Journal of Coastal Conservation 3: 35-40, 1997

Effects of nitrogen addition on the growth of the salt marsh grass *Elymus athericus*

Leendertse, Peter C.[1], **Rozema, Jelte & Andrea, Adrie**

Department of Ecology and Ecotoxicology, Faculty of Biology, Vrije Universiteit; De Boelelaan 1087, NL-1081 HV Amsterdam, The Netherlands; [1]*Present adress: Centrum voor Landbouw en Milieu (CLM), Postbus 10015, NL-3505 AA Utrecht, The Netherlands; Fax +31 20 4447123; E-mail jrozema@bio.vu.nl*

Abstract. Effects of nitrogen addition on the growth of the salt marsh grass *Elymus athericus* were studied under greenhouse conditions. The addition of inorganic nitrogen (in the form of nitrate or ammonium and ranging from 0 - 24 g N/m^2) stimulated the growth of *Elymus athericus* at the highest addition. Addition of nitrogen led to an increase of the soil nitrate concentrations both in the nitrate and ammonium treated soil in the first period of the experiment, whereas no differences were present at the end of the experiment. Ammonium in the ammonium treatments was transformed to nitrate within 15 days.

In another experiment the values of the stable isotope nitrogen-15 – expressed as δ^{15}N – in nitrogen compounds used as fertilizer, in salt marsh soil and of *Elymus athericus* were measured. The δ^{15}N of the N-compounds added (between – 3.2 and + 2.6 ‰) were lower than the soil (ca. +10 ‰) and plants (ca. + 8 ‰). During growth in water culture the δ^{15}N of the leaves, stems and roots of *Elymus athericus* decreased from + 9‰ to – 1 ‰. The latter value was close to the δ^{15}N of the N-compounds used in the water solution. Addition of N-compounds in soil culture, however, did not lead to such a decrease of the δ^{15}N of *Elymus athericus*. The difference in δ^{15}N between soil nitrogen and the N-compounds added may be too small to be used successfully in ecological studies of nitrogen fluxes in the salt marsh environment.

Keywords: Ammonium; δ^{15}N; Greenhouse; Nitrate, Nitrogen uptake; Plant growth; Water culture.

Introduction

During the last decades, the Wadden Sea has been loaded with large amounts of nitrogen and phosphorus of anthropogenic origin (van der Veer et al. 1989; de Jonge 1990), which will have reached the salt marshes in this area. Jefferies & Perkins (1977) and Valiela (1984) showed that fertilization of salt marsh vegetation with nitrogen leads to an increase in plant biomass production and to a reduction of the number of plant species.

One of the vegetation changes in the Wadden Sea area that may be related to enhanced nutrient input is the recent increase of the grass *Elymus athericus* (Link) Kerguélen. This species is mainly found in the flood mark zone (Beeftink 1977), where it profits from the high deposition of organic matter and the increased nutrient availability. However, Bakker (1989) reported a large increase in abundance of this plant species on the Oosterkwelder salt marsh on the Wadden island of Schiermonnikoog (northern Netherlands) in ungrazed areas outside the flood mark zones. Olff (1992) assumed that this spread of *E. athericus* is related to the accumulation of nitrogen in the soil due to sedimentation on the salt marsh. *E. athericus* may be a better competitor for light than other plant species with the progress of nitrogen accumulation in the soil (Olff 1992). However, as yet there is no experimental evidence that growth of this species is strongly stimulated by nitrogen.

It is difficult to separate effects of nitrogen on the vegetation caused by natural enrichment from that by anthropogenic sources. Part of the N-input into the Wadden Sea and the salt marshes will originate from industrial, agricultural and domestic sources. In some studies natural variation in nitrogen stable isotope ratios was used to trace the various N-sources (Heaton 1986; Lindau et al. 1989). The ratio ^{15}N/^{14}N is expressed relative to the ratio under standard conditions, i.e. atmospheric N_2 with a ^{15}N abundance of 0.3663%: $\delta^{15}N = 1000\ (R_{sample}/R_{standard}) - 1$, where R is the ^{15}N/^{14}N ratio.The ^{15}N abundance of atmospheric nitrogen is globally uniform and has a value of 0.3663% (δ^{15}N=0 ‰) (Junk & Svec 1958). In general, this ^{15}N isotope value is lower than in other N-pools, like soil and plants. Soil nitrogen shows more variation than atmospheric nitrogen, but on average a value of 0.3699% (δ^{15}N = + 9 ‰) is found (Shearer & Kohl 1989). Since N-compounds and fertilizers are in general synthesized from atmospheric nitrogen, their ^{15}N abundance is almost similar to the atmospheric value (Shearer et al. 1974).

In a situation where an N-fertilizer is added the ^{15}N value of the plant will approach the ^{15}N value of the fertilizer if all plant nitrogen is derived from the fertilizer. The difference in ^{15}N abundance between atmospheric nitrogen and other N-pools, like soil and plants, is used in ecological research for estimating the amount of nitrogen derived from atmospheric nitrogen fixation (Shearer & Kohl 1989). If a plant derives all its nitrogen

from nitrogen fixation, the ^{15}N value of the plant will be similar to the value of atmospheric nitrogen. If a plant derives all its nitrogen from the soil, the ^{15}N value will be similar to the value of the soil. From intermediate values the amounts derived from nitrogen fixation and from the soil can be calculated (Shearer & Kohl 1989). In theory, it should be possible to use the same approach to estimate the amount of nitrogen in plants derived from the natural soil source and from chemically synthesized N-compounds and fertilizers.

The growth and N-uptake of *E. athericus* was measured in a greenhouse with different levels of N-supply to test the response of the species to an increase in availability of inorganic nitrogen. *E. athericus* was expected to take up nitrogen mainly in the form of nitrate, since in the flood mark zone, where the species is frequently found, nitrate is the dominant inorganic nitrogen form (Rozema et al. 1982, 1983). To test whether *E. athericus* actually has a preference for either nitrate or ammonium as N-source, nitrogen was applied in both forms. In another experiment the $\delta^{15}N$ in soil, in plant parts of *E. athericus*, and in N-compounds was measured in order to investigate whether ^{15}N values can be used to differentiate between soil-derived and fertilizer-derived nitrogen in the plants.

Methods

Experiment 1

In February 1992 rhizomes of *Elymus athericus* and salt marsh soil were collected on a mainland salt marsh near Holwerd (Friesland, The Netherlands). The salt marsh soil had a total N-concentration of 0.19 %, a salinity of 12 g Cl^-/l and a pH (KCl) of 7.92. The rhizomes were transferred to commercial potting soil in a greenhouse under supplementary light (≥ 300 µmol m^{-2} s^{-1}, PAR) from 400 W Philips HPI/T lamps for a period of 14 h daily. Day temperature was 25 ± 2° and night temperature was 18 ± 1 °C; relative humidity varied from 60 to 75 %. After 14 days, 114 plants were transferred to 1.75 l pots (soil surface 147 cm^2) filled with the salt marsh soil, one plant per pot. After seven days six plants were randomly chosen for an initial harvest. The remaining 108 plants were randomly divided into nine groups of 12 plants each, and subjected to addition of nitrogen in the form of potassium nitrate or ammonium chloride at rates of 0, 3, 6, 12 and 24 g N/m^2. Water was added regularly to the pots to maintain a moisture content of 30 - 35% of fresh weight of the soil.

Six plants of each treatment were harvested on day 35 and day 70. The plants were separated into leaves, stems and roots. Roots were sampled by rinsing the soil through a 0.5 mm sieve. Dry weight of leaves, stems and roots was determined after drying at 80 °C for at least 48 h. The dried plant material was homogenized by use of a mill (Retsch Schwungmühle MM2) whereafter the N-concentration was measured on an automatic nitrogen analyser (Roboprep-CN/ Europascientific, Crewe, UK) for the treatments 0, 6 and 24 g N/m^2.

Soil samples of three pots per treatment were taken on day 15, 35 and 70 using a corer (∅ 3 cm). About 20 g of the fresh sample was weighed in 250 ml polyethylene pots and shaken for 1 h at 75 rpm with 50 ml of 1*M* KCl. The pots were incubated overnight (18 h, 12 °C), centrifuged for 15 min at 12000 g and filtered through a paper filter and a membrane filter (0.45 µm, Schleicher & Schuell). The solutions were frozen (– 25 °C) until analysis of nitrate and ammonium was performed using an auto-analyser (SKALAR SA 40). The remaining amount of each soil sample was oven-dried at 80 °C in order to determine the soil water content.

The data of biomass and N-concentrations of the plants and of the nitrate and ammonium concentrations of the soil were tested by One-way Analysis of Variance. Biomass and nitrogen data were log-transformed to obtain homogeneity of variances (Sokal & Rohlf 1981).

Experiment 2

Two soil (0 - 5 cm) and three leaf samples of *E. athericus* from three study sites in the Wadden Sea area (sampling and sites described in Leendertse 1995) were chosen in order to measure the ^{15}N abundance of soil and plants in the field situation. Furthermore 12 plants, precultured as described in experiment 1, were grown under similar greenhouse conditions in water culture. The plants were grown in 5 litres of a nutrient solution containing

3.0 mM KNO_3	2.0 mM $Ca(NO_3)_2.4H_2O$
1.0 mM $NH_4H_2PO_4$	0.5 mM $MgSO_4.7H_2O$
25 µM KCl	12.5 µM H_3BO_3
1.0 µM $MnSO_4.H_2O$	1.0 µM $ZnSO_4.7H_2O$
0.25 µM $CuSO_4.5H_2O$	0.25 µM $(NH_4)_6Mo_7O_{24}.4H_2O$

10 µM Fe(Na)EDTA.$3H_2O$; pH 5.5.

The solution was renewed weekly. Plants were harvested on days 0, 35 and 57 (four plants per harvest). The plants were separated in leaves, stems and roots and oven-dried at 80 °C for at least 24 h. Furthermore, a number of samples from experiment 1 was selected to measure the ^{15}N abundance (roots, leaves and stems of three plants of the control, the 6 g N/m^2 treatments and the 24 g N/m^2 treatments).

All plant and soil samples were homogenized by use of a mill (Retsch Schwungmühle MM2), 4 mg of each sample was weighed in tin cups and the ^{15}N abundance

Table 1. Soil ammonium and nitrate concentrations (KCl-extractable) (mg/kg dry soil) on day 0 and after 15, 35 and 70 days. Values are means of three replicates. Letters indicate significant differences within a column (Tukey's HSD test; $p < 0.05$). Added amounts of KNO_3 and NH_4Cl per kg dry soil on day 0 were derived from the total amount of dry soil per pot and the amount of nitrogen added per pot (treatment).

		Day 0	Day 15			Day 35			Day 70		
Treatment (g N/m^2)		added NO_3/NH_4	NO_3	NH_4	total	NO_3	NH_4	total	NO_3	NH_4	total
Control		0	2^a	3^a	5^a	1^a	8^a	9^a	1^a	2^{ab}	3^a
3 g	KNO_3	25	13^b	3^{ab}	16^b	1^a	6^a	7^a	1^a	2^{ab}	3^a
	NH_4Cl	25	15^b	3^{ab}	18^b	8^{ab}	3^a	11^a	1^a	5^{ab}	6^a
6 g	KNO_3	50	41^b	4^{ab}	45^b	15^{ab}	2^a	17^a	1^a	5^b	6^a
	NH_4Cl	50	21^b	4^{ab}	26^b	2^a	4^a	6^a	1^a	3^{ab}	4^a
12 g	KNO_3	100	97^c	3^{ab}	100^c	51^b	2^a	53^b	1^a	2^{ab}	3^a
	NH_4Cl	100	110^c	15^{abc}	125^c	59^b	3^a	62^b	7^a	2^{ab}	9^a
24 g	KNO_3	200	320^c	4^b	324^c	115^b	1^a	116^b	5^a	2^{ab}	7^a
	NH_4Cl	200	140^c	43^c	182^c	116^b	6^a	120^b	10^a	1^a	11^a

of the sample was measured on an automatic nitrogen and carbon analyser-isotope ratio mass spectrometer for solid and gas samples (Roboprep-CN and Tracermass, Europascientific, Crewe, UK). The results were expressed as $\delta^{15}N$ (‰). The ^{15}N abundance in the N-compounds KNO_3, NH_4Cl, $Ca(NO_3)_4.H_2O$ and NH_4NO_3 was also measured.

The data of ^{15}N abundance of the soil and leaves collected in the field were tested for significance of differences between sites by one way analysis of variance. The data of ^{15}N abundance of plants of the water culture and of the soil culture were tested for significance of differences in time by one way analysis of variance (Sokal & Rohlf 1981).

Results

Experiment 1

The addition of nitrogen led to an increase in inorganic nitrogen concentrations of the soil on day 15 and day 35 (Table 1; $p < 0.05$). On day 70 no differences between treatments in inorganic nitrogen concentrations were found. In both the ammonium and nitrate treatments the inorganic nitrogen of the soil was mainly present in the form of nitrate, indicating the transformation of ammonium to nitrate (nitrification) in the first weeks of the experiment.

There was a positive effect of the highest nitrogen addition of 24 g N/m^2 on the above-ground biomass of *E. athericus* after 35 days and 70 days ($p < 0.05$; Fig. 1). A significant increase in the nitrogen concentration of the leaves and stems of *E. athericus* was found on day 35 and day 70 at the highest nitrogen addition, both in the form of nitrate and of ammonium ($p < 0.05$; Table 2).

Experiment 2

The soil samples collected in the field had an average $\delta^{15}N$ value of +9.6 ‰ and there were no significant differences between sites (Table 3). The $\delta^{15}N$ of the leaves of *E. athericus* collected in the field had an average value of + 8.0 ‰ (Table 3). Only the $\delta^{15}N$ of the *E. athericus* leaves of the salt marsh at Terschelling were significantly lower compared to the other sites ($p < 0.05$).

The $\delta^{15}N$ of various chemically synthesized N-compounds (Table 4; between – 3.2 and +2.6 ‰) was significantly lower than the values found in the soil and leaves (Table 3; +9.6 ‰ and +8.0 ‰ respectively). There was a significant decrease in the $\delta^{15}N$ values of the plant parts of *E. athericus* cultivated in water culture from +9.1 ‰ at the start of the experiment to – 5.9 ‰ on day 35 and – 1.3 ‰ on day 57 ($p < 0.05$; Table 5). These values were close to the values of the N-compounds used in the nutrient solution (KNO_3: – 0.4 ‰ and $Ca(NO_3)_2.4H_2O$: – 3.2 ‰; Table 4). No difference was observed in the $\delta^{15}N$ of the plants between the nitrogen treatments in the soil culture (Table 6). Addition of

Table 2. Nitrogen concentrations (g N/kg dry weight) of the leaves, stems and roots of *Elymus athericus* 35 and 70 days after the start of the experiment. Values are means of three replications. Letters indicate significant differences within a column (Tukey's HSD test; $p < 0.05$).

		Day 35			Day 70		
Treatment (g N/m^2)		leaves	stems	roots	leaves	stems	roots
0 g	Control	28.0^a	23.1^{ab}	11.9^a	20.6^a	9.9^a	11.8^a
6 g	KNO_3	38.2^b	28.0^{ab}	19.4^a	18.2^a	10.0^a	10.8^a
	NH_4Cl	25.3^a	17.7^a	15.5^a	15.0^a	6.9^a	9.9^a
24 g	KNO_3	40.6^b	30.5^b	23.1^a	32.4^b	18.0^{ab}	16.2^a
	NH_4Cl	40.4^b	29.7^b	18.2^a	32.0^b	18.4^b	14.0^a

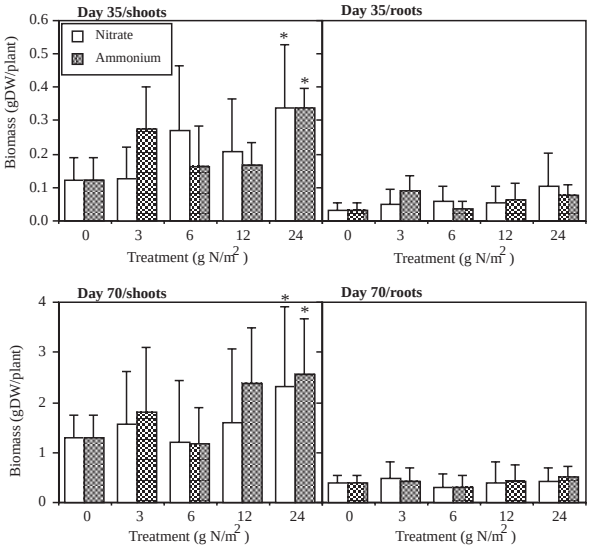

Fig. 1. Shoot and root biomass of *Elymus athericus* (g dry weight per plant) after 35 and 70 days of growth at increasing nitrogen addition in the form of nitrate or ammonium. Mean and standard deviations are given. The initial dry weight was 0.020 g for shoots and 0.0075 g for roots. Note that different Y-axes are used for 35 and 70 days. * indicates a significant difference from the control (Tukey's HSD test; $p < 0.05$).

nitrogen in the form of potassium nitrate (– 0.4 ‰) and ammonium chloride (+2.6 ‰), with lower δ^{15}N compared to the soil (+9.6 ‰) (Tables 3 and 4), did not lead to lower δ^{15}N in the plants (Table 6). On the contrary, there was an increase of δ^{15}N in the plants from +9.3 ‰ to +17.2 and about +28 ‰ during the experiment in the control and nitrogen treatments, respectively. These values were well above those of the original soil and the supplied inorganic N-compounds.

Table 3. δ^{15}N values (‰) of the top soil (0 - 5 cm) and of leaves of *Elymus athericus* at three different salt marsh sites in the Wadden Sea (as described in Leendertse 1995), and of soil and leaves at the start of experiment 1 (Friesland). Soil values are means and standard deviations of two samples collected in May 1991. Leaf values are means and standard deviations of three samples per site, one collected in May 1991 and two collected in September 1991. Leaf and soil samples at the start of experiment 1 were collected in February 1992. Letters indicate significant differences within a column (Tukey's HSD test; $p < 0.05$).

Salt marsh site	Soil	Leaves
Schiermonnikoog	+10.3 ± 0.1[a]	+8.1 ± 2.0[a]
Terschelling	+10.2 ± 0.1[a]	+4.2 ± 1.5[b]
Groningen	+6.4 ± 2.9[a]	+10.7 ± 1.5[a]
Friesland (exp. 1)	+11.5 ± 1.8[a]	+9.2 ± 0.6[a]
Mean of four sites	+9.6 ± 2.2	+8.0 ± 0.6

Table 4. δ^{15}N values (‰) – means and standard deviations of 2 - 6 replicates – of various inorganic nitrogen compounds.

Nitrogen compound	δ^{15}N (‰)
KNO_3	– 0.4 ± 2.2
NH_4Cl	+ 2.6 ± 2.6
$Ca(NO_3)_2.4H_2O$	– 3.2 ± 0.6
NH_4NO_3	+ 1.2 ± 0.6

Discussion

Effects of nitrogen in plants and soil

The increase of *Elymus athericus* on salt marshes in the Wadden Sea (Bakker 1989; Leendertse 1995) may be related to an increased supply of nitrogen to the salt marshes. In the present study, addition of inorganic nitrogen led to an increase in above-ground biomass of *E. athericus* in the highest treatment of 24 g N/m^2. In a field study on Schiermonnikoog, addition of 6 g N/m^2 also led to an increase in growth of *E. athericus* (Leendertse 1995). These results indicate that the aerial biomass of *E. athericus* is increased after addition of inorganic nitrogen. Not only the biomass but also the distribution of the species seems related to accumulation of nitrogen in the soil (Olff 1992). The yearly N-input to Wadden Sea salt marshes via flood water is estimated to be 50 - 200 g N/m^2 and via atmospheric deposition 3 g N/m^2 (Leendertse 1995). There is a large input of anthropogenic nitrogen into the Wadden Sea and the inorganic nitrogen concentrations in parts of the Wadden Sea have increased during the last decades due to anthropogenic input (van der Veer et al. 1989). It is not known, however, which part of the total yearly input into the salt marsh is from anthropogenic sources. Therefore, an effect of nitrogen on the plant species due to natural nitrogen enrichment cannot easily be separated from an effect due to anthropogenic N-input. A stimulation of growth of salt marsh plants after N-fertilization on salt marshes in Europe and the USA was also found for other plant species in a number of studies (e.g. Tyler 1967; Valiela & Teal 1974; Valiela 1984), which indicates that nitrogen is often a plant growth limiting factor in the salt marsh environment.

Table 5. δ^{15}N values (‰) (means and standard deviations of three replications) in *Elymus athericus* grown in water culture after 0, 35 and 70 days. Each plant value is the mean of root, stem and leaf – since there were no significant differences in δ^{15}N between these plant parts. Letters indicate significant differences within the row (Tukey's HSD test; $p < 0.05$).

	Day 0	Day 35	Day 57
δ^{15}N	+9.1 ± 2.1[a]	– 5.9 ± 1.1[b]	– 1.3 ± 1.4[c]

Table 6. δ^{15}N values (‰) in *Elymus athericus* grown in soil culture after 0, 35 and 70 days (experiment 1). Values are means and standard deviations of three replicates. Each plant value being the mean of root, stem and leaf since there were no significant differences in δ^{15}N between these plant parts. Letters indicate significant differences within a row (Tukey's HSD test; $p < 0.05$).

Treatment (g N/m^2)	Day 0	Day 35	Day 70
0 g Control	+9.3 ± 2.2^a	+14.7 ± 2.9^b	+17.2 ± 1.2^b
6 g KNO_3	+9.3 ± 2.2^a	+18.1 ±13.7^{ab}	+19.5 ± 0.6^b
NH_4Cl	+9.3 ± 2.2^a	+19.2 ± 0.7^b	+21.3 ± 1.3^b
24 g KNO_3	+9.3 ± 2.2^a	+9.5 ± 2.4^a	+25.6 ±13.2^b
NH_4Cl	+9.3 ± 2.2^a	+9.2 ± 4.5^a	+30.2 ± 8.8^b

E. athericus may show a preference for nitrate as N-source, since this is the dominant inorganic nitrogen form in the flood mark zone, where the species is found (Rozema et al. 1982, 1983). In the present study there were no differences in growth response and N-uptake of *E. athericus* between addition of inorganic nitrogen either as an ammonium or nitrate salt. Due to the rapid oxidation of ammonium to nitrate in the treatments with NH_4Cl (Table 1), however, the results cannot be used to test a preference of *E. athericus* for nitrate.

Addition of inorganic nitrogen initially led to a large increase of the inorganic nitrogen concentration in the soil. There was, however, a significant loss of inorganic nitrogen from the soil during the experiment. A first explanation for this loss may be the washing out of nitrate from the pots due to the addition of water. A second explanation may be the uptake of nitrogen by the plants. From biomass and nitrogen concentrations at the start and end of the experiment the uptake of nitrogen by plants can be estimated. In the control pots there was a plant uptake of 35 mg N per pot, whereas at the highest nitrogen addition (24 g N/m^2) this was 70 mg N per pot. This means an increase in uptake by the plants of 35 mg N per pot while the addition of nitrogen was 350 mg N per pot at the highest treatment. The conclusion is that the large decrease in ammonium and nitrate concentrations in the soil at the highest nitrogen addition cannot just be explained by increased N-uptake by the plants since the maximum uptake is only in the order of 10% of the added amount. A third explanation may be the transformation of nitrate to atmospheric nitrogen by the process of denitrification. The high nitrate concentrations at the beginning of the experiment were favourable for high denitrification rates, although the rapid oxidation of ammonium to nitrate suggest oxidized conditions in the soil. The shifts in ^{15}N values in the plants also suggest that denitrification was an important flux (see below).

Use of d^{15}N values in ecological studies

This study showed that the δ^{15}N values of the N-compounds analysed were close to the atmospheric value (0 ‰), as was also found by Shearer et al. (1974). The δ^{15}N values were also lower than the values of other N-pools like the soil and the plant parts of *E. athericus* (about +9 ‰). It might be possible to differentiate between the amount of nitrogen derived by the plants from the natural soil source and that derived from chemically synthesized N-compounds. Paerl & Fogel (1994) showed this for phytoplankton. In their growth experiments the δ^{15}N of phytoplankton decreased from +4.5 ‰ to +2 ‰ if rain water with a $\delta^{15}NH_4^+$ value of – 4 ‰ was added in bioassays with coastal water, indicating that the phytoplankton took up nitrogen from the rain water.

In a situation where only chemically synthesized N-compounds (e.g. fertilizers) are taken up, the δ^{15}N of the plants should decrease to the δ^{15}N value of the fertilizer. In the present study this was true for the water culture experiment, since the δ^{15}N of *E. athericus* decreased to the value of the N-compounds added to the nutrient solution. In water culture, the N-compounds added to the nutrient solution represent the only N-pool available for the plants. In soil culture, however, the situation is more complex. Although calculations make clear that the plants took up N from the added KNO_3 and NH_4Cl in the soil culture, the δ^{15}N of *E. athericus* did not decrease to the δ^{15}N of the fertilizer. Probably fractionation of ^{15}N/^{14}N played a role. Handley & Raven (1992) made clear that fractionation for the lighter N-isotope is found for many N-transformation processes. An important process showing relatively large fractionation is denitrification. The micro-organisms involved in denitrification show a preference for the lighter ^{14}N isotope (Handley & Raven 1992). In fact, this preference is assumed to be the main reason for the lower δ^{15}N in atmospheric N, compared to soil and plants. In the present study the N-concentrations in the control pots decreased between day 0 and day 35 from 2 to 1 mg N/kg dry weight and in the treatment with 6 g N/m^2 from 40 to 15 mg N/kg dry weight (Table 1). Due to N-transformations the δ^{15}N in the soil in both treatments might have increased, leading to an increase in δ^{15}N of *E. athericus* from 9 ‰ to 17 ‰ (Table 6). In the treatment with 24 g N/m^2 the nitrate concentrations in the soil after 35 days is still high (115 mg N/kg dry weight). This nitrate originated from the added potassium nitrate with a δ^{15}N of – 0.3 ‰.

The available nitrogen for *E. athericus* in this treatment may not have changed in δ^{15}N over this period, which explains why there was no shift in δ^{15}N in *E. athericus*. Between day 35 and day 70 the nitrate concentration in the treatment with 24 g N/m^2 decreased from 115 to 5 mg N/kg dry weight. This loss may have

led to the increase in $\delta^{15}N$ in the soil and, as was measured, in the plants. These results confirm the findings of Bergersen et al. (1988), who also reported an enrichment of plant $\delta^{15}N$, relative to the nitrate source, when growing soybeans in pots. In field experiments the shift to higher $\delta^{15}N$ in the soil due to denitrification might be less obvious, since fractionation effects are often masked by the complexities of nitrogen pathways in nature (Handley & Raven 1992), although Högberg (1990) found an increase in $\delta^{15}N$ in a heavily fertilized forest soil.

The small difference between the $\delta^{15}N$ value of N-compounds and plants may be useful for N-uptake studies in water cultures. It will be difficult to use the small differences in $\delta^{15}N$ value between soil nitrogen and N-compounds in ecological studies in the salt marsh environment since the $\delta^{15}N$ of nitrogen added to the soil or present in the soil can change due to fractionation during N-transformation processes. For studies in soil cultures or in the salt marsh artificially ^{15}N labelled N-compounds may be more useful. These labelled N-compounds are strongly enriched (with $\delta^{15}N$ values of at least +12650 ‰!) or depleted in ^{15}N (with $\delta^{15}N$ values of – 970 ‰) and differ much more from the $\delta^{15}N$ of natural pools like soils and plants than the normal N-compounds used in the present study. An example of the use of ^{15}N labelled $(NH_4)_2SO_4$ (with artificially enhanced ^{15}N values of +30 atom % ^{15}N i.e. $\delta^{15}N$ = 80900 ‰) is the study of Delaune et al. (1983) who described the N-cycle in a Louisiana Gulf Coast salt marsh.

Acknowledgements. We want to thank R.H. Zoomer for his assistance with the ammonium and nitrate analysis and G.W. Valkenburg (AB-DLO) for the ^{15}N analysis.

References

Bakker, J.P. 1989. *Nature management by grazing and cutting*. Kluwer, Dordrecht.

Beeftink, W.G. 1977. Salt marshes. In: Barnes, R.S.K. (ed.) *The coastline*, pp. 93-121. Wiley, New York, NY.

Bergersen, F.J., Peoples, M.B. & Turner, G.L. 1988. Isotopic discriminations during the accumulation of nitrogen by soybeans. *Aust. J. Plant Physiol*. 15: 407-420.

de Jonge, V.N. 1990. Response of the Dutch Wadden Sea ecosystem to phosphorus discharges from the River Rhine. *Hydrobiologia* 195: 49-62.

Delaune, R.D, Smith, C.J. & Patrick Jr., W.H. 1983. Nitrogen losses from a Louisiana gulf coast salt marsh. *Estuarine Coastal Shelf Sci.* 17: 133-141.

Handley, L.L. & Raven, J.A. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell Environ.* 15: 965-985.

Heaton, T.H.E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chem. Geol.* 59: 87-102.

Högberg, P. 1990. Forests losing large quantities of nitrogen have elevated 15N:14N ratios. *Oecologia* (*Berl.*) 84: 229-231.

Jefferies, R.L. & Perkins, N. 1977. The effects on the vegetation of the additions of inorganic nutrients to salt marsh soils at Stiffkey, Norfolk. *J. Ecol.* 65: 867-882.

Junk, G. & Svec, H.V. 1958. The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. *Geochim. Cosmochim. Acta* 14: 234-243.

Leendertse, P.C. 1995. *Impact of nutrients and heavy metals on salt marsh vegetation in the Wadden Sea*. Doctoral Thesis, Free University, Amsterdam.

Lindau, C.W., Delaune, R.D., Patrick Jr., W.H. & Lambremont, E.N. 1989. Assessment of stable nitrogen isotopes in fingerprinting surface water inorganic nitrogen sources. *Water Air Soil Pollut.* 48: 489-496.

Olff, H. 1992. *On the mechanisms of vegetation succession*. Doctoral Thesis, University of Groningen.

Paerl, H.W. & Fogel, M.L. 1994. Isotopic characterization of atmospheric nitrogen inputs as sources of enhanced primary production in coastal Atlantic Ocean waters. *Mar. Biol.* 119: 635-645.

Rozema, J., Bijl, F., Dueck, T. & Wesselman, H. 1982. Salt-spray stimulated growth in strand-line species. *Physiol. Plant.* 56: 204-210.

Rozema, J., Dueck, T., Wesselman, H. & Bijl, F. 1983. Nitrogen dependent growth stimulation by salt in strand-line species. *Acta Oecol. Oecol. Plant.* 4: 41-52.

Shearer, G. & Kohl, D.H. 1989. Estimates of nitrogen fixation in ecosystems: the need for and basis of the ^{15}N natural abundance method. In: Rundel, P.W., Ehrlinger, J.R. & Nogy, K.A. (eds.) *Stable isotopes in ecological research. Ecol. Stud.* 68: 342-374.

Shearer, G.B., Kohl, D.H. & Commoner, B. 1974. The precision of determinations of the natural abundance of nitrogen-15 in soils, fertilizers, and shelf chemicals. *Soil Sci.* 118: 308-316.

Sokal, R.R. & Rohlf, F.J. 1981. *Biometry*. 2nd ed. Freeman, San Francisco, CA.

Tyler, G. 1967. On the effect of phosphorus and nitrogen, supplied to baltic shore-meadow vegetation. *Bot. Not.* 120: 433-447.

Valiela, I. 1984. *Marine ecological processes*. Springer-Verlag, New York, NY.

Valiela, I. & Teal, J.M. 1974. Nutrient limitation in salt marsh vegetation. In: Reimold, R.J. & Queen, W.H. (eds.) *Ecology of halophytes*, pp. 547-563. Academic Press, London.

van der Veer, H.W., van Raaphorst, W. & Bergman, M.J.N. 1989. Eutrophication of the Dutch Wadden Sea: external nutrient loadings of the Marsdiep and Vliestroom Basin. *Helgol. Meeresunters.* 43: 501-515.

Received 2 November 1995;
Revision received 11 March 1996;
Accepted 22 March 1996.