

## Postprint: Responses of Seed Germination and Seedling Growth of *Suaeda salsa* with Different Nitrogen Substrates to Salinity and Nitrogen Input in the Yellow River Estuary

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### Abstract

From April to November 2014, *Suaeda salsa* wetlands on the high-tide beach of the northern coastal area of the Yellow River estuary were selected as the study site. Based on a field in-situ nitrogen load enhancement simulation experiment (N0, no additional nitrogen input; N1, low nitrogen input; N2, medium nitrogen input; N3, high nitrogen input), corresponding seeds with different nitrogen substrates (S0, S1, S2, and S3) were obtained to investigate the response of their germination rate and seedling growth status to the interactive effects of different salt stresses and nitrogen concentrations. The results showed that under different nitrogen loads, the nitrogen content in mature *Suaeda salsa* seeds generally exhibited the pattern  $S2 > S0 > S1 > S3$ , with medium nitrogen input being more conducive to nitrogen nutrient accumulation in seeds. Under the interaction of salt and nitrogen, the germination rates of the four types of nitrogen substrate seeds generally showed  $S2 > S1 > S0 > S3$  ( $P > 0.05$ ), with S2 having the highest germination rate under different salt stresses and the best seedling growth status. With increasing salt levels, the germination rates and seedling growth status of the four nitrogen substrate seeds were all inhibited to some extent; however, lower salt levels contributed to seedling length growth, and this inhibitory effect could be alleviated to some degree with increasing nitrogen input. Salt stress, nitrogen concentration, and seed type, as individual factors, all had significant effects on the germination rate, seedling length, fresh weight, and dry weight of *Suaeda salsa*. Except for seedling length, which was significantly affected by the interaction between nitrogen concentration and salt stress ( $P < 0.05$ ), the interactions of other factors did not have significant effects on the various ecological indicators. The study found that different nitrogen input treatments not only changed the nitrogen content of *Suaeda salsa*

seeds from their native environment, but also enabled these seeds with different nitrogen substrates to adopt different ecological adaptation strategies to environments with different salt stresses and nitrogen concentrations. *Suaeda salsa* seeds under medium nitrogen input (S2) were superior to seeds with other nitrogen substrates in both germination rate and seedling growth status. In the future, as nitrogen nutrient supply in the newly formed wetlands of the Yellow River estuary continues to increase, when wetland nitrogen nutrients reach medium nitrogen levels, it will be more conducive to *Suaeda salsa* seed germination and seedling growth; when nitrogen nutrients reach higher levels, *Suaeda salsa* seed germination and seedling growth may be inhibited to some extent.

## Full Text

### Responses of Germination and Seedling Growth of Different Nitrogen-Substrate Seeds of *Suaeda salsa* to Salinity Stress and Nitrogen Loading in the Newly Created Marshes of the Yellow River Estuary

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**Abstract:** From April to November 2014, an *in situ* nitrogen (N) loading experiment (N0, no N loading treatment; N1, low N loading treatment; N2, moderate N loading treatment; and N3, high N loading treatment) was conducted in a *Suaeda salsa* community in high marshes in the northern Yellow River estuary. Different N-substrate seeds were sampled in the corresponding N treatment plots (S0, S1, S2, and S3). The objective of this study was to explore the responses of germination and seedling growth of different N-substrate seeds of *S. salsa* to salinity stress and nitrogen loading. Results showed that the N content in seeds sampled from different N loading plots were in the order of S2 > S0 > S1 > S3, and compared to other N loading treatments, the N2 treatment was more favorable for N accumulation in seeds. The interaction of salinity stress and N concentrations showed substantial effects on germination rate of the four

N-substrate seeds, following the sequence of  $S2 > S1 > S0 > S3$  ( $>0.05$ ). Under different salinity stresses, the germination rate and seedling growth of S2 were the best. With increasing salinity, the germination rate and seedling growth of the four N-substrate seeds were generally inhibited, but at lower salinity, conditions were favorable for seedling elongation and the inhibitory effect could be alleviated by high N loading. The combined effect of salinity stress, N concentration, and seed types had significant impacts on germination rate, seedling length, and fresh and dry weight. Except for seedling length, the interaction of the three factors demonstrated no significant influences on the above ecological traits. This study found that the N loading treatments not only altered the nitrogen content of seeds in the primary environment, but also resulted in N-substrate seeds that presented different adaptation strategies to nitrogen loading and salinity stress. Compared to other N-substrate seeds, S2 showed great advantages in germination rate and seedling growth. In the future, nutrient loading will be increasingly utilized in the newly created marshes of the Yellow River estuary. It was concluded that, as the nutrient reached the N2 level, *S. salsa* germination and seedling growth would be greatly promoted. If nutrients reached higher levels, germination and seedling growth would, to some extent, be inhibited.

**Keywords:** N-substrate seed; nitrogen loading; salinity stress; *Suaeda salsa*; Yellow River estuary

## Introduction

Nitrogen, as one of the indicators of nutrient levels in estuarine wetlands, is often the most limiting nutrient, and its concentration directly affects wetland system productivity, vegetation germination, physiological metabolism, and other nutrient cycles. Nitrogen also plays an important role in improving the osmoregulation capacity and salt tolerance of halophytes. Salinity is another key factor affecting plant growth, significantly influencing various physiological processes of vegetation through osmotic stress, ion toxicity, and reactive oxygen metabolism imbalance. Whether seeds of plants in saline environments can germinate or grow normally is crucial for community construction in wetland ecosystems. The interactive effects of salinity and nitrogen on halophyte growth are complex, depending not only on plant type and growth stage differences but also on different salinity gradients and nitrogen nutrient types.

The Yellow River estuary wetland, formed by the interaction between the Bohai Sea and the Yellow River estuary, receives large amounts of nitrogen-containing materials from upstream. The flux of nutrients into the sea at the Yellow River estuary has reached  $1-2 \text{ g m}^{-2}$ , approaching its nitrogen deposition critical load and remaining at high levels. The nitrogen wet deposition in the Yellow River estuary area has increased from  $1.41 \times 10^{-4}$  to  $4.22 \times 10^{-4} \text{ g m}^{-2}$ , and nitrogen loading enhancement has become one of the most important factors altering estuarine wetlands. The impact on vegetation seed germination and seedling growth has become a current research hotspot.

*Suaeda salsa* is one of the most important halophytic vegetation types in the Yellow River estuary, serving as the main habitat for the Red-crowned Crane, egret, and Charadriiformes birds, and playing an important role in maintaining the normal succession of wetland systems. Although many scholars have conducted extensive work on the effects of salinity stress and nitrogen input on *S. salsa* seed germination characteristics, seedling osmotic regulation, ion accumulation, nutrient absorption capacity, and environmental adaptability of different seed forms, most of these studies have changed the growth environment of seeds collected from the field. Research on the germination and seedling development responses of different N-substrate seeds produced by *S. salsa* vegetation under different nitrogen loading influences to the interaction of different salinity and nitrogen concentrations is still rarely reported.

This study selected *S. salsa* wetlands in the high tidal flats of the northern Yellow River estuary as the research object. Through *in situ* nitrogen input simulation experiments, different N-substrate seeds were obtained to study their germination and seedling growth under different salinity and nitrogen inputs. The results help reveal the growth status and succession direction of *S. salsa* communities in tidal flat wetlands under current and future continuous nitrogen loading enhancement conditions in this area, and provide important scientific basis for the restoration and reconstruction of degraded wetlands.

## 1 Study Area Overview

The study area is located in the Shandong Yellow River Delta National Nature Reserve (37°40' -38°10' N, 118°41' -119°16' E), with a total area of 1928.2 km<sup>2</sup>, of which the intertidal area is 551.6 km<sup>2</sup>, accounting for 46.25% of the total protected land area. The area has a warm temperate monsoon continental climate with an annual precipitation of 383 mm, annual evaporation of 196 mm, and average temperature of 12.1°C. The soil types are mainly intrazonal fluvo-aquic soils and saline soils, with dominant vegetation types including *Suaeda salsa*, *Phragmites australis*, *Tamarix chinensis*, *Imperata cylindrica*, and *Apocynum venetum*.

## 2 Seed Collection and Storage

An *in situ* nitrogen input simulation experiment was conducted from late May to November. Based on existing data from the study area and the NITREX project, nitrogen input levels were set as: N0 (no additional nitrogen input, 9.0 g N m<sup>-2</sup>), representing the current actual nitrogen input; N1 (12.0 g N m<sup>-2</sup>), simulating future low external nitrogen increase; N2 (15.0 g N m<sup>-2</sup>), simulating future moderate external nitrogen increase; and N3 (18.0 g N m<sup>-2</sup>), simulating future high external nitrogen increase. Each treatment had three replicates (5 m × 10 m).

Nitrogen was applied as urea [CO(NH<sub>2</sub>)<sub>2</sub>] solution to simulate external nitrogen input intensity. Control plots were sprayed with equal amounts of water to

reduce the impact of added water on wetland ecological processes. *Suaeda salsa* seeds were collected from the N0, N1, N2, and N3 plots (designated as S0, S1, S2, and S3, respectively). Collected seeds were air-dried; some were stored in a refrigerator for later use, while others were oven-dried, ground, and analyzed for total nitrogen (TN) content using an elemental analyzer.

### 3 Experimental Design

The experiment employed a three-factor orthogonal design: three salinity concentrations (X: 0 mmol/L; Y: 300 mmol/L; Z: 600 mmol/L), four nitrogen concentrations (0: 0 mmol/L; 1: 1 mmol/L; 2: 5 mmol/L; 3: 10 mmol/L), and four N-substrate seed types (S0, S1, S2, S3). Salinity and nitrogen were provided by NaCl and NaNO<sub>3</sub>, respectively. Fifty plump seeds of each type were selected, rinsed with distilled water, blotted dry, and evenly placed in germination boxes (12 cm × 12 cm × 6 cm) containing two layers of germination paper. Different salinity and nitrogen treatment solutions were added, and the boxes were sealed with Parafilm to maintain water retention and air permeability. The experiment was conducted in an illuminated incubator (day/night temperature of 25°C/20°C) for 15 days. Germination was recorded daily, and seedling length (root, stem, and leaf) and fresh weight were measured. Seedlings were then oven-dried to determine dry weight and calculate leaf weight ratio, stem weight ratio, and root weight ratio.

### 4 Data Processing and Statistics

Origin 9.2 software was used for calculations and graphing, and SPSS 20.0 for statistical analysis. One-way ANOVA examined the effects of salinity, nitrogen concentration, and seed type individually on germination rate, fresh weight, dry weight, and organ growth of the four N-substrate seeds. Three-way ANOVA examined the interactive effects of salinity, nitrogen concentration, and seed type on these parameters.

### 5 Results and Analysis

#### 5.1 Effects of Salinity and Nitrogen Input on Germination of Different N-Substrate Seeds

Under different salinity and nitrogen conditions, the germination rates of the four N-substrate seeds showed varying patterns. Overall, germination rates decreased with increasing salinity, following the order S2 > S1 > S0 > S3. At the same salinity level, no significant differences were observed among seed types (>0.05). However, S2 seeds maintained consistently higher germination rates throughout the experiment. Under no-salt conditions, germination rates increased with nitrogen concentration, with high nitrogen showing significant promotion of S0 germination and moderate nitrogen promoting S3 germination. Under low-salt stress, germination rates gradually increased with nitrogen concentration, peaking at moderate nitrogen levels. Under high-salt stress,

although no significant differences existed among seeds at the same nitrogen concentration, S2 seeds showed superior germination rates (86.67%). Variance analysis indicated that nitrogen concentration and salinity stress individually had highly significant effects on germination rate ( $<0.01$ ), but their interaction was not significant.

### 5.2 Effects of Salinity and Nitrogen Input on Seedling Fresh and Dry Weight

The fresh and dry weight trends of seedlings from the four N-substrate seeds were consistent across different salinity and nitrogen treatments. Under the same nitrogen treatment, fresh and dry weights of different seed seedlings decreased with increasing salinity. Under the same salinity, weights generally increased with nitrogen concentration. Under no-salt conditions, except for S0 seedlings showing significantly higher fresh and dry weights than others ( $<0.05$ ), no significant differences existed among seedlings under other nitrogen treatments ( $>0.05$ ). With increasing nitrogen concentration, fresh and dry weights of all seed seedlings increased overall, with low nitrogen showing more pronounced promotion than high nitrogen for S2 seedlings. Under low-salt stress, no significant differences existed among seedlings in fresh/dry weight across different nitrogen concentrations ( $>0.05$ ). Under high-salt stress, fresh and dry weights of all seed seedlings increased with nitrogen concentration, with S2 seedlings showing the lowest weights under low nitrogen. Variance analysis showed that nitrogen concentration and salinity stress individually significantly affected seedling fresh and dry weight ( $<0.01$ ,  $<0.05$ ), but their interaction was not significant.

[Figure 2: see original paper] [Figure 3: see original paper] [Figure 4: see original paper]

### 5.3 Effects of Salinity and Nitrogen Input on Seedling Length

Under salinity and nitrogen influence, average seedling length showed low-salt  $>$  no-salt  $>$  high-salt, though differences among salinity levels for the same seed were not significant ( $>0.05$ ). Significant differentiation occurred among the four N-substrate seedling types during growth. Under no-salt conditions, except for S2 seedlings being significantly longer under moderate nitrogen, no significant differences existed among seedlings. Overall, seedling length followed S2  $>$  S0  $>$  S1  $>$  S3. With increasing nitrogen concentration, S0 and S2 seedling lengths decreased, while S1 and S3 lengths increased, peaking at moderate nitrogen (5.47 cm for S1, 5.86 cm for S2). Under low-salt stress, S2 seedling length peaked at moderate nitrogen (5.86 cm), while S1 peaked at low nitrogen (4.48 cm). Under high-salt stress, S2 seedling length increased significantly with nitrogen concentration, being markedly higher than other seeds under high nitrogen. S0 and S1 seedling lengths increased slightly with nitrogen concentration, while S3 showed little change. Variance analysis indicated that seed type and salinity individually had highly significant effects on seedling length ( $<0.01$ ), and the salinity-nitrogen interaction was also significant ( $<0.05$ ).

[Figure 5: see original paper]

#### 5.4 Root, Stem, and Leaf Weight Ratios

Under different salinity and nitrogen conditions, root weight ratio (RWR), stem weight ratio (SWR), and leaf weight ratio (LWR) of the four N-substrate seedlings showed some variation but without significant differences. Under the same nitrogen concentration, except for S0 and S1 seedlings showing consistent RWR under no-salt and low-salt stress, RWR of the other three seed types decreased with increasing salinity. LWR and RWR of all seed types increased with salinity, with LWR reaching maximum under high-salt stress. SWR of S0 and S2 increased with salinity, while SWR of S1 and S3 changed little. Under the same salinity stress, LWR of S0, S1, and S2 decreased with increasing nitrogen concentration, while LWR of S3 increased little. RWR of S0 and S2 decreased with nitrogen concentration, while RWR of S1 and S3 showed no clear response. SWR of S0 and S2 increased markedly with nitrogen concentration, while SWR of S1 and S3 increased slowly or showed no change.

[Figure 6: see original paper]

## 6 Discussion

### 6.1 Effects of Salinity and Nitrogen Input on Germination of Different N-Substrate Seeds

Nitrogen is a component of many important compounds in plants and a key factor limiting plant growth and primary productivity. Increased nitrogen content can enhance RuBisCO concentration, activity, and chlorophyll content, thereby increasing photosynthetic rate, but excessive nitrogen can cause nutritional imbalance and adversely affect photosynthesis. Studies have shown that when large amounts of nitrogen are input, plants absorb other nutrients relatively slowly, which is not conducive to growth and development.

The nitrogen content in mature *S. salsa* seeds under different nitrogen loading treatments followed  $S2 > S0 > S1 > S3$ , indicating that moderate nitrogen input is more conducive to nitrogen accumulation in seeds, while high nutrient input is not. Previous studies have shown that Yellow River estuary tidal flats are mainly nitrogen-limited. When large amounts of nitrogen are input, plant biomass increases greatly, but the nitrogen transferred to seeds at maturity is relatively low. Our field nitrogen addition studies found that seed development under moderate nitrogen input could advance by about 10 days compared to other treatments, suggesting that nitrogen in plants may accumulate in seeds earlier to promote maturation.

Although no significant differences existed in germination rates among the four N-substrate seeds at the same nitrogen concentration, the overall performance was  $S2 > S1 > S0 > S3$ , with S2 showing the highest germination rates under different salinity stresses. This may be related to the nitrogen content differences

among seeds. S2 seeds had the highest nitrogen content and thus the highest protein content, which is essential for cell growth and division, and protein hydrolysis into amino acids can provide raw materials for germination. S3 seeds had the lowest nitrogen content and thus the lowest germination rates.

All seed germination rates decreased with increasing salinity because higher salinity increases osmotic pressure in the medium, reducing cell water potential and the seed's ability to absorb nutrients. Salinity can also reduce soluble protein activity and dissolve or non-dissolve sugars, affecting germination rate. However, studies have confirmed that appropriate nitrogen addition can alleviate salt toxicity by increasing proline and other osmoregulatory substances, improving nutrient utilization and promoting germination. This study found that the inhibitory effect of salinity could be alleviated to some extent by increasing nitrogen concentration.

## 6.2 Effects of Salinity and Nitrogen Input on Seedlings from Different N-Substrate Seeds

Although fresh and dry weights of seedlings from the four N-substrate seeds generally decreased with increasing salinity, S2 seedlings showed significantly higher fresh and dry weights than other seeds, especially under no-salt and low-nitrogen interaction ( $<0.05$ ). This suggests S2 seeds can maintain better growth under different salinity stresses. Two main reasons may explain this: first, S2 seeds had the highest germination rate, giving them an initial growth advantage; second, S2 seeds had the highest nitrogen content and thus more sufficient nutrients to activate seed vigor. When seeds germinate and the embryo breaks through the seed coat, the nitrogen demand and utilization capacity for the germination environment are relatively low due to sufficient endogenous nitrogen.

Under low-salt stress, fresh and dry weights of S0 and S2 seedlings decreased with increasing nitrogen concentration, while S1 and S3 seedling weights increased. This may be related to different adaptation strategies of seedlings to salinity. Under low-salt stress, the fresh weight, dry weight, and length of S2 seedlings showed opposite trends, possibly because S2 seedlings' vacuoles can accumulate  $\text{Na}^+$  for osmotic regulation, absorbing more water to increase succulence. Studies have shown that salt stress can increase cell solution concentration, improving water absorption capacity. Under high-salt stress, fresh and dry weights of all seed seedlings increased with nitrogen concentration, with S2 seedlings showing the lowest weights under low nitrogen, indicating that nitrogen input can significantly alleviate high-salt toxicity. However, low nitrogen was insufficient to relieve salt stress on S0 and S2 seedlings, as low nitrogen content under salt stress is unfavorable for root nutrient absorption.

Comparing germination rates and seedling lengths, S2 seedlings showed lower germination rates under low nitrogen concentration, and their root systems were shorter, limiting nutrient absorption and resulting in poorer growth and

lower overall quality. The interaction of salinity and nitrogen concentration significantly affected seedling length ( $<0.05$ ), with low salt being more suitable for radial growth of *S. salsa* seedlings, consistent with previous studies. As a halophyte, *S. salsa* has strong adaptability to saline environments, and low salt can promote proline production in aboveground parts, increasing intracellular solutes and reducing osmotic potential. Under high-salt stress, nitrogen concentration increase significantly promoted S2 seedling length, especially under moderate nitrogen, which differed significantly from other nitrogen treatments. This may be because S2 seedlings developed in a nitrogen-deficient native environment, and when nitrogen supply increased, their growth changed significantly. Studies on barley have shown that under nitrogen starvation, glutamine synthetase activity increases, improving nitrogen utilization efficiency. S2 seedlings may have higher nitrogen utilization efficiency after long-term nitrogen deficiency, so when environmental nitrogen increases, their absorption and utilization efficiency increases significantly.

Variance analysis showed that nitrogen concentration and seed type individually caused significant differences in germination rate, fresh weight, and dry weight of *S. salsa* ( $<0.05$ ), but except for length, which was significantly affected by the nitrogen-salinity interaction, other factor interactions showed no significant effects. This may be because while single factors like salinity or nitrogen concentration can significantly affect plant growth, interactions among ions and different adaptation strategies of N-substrate seeds to nutrients may reduce or offset these significant effects.

Under different salinity and nitrogen conditions, root, stem, and leaf weight ratios of the four N-substrate seedlings showed variation but no significant differences. With increasing salinity, leaf length and leaf weight ratio of different seedlings increased, consistent with previous studies. Halophytes like *S. salsa* can adjust internal osmotic potential to absorb large amounts of water under salt stress, maintaining high water content to reduce ion toxicity. Under the same nitrogen concentration, except for S0 and S1 seedlings showing similar root weight ratios under no-salt and low-salt stress, root weight ratios of other seed types decreased with increasing salinity, indicating that S2 seedlings' root systems are more adaptable to salt stress and can supply more nutrients to aboveground parts. Except for S3 leaf weight ratio decreasing with nitrogen concentration, other seedling leaf weight ratios increased with nitrogen concentration, showing that nitrogen input can significantly promote leaf growth. S1 and S2 seedlings have special adaptation strategies to nutrient-poor environments in their native habitats, so their seedling length may not increase significantly with nitrogen in the short term, but leaf length and salt resistance improve to varying degrees.

## 7 Conclusion

The nitrogen content in mature *S. salsa* seeds under different nitrogen loading treatments followed  $S2 > S0 > S1 > S3$ , indicating that moderate nutrient input is more conducive to nitrogen accumulation in seeds, while high nutrient input is

not. Although the interaction of different salinity and nitrogen concentrations had no significant effects on germination rate, fresh weight, dry weight, and organ growth of the four N-substrate seeds ( $>0.05$ ), S2 seeds had higher germination rates and their seedlings showed the strongest adaptability to nitrogen-enriched environments under different salinity stresses. Salinity stress inhibited germination and seedling growth of different N-substrate seeds, but this inhibition could be alleviated with increasing nitrogen concentration. Low salt was more conducive to length increase of seedlings from the four N-substrate seeds. Nitrogen concentration and seed type individually had significant effects on germination rate, fresh weight, and dry weight of *S. salsa*, but except for length, which was significantly affected by the nitrogen-salinity interaction, other factor interactions showed no significant effects. When nitrogen nutrients in the newly created wetlands of the Yellow River estuary reach the moderate level (N2) in this study, *S. salsa* seed germination and seedling growth will be more favorable. When nitrogen nutrients reach higher levels, *S. salsa* germination and seedling growth may be inhibited to some extent.

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