (±SEM, One Way ANOVA, n=3).

	0 mol m ⁻³	50 mol m ⁻³	100 mol m ⁻³	200 mol m ⁻³
Fresh weight				
Shoot	0.151 ± 0.015	0.074 ± 0.006***	0.062 ± 0.007***	0.043 ± 0.004***
Root	0.175 ± 0.015	0.075 ± 0.010***	0.058 ± 0.004***	0.023 ± 0.003***
Water content				
Shoot	785 ± 9	791 ± 2 ^{n.s.}	767 ± 10 ^{n.s.}	762 ± 1 ^{n.s.}
Root	913 ± 1	912 ± 4 ^{n.s.}	905 ± 1 ^{n.s.}	874 ± 8***
RWR	538 ± 15	501 ± 24 ^{n.s.}	483 ± 15 ^{n.s.}	351 ± 13***

RWR decreased by 65% on 200 mol m⁻³ NaCl but was unaffected on 50 and 100 mol m⁻³ NaCl, respectively (Table 1). Water content in the root was the same in plants grown on 50 and 100 mol m⁻³ NaCl and decreased only by about 4% compared to controls in plants exposed to 200 mol m⁻³ NaCl (Table 1). In the shoot, water content was unaffected at all NaCl concentrations. Changes in water content can therefore not explain the large change in RWR. Obviously high salinity affected root growth more than shoot growth.

Since both RGR and RWR differed strongly between the NaCl treatments, it is difficult to interpret the effect of NaCl on NO₃⁻ NUR. If expressed on a root fresh weight basis the NO₃⁻ NUR was unaffected at 50 mol m⁻³ NaCl and decreased by 17% at 100 and 200 mol m⁻³ NaCl compared to control plants. If, however, expressed on a plant fresh weight basis NO₃⁻ NUR was decreased by 29, 27 and 46% at 50, 100 and

200 mol m⁻³ NaCl, respectively (Table 2). At the same time, however, RWR decreased due to a stronger reduction in root growth as compared to shoot growth. This reduction affected the factor root fresh weight as a reference value for the expression of NO₃⁻ NUR, leading, if not compensated for, to an overestimation of the actual uptake rates. The suggestion, that the changes in RWR led to an overestimation of the actual NO₃⁻ NUR at all NaCl treatments applied, is supported by the fact that NO₃⁻ NUR, if expressed on a plant fresh weight basis, was already considerably decreased at 50 mol m⁻³ NaCl. The reduction of NO₃⁻ NUR on a plant fresh weight basis was most likely the consequence of a lower RGR.

Table 2.

Growth and NO₃⁻ uptake in *Festuca rubra* on day 21 after transfer to nutrient solution supplied with 50, 100 and 200 mol m⁻³ NaCl and receiving no additional NaCl. RGR, relative growth rate based on plant fresh weight (g FW_{plant} g⁻¹ FW_{plant} day⁻¹), slopes of linear regression lines (± SEM, One Way ANOVA, n=9); NO₃⁻ NUR, NO₃⁻ net uptake rate expressed on a root (root fw) and on a plant fresh weight basis (plant fw), respectively (μmol g⁻¹ FW h⁻¹).

	0 mol m ⁻³	50 mol m ⁻³	100 mol m ⁻³	200 mol m ⁻³
RGR	0.183 ± 0.010	0.139 ± 0.012**	0.128 ± 0.010**	0.087 ± 0.006***
NO₃⁻ NUR				
Root FW	7.2	7.3	6.0	6.0
Plant FW	3.3	2.3	2.4	1.8

For a lower production of new plant biomass per unit existing plant biomass within a defined period – i.e. RGR – a correspondingly lower amount of N within the same period is required to meet the N ‘demand’ of a plant, resulting in a lower rate of NO₃⁻ being taken up per unit of existing biomass – i.e. NUR (CLEMENT & al. 1978 a,b). The N ‘demand’ of a given plant species is defined as the capacity of a plant to utilize, translocate or store NO₃⁻ or reduced N compounds at a given RGR and a given potential for NO₃⁻ net uptake. Utilization, translocation and storage capacity are determined by the capacity to translocate NO₃⁻ or reduced N compounds from root to shoot, to accumulate NO₃⁻ in the vacuoles and by the capacity of the NO₃⁻ assimilation pathway or the net rate of protein synthesis. Regulation of the NO₃⁻ NUR may then occur via feedback by NO₃⁻ itself, downstream metabolites of NO₃⁻ or other messengers, on the NO₃⁻ uptake system in the root plasma membrane (FORDE & CLARKSON 1999, TOURAINE & al. 2001). Eventually NO₃⁻ NUR will be adjusted via changes in NO₃⁻ efflux or, on a longer time scale, more likely in NO₃⁻ influx (DEANE-DRUMMOND 1986, LEE & DREW 1986, SIDDIQI & al. 1990).

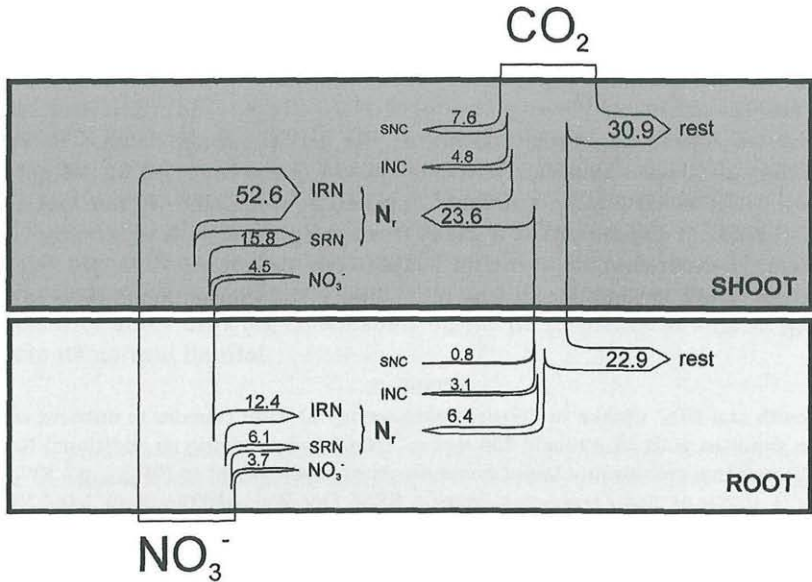


Fig. 1. Partitioning and allocation of C and N in seedlings of *Festuca rubra* as % of total C and N content with a RWR of $538 \text{ mg FW}_{\text{root}} \text{ g}^{-1} \text{ FW}_{\text{plant}}$. Plants were grown for 21 days on a nutrient solution at a RGR of $0.183 \text{ g FW}_{\text{plant}} \text{ g}^{-1} \text{ FW}_{\text{plant}} \text{ day}^{-1}$. For further explanation see text.

Allocation and partitioning of N

In addition to the above-described effect of NaCl on NO_3^- NUR, exposure to salinity led to changes in allocation and partitioning of N. Exposure to salinity had no effect on total N concentration, neither in the shoot nor in the root (Table 3). The N_r concentration in the shoot of NaCl treated plants was affected by less than 10% of the control value while in the root the N_r concentration increased by 14–48%, depending on the NaCl concentration in the nutrient solution (Table 3). In both root and shoot NO_3^- concentration was unaffected by exposure to 50 and 100 mol m^{-3} NaCl, respectively. On 200 mol m^{-3} NaCl, however, the NO_3^- concentration decreased by 57% and 58% in shoot and root, respectively (Table 3). Fig. 1 and 2 show the partitioning and allocation of N and C on day 21 after transfer to the nutrient solution in control plants and plants raised on 200 mol m^{-3} NaCl, respectively. Data from plants raised on 50 and 100 mol m^{-3} NaCl and data from harvesting days 7 and 14 from all NaCl treatments are not presented as figures. All values are expressed as percent of total N or total C. Exposure to salinity led to changes in the partitioning towards the single N fractions as well as allocation of N fractions between root and shoot. The amount of N diverted towards NO_3^- was nearly unaffected on 50 and 100 mol m^{-3} NaCl in both root and shoot (results not shown), but de-

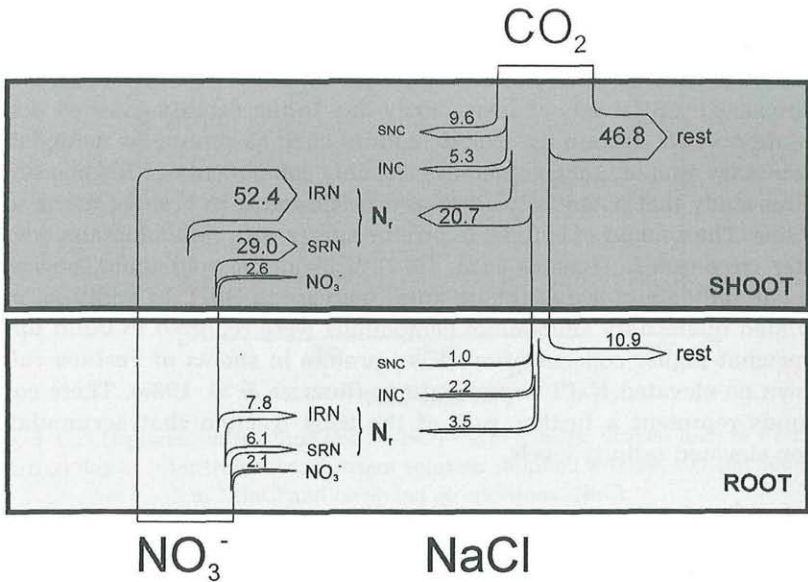


Fig. 2. Partitioning and allocation of C and N in seedlings of *Festuca rubra* as % of total C and N content with a RWR of $351 \text{ mg FW}_{\text{root}} \text{ g}^{-1} \text{ FW}_{\text{plant}}$. Plants were grown for 21 days on a nutrient solution containing $200 \text{ mol m}^{-3} \text{ NaCl}$ at a RGR of $0.087 \text{ g FW}_{\text{plant}} \text{ g}^{-1} \text{ FW}_{\text{plant}} \text{ day}^{-1}$. For further explanation see text.

creased in plants grown on $200 \text{ mol m}^{-3} \text{ NaCl}$ (Fig. 1 and 2). At the same time more N was fixed in the N_r fraction in the shoot, while less N was diverted to the N_r fraction in the root. The distribution between N_r and NO_3^- was unaffected on 50 and $100 \text{ mol m}^{-3} \text{ NaCl}$ in both root and shoot. On $200 \text{ mol m}^{-3} \text{ NaCl}$, however, $N_r:NO_3^-$ ratio in the root as well as in the shoot increased strongly, becoming 3 times higher on a whole plant basis (Fig. 3). The amount of N fixed in the IRN fraction of the shoot was only marginally affected by the salinity treatment. The SRN fraction was therefore responsible for most of the increase in N_r in the shoot, rising to 21, 20 and 29% of the plant total N on 50, 100 and $200 \text{ mol m}^{-3} \text{ NaCl}$, respectively. Consequently the IRN:SRN ratio in the shoot decreased at all salinity levels compared to control plants (Fig. 4a). In the root the amount of N fixed in SRN compounds remained at the control level of about 6% at all NaCl concentrations applied. The IRN:SRN in the root was unaffected at 50 and $100 \text{ mol m}^{-3} \text{ NaCl}$ but decreased by 35% on $200 \text{ mol m}^{-3} \text{ NaCl}$ (Fig. 4b). The concentration of free amino acids without proline remained unchanged in the shoot but increased more than 2 fold in the root at $200 \text{ mol m}^{-3} \text{ NaCl}$ (Fig. 5a).

The increasing allocation of N towards the shoot in relation to the whole plant in *Festuca rubra* was the consequence of a stronger inhibition

of root growth as compared to shoot growth and an increasing concentration of N_r containing compounds, mainly SRN, in the shoot as compared to the root of plants exposed to elevated salinity levels. The increasing N requirement by SRN was at least partly due to the salinity-induced accumulation of N_r containing osmotic solutes such as proline or methylated quaternary ammonium compounds. The only component of SRN measured in this study that potentially acted as osmotic solute in *Festuca rubra*, was proline. The amount of N fixed in proline agrees with results obtained with *Aster tripolium* L. (LARHER & al. 1982). This amino acid could, however, explain only about 4–10% of the total increase in SRN. In addition, methylated quaternary ammonium compounds were reported to build up at somewhat higher concentrations than proline in shoots of *Festuca rubra* grown on elevated NaCl concentrations (ROZEMA & al. 1985). These compounds represent a further part of the SRN fraction that accumulated upon elevated salinity levels.

Table 3.

Chemical composition ($\mu\text{mol g}^{-1}$ FW) of shoots and roots of *Festuca rubra* on day 21 after transfer to nutrient solution supplied with 50, 100 and 200 mol m^{-3} NaCl and receiving no additional NaCl. Total C; total N; NO_3^- ; amino acids (without proline); proline. Mean values (\pm SEM, One Way ANOVA, $n=3$). N_r , reduced nitrogen calculated from the difference between mean total N and NO_3^- concentration.

	0 mol m^{-3}	50 mol m^{-3}	100 mol m^{-3}	200 mol m^{-3}
Shoot				
Total C	6194 \pm 142	6355 \pm 43 ^{n.s.}	6858 \pm 163*	7754 \pm 217***
Total N	618 \pm 17	568 \pm 36 ^{n.s.}	640 \pm 13 ^{n.s.}	535 \pm 17 ^{n.s.}
NO_3^-	38 \pm 3	36 \pm 3 ^{n.s.}	31 \pm 2 ^{n.s.}	17 \pm 5**
Amino acids	51 \pm 3	52 \pm 3 ^{n.s.}	44 \pm 3 ^{n.s.}	55 \pm 1 ^{n.s.}
Proline	6.5 \pm 0.2	7.3 \pm 0.3 ^{n.s.}	7.1 \pm 0.7 ^{n.s.}	11.8 \pm 0.5***
N_r	580	534	610	519
Root				
Total C	2685 \pm 55	3080 \pm 88*	3199 \pm 58*	3908 \pm 152***
Total N	190 \pm 8	230 \pm 15 ^{n.s.}	201 \pm 23 ^{n.s.}	212 \pm 9 ^{n.s.}
NO_3^-	66 \pm 8	64 \pm 4 ^{n.s.}	60 \pm 2 ^{n.s.}	28 \pm 3**
Amino acids	13 \pm 1	9 \pm 2 ^{n.s.}	15 \pm 1 ^{n.s.}	29 \pm 3***
Proline	1.2 \pm 0.2	1.2 \pm 0.1 ^{n.s.}	1.7 \pm 0.3 ^{n.s.}	4.0 \pm 0.5***
N_r	124	166	141	184

The pool of free amino acids not including proline may also have been responsible for part of the increase in SRN as compared to IRN. Estimates, however, are difficult because the actual amount of N present in amino acids is unknown and it is unclear whether the composition of the amino acid pool changed upon exposure to salinity. Since not all free amino acids contain the same amount of N, a change in the composition of free amino

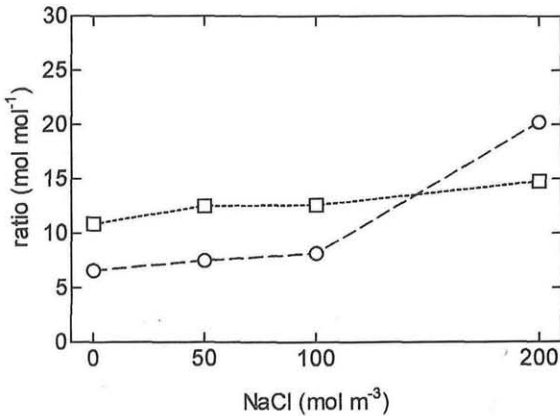


Fig. 3. C:N (squares, dotted line) and $N_r:NO_3^-$ ratio (circles, broken line) in *Festuca rubra* on day 21 after transfer to nutrient solution supplied with 50, 100 and 200 mol m^{-3} NaCl and receiving no additional NaCl.

acids would have affected the amount of N fixed in this fraction. Assuming that one mol of free amino acid not including proline contains one mol N (in reality this ratio must be higher) and that the composition of these amino acids does not change upon salinity, this fraction would require about 8% of total N in control plants and plants raised on 50 and 100 mol m^{-3} and 11% in plants raised on 200 mol m^{-3} NaCl, respectively. Similarly, the proline concentration in shoot and root was unaffected on 50 and 100 mol m^{-3} NaCl but increased 1.8 and 3.3 fold, respectively, in shoot and root of plants raised on 200 mol m^{-3} NaCl (Fig. 5b). The amount of proline present in the whole plant requires around 1% in control plants and plants grown on 50 and 100 mol m^{-3} and 2% of total N at 200 mol m^{-3} NaCl, respectively. The shoot:root ratio of total N increased by 59, 41 and 93% in plants grown on 50, 100 and 200 mol m^{-3} NaCl, respectively, compared to plants receiving no additional NaCl (Fig. 6). Likewise, shoot:root distribution of N_r increased by 73, 41 and 57% at 50, 100 and 200 mol m^{-3} NaCl, respectively, while shoot:root distribution of NO_3^- was unaffected (results not shown).

Taken together, exposure of *Festuca rubra* to elevated NaCl concentrations led to a slight increase in the 'demand' for N as a result of salinity induced accumulation of N_r containing osmotic solutes. A quantitative estimation of this increased 'demand', however, is difficult. Not all N_r containing compounds that presumably act as osmotic solutes have been assessed in the present study. Moreover, the N 'demand' for free amino acids not including proline is unknown. Changes in the concentration of this fraction are more likely the consequence of a salinity induced inhibition in net protein synthesis rather than due to a specific role in the maintenance of the

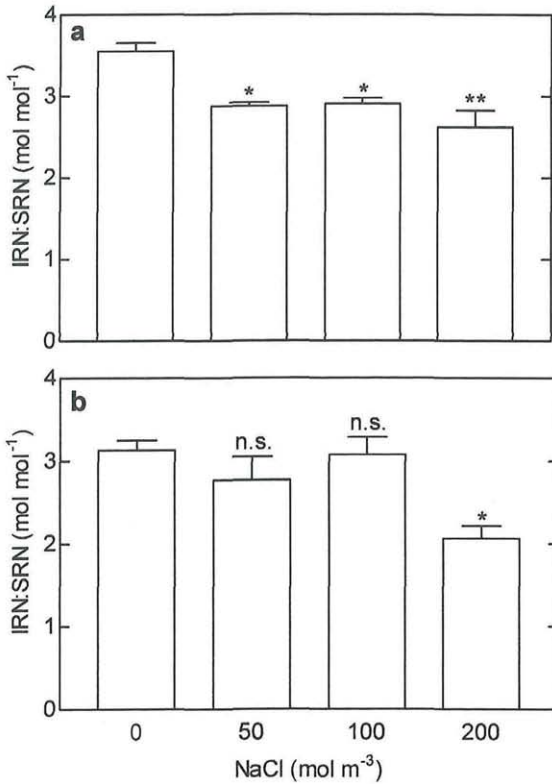


Fig. 4. IRN:SRN ratio shoot (a) and root (b) in *Festuca rubra* on day 21 after transfer to nutrient solution supplied with 50, 100 and 200 mol m⁻³ NaCl and receiving no additional NaCl. Mean values (±SEM, One Way ANOVA, n=3).

osmotic potential within the cell. In the case of such physiological disorders utilization of NO₃⁻ does not match with the RGR. The concept of N 'demand' is therefore not just related to the net rate of protein synthesis and the treatments are difficult to compare with control plants.

Allocation and partitioning of C

As for N, exposure to salinity led to enhanced C allocation to the shoot as a result of a stronger reduction of root growth as compared to shoot growth. No major changes, however, occurred in the partitioning of C towards the single fractions. In the shoot of plants grown on 50 mol m⁻³ NaCl total C concentration was unchanged but increased on 100 and 200 mol m⁻³ NaCl by 11 and 25% compared to control plants. In the root total C concentration increased by 15, 19 and 46% compared to controls at 50, 100 and 200 mol m⁻³ NaCl, respectively (Table 3). Fig. 1 and 2 show the parti-

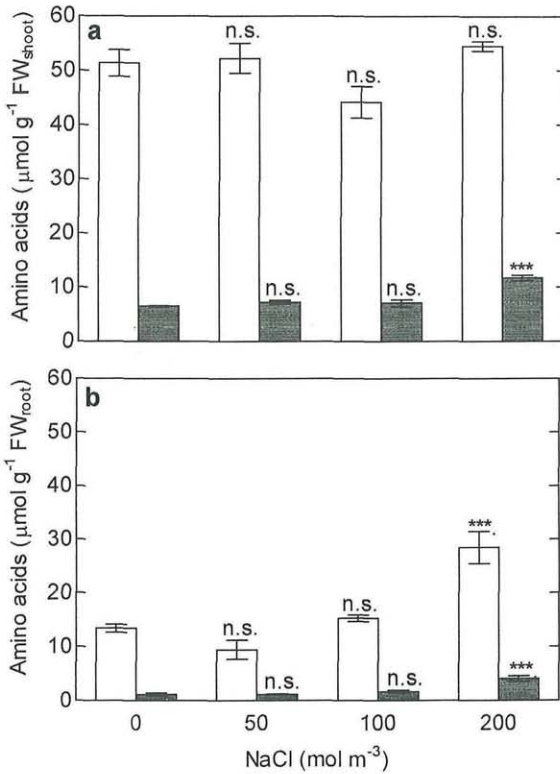


Fig. 5. Concentration of free amino acids without proline (white bars) and proline (grey bars) in shoot (a) and root (b) in *Festuca rubra* on day 21 after transfer to nutrient solution supplied with 50, 100 and 200 mol m⁻³ NaCl and receiving no additional NaCl. Mean values (±SEM, One Way ANOVA, n=3).

tioning and allocation C on day 21 after transfer to the nutrient solution in control plants and plants raised on 200 mol m⁻³ NaCl, respectively. Data from 50 and 100 mol m⁻³ NaCl are not presented as figures. Expressed as percentage of plant total C, 67% of C was present in the shoot and salinity treatment increased this value to 78, 72 and 82% in plants raised on 50, 100 and 200 mol m⁻³ NaCl, respectively. Assuming a protein C:N ratio of 3.75 (FLORKIN & STOTZ 1963), about 30% of total C would be present in Nr containing compounds of control plants and exposure to NaCl lowered this only slightly, mainly due a relative decrease in the Nr content of the root as compared to the whole plant. INC required 5% of total C in the shoot and 3% in the root and exposure to NaCl had no strong effect on the partitioning of C towards this fraction. The SNC fraction of shoot and root contained 8 and 1% of total C, respectively. In the shoot of plants raised on 50, 100 and 200 mol m⁻³ NaCl the C requirement for SNC increased only

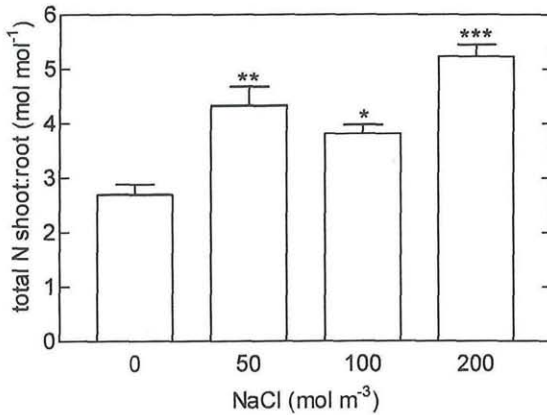


Fig. 6. Shoot:root distribution of total N in *Festuca rubra* on day 21 after transfer to nutrient solution supplied with 50, 100 and 200 mol m⁻³ NaCl and receiving no additional NaCl. Mean values (\pm SEM, One Way ANOVA, n=3).

slightly to 11, 9 and 10%, respectively, while in the root no change in C requirement occurred. The higher C requirement of the SNC fraction agrees with the general observation that in *Monocotyledoneae* soluble sugars, mainly glucose, fructose and saccharose contribute more to the osmotic potential than in *Dicotyledoneae* (ALBERT & POPP 1978, GORHAM & al. 1980). Relative to the total C content of the whole plant, however, this increase was negligible. The part of the C budget that could not be explained was 54% in control plants and increased only slightly in plants grown on 200 mol m⁻³ NaCl to about 58% (Fig. 2)

The C:N ratio based on the whole plant was enhanced at all salinity levels (One Way ANOVA, P°.001), reaching an amount that was 15, 16 and 36% higher than in control plants (Fig. 3), supporting the suggestion that NO₃⁻ uptake and reduction were not limited by a decreased availability of C (RUBINIGG 2002).

Salinity and N 'demand'

It is concluded that exposure of *Festuca rubra* to elevated NaCl concentration in the nutrient solution led to a strong reduction in RGR resulting in a lower N 'demand' for plant growth and consequently in a lower NO₃⁻ NUR. The stronger inhibition of root growth as compared to shoot growth led to an overestimation of the actual NO₃⁻ NUR if expressed on a root fresh weight basis and made it therefore difficult to compare the uptake rates from NaCl treated plants with control plants. The accumulation of N_r containing osmotic solutes resulted in a slightly enhanced N 'demand'. The requirement for soluble sugars involved in osmotic adjustment at elevated salinity levels did not require significant amounts of the C pool.

The present case shows that changes in RGR and RWR must be included in long-term studies on the effects of NaCl salinity on the NO_3^- uptake system for a correct interpretation of the results. Moreover, it shows the necessity of using a more salt tolerant plant species such as *Plantago maritima* to study direct effects of NaCl on the NO_3^- uptake system which are not the result of a possible 'demand'-driven downregulation via growth.

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