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EFFECTS OF DIFFERENT NITROGEN FORMS ON THE NITROGEN DISTRIBUTION AND UTILIZATION OF *TAMARIX RAMOSISSIMA* SEEDLINGS UNDER WATER STRESS

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Abstract

In order to explore the influence of drought stress and nitrogen supplementation on changes in growth, photosynthetic physiological traits, and nitrogen distribution and utilization in *Tamarix ramosissima* seedlings, four soil watering regimes (D_1-20%, D_2-35%, D_3-50%, and D_4-75% of relative field capacity) and supplementation with two nitrogen forms (^{15}N-KNO_3 and ^{15}N-NH_4Cl) were evaluated. The results indicated that 1) water was the main factor influencing seedling growth. ^{15}N-NH_4Cl was more easily absorbed by the roots for total biomass accumulation, while ^{15}N-KNO_3 absorption for total biomass accumulationwas promoted under D_4. Under water deficient conditions, ^{15}N-KNO_3 increased the root/shoot ratio significantly, and this ratio was significantly increased by ^{15}N-NH_4Cl under D_3. 2) The two different nitrogen forms significantly influenced the chlorophyll a content when the seedlings were subjected to severe water stress, while ^{15}N-NH_4Cl had a significant effect on the chlorophyll b content under D_2 and D_3. Nitrogen improved the photosystem (PS) II actual photochemical efficiency ($\varphi_{PS(II)}$) of the seedlings, with ^{15}N-NH_4Cl significantly effecting $\varphi_{PS(II)}$ under D_1 and D_3, and ^{15}N-KNO_3 being more influential under D_2. 3) The nitrogen absorption capacity was diminished under D_1 and D_4, and followed a trend of an initial increase followed by a decrease along with the increase in soil moisture. The distribution of ^{15}N-KNO_3 to the seedlings was higher in comparison to ^{15}N-NH_4Cl, whereas the utilization of ^{15}N-NH_4Cl was higher. The interactive effects of water and nitrogen thus influence the ability of seedlings to adapt to undesirable surroundings.

Key words: *Tamarix ramosissima* seedlings, Nitrogen, Water.

Introduction

The nitrogen cycle is an important component of the ecosystem, particularly in arid desert regions. In addition to water, which is somewhat limiting for plant growth, nitrogen is one of the primary growth limiting factors (James, 2005; Delgado-Baquerizo *et al*., 2013), and drought will inhibit the absorption and utilization of nitrogen in plants (Everard *et al*., 2010). The Tarim River is the largest inland river in China that crosses one of the districts exposed to extreme drought. Annual evaporation in downstream of the river and desertification, and the nutrient content especially the nitrogen pigment occurs far below its Upper and Middle River (Yuan *et al*., 2006; Li *et al*., 2015). While intermittent eco-water conveyance engineering since 2000 has resulted in a modest recovery of the downstream riparian vegetation on both sides of the river, but in the overflow zone formed by the water conveyance, water and nutrients remain the key limiting factors for plant growth (Huang *et al*., 2013).

Tamarix ramosissima occurs on the lower reaches of the Tarim River, and is able adapt to changes in the underground water on both sides of the river in order to ensure survival. The adaptability of this species has resulted in it being widely distributed across various environmental gradients (Li *et al*., 2013; Polacik *et al*., 2013). Studies have shown that the increased soil fertility under *Tamarisk* plants is associated with the nitrogen content (Taniguchi *et al*., 2015). A comparison of the non-leguminous plants *Tamarix chinensis* and *Karelinia caspica* with the legume *Alhagi* indicated that the nitrogen distribution in the non-legume leaves was lower, with lower amounts being allocated to photosynthetic systems but with a more efficient nitrogen distribution mechanism. Non-leguminous plants have a higher maximum net photosynthetic rate and photosynthetic nitrogen use efficiency (Zhu *et al*., 2010). The ratio of nitrogen and phosphorus in *T. ramosissima* is relatively stable, which allows this species to grow in desert regions (Li *et al*., 2011; Evans *et al*., 2013). Currently, *T. ramosissima* seedlings demonstrate slow regeneration when exposed to a combination of water and nitrogen stress in the Tarim River. Overcoming this sensitive period of seedling growth is thus essential for maintaining and encouraging the functional recovery of downstream plants. However, only limited relevant research is currently available. Exploring the absorption, distribution, and utilization of nitrogen in *T. ramosissima* seedlings under water stress, as well as how this affects seedling biomass and photosynthesis, could inform the survival of this plant during the sensitive seedling stage. Furthermore, this should provide an essential theoretical foundation for elucidating the ecological environment in the lower reaches of the Tarim River, which could also be applied to the study of the ecological processes in similar areas.

Materials and Methods

Plant material and experimental design: In April 2015, *T. ramosissima* seedlings were collected from the lower reaches of the Tarim River, and sand collected from the *T. chinensis* community in the Tarim River was used for indoor germination. When the germinating seedlings had reached 5 cm, they were transplanted into pots (30 cm × 30 cm). Each pot contained 10 slots. According to the nitrogen treatment conditions, up to 8 mL of ^{15}N-KNO$_3$ and ^{15}N-NH$_4$Cl was added to each slot (each pot contained ^{15}N in a proportion of 30 mg/kg), whereas the same volume of distilled water was added to the control group (Table 1). The tests were conducted in a Greenhouse. The average relative humidity was 20.5~34.56% and the mean temperature was 31.4~38.96°C. The test soil field capacity was 22.75%, the total amount of phosphorus was 0.467 g/kg on average, and the total amount of potassium was 21 g/kg on average. The total nitrogen levels averaged 0.33 g/kg, with an effective average nitrogen content of 12 mg/kg.

The soil water content was measured using a WET-2 portable moisture instrument, and the weighing method maintained the soil water content. The experiment consisted of three soil-watering regimes at a field capacity of 20%, 35%, 50%, and 75% (Table 1). Each treatment combination was replicated four times. Sampling began with being nitrogen added after 90 days. The samples were prepared in accordance with the ground and underground parts (Fig. 1), wrapped in drying box for 15 min at 105°C, dried to constant weight at 70°C, and then ground. The stable isotope ratio mass spectrometry method for the determination of the δ^{15}N values was used and the total nitrogen content was measured according to the Institute of Botany, Chinese Academy of Sciences in beijing.

Table 1. Design of experiments.

Soil moisture content	**20% (D_1)**	**35% (D_2)**	**50% (D_3)**	**75% (D_4)**
N_0- Distilled water	N_0D_1	N_0D_2	N_0D_3	N_0D_4
N_1- KNO_3(Potassium nitrate)	N_1D_1	N_1D_2	N_1D_3	N_1D_4
N_2-NH_4Cl(Ammonium chloride)	N_2D_1	N_2D_2	N_2D_3	N_2D_4

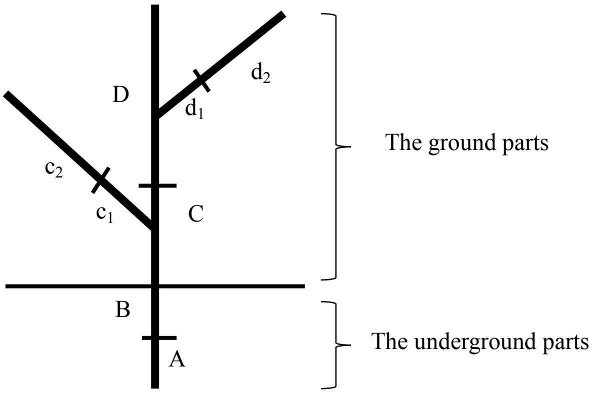

Fig. 1. The sampling distribution map of *Tamarix ramosissima* seedlings.

Analysis Methods

Biomass measurement and root/shoot ratio: seedling biomass was determined using a direct measurement method, and the root/shoot ratio was calculated according to the following formula:

$$\text{Root/shoot ratio (R/S)} = \frac{\text{Belowground part biomass}}{\text{Aboveground part biomass}}$$

Chlorophyll fluorescence: Chlorophyll fluorescence was determined using a Mini-Pam portable modulated chlorophyll fluorometer, whereas the maximum fluorescence (Fm') and the steady-state fluorescence (Fs) were determined during light adaptation at 8:00~20:00, and an average of four hours is measured.each measurement being repeated six times. The initial fluorescence (Fo) and maximum fluorescence (Fm) were measured at 4:00 in the morning in the dark. The actual photochemical efficiency ($\varphi_{PS(II)}$) was calculated using the following formula:

$$\varphi_{PS(II)} = (Fm'-F')/Fm'$$

Chlorophyll content: Clippings of the *T. ramosissima* seedlings were randomly selected and combined, and then soaked in a solution of ethanol: acetone: water =4.5:4.5:1 until there was no longer any green residue. The UV absorption of the extracts was measured using a UV spectrophotometer at wavelengths of 646 and 663 nm and compared to a blank measurement. Chlorophyll a, chlorophyll b, and the total chlorophyll content were calculated according to the formulae:

$$C_a = 12.21A_{663} - 2.81A_{646}$$
$$C_b = 20.13A_{646} - 5.03A_{663},$$

where A represents absorbance, and C_a, and C_b respectively represent the chlorophyll a and chlorophyll b concentration (mg/L).

Ndff, distribution, and utilization: Seedling Ndff (Pan, 2010; Wang *et al.*, 2013) was calculated as follows:

$$\text{Ndff (\%)} = \frac{[\text{The } ^{15}\text{N abundance in the sample (\%)} - \text{Natural abundance (0.365\%)}]}{[\text{The } ^{15}\text{N abundance in fertilizers (\%)} - \text{Natural abundance (0.365\%)}]} \times 100$$

$$\text{Distribution} = \frac{^{15}\text{N uptake of nitrogen from the seedlings in the part (mg)}}{\text{Total } ^{15}\text{N uptake of nitrogen (mg)}} \times 100$$

$$\text{Utilization} = \frac{[\text{Ndff} \times \text{Organ of total nitrogen (g)}]/}{\text{Amount of fertilizer applied (g}} \times 100$$

Total nitrogen (g) = Dry weight (g) ×Total N content N%

^{15}N uptake (mg) = Total nitrogen (g) × Ndff % × 1000

Data analysis: Differences between the treatments were compared using the least significant difference (LSD) multiple range test at a significance level of 0.05. All the statistical analyses were performed in SPSS19.0. Data were calculated as mean ± standard error (SE).

Results

Growth characteristics: Water was determined to be a crucial factor influencing biomass accumulation in the seedlings. As the soil moisture increased, the total biomass increased significantly, whereas excessive water (D_4) resulted in a decrease in the total biomass to 9.59 g. This indicated that excessive moisture was not conducive to seedling growth. Total biomass only accumulated significantly under D_4 with ^{15}N-KNO_3, increasing to 17.99 g. However, the total biomass under D_2 and D_3 was lower than the control group. Under D_1, D_2, and D_3, ^{15}N-NH_4Cl resulted in an increase in the total biomass, but the effect was not significant. Under D_4, both nitrogen forms significantly increased total biomass accumulation (N_1 was 17.99 g, N_2 was 15.22 g) (Fig. 2A). ^{15}N-KNO_3 resulted in a significant increase in the root/shoot ratio under D_1, while ^{15}N-NH_4Cl resulted in a significant decrease in the root/shoot ratio (Fig. 2B).

Physiological responses: The *T. ramosissima* seedlings were exposed to severe water limitation (D_1). The two forms of nitrogen had a significant influence on the chlorophyll a content (N_1 was 7.78 mg/L, N_2 was 6.71 mg/L), whereas only ^{15}N-NH_4Cl had a significant impact on the chlorophyll b content (3.40 mg/L) (Fig. 3). Under D_2, only ^{15}N-NH_4Cl resulted in a significant increase in chlorophyll b content (4.56 mg/L).

The chlorophyll a and b contents of the remaining treatment group were significantly reduced in comparison to the control group.Chlorophyll fluorescence was detected at different times of the day with the soil moisture increase. The real fluorescence yield $\varphi_{PS(II)}$ values of the plants are reflected in the ability of photosystem (PS) II to efficiently capture light energy under light conditions. With the increase in water, $\varphi_{PS(II)}$ showed an increasing trend, and treatment with ^{15}N-KNO_3 and ^{15}N-NH_4Cl resulted in an increase in the $\varphi_{PS(II)}$ values of the seedlings (Fig. 4A). The $\varphi_{PS(II)}$ values were less affected by nitrogen species at 8:00 in the morning, with the $\varphi_{PS(II)}$ almost coinciding. Under D_2, ^{15}N-KNO_3 was higher than ^{15}N-NH_4Cl (N_1 was 0.48, 0.37, and 0.62, respectively) at 12:00, 16:00, and 18:00. Under D_1, D_3, and D_4, ^{15}N-NH_4Cl increased more than the ^{15}N-KNO_3 treatment groups in terms of $\varphi_{PS(II)}$ value.

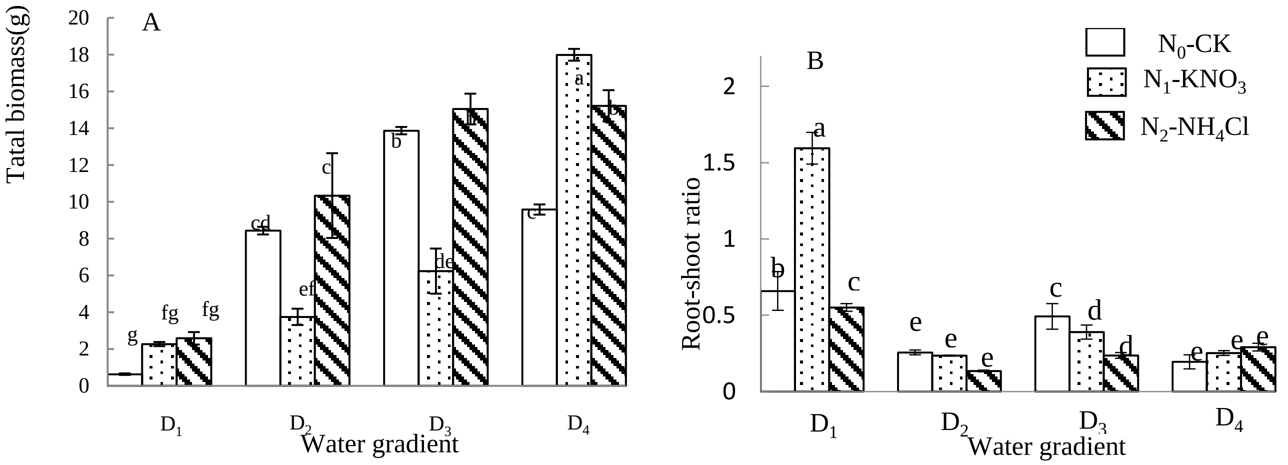

Fig. 2. The effect of nitrogen on total biomass and root-shoot ratio of *T. ramosissima* seedlings under different soil water.

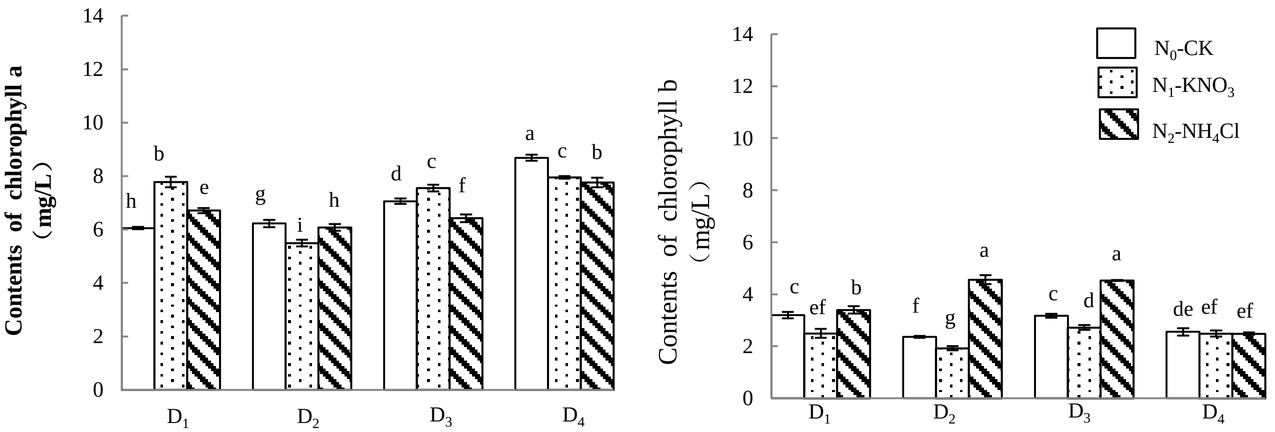

Fig. 3. The effect of nitrogen on the chlorophyll concentration of *T. ramosissima* seedlings under different soil water.

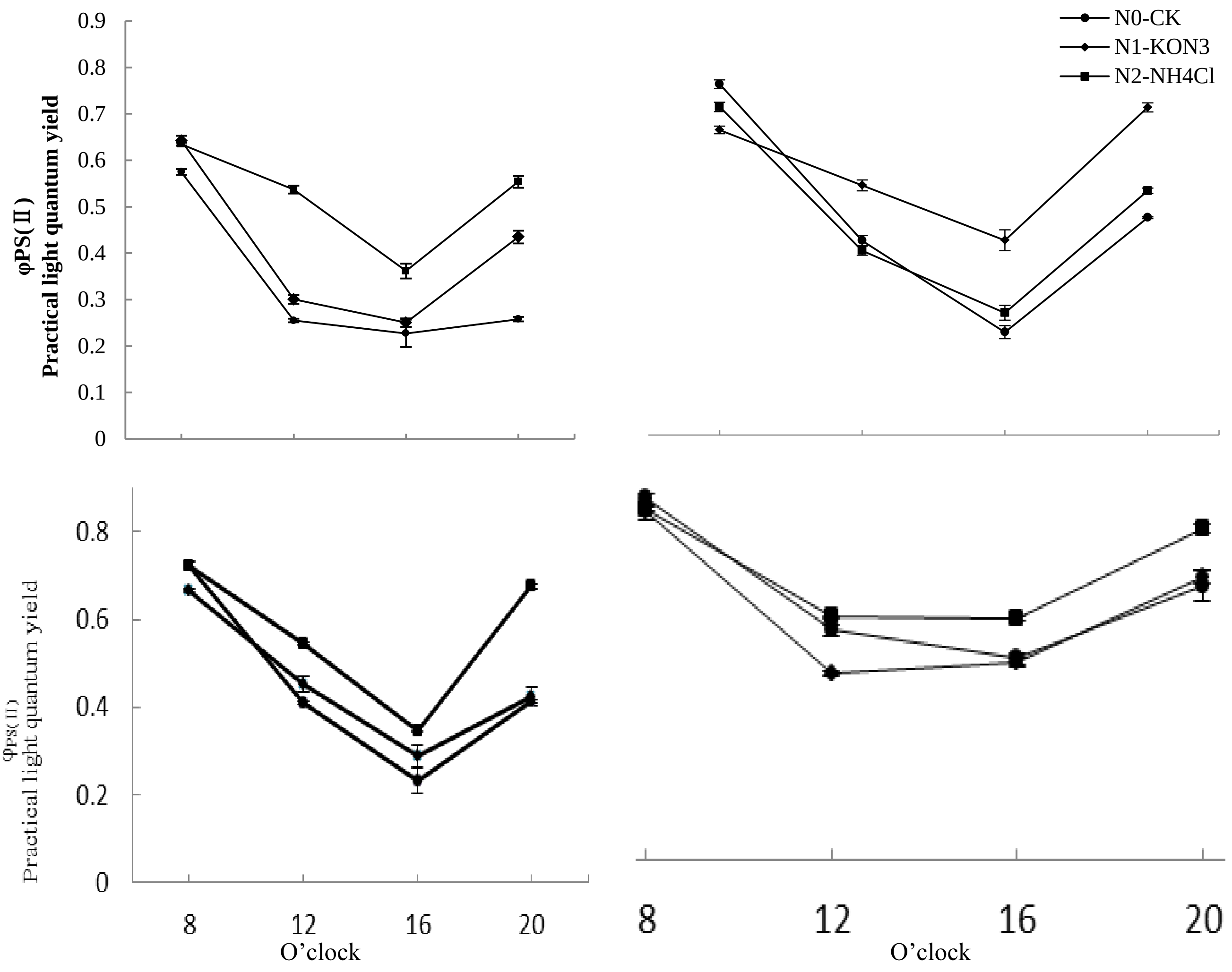

Fig. 4 The daily change of nitrogen on $\varphi_{PS(II)}$ of *T. ramosissima* seedlings under different o'clock.

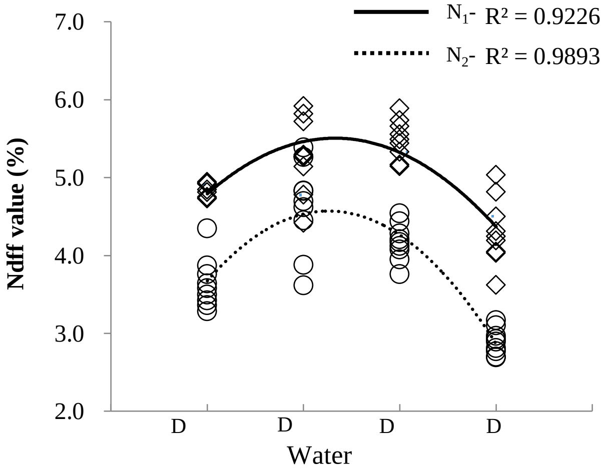

Fig. 5. The effect of nitrogen on the Ndff of *T. ramosissima* seedlings under different soil water.

Ndff of the *T. ramosissima* seedlings: Soil moisture directly affected the nitrogen absorption and requisition ability of each of the plant parts. ^{15}N-KNO$_3$ and ^{15}N-NH$_4$Cl differentially impacted the Ndff of *T. ramosissima*. With the increase in soil moisture, the Ndff trend in the seedlings first increased and then decreased (Table 2). ^{15}N-KNO$_3$ resulted in a significant increase in nitrogen absorption in the seedlings (Fig. 5, Table 2).

The Ndff values of the *T. ramosissima* seedlings also differed under the same soil moisture gradient. For ^{15}N-KNO$_3$, the Ndff trend was more obvious in the main A and C branches than B and D. However, under increased soil water content, the Ndff trend in these four parts first increased and then decreased (Fig. 6). Ndff was significantly higher in D than the other three parts under D$_2$ and D$_3$.

For ^{15}N-NH$_4$Cl, Ndff only decreased in part B with the increase in soil moisture. The Ndff of parts A, C, and D changed significantly, and part D exhibited strong absorption under D$_2$ and D$_3$. When the soil moisture increased to D$_4$, Ndff in part D decreased significantly, at which point the Ndff was at its minimum value (^{15}N-NH$_4$Cl was only 2.69%) (Table 2, Fig. 6).

The Ndff of the aerial parts of the burgeon and collateral of seedlings increased and then decreased, but the nitrogen absorption and requisition capacity differed between ^{15}N-KNO$_3$ and ^{15}N-NH$_4$Cl (Fig. 6). For ^{15}N-KNO$_3$, the change in Ndff of part d2 was the most significant, while C exhibited the opposite trend. The Ndff of d2 reached maximum values of 5.92% and 5.89%, respectively, at D$_2$ and D$_3$. However, when the water increased to D_4, the Ndff of c1 (5.04%) was significantly greater than the other parts. For ^{15}N-NH_4Cl, C still exhibited the lowest Ndff, while the trend of c2 was higher. Meanwhile, the Ndff of c1 (only 3.42%) was significantly lower than the Ndff of the other parts under D_1, and the Ndff values of c2 and C were significantly higher than the others under D_4.

The Ndff of the seedlings during the growth season between 0 and 90 days did not change significantly under the D_1 and D_2 treatments, but decreased under D_3 and D_4, as indicated in Fig. 7. Meanwhile, the Ndff of the seedlings decreased significantly during 30–60 days. Between 0 and 60 days, the Ndff of the seedlings was the lowest under D_1 and the highest under D_4. However, at 90 days, the Ndff of the seedlings was lowest under D_4 and highest under D_3 and D_2 (Fig. 7).

Utilization and distribution: The two nitrogen forms had a significant influence on the nitrogen distribution in the various parts of the *T. ramosissima* seedlings as the soil water content increased ($p<0.05$) (Table 3). For ^{15}N-KNO_3, each of the sampled parts showed a trend of first increasing and then decreasing with the increase in water, and a slow decreasing trend was observed for ^{15}N-NH_4Cl. The distribution of the two nitrogen in C, c1 and c2 was higher, and the distribution of A and B was higher under D_1. For ^{15}N-KNO_3, the distribution of the burgeon of seedlings was in the order of C>D under different water gradient. For ^{15}N-NH_4Cl, the distribution of the halves of seedlings was in the order of C>D under D1, D2, and D3. The distribution of the two types of nitrogen in the collateral of the seedlings was higher than in the primary branches (C was lower than c1 and c2, D was lower than d1 and d2).

Table 2. The Ndff value of *T. ramosissima* seedlings under different water treatments (%).

Water gradient	D_1		D_2		D_3		D_4	
Nitrogen treatment	N_1	N_2	N_1	N_2	N_1	N_2	N_1	N_2
A	4.94fC	3.28bB	4.78eB	4.45dB	5.33dB	3.95cB	4.50dD	2.70aA
B	4.73eA	4.35dD	5.27gC	3.88bA	5.14fA	4.08cC	4.20cC	2.93aC
C	4.75deABC	3.87bcCD	4.42cdA	3.62abA	5.17eA	4.44cdF	4.05bcB	3.17aD
c1	4.93deBC	3.42aA	5.26fC	4.83dC	5.66gDE	4.54cF	5.04eF	2.97bC
c2	4.74dAB	3.76bBC	5.14eC	5.39fD	5.44fBC	4.28cE	4.82dE	3.11aD
D	4.81dABC	3.36bBC	5.82gD	5.27eD	5.56fCD	3.76cA	3.62cA	2.69aA
d1	4.92dABC	3.50bBC	5.72eD	4.83dC	5.74eE	4.21cDE	4.25cC	2.81aB
d2	4.93eABC	3.58bBC	5.92fD	4.70dBC	5.89fF	4.12cCD	4.03cB	2.77aAB

*In the table, small-letter means significant difference in the same line ($p<0.05$), capital-letters means significant difference in the same column ($p<0.05$). Following the same

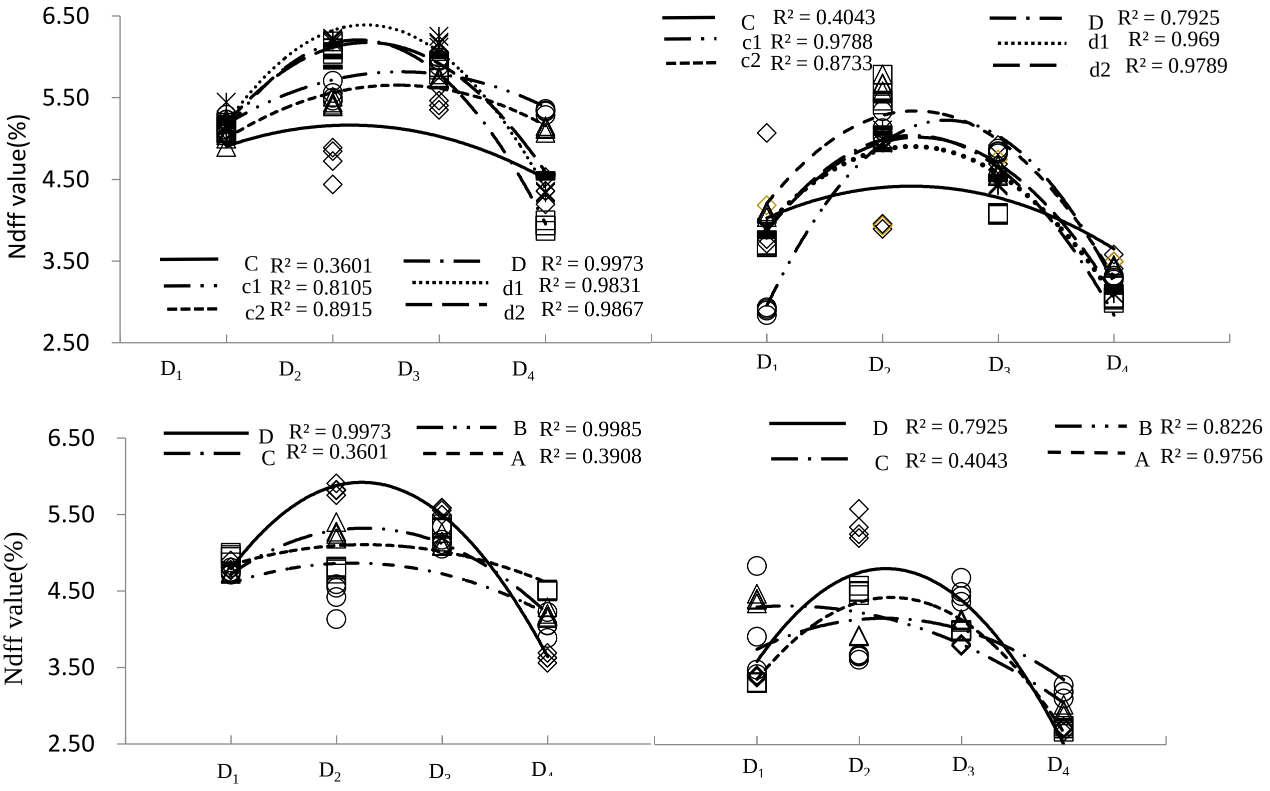

Fig. 6. The effect of nitrogen on the Ndff of *T. ramosissima* seedlings in each part under different soil water.

Table 3. The distribution of *T. ramosissima* seedlings under different water treatments (%).

Water gradient	D_1		D_2		D_3		D_4	
Nitrogen treatment	N_1	N_2	N_1	N_2	N_1	N_2	N_1	N_2
10-20A	14.68aA	5.17dE	4.05bF	4.18cF	5.35bF	3.64dF	4.81eEF	5.38bF
0-10B	14.69bA	16.85aB	6.93 eE	8.66dE	6.83 eE	12.98cC	5.74 fE	14.86bC
BC	14.86cA	21.93aA	8.27Ed	14.58cC	15.45cC	19.86bA	10.92dD	9.19eE
B2c1	6.73fD	10.57eD	25.00aB	11.21eD	15.89dC	16.54dB	21.98bB	18.43cB
B1c2	8.94fC	11.62eD	27.00bA	28.89aA	18.19dA	19.92cA	25.91bA	20.39cA
AD	11.81aB	4.80eE	6.60cE	3.92fF	7.32bE	5.49dE	4.16fF	4.70eF
A2d1	15.64bA	16.12bB	11.42dC	20.10aB	13.70cD	10.19eD	16.28bC	13.49cD
A1d2	12.66cB	12.94bcC	10.72dC	8.45eE	17.27aB	11.37dCD	10.20dD	13.56bD

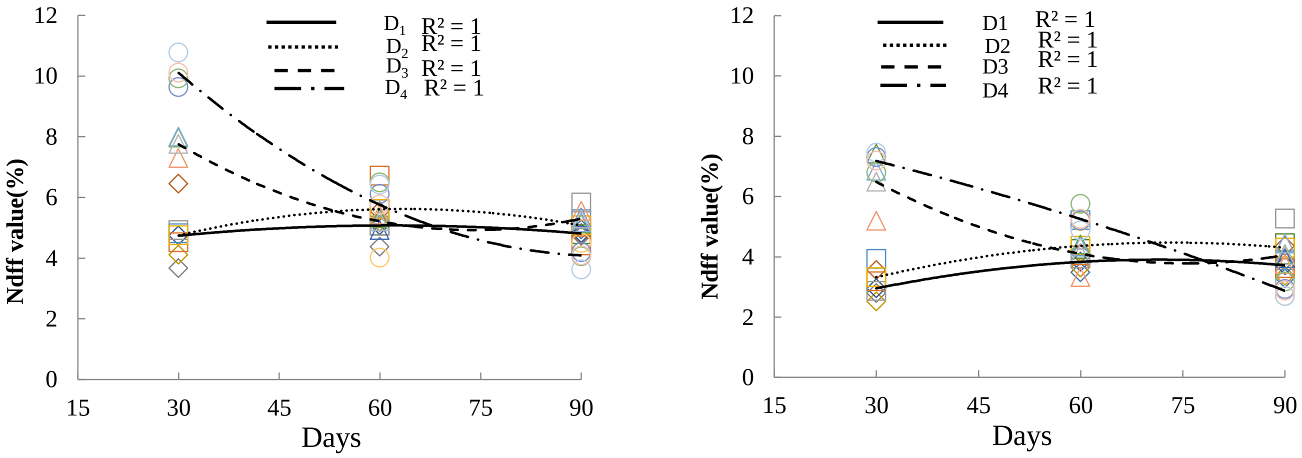

Fig. 7. The effect of nitrogen on the Ndff of *T. ramosissima* seedlings in different days.

Soil moisture content influenced the nitrogen utilization of the seedlings, and the seedlings showed different nitrogen utilization trends with the increase in soil water content. For ^{15}N-KNO_3, the seedlings showed a gradual increasing trend, while for ^{15}N-NH_4Cl, the seedlings exhibited a trend of first increasing and then decreasing. The seedlings only demonstrated a higher utilization of nitrate under D_4. The seedlings exhibited a better utilization of ammonium nitrogen under D_1, D_2, and D_3, and the utilization rate was the highest for all parts under D_3 (Table 4). For ^{15}N-KNO_3, the utilization of B Fig. 6. The effect of nitrogen on the Ndff of *T. ramosissima* seedlings in different days (29.71%) was higher under D_1, and an increase in the soil water content resulted in a higher utilization in C. For ^{15}N-NH_4Cl, the efficiency of B was highest under D_1 and D_4 (29.71% and 27.54%, respectively), but the utilization of C was highest under D_2 and D_3. Compared with the utilization of the main branches and the branches of the seedlings, we found that with the increase in soil water content, the utilization of c1 was the highest under ^{15}N-KNO_3, while the utilization of D_2 was the lowest. The collateral c2 of the seedlings showed a higher utilization under ^{15}N-NH_4Cl and the utilization of d2 was lower at soil water contents of D_2, D_3, and D_4.

Discussion

Nitrogen is an important element for plant growth. The efficient use of nitrogen can improve drought resistance in arid regions (James *et al*., 2011; Kang *et al*., 2014), and the effects of different nitrogen forms on plant growth differ (Ma *et al*., 2016). In this study, low (D_1) or high (D_4) soil moisture contents did not encourage *T. ramosissima* seedling growth. However, nitrogen application under drought stress (D_1) resulted in an increase in the root/shoot ratio and the growth of the belowground parts, the latter being conducive for water and nutrient absorption. Additionally, additional nitrogen can increase the chlorophyll a content of the aerial parts and $\varphi_{PS(II)}$, and can also ensure the efficient photosynthesis of seedlings in an arid environment, as observed by Wan Hongwei (2008), Yang Bingli (2015), and others. In contrast, when the soil moisture was increased to a suitable amount (D_3), the root/shoot ratio decreased and the aboveground growth increased significantly, and the chlorophyll content and photosynthetic efficiency were also respectively enhanced. The aerial parts of the seedlings grew significantly, but the chlorophyll a content and photosynthetic efficiency did not increase significantly under excessive water application (D_4). This suggested that nitrogen and photosynthetic pigment or efficiency do exhibit a linear relationship when the relative moisture content of the soil is 75% (Nakaji *et al*., 2001). By comparing seedling growth and the physiological indices under different nitrogen concentrations, we also found that ^{15}N-KNO_3 had a greater impact on the root/shoot ratio and biomass accumulation in the seedlings, and also influenced the chlorophyll a content. ^{15}N-NH_4Cl had a greater impact on the chlorophyll b content and the $\varphi_{PS(II)}$ of the seedlings (Mihailovic *et al*.,1992; Zhang *et al*., 2010).

Soil moisture significantly influenced nitrogen absorption in the *T. ramosissima* seedlings, and different nitrogen concentrations had notably different effects. In this experiment, when the soil water content increased from D_1 to D_4, the Ndff of all the sampled parts first increased and then decreased, and the Ndff of ^{15}N-KNO_3 was greater than that of ^{15}N-NH_4Cl, indicating that the seedlings have better nitrate absorption. This also showed that the absorption and requisition ability of the seedlings towards ^{15}N-KNO_3 was better than that of ^{15}N-NH_4Cl (Li *et al*., 2013; Bagherzadeh *et al*., 2008). Compared with the aerial parts of *T. ramosissima*, the capacity of the roots to absorb and retain nitrogen was high under heavy drought stress (D1). This result is consistent with the belowground parts of the seedlings accumulating more carbon during drought, and also demonstrates that root growth is an important strategy for the adaptation of the seedlings to arid environments. With a reduction in drought stress, the aerial parts of the seedlings showed greater nitrogen absorption, and the younger parts, such as the lateral branches (collateral c1, c2, d1, and d2), were more likely to absorb nitrogen (Wang *et al*., 2012; Dong *et al*., 2002).

The distribution and utilization of nitrogen in the aboveground and belowground parts of the seedlings differed following nitrogen absorption (Khalifa K., 2005). Under high drought stress (D_1), the distribution of ^{15}N-NH_4Cl was higher than that of ^{15}N-KNO_3, and the roots exhibited high nitrogen accumulation. However, an increase in soil moisture resulted in a greater increase in the distribution of ^{15}N-KNO_3 than ^{15}N-NH_4Cl [Du *et al*., 2015]. The distribution of ^{15}N-NH_4Cl in the roots (A) was highest, while the principal branches of the aboveground parts (C) had a higher proportion of ^{15}N-KNO_3. Although the distribution of ^{15}N-KNO_3 in the seedlings was higher, the utilization slowly declined as the soil water content increased. However, ^{15}N-NH_4Cl utilization was higher than that of nitrate nitrogen, and presented a trend of an initial increase followed by a decrease, which corroborates the findings of Chu Wu (2005), Wenyi Dong (2009), and others. Generally, the seedling roots exhibited high distribution and utilization of nitrogen under soil moisture limitation, whereas the aboveground parts of the seedlings showed the same trend when the soil moisture increased.

Arid ecosystems typically lack water and nutrients, especially nitrogen. The growth and development of plants in arid regions is thus severely restricted (Khasanova *et al*., 2013; Drenovsky and Richards, 2004). While the relationship between drought stress and nitrogen is complex, it is understood that drought inhibits the absorption and utilization of nitrogen in plants, while nitrogen, to some extent, helps plants to improve their adaptation towards drought stress (Pavon *et al.,* 2005; Meyer *et al*., 2015; Sperry *et al*., 2006). The lower reaches of the Tarim River are extremely arid, with soils that have a low nutrient content, particularly nitrogen (Wang *et al*., 2015; Chen *et al*., 2006; Li *et al*., 2015). Adequate water and nutrient supply for *T. ramosissima* seedlings during their initial period of vulnerability is especially critical. We found that drought limits the absorption and utilization of nitrogen by the seedlings, and the effective absorption of nitrogen can help plants adapt to arid environments. However, too much water (D_3 and D_4; particularly D4) is not conducive to nitrogen absorption and utilization. Excessive water resulted in a sharp decline in the capacity of the seedlings to absorb nitrogen during the first 30−60 d, and thus water was determined to be a key factor affecting nitrogen absorption and utilization efficiency. The lower reaches of the Tarim River are particularly deficient in nutrients, particularly nitrogen. The nitrogen content increased as a result of overflow from eco-water conveyance engineering systems, but irrigation and increased duration were not conducive for seedlings to grow in the wild. Rising temperatures have resulted in rapid decreases in the surface water, and the nitrogen content in the overflowing water is lower than the non-overflowing water due to leaching and deposition. Both water and nutrient stress present challenges to seedling survival (Fu *et al.*, 2013; Zhou *et al*., 2010). In addition, the use of nitrogen fertilizer on farms has increased rapidly in recent years on both sides of the middle reaches of the Tarim River, with some of the nitrogen being deposited in the river (Ji *et al*., 2000; Li *et al*., 2006; Ma *et al*., 2012). Therefore, as the water is carried downstream, the soil nitrogen content of the overflowing water increases, thereby facilitating plant germination and growth, which is conducive to the recovery of the vegetation.

Table 4. The utilisation of *T. ramosissima* seedlings under different water treatments (%).

Water gradient	**D_1**		**D_2**		**D_3**		**D_4**	
Nitrogen treatment	**N_1**	**N_2**	**N_1**	**N_2**	**N_1**	**N_2**	**N_1**	**N_2**
A	6.79cD	4.41aA	5.56bA	9.50dA	10.47fA	10.86gA	11.81hB	9.98eB
B	14.35bE	29.71eF	9.52aB	19.65cB	13.36bB	38.76fE	14.08bC	27.54dE
C	6.87aD	18.69cE	11.36bC	23.48dC	30.24fD	53.06gG	26.81eE	17.03cC
c1	3.11aA	9.00bB	34.34eE	25.46cD	31.09dD	49.38fF	53.96gH	34.15eF
c2	4.13aB	9.91bB	16.43cD	35.52dE	35.60dF	48.48fF	38.47eG	37.79eG
D	5.46bC	4.09aA	9.07cB	8.90cA	14.33eB	16.40fB	10.22dA	8.71cA
d1	7.23aD	13.74bD	15.69cD	45.65hF	26.80eC	30.43fC	33.96gF	25.00dD
d2	5.85aC	11.03bC	14.73cD	19.19dB	33.78fE	33.94fD	25.05eD	25.12eD

Conclusions

In summary, soil moisture affected all of the sampled parts of the *T. ramosissima* seedlings with regards to nitrogen absorption and utilization, and drought or excessive moisture were not conducive to seedling growth. Therefore, water and nitrogen are key factors influencing the early growth of *T. ramosissima* seedlings, and suitable moisture is required for effective nitrogen use. These factors facilitate plant adaptation to arid conditions and survival through the vulnerable growth period.

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