

Water and nitrogen supply effects on four desert shrubs with potential use for rehabilitation activities

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Abstract Desert evergreen shrubs, which are adapted to low-fertility ecosystems, generally exhibit limited responses to increased nutrient availability and tend to absorb and store nutrients rather than synthesize new tissues. The objective of this work was to analyze the effect of nitrogen fertilization combined with soil water availability on growth, nitrogen content, and nitrogen use efficiency on four shrubs (*Atriplex lampa*, *Capparis atamisquea*, *Larrea cuneifolia*, and *Senecio subulatus*) from the Monte Desert. In a 120-day glasshouse experiment in Mendoza, Argentina, we compared the effects of three levels of nitrogen fertilization combined with two levels of water availability on seedling biomass,

nitrogen content, water potential, and nitrogen use efficiency. Fertilization induced a higher biomass on *A. lampa* under high water availability and on *C. atamisquea* regardless of water level. Shoot:root ratios of these two species were lower under water stress without fertilization. On the other hand, *L. cuneifolia* presented lower root biomass and lower water potential with N fertilization. All species when fertilized exhibited higher nitrogen content and lower nitrogen use efficiency. Also, *A. lampa* and *L. cuneifolia* presented higher nitrogen content under water stress conditions. In conclusion, some desert shrubs (*A. lampa* and *C. atamisquea*) were able to take advantage of increased nitrogen availability producing more biomass. Understanding seedlings response to nitrogen and water availability on arid lands is critically important to develop adequate revegetation techniques of degraded areas.

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Introduction

In general, an increase in the availability of a limiting soil nutrient or other resource results in an increase in plant biomass, but the specific growth responses to soil nutrients vary broadly across the plant kingdom (Chapin 1980; Chapin et al. 1986). Among perennial species, the individual growth responses tend to correspond to the native fertility (Chapin 1980; Grime 2006). Species adapted to low-fertility ecosystems, generally exhibit low maximum potential growth rates, tissue nutrient concentration, shoot-to-root ratio, and foliar abscission, and exhibit limited responses to increased nutrient supply (Chapin 1980; Drenovsky and Richards 2004; Padgett and Allen 1999; Vilela et al. 2003; Yahdjian et al. 2014). These species generally absorb and store nutrients rather than synthesize new tissues in response to increased availability. The stress resistance syndrome theory proposes something similar: plants adapted to low-resource environments (infertile, dry or saline soils, etc.) grow slowly even when provided with an optimal supply of resources as they are mainly diverted to defense or storage (Chapin 1980).

Although we have a good understanding of the dynamics of N availability in arid soils, little is known about the response of different desert plant species to changes in N availability. Nitrogen use efficiency (biomass produced per unit nitrogen taken up from the soil) is high in desert shrubs, and it can reduce the dependence of plant growth on soil nutrient availability (Lajtha 1987). However, some authors had observed greater growth and establishment with increasing nitrogen availabilities in shrubs from ecosystems with low soil fertility (Clemente et al. 2004; Donovan and Richards 2000; Fisher et al. 1988; Lajtha and Klein 1988). Bisigato and Bertiller (1999) found in species that tend to establish in bare soils, with low nutrient availability, low responses to nitrogen supply, unlike species that colonize beneath vegetation patches. In deserts, nutrients are concentrated in these patches, also called “fertility islands,” where some species can take advantage of these and other improved microclimatic conditions (like

increase water availability and protection against herbivory) and get established (Bosco et al. 2015; Callaway and Pugnaire 1999; Passera and Borsetto 1989; Pugnaire et al. 1996; Rossi and Villagra 2003).

Degraded arid lands are associated with significantly altered soil conditions, with soil surface completely replaced and changes in soil components. In such places, both the availability and distribution of belowground resources may be affected, and the ability of plants to acquire the critical resources essential for growth may be altered too (Ehleringer and Sandquist 2006). A common practice to restore these environments is to provide some of the most critical macronutrients for the growth of plants as fertilizers (Bradshaw 2004; Cione et al. 2002). Fertilization in the nursery has an important influence on the morphology and physiology of the plant and in later field survival.

Plants that arrive at the field with high N content can have an advantage in degraded dry regions since the absorption and assimilation of nitrogen and the subsequent metabolism of its products are affected by water stress due to reduced transpiration rates, impaired active transport, and membrane permeability, and a decrease in the diffusion rate of nutrients in the soil to the absorbing root surface (Erskine et al. 1996; Hu and Schmidhalter 2005). During periods of high water availability, this higher N in plants can be utilized to support further growth. On the other hand, additional N can be linked to increased plant susceptibility to drought, if physiological (water potential, stomatal conductance, etc.) or morphological (shoot:root ratio, leaf area index, etc.) traits are altered (Pivovarov et al. 2016; Ward et al. 2015), ultimately resulting in increased mortality (Allen et al. 2010). Indeed, both sources of stresses (drought and nitrogen limitation) are difficult to disentangle.

The Monte region is located in western Argentina, covering approximately 460,000 km² (Rundel et al. 2007). This region experiences water deficit for most of the year and has an average annual rainfall ranging from 30 to 350 mm, with a mean temperature between 13 and 18 °C (Morello 1958; Rundel et al. 2007). Soil nutrient availability in these areas is strongly coupled with the quantity and quality of litter-fall (Alvarez et al. 2009; Carrera et al. 2009) along with the decomposition/mineralization pathway controlled by the unpredictable water inputs.

Some areas of the Monte Desert present moderate to severe degree of native ecosystem degradation

(Abraham 2002; Roig et al. 1991). To reverse these processes, revegetation programs are the starting point for reaching ecosystem restoration. Many efforts conducted on restoring degraded areas in Argentina fail because of the lack of basic knowledge of the interaction between soil properties and vegetation (Rovere 2015). Particularly, in arid regions, it is essential to understand plant responses (biomass, N content, shoot/root, water potential, etc.) to nitrogen and water availability, and the interaction between them.

In order to analyze plant responses to the availability of both resources (nitrogen and water), we selected four shrub species from the Monte Desert that are widely spread over the region and are broadly used in revegetation programs in arid zones: *Atriplex lampa*, *Capparis atamisquea*, *Larrea cuneifolia*, and *Senecio subulatus*. Little information about these species is available, so the present study would provide new insights into nursery techniques (fertilization requirements, preconditioning treatments, seedling quality, etc.), and the factors that influence seedling growth and their ecophysiology, in order to design appropriate revegetation strategies.

This study aims to analyze the effect of nitrogen supply combined with soil water availability on growth, water potential, nitrogen content, and nutrient use efficiency of four native shrubs from a dry region. We hypothesized that since the four species are adapted to low-fertility arid environments, plants would respond to nitrogen addition with increases in nitrogen content and decreases in nitrogen use efficiency (NUE) but biomass would not be affected compared to the unfertilized control. Under low water and low nitrogen availability, plants would present lower shoot-to-root ratio and water potential.

Materials and methods

Species and seed collection

Atriplex lampa Moq. (Amaranthaceae) is a 0.5- to 2.5-m-high C4 forage shrub with high (> 15%) protein content (Silva Colomer and Passera 1990; Wainstein and Gonzales 1969). It frequently occupies loose or sandy soils and tolerates high soil salinity concentrations (Ruiz Leal 1972). *Capparis atamisquea* Kuntze (Capparaceae) is a 2- to 3-m-high C3 forage shrub

with small leathery leaves, densely pubescent on their abaxial face (Roig 1980), and has a facultative phreatophyte behavior (Jobbágy et al. 2011). *Larrea cuneifolia* Cav. (Zygophyllaceae) is a microphyllous C3 shrub with resinous cuticles and small stomata showing high resistance and closure at midday during dry seasons (Barbour et al. 1974; Morello 1955). It can tolerate very hot environments by physically evading the midday sun and intercepting early morning and late afternoon light with its erect, east-facing leaves and branches (Ezcurra et al. 1991). *Senecio subulatus* Don ex Hooker et Arnot (Asteraceae), is a 1- to 1.5-m C3 shrub with a dense canopy and perennial foliage (Roig 1980). Images of the four species are available on Online Resource 1.

Seeds of *C. atamisquea* were collected from the Telteca Natural Reserve, NE Mendoza (32°14'56"S; 67°49'05"W) and *A. lampa*, *L. cuneifolia*, and *S. subulatus* from a natural shrubland located in Agrelo, SW Mendoza (33°07'01"S; 68°52'46"W). They were stored in paper bags at room temperature until experiments were performed. To obtain the seeds of *A. lampa*, *C. atamisquea*, and *L. cuneifolia*, fruits were rubbed between two rubber sheets, and only well-formed seeds were used in the experiments. Seeds of *L. cuneifolia* were washed with running water for 48 h in order to break their chemical dormancy. Prior to sowing, both types of seeds were disinfected with a 15% solution of commercial hypochlorite (60 g Cl/I) for 7 min and then repeatedly washed with sterile water.

Experimental set-up

The experiment was performed in the glasshouse of IADIZA (Instituto Argentino de Investigaciones de las Zonas Áridas 32°53'S; 68°57'W), Mendoza, Argentina, from January to April 2013, over a period of 120 days. Seeds were sown in a terrine and then transplanted to plastic pots 10 cm in diameter and 5 L of capacity filled with 3.6 kg of air-dried sandy loam soil (EC = 2972.5 μ S/cm 25 °C, pH 7.95, N = 357 ppm, P = 2.03 ppm, K = 156 ppm, organic matter = 1.47%). Field capacity (FC) of the soil was 18.1%. Pots were watered as necessary until the start of the experiment. Daily maximum and minimum temperatures during 2013 were 35.4 °C (\pm 5.3) and 19.8 °C (\pm 3.5); minimum relative humidity was 19.9% (\pm 8.7) and maximum 54.1% (\pm 14.1), and

mean PAR at midday was 1100 $\mu\text{mol}/\text{m}^2/\text{s}$. A foam pad was placed around all pots to avoid overheating from the sun. The plants were 6 months old at the beginning of the experiment.

Fertilization and water treatments

The experiment was set up in a completely randomized design with two factors (nitrogen and water). Fertilization levels were selected in accordance with Vilela et al. (2003), who applied some fertilization treatments to other native shrubs from the Monte. Nitrogen supply had three levels: 0 mg N per seedling (F1—control), 32 mg N per seedling (F2), and 64 mg N per seedling (F3). All N levels were provided by ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) in one application at the beginning of the experiment. Soil water availability had two levels: high water availability (W1—control) was watered when soil water content decreased by 50% from field capacity (FC) (being watered up to FC 14 times, except on *C. atamisquea* being watered 10 times), and low water availability (W2—water stress) when it decreased by 80% from FC (being watered up to FC 9 times, except on *C. atamisquea* being watered 4 times). The amount of water to be replenished to FC at each irrigation date was determined from the gross weight of pots (without leach from the pots). We used 120 plants of each species distributed among the six combinations of water and nitrogen levels (20 replicates for each treatment combination). Plants were randomly assigned to one of these six treatments.

Leaf water potential

Water potential (Ψ , MPa) was measured on the main shoot immediately after being excised at predawn (pd), 4:00–5:00 h, before re-watering. Measurements were made using a pressure chamber (Biocontrol, Buenos Aires, Argentina), based on Scholander et al. (1965). For these measurements, five plants per treatment per species were randomly selected in February and March.

Growth

Ten plants per treatment were randomly selected to determine sapling height and stem basal diameter every 15–20 days, starting at day 0. At the end of the

experiment, these same 10 plants were used to determine shoot (SB), root (RB), and total (TB) biomass after oven-drying them to constant weight at 60 °C for 72 h. Fallen leaves were collected throughout the experiment and added to live leaves to make the measurements.

We calculated the shoot-to-root ratio by the dry weight of shoot and root (S/R) using data from growth parameters.

Nitrogen status

Nitrogen determination was based on the Kjeldahl method (grams of nitrogen per 100 g of dry matter). It was determined in the same plants used for growth determinations. Five shoots and roots dry samples (1000 mg) per treatment per species (60 samples per species) were milled and then measured separately in the laboratory of fitoquímica (IADIZA). With these data, we calculated Nitrogen ($\text{N} = \text{SN} + \text{RN}$) for each plant.

Nitrogen use efficiency

Nitrogen use efficiency was defined according to Lajtha and Klein (1988) as $\text{NUE} = \text{plant biomass (g)}/\text{plant N (g)}$. Plant N (g) was calculated as $\text{TB (g)} * \text{N (g)}/100 \text{ (g)}$.

Statistical analysis

Growth and water potential data were subjected to a two-way analysis of variance (ANOVA), with water and fertilization as factors. Nitrogen and NUE data were subjected to a three-way ANOVA, including species as a third factor. Tukey's test was used for comparison of means. Statistical analysis was performed with R, with stats and car packages (R Core Team 2014). Because the data on shoot-to-root ratio did not meet the ANOVA assumption of variance homogeneity, an $\ln(x + 1)$ transformation was applied.

Results

Total (TB), shoot (SB), and root biomass (RB) were significantly affected by water and nitrogen availabilities on *A. lampa* seedlings and their interaction was

significant (except for SB). Water availability level affected TB and RB response to fertilization treatments. This species, with 32 and 64 mg N/seedling under high water availability, showed higher TB and RB (higher only with 64 mg N), and under water stress (W2) conditions biomass was not affected (Fig. 1). Shoot biomass did not present interaction between factors, and was higher under 32 and 64 mg N/seedling and under high water availability. *Capparis atamisquea* seedlings accumulated more biomass (TB, SB, and RB) under F3 (64 mg N/seedling), regardless of water level (Fig. 1). Thus, these two species did not show a conservative behavior expected in plants from low-resource environments.

Total biomass was unresponsive to nitrogen availability in *L. cuneifolia* seedlings, but under the water stress treatment (W2) TB was lower than under W1 (Fig. 1). On the other hand, shoot biomass did not

differ between treatments and root biomass was significantly affected by both water and nitrogen levels (fertilization \times water interaction $F = 5.36$, $P = 0.0079$). Under high water availability and no fertilization (W1:F1), *L. cuneifolia* seedlings presented a higher RB than the other fertilization levels, while there were no differences under water stress conditions, meaning that fertilization had a negative effect in root development for this species. *S. subulatus* seedlings showed differences among water levels in TB and SB, with higher biomass accumulation in seedlings under high water availability (Fig. 1).

Shoot-to-root ratio (S/R) was significantly affected by fertilization and water availability in the four species. Interactions between nitrogen and water supply were found except on *S. subulatus* ($F = 4.27$, $P = 0.0193$ for *A. lampa*; $F = 4.91$, $P = 0.0111$ for *C. atamisquea*; $F = 10.9$, $P = 0.0001$ for *L. cuneifolia*;

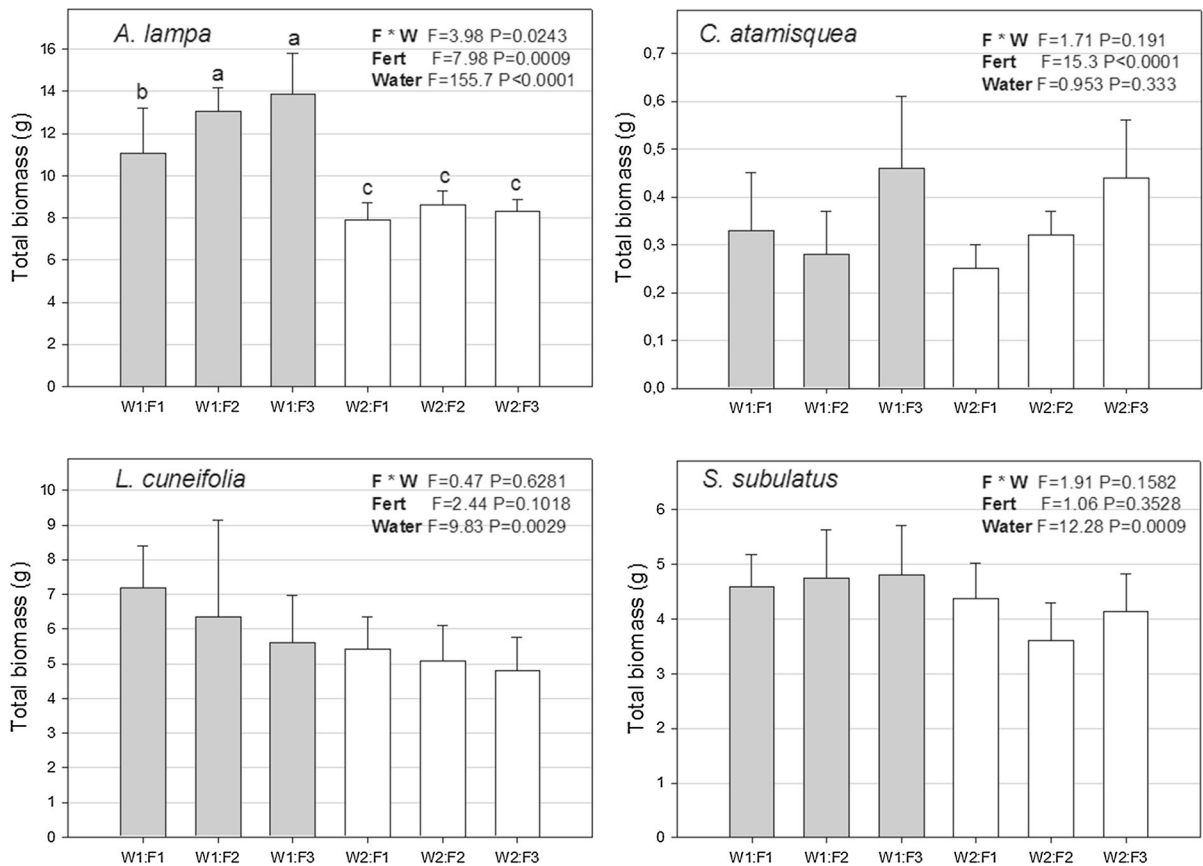


Fig. 1 Total biomass of *A. lampa*, *C. atamisquea*, *L. cuneifolia*, and *S. subulatus* plants at the end of the experiment. W1: high water availability (gray bars), W2: low water availability (white bars). F1: 0 mg N/seedling; F2: 32 mg N/seedling and F3:

64 mg N/seedling. Different letters among treatments for each species indicate significant differences at $P < 0.05$ for total biomass. ANOVA results are shown. Vertical lines indicate ± 1 SE of the mean

$F = 2.47$, $P = 0.0939$ for *S. subulatus*). *Atriplex lampa* and *C. atamisquea* seedlings without fertilization assigned more biomass to the root than to the shoot (lower S/R) under water stress (W2:F1) than seedlings under high water availability (W1:F1), while no significant differences were found among fertilized seedlings (Table 1). For unfertilized *L. cuneifolia*, the opposite was observed: greater biomass was assigned to the root (lower S/R) under high water availability (W1:F1) than under water stress conditions, and fertilized seedlings did not present differences in this ratio (Table 1). In *S. subulatus* seedlings, this ratio was significantly lower in seedlings without fertilization than under 32 mg N/seedling (Fertilization $F = 6.38$, $P = 0.00324$) (Table 1). Overall, the S/R ratio was significantly higher in *A. lampa* compared to the other three species ($F = 124.44$, $P < 0.0001$), and therefore *A. lampa* seedlings allocated a higher proportion of assimilates to the shoot.

Height and stem diameter were unresponsive to nitrogen and water availabilities, both in *L. cuneifolia* and *S. subulatus* seedlings. On the other hand, the low water availability treatment (W2) significantly reduced height and stem diameter in *A. lampa* (Figs. 2, 3), and fertilization treatments had no effect on them. *C. atamisquea* seedlings presented higher height and stem diameter when fertilized with 64 mg N/seedling (Figs. 2, 3).

Water potential at predawn (Ψ_{pd}) was significantly lower under water stress conditions for the four species ($P < 0.0001$ for all four species, Fig. 4), while fertilization also affected *L. cuneifolia*: seedlings under F3 had lower Ψ_{pd} than seedlings without fertilization (fertilization factor $F = 3.68$, $P = 0.0338$) (Fig. 4). Overall, *A. lampa* seedlings had the lowest Ψ_{pd} values and *S. subulatus* the highest.

Measurements of nitrogen content on *C. atamisquea* were not included in the statistical analysis since there were no replicates. Biomass obtained from individual seedlings was too little to measure N with the Kjeldahl method, and therefore biomass per treatment was pooled (Fig. 5). Nitrogen and Nitrogen Use Efficiency (NUE) were significantly affected by water and nitrogen availability and differed among *A. lampa*, *L. cuneifolia*, and *S. subulatus*, but the three-way interaction was not significant. Interactions between water level and species were found in N and NUE ($F = 4.54$, $P = 0.0139$; $F = 14.37$, $P < 0.0001$, respectively). This interaction showed

Table 1 Shoot-to-root ratio for *A. lampa*, *C. atamisquea*, *L. cuneifolia*, and *S. subulatus* seedlings under water (W1: high water availability, W2: low water availability) and fertilization (F1: 0 mg N/seedling, F2: 32 mg N/seedling, F3: 64 mg N/seedling) treatments

Treatment	<i>A. lampa</i>		<i>C. atamisquea</i>		<i>L. cuneifolia</i>		<i>S. subulatus</i>	
	W1	W2	W1	W2	W1	W2	W1	W2
F1	6.17 a (2.13)	4.19 b (1.59)	3.16 a (0.72)	1.57 d (0.44)	1.45 b (0.26)	2.54 a (0.37)	1.9 (0.62)	1.81 (0.5)
F2	5.67 ab (1.55)	5.04 ab (1.38)	2.5 abc (0.61)	1.71 cd (0.47)	2.68 a (1.24)	2.35 a (0.27)	2.47 (0.67)	2.49 (0.44)
F3	4.77 ab (1.61)	5.47 a (1.94)	2.52 ab (0.69)	2.11 bcd (0.59)	2.50 a (0.24)	2.77 a (0.47)	2.43 (0.77)	1.71 (0.56)

Different letters among water and fertilization treatments for each species indicate significant differences at $P < 0.05$ (SE between parentheses)

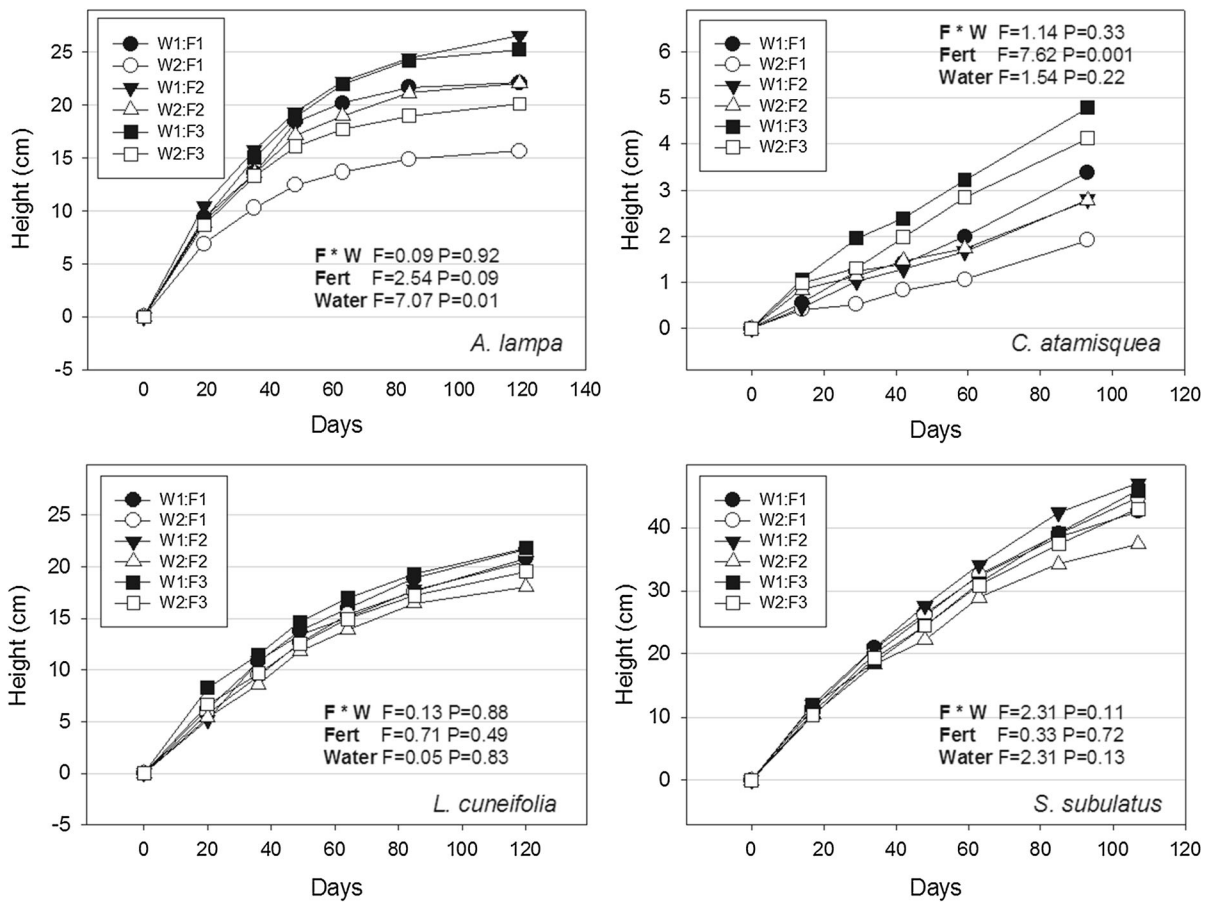


Fig. 2 Average seedling height growth imposed by different irrigation and fertilization levels along the experiment for the four species. W1: high water availability, W2: low water

availability. F1: 0 mg N/seedling; F2: 32 mg N/seedling; and F3: 64 mg N/seedling

that *A. lampa* seedlings had a significantly lower N content than the other two species under both irrigation treatments (Fig. 5). Furthermore, *A. lampa* and *L. cuneifolia* presented higher N under low than under high water availability, and *S. subulatus* did not show significant differences between both water levels. When species were analyzed individually, it was found that seedlings absorbed more N with 32 and 64 mg N/seedling, under both water levels ($P < 0.0001$ for the three species; Fig. 5).

were analyzed individually, fertilization treatment with 64 mg N/seedling produced a lower NUE in the three species ($P < 0.0001$ for the three species, Fig. 6).

The significant interaction found between water level and species on NUE also showed that irrigation treatments did not produce any difference on *S. subulatus* seedlings, while the other two species presented a lower efficiency under low water availability. *A. lampa* seedlings were more efficient in nitrogen use than the other two species. When species

Discussion

The hypothesis evaluated here was that since *Atriplex lampa*, *Capparis atamisquea*, *Larrea cuneifolia*, and *Senecio subulatus* are adapted to arid environments, plants would respond to nitrogen addition with increases in nitrogen content and decreases in nitrogen use efficiency (NUE) but biomass would not be affected compared to the unfertilized control. Under low water and low nitrogen availability, plants would present lower shoot-to-root ratio and water potential.

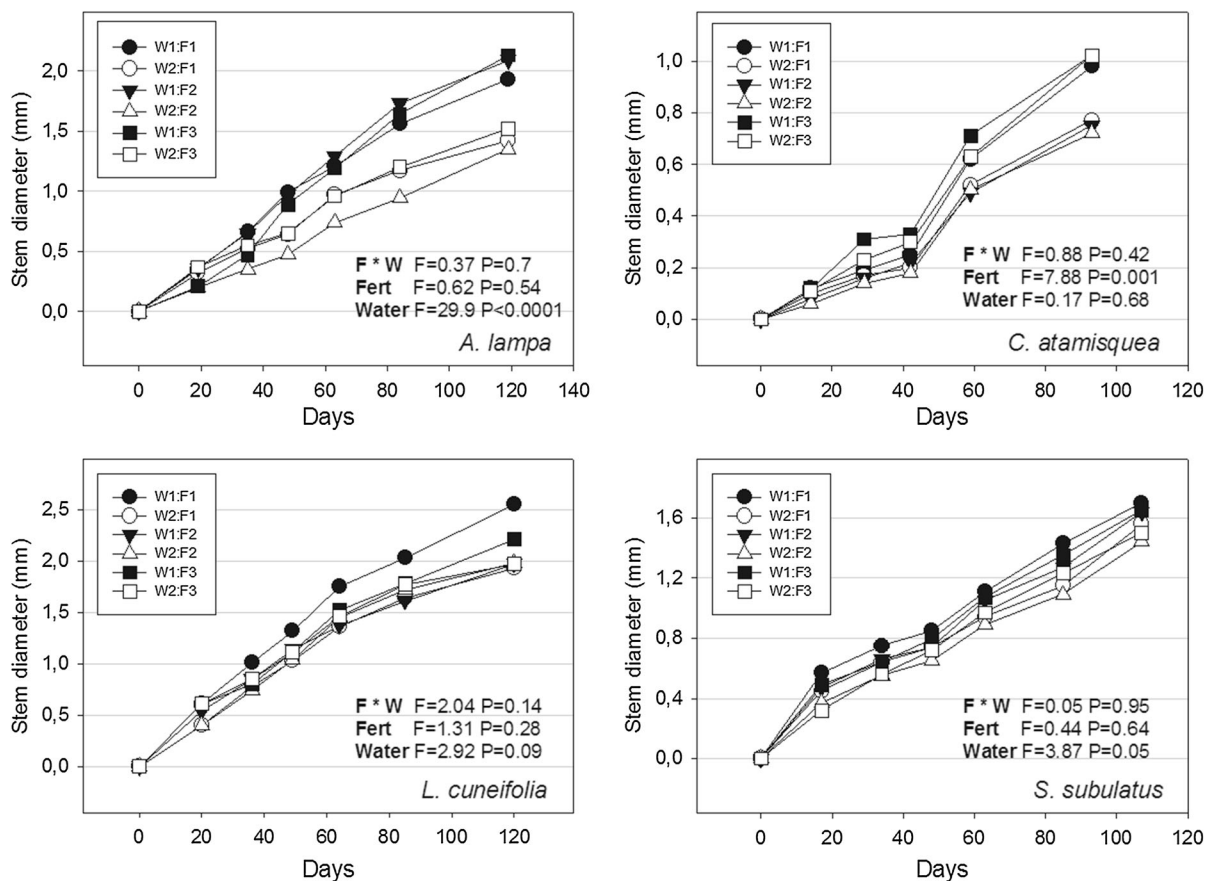


Fig. 3 Average seedling stem basal diameter growth imposed by different irrigation and fertilization levels along the experiment for the four species. W1: high water availability,

W2: low water availability. F1: 0 mg N/seedling; F2: 32 mg N/seedling; and F3: 64 mg N/seedling

As predicted, *L. cuneifolia* and *S. subulatus* seedlings did not present a greater biomass accumulation in response to nitrogen supply but did absorb and store nitrogen, and showed a lower Nitrogen Use Efficiency. Contrary to expectations, *A. lampa* produced higher biomass in response to higher N and water supply, and among the four species it had the lowest N content and the highest NUE. *C. atamisquea* also responded to fertilization by increasing biomass production, presenting higher stem basal diameter and height, regardless of water availability. Finally, while lower water potential under water stress conditions was observed in all four species, only in *A. lampa* and *C. atamisquea* lower S/R ratio was observed. In summary, these results indicate that despite their adaptation to arid, low-nutrient environments, these shrubs

differ in their response to combined nutrient addition and water availability.

Even though the higher biomass shown by *C. atamisquea* with higher N was contrary to expectations, these results are consistent with the idea that under natural conditions, emergence and plant performance of this species in the Monte region may be promoted beneath vegetation patches (Cesca et al. 2012; Páez and Marco 2000; Rossi and Villagra 2003). In these patches, N mineralization occurs at a higher rate than in bare soil, and greater nitrogen content is observed (Mazzarino et al. 1998).

Capparis atamisquea also developed a lower shoot-to-root (S/R) ratio under water stress conditions. This may actually be induced by water stress, which can affect leaf expansion and reduce carbon and energy uptake. Therefore, a greater proportion of the plant's

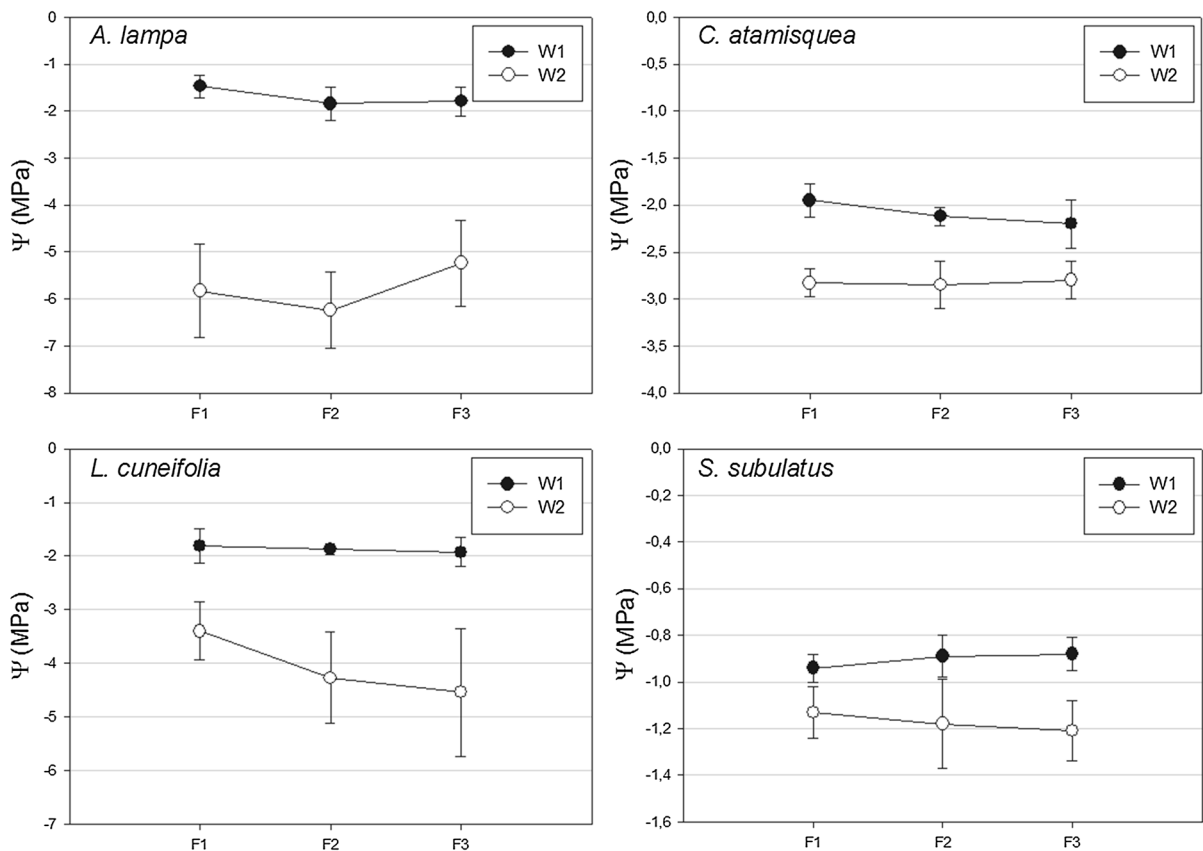


Fig. 4 Predawn water potential (MPa) for *A. lampa*, *C. atamisquea*, *L. cuneifolia*, and *S. subulatus* under different fertilization levels (F1: 0 mg N/seedling, F2: 32 mg N/seedling,

F3: 64 mg N/seedling) at each water level (W1: high water availability, W2: low water availability). Vertical lines indicate ± 1 SE of the mean

assimilates can be distributed to the root system, where they can support further root growth while maintaining a better water status, increase nitrogen capture, and return carbon and nutrient contents to a balance more favorable to storage (Bradshaw 1987; Patterson et al. 1997; Taiz and Zeiger 2006; Vilela et al. 2003). *Atriplex lampa* also presented a lower S/R ratio under water stress conditions. This response has been previously described in both species (Fernández et al. 2016; Passera and Allegretti 1996) and in other shrubs of the Monte region (Vilela et al. 2003; Villagra and Cavagnaro 2006). On the other hand, S/R ratio of *L. cuneifolia* seedlings was lower under high water availability and without fertilization, contrary to previous studies, where it was lower under water stress conditions (Fernández et al. 2016). This difference was caused by high root biomass obtained in seedlings under high water availability. In general, fertilization

induces higher S/R ratios (Bradshaw 1983, 1984; Garnier et al. 1989; Villar-Salvador et al. 2010), while water stress reduces this ratio, but in the presence of both, water stress tends to prevail over fertilization (Faustino et al. 2011; Graciano et al. 2006), although on *L. cuneifolia* we could not observe this pattern.

High levels of nutrients can have positive effects on the growth of some species and negative effects on others (Bradshaw 2004); this is especially important for species adapted to ecosystems with low soil fertility. As mentioned before, additional N can be linked to increased plant susceptibility to drought, if physiological (water potential, stomatal conductance, etc.) or morphological (shoot-to-root ratio, leaf area index, etc.) traits are altered (Pivovarov et al. 2016; Ward et al. 2015). Here, nitrogen fertilization had a negative effect on root biomass accumulation in *L. cuneifolia*, being lower on fertilized seedlings under

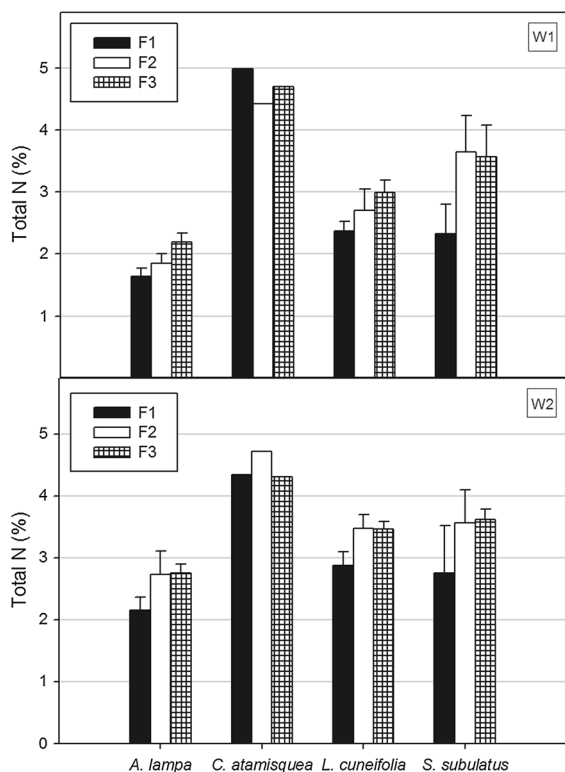


Fig. 5 Effect of nitrogen addition level (F1, F2, and F3, 0, 32, and 64 mg N/seedling, respectively) on plant nitrogen (nitrogen in grams of N per 100 g of dry matter) for *A. lampa*, *C. atamisquea*, *L. cuneifolia*, and *S. subulatus* at each water level (W1 and W2, high and low, respectively). Bar height indicates mean ± 1 SE

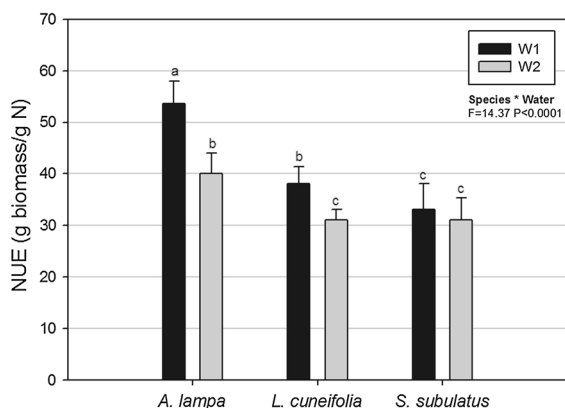


Fig. 6 Effect of water availability on Nitrogen Use Efficiency (grams of biomass/grams of nitrogen) for *A. lampa*, *L. cuneifolia*, and *S. subulatus*. Water treatments were (W1): high water availability, (W2): low water availability. Different letters among treatments and species indicate significant differences at $P < 0.05$. Vertical lines indicate ± 1 SE of the mean

high water availability, which in turn, presented a lower Ψ_{pd} than seedlings without fertilization. Since fertilization reduced root biomass accumulation, this could affect the availability of water for the aerial part of the plant and lowered the Ψ_{pd} (Faustino et al. 2011). These types of responses have already been observed in other species of the genus *Larrea*. Meinzer et al. (1988) observed in *L. tridentata* from North America lower Ψ on fertilized seedlings than the control without fertilizer. Also, in a study carried out in a nursery, *L. divaricata* from the Monte Desert, when provided with 25 kg N/ha (NO_3NH_4), had lower seedling survival rate (Bisigato and Bertiller 1999).

As it was expected, under both water availabilities, *A. lampa*, *L. cuneifolia* and *S. subulatus* seedlings absorbed and stored more nitrogen with the increasing availability. These reserves could be used to support further growth after soil reserves are depleted (Chapin 1980; Padgett and Allen 1999). The higher nitrogen content was accompanied with a higher biomass accumulation in *A. lampa* seedlings under high water availability, despite the fact that this species tends to colonize bare soil with low nitrogen content. But under water stress conditions it did not respond to nitrogen supply, suggesting that water limits growth to a greater extent than nitrogen, as observed in other species (Graciano et al. 2006; Vilela et al. 2003). This lack of biomass response to N addition is related to the traits that allow these species to cope with water stress. Low photosynthetic rates, low relative growth rates, and low stomatal conductance are some traits that allow them to continue growing with very low soil water content, as we have seen in these species when soil water content decreased by 80% from FC they survived and continued growing. These same traits prevent them from investing newly acquired nutrients on growth, i.e., they have lower nutrient tissue requirements and present lower plasticity (Sala et al. 2012; Yahdjian et al. 2014).

In many desert species, a higher concentration of nitrogen has been observed under water stress conditions due to an accumulation of nitrate ions, which may contribute to osmotic adjustment at low water potentials. Compatible solutes including proline, amino acids, and betaines also tend to increase markedly in water-stressed tissues of some species (Rhodes and Hanson 1993; Erskine et al. 1996). Under water stress conditions, we could observe higher nitrogen content in *A. lampa* and *L. cuneifolia*

seedlings when fertilized. This could be related to the fact that both species perform osmotic adjustment to cope with water stress (Fernández et al. 2016; Passera and Allegretti 1996). Similar responses have been seen in *Prosopis* species from the Monte region (Vilela et al. 2003). Trione and Passera (1993) could not observe these different nitrogen concentrations between seedlings with different water regimes in *A. lampa* seedlings; they only observed lower foliar nitrogen content at the beginning of the experiment.

A high efficiency of nitrogen use (NUE) could be an adaptation to low soil fertility (Lajtha 1987; Sala et al. 2012). In this study, efficiency was defined as the quantity of dry matter produced per g nutrient, which is the inverse of tissue concentration, and could be affected by several processes with different potentials to contribute to future productivity (Chapin 1980). In fact, we observed a lower NUE in the seedlings of the three species that were fertilized, which could be related to luxury consumption, rather than a lower efficiency. So, a more useful measure of efficiency might be respiration, photosynthetic, or net assimilation rate per g nutrient (Chapin 1980; Sala et al. 2012). *Atriplex lampa* seedlings presented the highest NUE. Since this is a C4 species, it has low N requirements, and high N use efficiency due to the lower use of photosynthetic enzymes of carboxylation with respect to C3 species (Brown 1978; Carrera et al. 2009).

Higher nitrogen was related to negative impacts on root growth in *L. cuneifolia*, and lack of a response of the S/R ratio to drought in *A. lampa* and *C. atamisquea*; similar negative effects of combined N addition and drought on root development have previously been described for other woody species like *Fagus sylvatica* (Dziedek et al. 2016), *Calluna vulgaris* (Meyer-Grünefeldt et al. 2015), and grasses (Friedrich et al. 2012). It has been proposed that this outcome is stronger in N limited plants since it is more likely that they will respond with increased shoot biomass to N addition which will determine greater imbalance in shoot-to-root ratio and greater sensitivity to drought. Within this study, this mechanism would only apply partially to *A. lampa*, which responded to greater N with shoot and root biomass increase with high water availability. In the case of *C. atamisquea*, seedlings showed higher shoot and root biomass with high nitrogen, regardless of water level, meaning that an increase in shoot biomass due to higher N availability is an unlikely cause of the lack of response

of the shoot/root ratio to drought. Together these results support findings of other studies which alert that alterations in the N cycle through pollution, livestock raising, and the introduction of exotic species among others can impact plant species' response to drought (Gessler et al. 2017; Pivovarov et al. 2016) and the competitive balance among species (Cabal et al. 2017; Zong et al. 2016).

In addition, understanding seedlings' response to nitrogen and water availabilities in drylands is critically important to develop adequate revegetation techniques of degraded areas. Generally, nurseries from these regions do not apply fertilizers since it is well known that native species are adapted to low soil fertility ecosystems. Nonetheless, in some desert species fertilization treatments increase survival and growth after field transplantation, as in many cases nitrogen reserves contribute to future productivity (Villar-Salvador 2003; Villar-Salvador et al. 2010, 2012). Therefore, to apply appropriate restoration techniques on these species in the future, it would be necessary to take this study into account and evaluate the effect of the fertilization treatments on the survival and growth after transplantation.

In conclusion, the individual responses to the combination of N and water availability are variable among desert species. Some desert shrubs (*A. lampa* and *C. atamisquea*) were able to take advantage of increased nitrogen availability by producing more biomass, while others (*L. cuneifolia* and *S. subulatus*) did not produce more biomass but absorbed more N. On the other hand, negative effects in the partition of assimilates in some of them highlight the importance of understanding the interaction between both resources in each desert shrubs species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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