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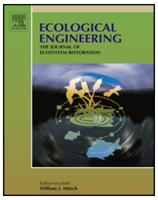

Short communication

Selective uptake of nitrogen by *Suaeda salsa* under drought and salt stresses and nitrogen fertilization using ^{15}N

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ABSTRACT

Plant nitrogen uptake can be influenced by drought and salt stresses and nitrogen fertilization; however, the selective uptake of different forms of nitrogen is poorly understood. We conducted a short-term ^{15}N-labeling experiment to investigate the preferences of *Suaeda salsa* for nitrate, ammonium and amino acid. Our results indicated that nitrate and glycine, especially nitrate, were major nitrogen sources and glycine was absorbed after decompostion. Drought and salt stresses had no significant effect on the uptake of ammonium ($p > 0.05$). However, nitrate uptake was strongly related to water content and nitrogen application ($p < 0.05$) instead of salinity ($p > 0.05$). Drought and nitrogen application reduced the uptake of nitrate by almost 65.73% and 90.29%, respectively, suggesting appropriate soil water content and nitrogen deficiency could promote nitrate uptake. The uptake of glycine was mainly influenced by water content, and drought stress decreased glycine uptake by almost 89.74%.

1. Introduction

Nitrogen (N) is one of the most limiting elements for plants in coastal wetlands (Mitsch and Gosselink, 2015). Generally, nitrate and ammonium are the two main forms of N assimilated by plants, and their relative availability has been considered to be an important factor controlling the distribution of plant species (Mitsch and Gosselink, 2015). However, some studies have indicated that plants can take up organic N directly or indirectly, especially simple forms such as amino acid (Schimel and Bennett, 2004). Some studies have even exhibited that amino acids can be assimilated by plants as fast as, or faster than, inorganic N sources (Persson et al., 2003). Species vary in their preferences for different forms of N, and environmental factors may exert a significant impact on these preferences (Buckeridge and Jefferies, 2007). Previous studies have reported that the selection of N forms is highly influenced by species at different succession stages (Warren, 2009). Nordin et al. (2001) showed correlations between amino acids and nitrate levels in soil and plant preferences for these N forms. Miller and Bowman (2003) studied alpine plant uptake of organic and inorganic N along a moisture gradient, and reported that all species appeared to take up glycine at rates comparable to or greater than those of ammonium or nitrate, and species from the same community showed different uptake rates of ammonium, nitrate and glycine. Shao et al. (2015) found that the salt stress-induced growth inhibition of cucumber seedlings might disrupt N uptake and decrease the activities of enzymes. However, in coastal wetlands, little information is available on organic and inorganic nitrogen uptake by plants under drought, salt stresses and N fertilization. Isotope tracer technique can improve the understanding of plant N preference, which in turn is expected to aid in the prediction of plant growth and evolutionary trends and contribute to coastal wetland health and management.

The primary objectives of this work were to reveal the main sources of inorganic and organic N for *S. salsa* as well as the preferences of *S. salsa* for N under drought and salt stresses, and N fertilization using the ^{15}N isotope tracer technique.

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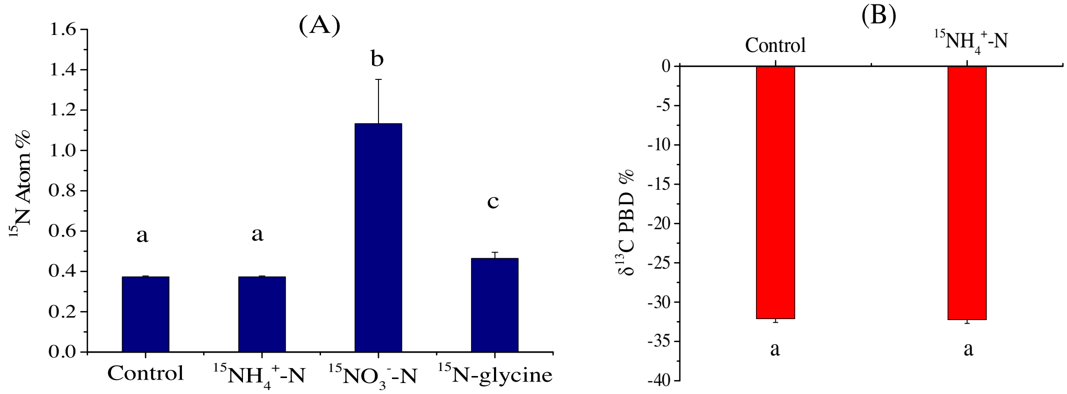

Fig 1. ^{15}N uptake by *S. salsa* under different N application treatments (A) and ^{13}C uptake under glycine application treatment (B) (abc represent the differences in ^{15}N uptake under different ^{15}N forms application).

2. Materials and methods

2.1. Site description

The Yellow River Delta National Reserve (118°33′ ~ 119°20′E, 37°35′ ~ 38°12′N) is located in Dongying City, Shandong Province. It is the most complete and youngest wetland in the warm temperate zone, with a typical continental monsoon climate and distinctive seasons. Its annual average temperature and frost-free period are 12.3 °C and 196 days, respectively. The annual evaporation is 1962 mm and annual precipitation is 551.6 mm, with approximately 70% of precipitation and rainfall occurring between June and August. The shallow salt groundwater, loose rock class and high evaporation in the Yellow River Delta subject the region to the risk of salinization, and the seasonal fresh water deficiency would aggravate the environmental stresses of drought and salinization on plants. In addition, to solve the sedimentation of the Yellow River, the flow-sediment regulation engineering has been carried out in June or July since 2002, and it brings plenty of freshwater to the Yellow River Delta during this period. The subsequent freshwater deficit would cause the region suffering from drought. The dominate soil types are fluvo-aquic soil and salt soil (Bai et al., 2012) and the dominate vegetation is *Suaeda salsa*, *Tamarix chinensis* and *Phragmite australis*. As a pioneer plant in the Yellow River Delta, *Suaeda salsa* is an euhalophytic herb that naturally grows on highly salt soil and it has strong adaptations to environmental stresses.

2.2. Experimental design and statistical analysis

An outdoor shed (2 m height and 12 m^2 area) at the Yellow River Delta management station was used as the test site in April 2012. Soils for experiment were collected at a depth of 15 cm near the test site. *S. salsa* seedlings with similar growth characteristics were collected around the soil sampling site. Soil samples were brought to the laboratory and air-dried at room temperature, and then grounded through a 2-mm sieve to remove the coarse plant residues and stones. Plants were divided into three groups and transplanted to the plastic pots filled with 2 kg air-dried soils. The salinity, total nitrogen, and water content of initial experimental soils are 1.87 g/kg, 209 mg/kg, and 26%, respectively. The particle size of soil is mainly composed of sand (about approximately 75.84%). After the plant adaptive phase, experiment was carried out with two moisture treatments (i.e., 26% and 14%), four salinity treatments (i.e., 8 g/kg, 6 g/kg, 4 g/kg, and 2 g/kg) and two N-application treatments (i.e., 0 N mg/kg and 200 N mg/kg). Each treatment included four replicates. The isotopic labeled substrates were $(^{15}NH_4)_2SO_4$ (98 atom%), $K^{15}NO_3$(98 atom%) and Glycine-2-^{13}C, ^{15}N(98 atom% ^{15}N; 99 atom% ^{13}C) (Sigma-Aldrich company). We injected 1.0 mmol/L N solutions (0.6 mmol/L ^{15}N fertilizer in treatment + 0.2 mmol/L ^{14}N of one of the two remaining N fertilizer + 0.2 mmol/L ^{14}N of the remaining N fertilizer) into the soil at depths of 5 cm and 10 cm in the four corners of a 2 × 2 cm^2 area around the plant. The injection concentration ensured the detection of plant uptake and prevented the plant from being injured, and the injection procedure ensured the label was spread evenly throughout the soil. The plants were harvested after 6 h.

Plant samples were sent to the Nanjing Normal University for ^{13}C and ^{15}N analysis by isotope ratio mass spectrometry (IRMS) at the SerCon 20–22 Stable Isotope Facility. Soil salinity was measured using a salinity meter, respectively (soil/water, 1:5), and soil water content was determined by drying soil samples at 105 °C for 24 h in an oven. The grain size fractions of soil were measured on a Laser Size Analyzer (Microtrac S3500) and classified into clay (<0.002 mm), silt (0.002–0.02 mm), and sand (0.02–2 mm). The contents of total nitrogen of soil were determined by CHNOS Elemental Analyzer (Vario EL, German). One-way variance analysis (ANOVA) was conducted to identify the significant effects of the water content, salinity and nitrogen application on the plant nitrogen uptake. Statistical analysis was carried out using SPSS 16.0 software package. The difference was considered to be significant if $p < 0.05$.

3. Results and discussion

3.1. Different forms of nitrogen absorbed by S. salsa

S. salsa showed significant uptake of nitrate and glycine ($p < 0.05$) other than ammonium ($p > 0.05$) (Fig. 1A), indicating that nitrate and amino acids, especially nitrate, were the main sources of N for *S. salsa*. This result is partly consistent with results of Goloran et al. (2015), who presented *H. violacea* and *L. rigidum* mainly absorbed nitrate, which might be explained by low toxicity of nitrate and species preference. In addition, Moneta et al. (2014) studied the seasonal uptake of N and observed ammonium was preferentially assimilated, whereas nitrate was preferred in spring and summer, owing to the competition between plants and microbes. However, other researchers have reported that ammonium was the main inorganic source of N in coastal wetlands, and this preference was related to the amounts of different forms of N in soil (Kirk and Kronzucker, 2005). Nordin et al. (2001) reported that in a boreal forest, all plants preferred ammonium, but ammonium and nitrate showed equivalent plant uptake when their abundances were equal. One probable explanation for this inconsistency is associated with species and seasonal variations. The ^{13}C DeltaPBD% in the ^{15}N-Glycine treatment was not significantly different from the