Salt tolerance of *Elytrigia atherica*

Effects of nitrogen on salt tolerance of *Elytrigia atherica*

Iris Bakker
Supervisor: Roos Veeneklaas
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Abstract

Recently *Elytrigia atherica* has extended its range to a lower range on the salt marsh. These lower salt marshes have a higher soil salinity. Simultaneously, the nitrogen content of the soil has increased. It is thought that a strategy for halophytes lies in the ability to accumulate nitrogen containing metabolite compounds. This implies a close relation between nitrogen and salt tolerance. Because *E. atherica* takes over when the nutrient availability increases like in the high marsh and the implication of the close relation of nitrogen and salt tolerance, we hypothesised that E. atherica can tolerate more salt when more nitrogen is available. We also hypothesised that *E. atherica* would accumulate nitrogen under saline conditions as this is a proven strategy for many halophytes.
In this study we analysed the reaction of *E. atherica* to the combination of increasing salt and nitrogen.
No influence of nitrogen on the salt tolerance of *E. atherica* was found. Salt however did have a great influence on the growth rate and survival.

Introduction

Salinity is an important stress for plants in both natural and agricultural environments. It is one of the most important constraints for plants worldwide as it restricts growth and development (Orcutt & Nilsen,2000, Di Martino et al.2003).Salt stress involves osmotic and ionic stress. Osmotic stress limits absorption of water from the soil and ionic stress results from high concentrations of (possibly) toxic salts within plant cells (Orcutt & Nilsen, 2000, Naidoo & Naidoo, 2001, Di Martino et al., 2003).

Saline conditions occur naturally along the coastline under the influence of periodic flooding or wind salt spray (Rubinigg, 2002). Along the coastline, salt marshes emerge. Salt marshes develop along sheltered coasts on land, which still lie within the tidal limits of the sea (Pigott,1969).Salt marshes are regularly flooded with seawater, creating a saline environment. The major component of seawater is NaCl (fig 1), which is toxic for plants. In salt marshes, plants have adapted to (toxic) saline conditions. The halophytes in salt marshes are tolerant to regular tidal floodings (Di Martino et al., 2003).

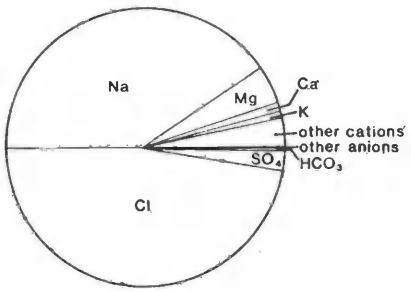


fig 1. Ionic composition of seawater. Jeffrey, 1987

Salt tolerance is associated with the ability to maintain a homeostatic ion concentration in the cytoplasm (Orcutt & Nilsen, 2000).
The metabolic properties of a cell depend on integrity of the cytoplasm. The enzymes of halophytes are no less sensitive to NaCl as non-halophytes. So halophytes must have some way of maintaining a low water potential (Jeffrey, 1987, Orcutt & Nilsen. 2000, Naidoo & Naidoo, 2001). There are several ways to obtain salt tolerance.

A first example is exclusion of salt. It is a simple way to obtain a certain tolerance. The exclusion is regulated by the transport of ions inside and outside the cell. Plants may discriminate against Na^+, and take K^+ in instead. Most halophytes have a high Na^+ /K^+ ratio. Halophytes on average, however do not use exclusion as a method to obtain salt tolerance.

Another way to cope with salinity is reabsorption of salts. Sodium is absorped by the plant, but then Na^+ is reabsorped from the transpiration stream and relocated to the roots where it is stored. This is only found in species with low salt tolerance, because on the long run the amount of Na+ in the roots would become toxic (Orcutt & Nilsen, 2000).

A third mechanism for salt tolerance is by using salt glands/bladders (Jefferies & Perkins, 1977, Orcutt & Nilsen, 2000). The structure of salt glands/bladders is designed for excluding salt from tissue. Most grasses have salt glands. The structures exclude the salt to the surface of leaves.

Plants also use succulence, which means they harbour a high proportion of water to dry weight. They have a higher ratio of vacuoles and relatively bigger vacuoles) as other plants. Cytoplasmic toxicity rapidly develops when the central vacuole becomes saturated with salt. Thus plants with high succulence can store more salt as they have more and bigger vacuoles as other plants. This, however, cannot be the only mechanism as the vacuole has a limited content.

A mechanism comparable to succulence is compartmentation. Plants compartmentalize the salt. The most likely place where the salts are stored is the vacuole. The salt is usually stored in older leaves. Halophytes with a relative fast growth rate thus have relative high salt tolerance if they use this mechanism, the salt disappears with the dying old leaves (Orcutt & Nilsen, 2000).

Several authors suggest that compatible metabolite compounds are used for the salt tolerance by lowering the waterpotential (Stewart et al. 1979, Jeffrey, 1987, Orcutt & Nilsen, 2000, Naidoo & Naidoo, 2001, Di Martino et al. 2003). Compatible metabolite compounds should not interfere with normal biochemical reactions. All the compounds are molecules that occur naturally in plants. Under saline conditions however they increase strongly above the normal levels and all are nitrogenous, what means they contain nitrogen. This indicates a close relationship between nitrogen metabolism and salt tolerance. Organic and inorganic solutes (compounds) occur. Inorganic solutes are found inside the vacuole and the organic solutes are found within the cytoplasm (Naidoo & Naidoo, 2001).

Elytrigia atherica is a tall grass, which is normally found on the high marshes along the North Sea coast. Its range spans from Southern Denmark to Northern Portugal. It reproduces both clonally and sexually. *E. atherica* is spreading from the high marsh to the lower marsh (v. Wijnen, 1999). The low marsh is more saline then the high marsh. *E.atherica* should have problems with growing on the lower marsh soil, because of the higher salinity.

The last decennia ,an increase in nitrogen accumulation on the entire salt marsh of Schiermonnikoog is observed. This might be caused by pollution by acid rain and inundation with seawater (Pigott, 1969, v. Wijnen, 1999).

The spread of *E. atherica* might be related to the increase of available nitrogen on the lower marshes. Apparently with accumulation of nitrogen on the high marsh, tall growing species take over on the salt marsh (Olff et al.,1997, v. Wijnen & Bakker, 1997, v. Wijnen, 1999). *E. atherica* takes over as a result of nitrogen accumulation at later stages of succession (Leendertse et al. 1997). More nitrogen is present in the sediment of late successional stages where more clay has accumulated over the past years. Sedimentation increases the nitrogen content of the soil and the older/ more mature the marsh, the thicker the sedimentation layer and hence the nitrogen pool.

E. atherica became established at sites with a thick clay layer (v. Wijnen, 1999).

One of the mechanisms to tolerate salt is by accumulating compatible metabolite compounds, which are all nitrogenous. *Triglochin bulbosa* and *Plantago maritima* can tolerate more salt when offered more nitrogen and its compatible metabolite compounds increase in number (Sheehy Skeffington & Jeffrey, 1985, Naidoo & Naidoo, 2001).
Stewart et al. (1979) state that *E. atherica* also accumulates a compatible metabolite compound (in *E. atherica's* case glycine betaine), just like *T. bulbosa* and *P. maritima*. This solute is nitrogenous, so the plant should be able to make more solutes when offered more nitrogen, thus creating a higher salt tolerance.

The present experiment is to see whether *E. atherica* spreads to the lower marsh due to an increase of the nitrogen availability, which may affect the salt tolerance of *E.atherica*. Our hypothesis is that nitrogen has a positive influence on the salt tolerance of *E. atherica*. Our second hypothesis is that plants accumulate nitrogen when growing on higher nitrogen levels.

We will try to answer those questions by growing plants in different nitrogen and salinity levels. We will test whether higher nitrogen availability gives *Elytrigia* the possibility to withstand more salt and whether we see an increase of internal nitrogen concentration as the plant is supposed to accumulate more nitrogenous compounds in saline conditions.

Material and Method

We want to test whether nitrogen has a positive influence on the salt tolerance of *E. atherica*. We also want to test whether *E. atherica* accumulates more nitrogen when grown on higher nitrogen levels.
We will do this by placing *E. atherica* plants per five in buckets with three levels of salinity (0, 50, 100% salinity of seawater) in combination with three nitrogen levels (5, 10, 20 mg/l) (table 1). We have five replicates per combination of salt and nitrogen, a total of 45 buckets. We will weight and measure the plants (in cm) to obtain data about growth and with that salt tolerance.

	5 mg N/l	10 mg N/l	20 mg N/l
0 % salt seawater	5 +	5 +	5 ++
50% salt seawater	5 -	5 +-	5 +
100% salt seawater	5 _	5 -	5 +-

Table 1. Number of buckets per treatment combination and the expectation for the experiment. Plus means plants will problems, minus means plants will have problems.

High levels of salinity

We germinated seeds from Schiermonnikoog (collected in September 2002) on vermiculite. We let them grow for seven weeks and added a little POKON during this period. After the seven weeks we placed them in a ten times diluted Hoagland solution (Hoagland,1950), in order to get them used to growing in fluid. Because experience has taught us that when working with soil, salt and nutrients tend to accumulate and exceed the concentration needed, a hydroponic (Hoagland) solution was chosen.

After one week, we placed these plants in 45 buckets, five per bucket (30*30 cm). The plants were placed in a plank that had five holes. The plants stayed with the shoot above the water level because they were supported by rubber corks. The buckets were aerated to prevent algae growth (for drawing see fig 2).

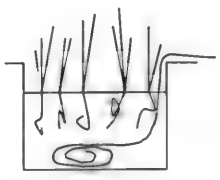


Fig 2. bucket with plants and aeration

The buckets contained a Hoagland solution with a minimal nitrogen (5 mg/l N) quantity and were randomly divided between two climate chambers with a temperature of 20 °C and a humidity of 50-70%. We slowly increased the

salinity to the required levels, by adding 25% of the maximum salt every day, over the period of one week. We increased the salinity slowly to prevent osmotic shock. The salinity was increased by adding only salt (NaCl), without changing the recipe of the Hoagland solution. The salinities were 0, 50 and 100 % that of waddenseawater (30 PSU), see table 1. On the first day we added salt to reach 25 % salinity of Waddenseawater, on the second day we raised this to 50%, and for the plants that were to grow in 100% salinity, we raised the salinity on the third day to 75% and on the fourth day to 100%.

The nitrogen levels were set at 5 mg/l, 10 mg/l and 20 mg/l (table 1). In the field on Schiermonnikoog the average nitrogen content of the soil lies around 10 mg/l (v. Wijnen, 1999). We doubled that amount to have a level with high nitrogen content. And because all plants need nitrogen for growth under any condition, our control was set at 5 mg/l, in stead of 0 mg/l. These were added to the buckets in the form of KNO_3. A combination of one salinity level and one nitrogen level is one treatment. We had 9 treatments and each treatment had 5 replicates (table 1), thus 45 buckets.
Three days after the highest salinity was reached, the plants were dabbed dry, measured (root length and shoot length in centimetres) and weighted (in grams). This was week 0.

Every week, the buckets were cleaned, the solution replaced, and new KNO_3 and salt were added . The plants were weighted and measured at the same time as the cleaning. We measured weight (grams), shoot length (cm) and root length (cm). If the salinity, which was measured once every three days, got higher during the week, demineralised water was added to decrease salinity. The climate chambers were checked every day to see if they still functioned.
The experiment ran for three weeks and after these three weeks, the plants were measured for the last time.

Low levels of salinity

The preliminary results from the first experiment showed that the salinities used there were too high for the *E. atherica* plants in that experiment. We decided to lower the salinity levels to 0, 15, 30 % seawater. The preliminary results also showed the possibility that 20 mg/l nitrogen was too high and we lowered this value to 15 mg/l (table 2).

	5 mg N/l	10 mg N/l	15 mg N/l
0% salt seawater	5 +	5 +	5 ++
15% salt seawater	5 -	5 +-	5 +
30% salt seawater	5 –	5 -	5 +-

Table 2. Number of buckets per treatment combination and the expectation for the experiment. Plus means plants will problems, minus means plants will have problems.

The second experiment was done a month after the first had ended and no POKON was added to the vermiculite. The plants grew for 7 weeks in the greenhouse. The experiment was largely done in the same way as the first experiment. The plants had some time to adjust to the salt to prevent osmotic stress and grew on a hydroponic (Hoagland) solution with minimal nitrogen (5 mg/l). The plants were measured weekly for weight, root length and shoot length. The solution was replaced and buckets were cleaned at the same time. The experiment lasted 4 weeks.

After the second experiment, the plants were dried and grinded. Then we had a nitrogen content analysis done by the laboratory, as described by Buurman (1996).

Data collection

The collected data of the plants were ordered according to week, N-level and salt-level (treatment).

Data was analysed per week per treatment. Each treatment consisted out of a combination of a nitrogen level and a salinity level. See table 1 and 2.
The total weight of the plants in one bucket in week 0 was subtracted from the total weight of the plants in that same bucket in week 1, 2 and 3 (and 4). This difference was averaged for each treatment. This is the relative growth rate in grams per treatment for that week in respect to week 0. The root length and shoot length did not show any significant changes in the preliminary results and were excluded from further analysis.
For the mortality graphs, we took the total number of dead plants per treatment divided per treatment.

Data analysis

For both experiments we used the data of the second week for the statistical analysis. The possible effects of nitrogen and salt are already visible in that week and the mortality is not very high yet. On each set of data we performed a normality test (Shapiro-Wilk). This was in order to see if an ANOVA was appropriate. (An ANOVA was applied here as the experiment is parametric in design). To achieve normality, the data needed to be log-transformed (Zar, fourth edition).

Then homogeneity of variance was tested with Levene's test. Here the data was also log-transformed (Zar, fourth edition). The parameters were tested with a univariate ANOVA test, with salt and nitrogen as independent factors (Field, 2000).

A Tukey- Kramer-post hoc test and a Newman-Keuls-post hoc test were used to distinguish between the levels of one factor.
All tests were performed in SPSS 11.0 and 12.0 (SPSS Inc).

Results

High levels of salinity

Salt does have a significant influence on the growth rate ($P<0.01$) (table 3 appendix). Influence of salinity was detected in the first week. The growth rate was lowest, even negative, when salinity was highest (100 % seawater) (fig. 3).

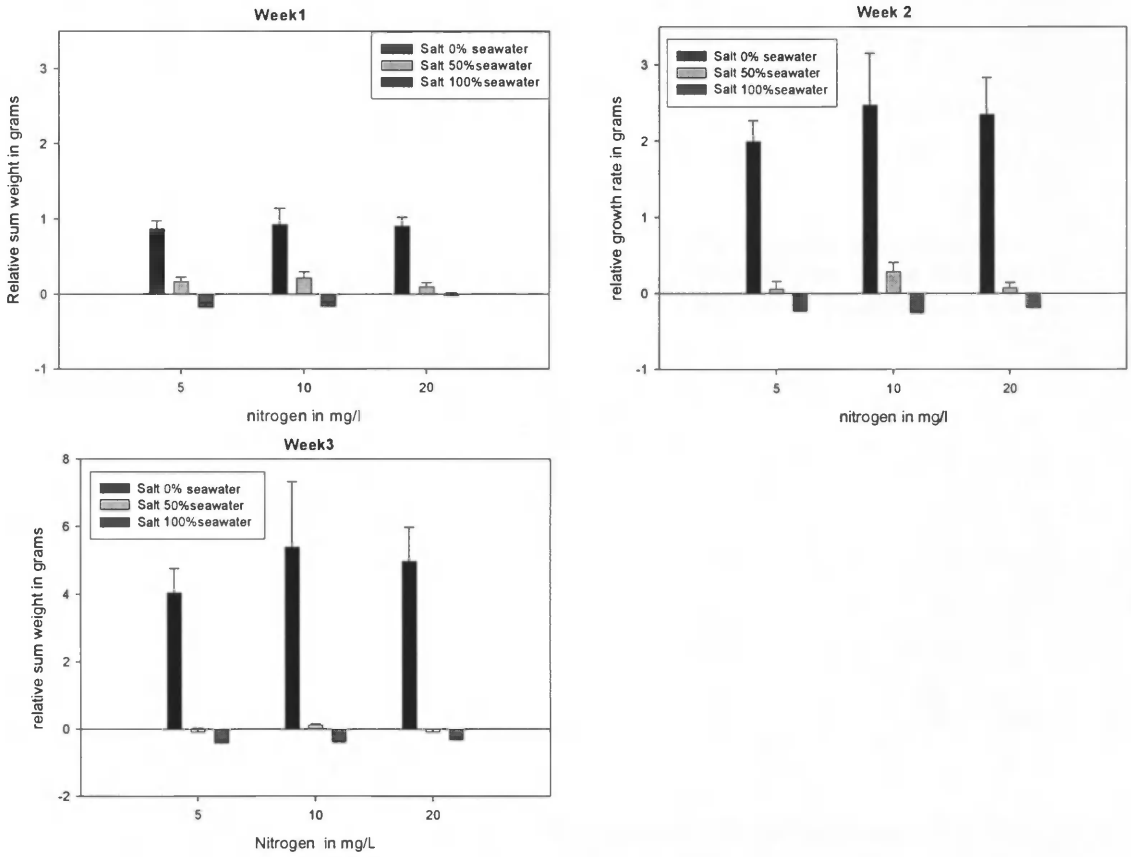


Fig 3. experiment1; relative growth rates per week of plants grown at different nitrogen concentrations and different salinities

Nitrogen does not have a significant influence on the growth rate and the different levels show more or less the same pattern (fig. 3). No significant interaction between salt and nitrogen was found. When the data was tested without interaction the results remained the same.

No deaths occurred in the first week. The mortality does increase in time, although no deaths occur at the level of 0 % salinity. The highest mortality occurs at 100 % salinity and 5 mg/l nitrogen after three weeks. In the second week , the highest mortality occurs at 50 % salinity and 20 mg/l nitrogen (fig. 4).

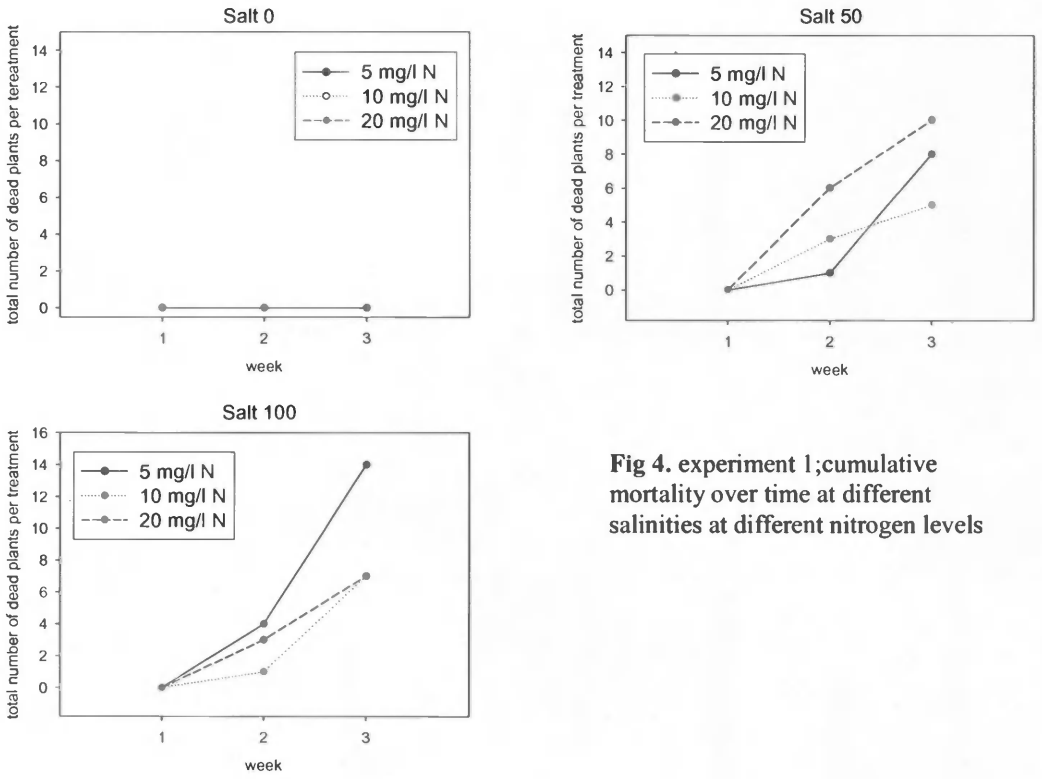


Fig 4. experiment 1;cumulative mortality over time at different salinities at different nitrogen levels

Low levels of salinity

Salt does have a significant influence on the growth rate ($P<0.01$) (table 4 appendix). Influence of salt was detected in the first week. Nitrogen appears to have an influence on the growth rate at 100 % salinity. The growth rate is above 0 at 15 mg/l nitrogen, whereas the other two levels have a negative growth rate (fig. 5). This is not significant. We found no significant interaction between salt and nitrogen. When the data was tested without interaction the results stayed the same.

The mortality increases in time. The highest mortality was found at 30 % salinity and 10 mg/l nitrogen. On average the 10 mg/l nitrogen has the highest mortality at each salt level and week (fig. 6). No time was left to analyse the mortality results statistically.

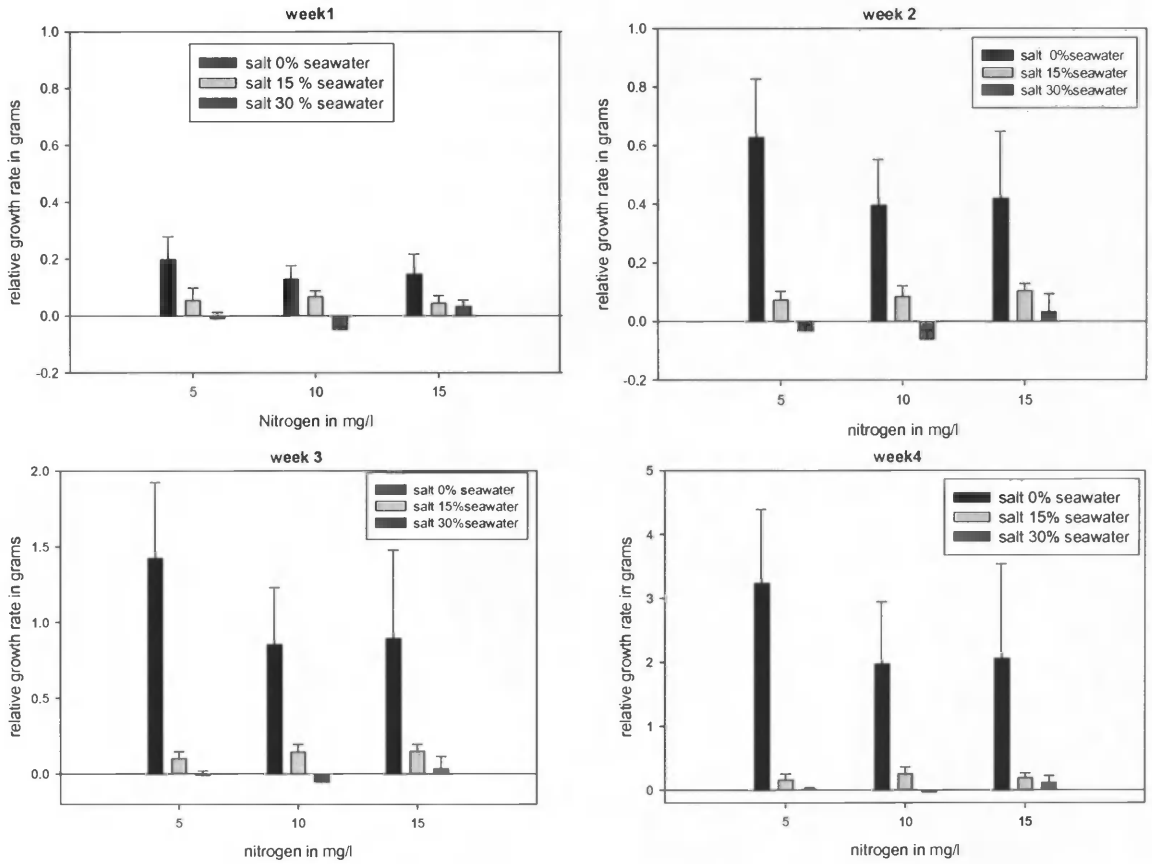
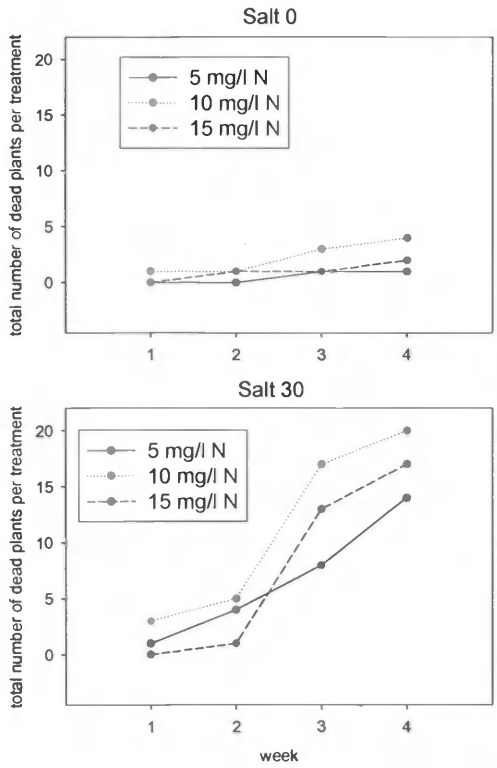


Fig 5 experiment 2; relative growth rates per week of plants grown at different nitrogen concentrations and different salinities



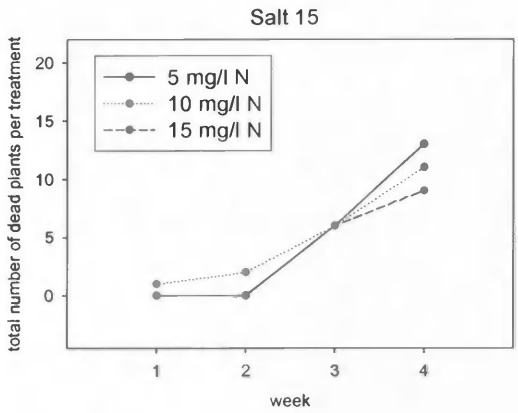


Fig 6 experiment 2; cumulative mortality over time at different salinities at different nitrogen levels

Nitrogen content

No strong differences in the pattern of the nitrogen content were found between the different nitrogen levels. The content at the higher salinity levels seems to be a little higher at 10 mg/l nitrogen than the other two nitrogen levels. The 0 % salinity has a higher nitrogen content as the other two levels. The 15 % and 30 % salinity do not differ much from each other. No significance was found, neither for salt not for nitrogen nor for an interaction between the two.

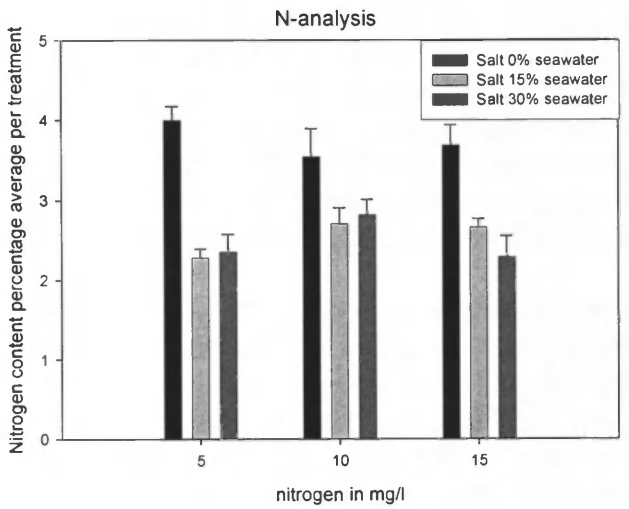


Fig 7. nitrogen content analysis, bars represent the average content of nitrogen per treatment in percent of total plant

Conclusion and Discussion

Our hypothesis was that nitrogen has a positive influence on the salt tolerance of *E. atherica*. Our second hypothesis was that *E. atherica* accumulates nitrogen at higher levels of nitrogen under saline conditions.

In this study, no effect of nitrogen on the salt tolerance of *E.atherica* was found. We conclude this for several reasons. First, no effect of nitrogen on the growth rate was found in either of the experiments. We expected to find a higher growth rate at high salinity at higher nitrogen levels. We expected that because the compatible compounds contain nitrogen. E. atherica is thought to accumulate a compatible compound. If the plants receive more nitrogen they should be able to tolerate more salt and have a higher growth rate than with less nitrogen. The growth rate however was not significant better at higher nitrogen levels in either of the experiments. The difference between the experiments is the height of the growth rate. In the first experiment the growth rate was higher. The difference might lie in the fact that our plants appeared to be weaker in the second experiment.

Secondly, there was no significant interaction between nitrogen and salt. Salt and nitrogen did not influence each other. A third reason is that salt did have a strong effect on the growth rate. The growth rate became lower with increasing salt. The nitrogen had no positive effect. The plants grew best without salt. In the first experiment there was a slightly higher growth rate if you added nitrogen but in the second experiment the lowest concentration of nitrogen had the highest growth rate, but none was significant.

A reason for concluding we found no accumulation of nitrogen, is that there is no significant higher nitrogen content of the plants grown at higher nitrogen and salt levels. The nitrogen content even decreased at higher salt levels. We expected an accumulation of nitrogen as the compatible compounds, which are thought to enhance salt tolerance, contain nitrogen (Naidoo & Naidoo, 2001, Di Martino et al., 2003). The pattern of the bars of the nitrogen content graph remains the same among the different nitrogen levels.
Our results are different than the results of some other studies (Sheehy Skeffington & Jeffrey, 1985, Leendertse et al., 1997, Naidoo & Naidoo, 2001, Di Martino et al., 2003). These studies did find an effect of nitrogen on the salt tolerance of plants or an increase in nitrogen content or compatible metabolite compounds, whereas we did not.

There are a few explanations for our results. While they diverge from some studies, other studies actually confirm them, at least partially. A first explanation for the decrease of nitrogen content with increasing salinity and the fact that we found no influence of nitrogen on the growth rate, is that some plants have a decreased nitrate net uptake rate under elevated levels of NaCl. The decrease in uptake rate is due to a reduced nitrate influx (Rubinigg, 2002). This means that at a higher salinity, less nitrogen is take in by the plants. This might be the case for *E.atherica*. A second reason for the decrease of nitrogen content and the fact that we found no influence of nitrogen on the growth rate, is given by

a different study. In that study a majority of the studied species showed no marked change in capacity to assimilate nitrogen when grown in saline conditions. So the *E. atherica* plants in our study were possibly not able to use more nitrogen then when the plants were grown without salt. And therefore also not able to increase their salt tolerance. However, *Sueda maritima* and *Atriplex littoralis* did have a decrease in nitrate and soluble organic nitrogen (Stewart et al, 1979). In our study we used KNO_3 to add (available) nitrogen to the solution, what might explain why we found no nitrogen accumulation in *E.atherica* and why the nitrogen did not enhance the salt tolerance. There is the possibility that *E. atherica* decreases its internal nitrate just as S. maritima and A. littoralis. *E.atherica* might need another source of nitrogen than nitrate.

Ammonium could be a good alternative nitrogen source. Sheehy Skeffington & Jeffrey (1985), used ammonium nitrate in their study. They did find an increase in growth under saline conditions when they added nitrogen in that form. In another (field) study, the number of plants on plots with addition of ammonium was higher than plots with nitrate added (Jefferies & Perkins, 1977). Populations in culture show a relative poor growth response to additions of nitrogen (Jefferies, 1977). Valiela and Teal (1974) added urea to their plots, because this is easily hydrolyzed to ammonium, a form readily available to plants. Their plants did have a higher nitrogen content when more urea was added. They also found that the ammonium contents of sediment water was steadily depleted during the growing season. This could mean that the plants use the ammonium. They found only small amounts of NO_2 and NO_3. It is suggested that this small amount merely reflects the reduced condition of sediment. We suggest to test whether *E. atherica* responds more to nitrate or to ammonium as others authors are still in doubt (Leendertse et al. 1997). But with all in mind, we do suggest using a form of ammonium instead of nitrate (Valiela & Teal, 1974, Jefferies & Perkins, 1977, Sheehy Skeffington & Jeffrey, 1985).

A high mortality rate was found with increasing salinity despite the added nitrogen. The salinity might have had such a strong effect that the *E. atherica* plants used in our study can not cope anymore. Rozema et al. (1983) showed that with an increase of salinity (same concentration range as in the present study) a decrease in growth rate of *E.atherica* occurs which is in agreement with our study. They however did not incorporate the effect of nitrogen on salinity stress in their study. *E. atherica* grew best in our study under sweet condition, like most other halophytes (Rozema, 1978).

A last explanation for our results might be given by the age and state of our plants. Most other authors have used older or even mature plants. There is the possibility that young *E. atherica* plants can not take the salinities used in the experiment. In the second experiment the plants appeared weak in comparison to the first experiment and showed even higher mortality than the plants in the first experiment. This suggests that young and weak plants have to little salt tolerance to cope with the salinity used in this study.

Although nitrogen does not seem to influence the salt tolerance, it may still contribute to the spreading of *E. atherica* to the lower marshes. Abiotic factors

such as salinity and water logging, did not prevent *E. atherica* from invading the lower marsh (Bockelmann and Neuhaus, 1999). Nitrogen is needed for plant growth and maintenance and the major limiting factor for plants on salt marshes (Jefferies and Perkins, 1977, Stewart et al., 1979, v. Wijnen, 1999). Bockelmann and Neuhaus (1999) suggested that *E. atherica* uses the increasing nitrogen levels for enhancing its competitive ability. And in a recent study the authors state that competitive processes are important for the spread of plants (Levine et al., 1998). This suggestion about the competitive ability is confirmed by a greenhouse experiment (Kuijper, 2004). In this study *E. atherica* was grown together with *Festuca rubra*, a grass from the lower marsh. When the nitrogen levels were increased, the competitive balance was shifted towards *E. atherica*. With high nutrient levels, the competition shifts towards above ground biomass and competition for light becomes more important (Emery et al., 2001). *E. atherica* is a tall grass with a large amount of above ground biomass, which can receive a high amount of sunlight. And can thus win competition for light.

Whether nitrogen increases *E. atherica's* salt tolerance or it enhances the competition abilities, the spread of the plant might change the composition of the salt marsh communities. Vitousek et al.(1997) state that recent human activities have doubled the (fixed) nitrogen supply on the planet and that coastal waters have experienced heavy eutrophication. With more available nitrogen in the soil, the plants adapted to low nitrogen supplies will disappear (Levine et al. 1998). It is therefore vital to discover how *E. atherica* (and other salt marsh plants) react to the increasing nitrogen levels. Further research on this subject is important.

With more knowledge of how biotic and abiotic factors interact to structure the salt marsh species composition, we will be able to better predict the effect of human activities and develop effective strategies for conserving the salt marshes for future generations.

Recommendations for future research

In future experiment it is advisable to further test the competition hypothesis. It is important to know how nitrogen influences *E. atherica* for possible conservational purposes. With more knowledge better decisions can be made to conserve the salt marsh for future generations.

However salt tolerance studies should still be performed as it is still a possible reason for the spread of E. atherica. First, we suggest to test whether *E. atherica* responds more to nitrate or to ammonium as others authors are still in doubt (Leendertse et al. 1997).

Second, older, maybe mature plants should be used, which have been germinated/ grown in lower densities with additional nutrients as vermiculite had none. This way the plants are stronger and can probably tolerate more salt.

A third recommendation is to let plants get used to the salinity over longer periods of time or to let them germinate with salt water to prevent osmotic shock. The plants in this study were grown under salt free conditions and therefore not used to growing on salt.

Future researchers may want to analyze the mortality as well as an influence nitrogen might be found here too.

For the nitrogen analysis, we suggest dividing the root and shoot. It is possible that *E. atherica* allocates its nitrogen more to one than the other. And it is advisable as well to test not the total nitrogen content but the levels of compatible metabolite compounds. *E. atherica* might not increase its total nitrogen content but reallocate the nitrogen it already contains to the compatible solutes.

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Appendix

Hoagland's hydroponic solution

Stock 60 Litre

0.5 M K2SO4	5ml/ L
1 M MgSO4	2 ml/ L
1 M NaH2PO4	1 ml/ L
0.01 M CaSO4	0.29gram/ L
Micronutrients	0.25 ml/ L
Fe3+EDTA	0.25 ml/ L
1 M KNO3	0.25 ml/ L

Micronutrients

H3BO3	2.86 gram/L
MnCl2.4H2O	1.81 gram/L
ZnSO4.7H2O	0.22 gram/L
CuSO4.5H2O	0.08 gram/L
Na2MoO4.2H2O	0.126gram/L

Statistical analysis

Table 4.

	M.S.	df	F	P
Salt	0.424	2	104.651	**0.000**
N	0.002	2	0.386	0.682
Salt * N	0.005	4	1.222	0.318

Statistical analysis of the (relative) growth rate of experiment 1.

Table 5.

	M.S.	df	F	P
Salt	0.111	2	20.937	**0.000**
N	0.002	2	0.419	0.661
Salt * N	0.003	4	0.644	0.634

Statistical analysis of the (relative) growth rate of experiment 2.