Effects of NaCl salinity on $^{15}$N-nitrate fluxes and specific root length in the halophyte Plantago maritima L.

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Abstract
The effect of salinity on nitrate influx, efflux, nitrate net uptake rate and net nitrogen translocation to the shoot was assessed in a $^{15}$N steady state labelling experiment in the halophyte Plantago maritima L. raised for 14 days on solution supplied with 50, 100 and 200 mol m$^{-3}$ sodium chloride or without sodium chloride. Additionally, salinity induced changes in root morphology were determined. Specific root length increased upon exposure to elevated sodium chloride concentrations due to variations in biomass allocation and length growth of the tap root. Changes in root morphology, however, had a minor effect on nitrate fluxes when expressed on a root fresh weight basis. The decreased rate of nitrate net uptake in plants grown on elevated levels of sodium chloride was almost entirely due to a decrease in nitrate influx. Expressed as a proportion of influx, nitrate efflux remained unchanged and was even lower at the highest salinity level. At all sodium chloride concentrations applied the initial rate of nitrogen net translocation to the shoot decreased relative to the rate of nitrate net uptake. It is concluded that under steady state conditions the negative effect of sodium chloride on the rate of nitrate net uptake at non growth-limiting salinity levels was due to the interaction between sodium chloride and nitrate transporters in the root plasma membrane and/or processes mediating the translocation of nitrogen compounds, possibly nitrate, to the shoot.

Abbreviations: iHATS – inducible high affinity transport system; cHATS – constitutive high affinity transport system; LATS – low affinity transport system; NUR – net uptake rate; N$_r$ – reduced nitrogen; RGR – relative growth rate; RWC – root water content; RWR – root weight ratio; SRL – specific root length

Introduction
High levels of NaCl in the root zone decreased the NO$_3^-$ net uptake rate (NUR) in a range of vascular plants (Gouia et al., 1994; Luque and Bingham, 1981; Martinez and Cerdá, 1989; Peuke et al., 1996). The physiological background of this interaction, however, is still unclear (Grattan and Grieve, 1999; Ullrich, 2001). Net uptake of NO$_3^-$ across the root plasma membrane is the result of two simultaneous and probably independent processes, which are regulated by different control mechanisms, influx and efflux (Forde and Clarkson, 1999; Jackson et al., 1976; Morgan et al., 1973; Touraine et al., 2001). It is generally assumed that NO$_3^-$ influx is performed by at least three structurally and functionally distinct transport systems, which are classified according to their kinetic properties as inducible and constitutive high affinity (iHATS and cHATS, respectively) and as constitutive low affinity transport system (LATS). Expression and kinetic properties of these transporters are regulated

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by the external NO$_3^-$ concentration and the internal N demand of the plant (Forde and Clarkson, 1999). Despite the quantitative importance of NO$_3^-$ influx, particularly in plants growing on optimal NO$_3^-$ supply, little is known about its nature and function (Aslam et al., 1996a; Clarkson, 1998; Miller and Smith, 1996; Schmidt and Schroeder, 1994). NO$_3^-$ efflux possibly regulates the cytoplasmic NO$_3^-$ concentration in the root which further depends on the rate of NO$_3^-$ influx, reduction, accumulation in the root vacuole and translocation to the shoot (Deane-Drummond and Glass, 1983; Miller and Smith, 1996).

Most studies that aimed to characterize the interaction between high external NaCl concentrations and the NO$_3^-$ uptake system have not determined influx and efflux separately (Aslam et al., 1984; Botella et al., 1994; Cerezo et al., 1992, 1999; Hawkins and Lewis, 1993; Peuke and Jeschke, 1999; Ward et al., 1986). Wieneke (1995) studied the effect of NaCl salinity on NO$_3^-$ influx and efflux separately (Aslam et al., 1984; Botella et al., 1994; Cerezo et al., 1992, 1999; Hawkins and Lewis, 1993; Peuke and Jeschke, 1999; Ward et al., 1986). Wieneke (1995) studied the effect of suddenly imposed NaCl salinity on NO$_3^-$ influx and efflux in *Cucurbita maxima*. However, in studies involving transfer of relatively salt-sensitive plant species to solutions with high salt concentrations, the consequences of drastic salt stress might superimpose interactions that become significant on a longer time scale (Billard and Boucaud, 1980, 1982; Cakirlar and Bowling, 1981; Hamza, 1980; Klobus et al., 1988; Niu et al., 1995; Suhayda et al., 1990). There are indications that the rate of NO$_3^-$ translocation to the shoot decreases in the presence of NaCl. The nature of this interaction is unclear (Cramer et al., 1995; Gao et al., 1996; Peuke et al., 1996). Cramer and co-workers, however, suggested that the decreased rate of NO$_3^-$ translocation to the shoot might lead to higher NO$_3^-$ efflux from the root (Cramer et al., 1995).

In the present study we have determined NO$_3^-$ fluxes in a steady state system in the halophyte *Plantago maritima* raised for 2 weeks on nutrient solution containing high concentrations of NaCl. Nitrate influx, efflux, NUR and the rate of N net translocation to the shoot were determined in labelling experiments using the $^{15}$N isotope as a tracer. Finally, changes in the morphology of the root system, which had previously been described for *Gossypium hirsutum* (Kurth et al., 1986; Reinhardt and Rost, 1995), *Raphanus sativus* (Waisel and Breckle, 1987) and *Plantago maritima* (Rubinigg, 2002), as well as reports on alterations in biomass partitioning between root and shoot of plants exposed to NaCl salinity (Kafkafi and Bernstein, 1996) led us to assess the suitability of using root fresh weight as reference value for the expression of N fluxes.

**Materials and methods**

**Plant culture**

Seeds of *Plantago maritima* L. were collected from a natural population on the Island of Schiermonnikoog, The Netherlands. Plants were germinated on vermiculite in a climate room with a day/night temperature of 20/20°C, RH of 65%, a photoperiod of 12 h day$^{-1}$ and a light intensity of 550 µmol m$^{-2}$ s$^{-1}$ at the plant level (PAR 400–700 nm, Quantum Sensor, SKP215, Skye Llandrindod Wells, UK) supplied by fluorescence lamps (F96T12/CW/VHO, 215 W, Sylvania, USA). On day 16 after sowing seedlings which had formed two primary leaves 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 of the following composition (in mol m$^{-3}$): KNO$_3$, 3.75; CaCl$_2$, 1.25; MgSO$_4$, 0.5; KH$_2$PO$_4$, 0.2; FeEDTA, 0.0225. Micronutrients were added according to Smakman and 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 NO$_3^-$ depletion did not exceed 10% of the initial amount. Solutions were replaced weekly. All measurements were performed on day 14 after transfer to the treatment solution.

**NUR, influx and efflux measured with $^{15}$N-nitrate**

Plastic disks supporting six plants each were transferred from the treatment solution to PVC beakers containing aerated labelling solution. Beakers for influx measurements had a volume of 300 cm$^3$ containing 99% enriched K$^{15}$NO$_3$ (Campro, The Netherlands). Beakers for NUR measurements had a volume of 1100 cm$^3$, containing 9.9% enriched K$^{15}$NO$_3$. Treatment solutions and labelling solutions had the same composition and pH. Based on earlier studies (Clarkson et al., 1996; Ter Steege, 1996) labelling periods of 5 min and 2 h were used to determine $^{15}$NO$_3^-$ influx and $^{15}$NO$_3^-$ NUR, respectively. At the end of the labelling period, the plants were subjected to a washing procedure during which roots were