






RESEARCH PAPER

Involvement of soluble proteins in growth and metabolic adjustments of drought-stressed *Calligonum mongolicum* seedlings under nitrogen addition

Z. Zhang^{1,2,3,4} , A. Tariq^{1,2,3} , F. Zeng^{1,2,3} , X. Chai^{1,2,3,4}  & C. Graciano⁵ 

1 Xinjiang Key Laboratory of Desert Plant Root Ecology and Vegetation Restoration, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China

2 State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi, China

3 Cele National Station of Observation and Research for Desert-Grassland Ecosystems, Cele, China

4 University of Chinese Academy of Sciences, Beijing, China

5 Instituto de Fisiología Vegetal, Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata, Buenos Aires, Argentina

Keywords

Elemental stoichiometry; global change; N addition; network analysis; resource allocation.

Correspondence

A. Tariq and F. Zeng, Xinjiang Key laboratory of Desert Plant Roots Ecology and Vegetation Restoration, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China.

E-mail: akash.malik786@mailsucas.ac.cn; zengfj@ms.xjb.ac.cn

Editor

R. Leegood

Received: 7 August 2020; Accepted: 24 September 2020

doi:10.1111/plb.13190

- The planting of seedlings is the most effective measure for vegetation restoration. However, this practice is challenging in desert ecosystems where water and nutrients are scarce. *Calligonum mongolicum* is a sand-fixing pioneer shrub species, and its adaptive strategy for nitrogen (N) deposition and drought is poorly understood.
- Thus, in a pot experiment, we studied the impacts of four N levels (0, 3, 6, 9 gN·m⁻²·year⁻¹) under drought or a well-watered regime on multiple eco-physiological responses of 1-year-old *C. mongolicum* seedlings.
- Compared to well-watered conditions, drought considerably influenced seedling growth by impairing photosynthesis, osmolyte accumulation and activity of superoxide dismutase and enzymes related to N metabolism. Nitrogen addition improved the productivity of drought-stressed seedlings, as revealed by increased water use efficiency, enhanced superoxide dismutase and nitrite reductase activity and elevated N and phosphorus (P) levels in seedlings. Nevertheless, the addition of moderate to high levels of N (6–9 gN·m⁻²·year⁻¹) impaired net photosynthesis, osmolyte accumulation and nitrate reductase activity. N addition and water regimes did not markedly change the N:P ratios of aboveground parts; while more biomass and nutrients were allocated to fine roots to assimilate the insufficient resources. Soluble protein in assimilating shoots might play a vital role in adaptation to the desert environment.
- The response of *C. mongolicum* seedlings to N addition and drought involved an interdependency between soluble protein and morphological, physiological and biochemical processes. These findings provide an important reference for vegetation restoration in arid lands under global change.

INTRODUCTION

Atmospheric nitrogen (N) derived from anthropogenic activities (e.g. fossil fuel consumption and chemical fertilizer application) has been increasing in recent decades, including in northwestern China (Liu *et al.* 2013; Yue *et al.* 2019). Moreover, drought events have become more frequent (IPCC 2013). In this regard, desert plant species might be more responsive to climate change than species in other ecosystems, because they may already be at their physiological limits (Vale & Brito 2015). To date, these two drivers have profoundly altered the outcome of plant–soil interactions, leading to negative cascading effects, such as vegetation loss and land degradation, in many desert ecosystems throughout the world (D’Odorico *et al.* 2013; Yu & Wang 2018; Zhao *et al.* 2020).

In N-limiting ecosystems, high soil N availability after the addition of N could improve net primary production and alter stoichiometric homeostasis, which might shift such ecosystems towards phosphorus (P) limitation (Crowley *et al.* 2012). Thus,

relative changes in N and P may result in a series of modifications to plant performance, as has been widely observed in terms of biomass allocation among organs, photosynthesis, osmotic adjustment and morphological plasticity (Poorter *et al.* 2012; Sun *et al.* 2018; Tariq *et al.* 2019; Yang *et al.* 2014). Nevertheless, water deficit limits the mobility of nutrient elements in dehydrated soils, uptake by roots and vertical migration to leaves, generating asymmetric divergences in nutrient concentrations and allocation among different tissues (Gonzalez-dugo *et al.* 2012; Kou *et al.* 2018; Yuan & Chen 2009). In response to changes in soil nutrient availability, plants maximize their growth by optimally allocating resources. Desert plants allocate more N to generate proteins, which can maintain photosynthetic uptake (Fisher *et al.* 1988), store photosynthates and serve as chemical defence for tissues (Yang *et al.* 2014). As the main component of RNA, P is mainly involved in the synthesis of proteins. Therefore, theoretically, the extent of N constraint in plants would be exacerbated by drought, which would negatively impact plant growth, especially in arid

lands. However, how the impact of N deposition interacts with the effects of drought on xerophyte performance in desert ecosystems remains mostly unknown.

Different plant organs often exhibit different responses to changing environments. For example, roots, especially small non-woody absorptive types in the fine root system, are more sensitive to variable soil nutrient availability (Huang *et al.* 2015), while foliage may respond weakly or be insensitive to this (Schachtman & Goodger 2008). Stems provide support and hydraulic pathways. Given the diverse roles and responses of different plant tissues, there are differences in the requirements for N and P among organs. Plants can coordinate above-ground and belowground tissues concerning the absorption and allocation of resources to adapt to adverse environments (Liu *et al.* 2010). For example, plants in desert ecosystems seem to allocate more N to foliage to exploit light resources (Fisher *et al.* 1988) and improve water use efficiency (WUE) at the cost of reduced N use efficiency (Wright *et al.* 2003); that is, plant traits are not independent of each other. Hence, exploring the interdependent response patterns of morphological features, resource allocation and physiological functions will facilitate a better understanding of the adaptive strategies used by desert plants under global change.

Calligonum mongolicum, one of the most widely used plantation shrubs for afforestation and dune stabilization, is mainly distributed in the desert–oasis ecotone in the drylands of northwestern China (Yu & Wang 2018). Its assimilating shoots (branchlets with reduced leaves) serve as nutritious forage for sheep and camels (Xu *et al.* 2017a). Previous studies have focused on its root ecology (Xu *et al.* 2017b) and physiological responses to environmental disturbance (*e.g.* sand burial and wind erosion) (Fan *et al.* 2018; Wang *et al.* 2019). However, available data on the responses of *C. mongolicum* seedlings to N deposition and drought are scarce. The planting of seedlings has been widely implemented for vegetation restoration in arid regions, given that natural revegetation is challenging (Bhat-tachan *et al.* 2014; Dye & Versfeld 2007). Therefore, an evaluation of the effects of N deposition and drought on the performance of *C. mongolicum* seedlings is urgently needed to develop conservation strategies to mitigate the negative impacts of future climate change. Thus, we studied the effects of N addition and drought on the growth of *C. mongolicum* seedlings, as well as their adaptive strategy, *i.e.* the interdependent response patterns of morphological features, resource allocation and physiological functions.

MATERIAL AND METHODS

Study region and experimental design

This study was carried out in the Cele National Station of Observation and Research for Desert–Grassland Ecosystems (37°00′56″ N, 80°43′81″ E), Chinese Academy of Sciences, located at the southern edge of the Taklamakan Desert in Xinjiang, China. This area has an arid, warm temperate desert climate with an average annual temperature of 11.9 °C, mean annual precipitation of only 35 mm and average yearly potential evaporation of 2600 mm (Liu *et al.*, 2016).

A pot experiment was conducted in an outdoor nursery from May to August 2019. Seeds of *C. mongolicum* were randomly collected from an afforestation site in the desert–oasis

ecotone (36°17′–39°30′ N, 80°03′–82°10′ E). In May 2019, at least five seeds were sown per pot (90-l volume, 40 cm bottom diameter, 50 cm top diameter, 62 cm height) and then the soil was watered to field capacity (–18%). Homogenized topsoil (70 kg) from this area (0–30 cm depth; aeolian loamy sand with the following physicochemical traits: 0.23 g·kg⁻¹ total N; 2.99 g·kg⁻¹ organic C; 23.11 g·kg⁻¹ total K; 0.60 g·kg⁻¹ total P) was used in this research. After 4 weeks of growth with routine management, healthy and uniform seedlings (10.2 ± 3.4 cm height, 0.73 ± 0.13 mm ground basal diameter, 2–3 assimilating shoots) were selected down to one plant per pot.

Because we only focused on seedling growth rather than survival, the plants were watered three times a week. We controlled the amount of water delivered to individual pots, as well as the time of watering (19:00 h). For the first 4 weeks, all pots received 1.5 l of water. For the following period, the well-watered group continued to receive 1.5 l of water, whereas drought pots received 0.5 l of water three times a week. Each pot was placed on a plastic plate to eliminate nutrient transfer to other pots. A transparent plastic film was placed over the pots when it rained. Due to the rapid development of agriculture and industry, there is prominent N deposition (3.5 gN·m⁻²·year⁻¹) in northwestern China (Yue *et al.* 2019), and this rate is expected to double by 2050 (Galloway *et al.* 2008). Extreme N deposition events have occurred in the North China Plain, where the rate is 9.0 gN·m⁻²·year⁻¹ (Liu *et al.*, 2013). Therefore, in this study, we conducted four N treatments (N0, 0 gN·m⁻²·year⁻¹; N1, 3.0 gN·m⁻²·year⁻¹; N2, 6.0 gN·m⁻²·year⁻¹; N3, 9.0 gN·m⁻²·year⁻¹), with six replications of each treatment under both water regimes. From June to August 2019, N addition (solid urea) was added to the upper soil surface after watering.

Sample collection

Plant samples were collected in late August 2019 (mid-late growth period). Three to five *C. mongolicum* seedlings of similar growth status were chosen, and four to six mature assimilating shoots were held together in a plane to determine gas exchange and chlorophyll fluorescence. Then the shoots were picked, put in ziplock bags and immediately stored at –20 °C for laboratory analysis.

Gas exchange and chlorophyll fluorescence measurements

A portable open-flow gas exchange system (LI-6400; LI-COR, Lincoln, NE, USA) was used to measure the gas exchange: net photosynthesis rate (P_n), stomatal conductance (g_s), intercellular CO₂ concentration (C_i) and transpiration rate (E). The relative humidity of the air, CO₂ concentration and photon flux density in the leaf chamber was controlled at 30–40%, 400 μmol·mol⁻¹ and 1500 μmol·m⁻²·s⁻¹, respectively. The WUE was calculated with the equation: P_n/E . A handy plant efficiency analyser (Hansatech, Wimborne, UK) was used to detect maximum quantum efficiency of photosystem II (F_v/F_m). A saturation pulse of 1500 μmol·m⁻²·s⁻¹ was applied for 0.8 s after 30 min dark adaptation at air temperature, and the maximum fluorescence yield (F_m) and intrinsic quantum efficiency of photosystem II (PSII) photochemistry (F_v/F_m) were recorded.

Measurement of photosynthetic pigments

Following the method of Lichtenthaler & Wellburn (1983), we used 95% (v/v) ethanol to extract chlorophyll from the samples (0.1–0.3 g fresh leaves) and measured the absorption of the filtrate at 665 nm and 649 nm. The chlorophyll content ($\text{mg}\cdot\text{g}^{-1}\cdot\text{FW}$) was calculated with the following equations

$$\text{Chl } a = 13.98 A_{665} - 6.88 A_{649} \quad (1)$$

$$\text{Chl } b = 24.96 A_{649} - 7.32 A_{665} \quad (2)$$

$$\text{Chl} = \text{Chl } a + \text{Chl } b \quad (3)$$

$$\text{Chl } a/b = \text{Chl } a/\text{Chl } b \quad (4)$$

Measurement of biochemical parameters

The concentrations of soluble protein (SP) in 0.1–0.3 g fresh assimilating shoots was determined following the Bradford (1976) method, using Coomassie brilliant blue G-250, with bovine serum albumin as standard. The concentration of soluble sugars (SS) was measured using the anthracnose method, with glucose as standard (Yemm & Willis 1954). The concentration of free proline (Pro) extracted in aqueous sulphosalicylic acid was measured using ninhydrin (Bates *et al.* 1973). We measured malondialdehyde (MDA) concentrations to estimate lipid peroxidation (Heath & Packer 1965), using the thiobarbituric acid test with some modifications. The activity of superoxide dismutase (SOD) in leaves was measured at 560 nm according to the reduction in percentage nitroblue tetrazolium following the method of Giannopolitis & Ries (1977).

Measurement of assimilating shoot traits and biomass allocation

The fresh weight (FW) of assimilating shoots was measured, then the samples were immersed in distilled water at 4 °C for 4 h in the dark to obtain turgid weight (TW). After that, all samples were placed in an oven at 70 °C for 24 h to obtain dry weight (DW). Finally, the relative water content of assimilating shoots (SRWC) was calculated using the following equation:

$$\text{SRWC} = [(\text{FW} - \text{DW})/(\text{TW} - \text{DW})] \quad (5)$$

After scanning a picture (300 dpi) with a scanner (LiDE200; Canon, Tokyo, Japan), the length and diameter of assimilating shoots was determined using ImageJ software (National Institutes of Health, USA; version 1.52a). Specific assimilating shoot length (length of an assimilating shoot divided by its dry mass) was calculated.

Biomass allocation among organs was calculated for the biomass of a single organ divided by the total plant biomass. Total plant biomass was determined as the sum of the dry weight of aboveground and belowground biomass, and R/S (root/shoot) ratios were calculated as the ratio of biomass of aboveground and belowground parts. We separated roots into three categories based on root diameter: tap root/axial root (developed

from the radicle), coarse root (lateral root >2 mm in diameter) and fine root (lateral root <2 mm in diameter).

Chemical analysis and nutrient allocation

Powdered organ samples (0.2 g DW) were digested with concentrated H_2SO_4 . Then N and P concentrations in the digests were determined using a Kjeldahl Nitrogen Analyzer (K1160; Jinan Hanon Instruments, Jinan, Shandong, China) and an inductively coupled plasma-optical emission spectrometer (iCAP 6300; ThermoFisher Scientific, Waltham, MA, USA), respectively. N (or P) mass in specific organs was calculated according to Equation. (6); N (or P) distribution among organs was calculated as the value of a single organ divided by the total N and P mass of the whole plant.

$$\begin{aligned} \text{N(or P) mass in specific organ} \\ = \text{N(or P) concentration} \times \text{specific organ biomass} \end{aligned} \quad (6)$$

Measurement of soil properties

After removing plant aboveground parts, three layers of potted soil samples (upper, 0–20 cm; middle, 20–40 cm; bottom, 40–60 cm) were collected and repeated three times, then divided into three subsamples. The first subsample was dried at 105 °C to determine soil water content. After air drying, the second subsample was sifted through a 0.15-mm sieve before determining its chemical properties. Soil organic carbon was measured using the $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ oxidation method. Total N concentration was determined using a Kjeldahl Nitrogen Analyzer. Available N was measured using the alkali hydrolyzable method. Total P and K were determined *via* inductively coupled plasma-optical emission spectrometry after digestion of the samples in concentrated HNO_3 . Available N was extracted with a KCl solution and determined on a continuous-flow autoanalyzer (AutoAnalyzer 3, Seal Analytical, Hampshire, UK). Available P was extracted with $\text{HCl}/\text{NH}_4\text{F}$ and determined colorimetrically *via* ascorbic acid molybdate analysis on a continuous-flow autoanalyzer. Extraction with NH_4OAc was used to measure available K. Soil pH was measured at a soil:water ratio of 1:2.5 (w/v) using a pH meter (PHSJ-6L; INESA Scientific Instrument, Shanghai, China). Electrical conductivity (EC) was measured at a soil:water ratio of 1:5 (w/v) using an EC meter (DDSJ-319L; INESA Scientific Instrument, Shanghai, China). The third subsample was used to determine mineral soil N (NO_3^- -N and NH_4^+ -N) spectrophotometrically following Bremner & Mulvaney (1982).

Statistical analyses

All statistical analyses were conducted using R (R Core Team 2013). The normality of raw data was checked using the Shapiro-Wilk normality test. Analysis of variance (ANOVA) and least significant difference (LSD) test or Tukey's test were performed to test the significance of the effect of N and water addition on plant ecophysiological parameters and soil properties. Pearson's correlation coefficients between plant-soil and plant trait-trait factors were conducted and visualized using the 'corplot' and 'ggcorrplot' R packages, respectively (Kassambara

2019; Taiyun & Viliam 2017). The P -values of the correlation matrix were adjusted using the Benjamini and Hochberg false discovery rate (FDR) control procedure (Benjamini *et al.* 2006). We used the plant trait network (PTN) to explore the relationships among a series of plant traits and their significance (He *et al.* 2020). First, we calculated the matrix of trait–trait relationships and quantified the correlation coefficient (r) using Spearman's method with the 'psych' R package (Revelle 2018). Second, to remove spurious correlations among traits, we controlled the threshold of adjacency matrix as $|r| > 0.5$ and $P < 0.05$ (Kleyer *et al.* 2019). Finally, we visualized the PTN and its topological parameters using the 'igraph' R package (Csardi & Nepusz 2006).

RESULTS

Gas exchange, chlorophyll and fluorescence parameters

Nitrogen addition and water regime altered the photosynthetic physiology of *C. mongolicum* (Figs 1 and 2; Figure S1). We found that N addition, water addition and their interaction significantly influenced gas exchange of *C. mongolicum* seedlings (Fig. 1). Drought significantly reduced the P_n of seedlings. Compared to N0 plants, N1 ($3.0 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) markedly improved P_n in both water regimes; N2 ($6.0 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) and N3 ($9.0 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$) caused a decline in P_n of drought-stressed seedlings but did not influence P_n in the well-watered regime. For drought-stressed seedlings, N addition significantly reduced g_s and E but increased WUE compare to the control treatment.

Nitrogen addition, water regime and their interaction significantly influenced Chl a , b and $a + b$ content (Figure S1). It

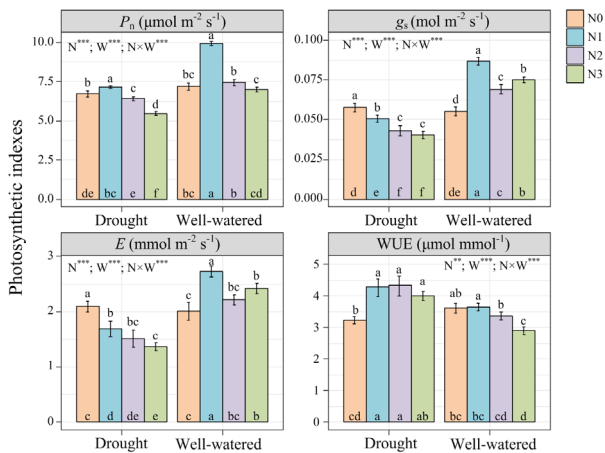


Fig. 1. Effects of nitrogen addition (N) and water (W) treatments on gas exchange parameters of *Calligonum mongolicum* seedlings. N0, control; N1, $3 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$; N2, $6 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$; N3, $9 \text{ g}\cdot\text{N}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$. P_n , net photosynthesis rate; g_s , stomatal conductance; E , transpiration rate; WUE, water use efficiency. Bars show mean \pm SE ($n = 5$). Different letters above bars indicate significant differences ($P < 0.05$) among the four N treatments in each of the two water regimes according to the LSD test. Letters below bars indicate significant differences ($P < 0.05$) among all treatments. A summary of the P -values of a two-way ANOVA addressing the effects of N and W treatments is provided in each panel. * $P < 0.05$, ** 0.01 , *** 0.001 indicate significant F -values.

also significantly reduced Chl a and $a + b$ of drought-stressed plants, and caused a decreasing trend in well-watered regimes. The Chl a/b was only influenced by N addition. Both N input and water regime had independent effects on F_v/F_m (Fig. 2). The F_v/F_m was unaffected by drought or N addition in drought. Only the N1 and N2 treatments significantly elevated the F_v/F_m of well-watered seedlings.

Osmolytes, antioxidant enzyme activity and N assimilation-related enzymes

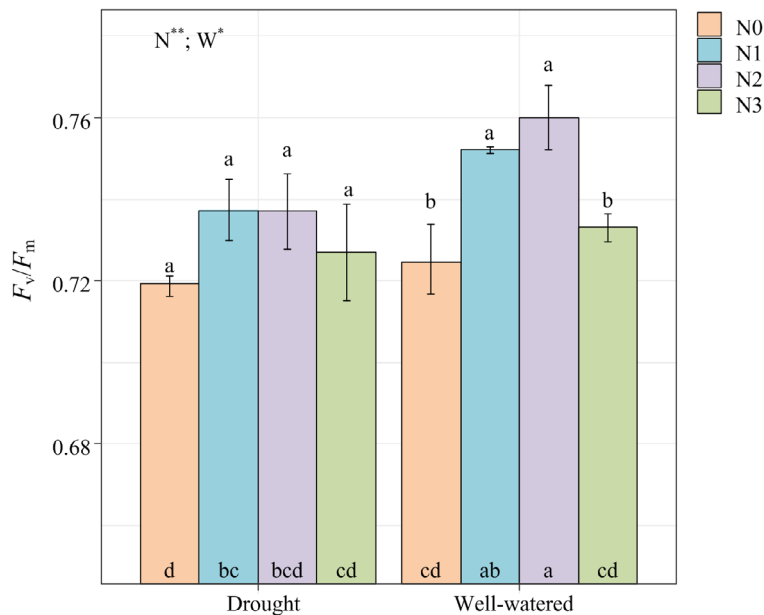
The biochemical indices of *C. mongolicum* were changed by N addition and water treatments (Fig. 3). Both N2 and N3 significantly decreased soluble protein content under both water regimes. Soluble sugar levels were impacted by both N addition and N–water interactions. Drought decreased soluble sugar content compared to well-watered seedlings, which had the highest content, while additional N inputs decreased soluble sugar levels further (except for N1 under drought conditions). N addition, water regime and their interaction jointly influenced free Pro and SOD levels. Under water deficit, N1 and N2 treatment significantly improved Pro content, while a high N load caused a decline in Pro under well-watered conditions. Drought-stressed seedlings had lower SOD activity, while N inputs significantly improved activity in both water regimes, except for the N3 treatment under well-watered conditions. N addition and water condition independently influenced MDA content. N inputs significantly improved MDA levels, regardless of water regime. Nitrite reductase (NiR) and nitrate reductase (NR) activities were significantly influenced by N addition, water regime and their interaction. The N0 plants under drought conditions had higher NiR levels, which increased further with N addition. Only N1 significantly increased NR levels in both water regimes.

Growth and biomass

The morphological traits of *C. mongolicum* also varied by N addition and water treatment (Table 1). The assimilating shoot water content improved only after N addition ($F = 14.02$, $P < 0.001$; Table 1), whereas specific assimilating shoot length was significantly affected by both N addition ($F = 164.59$, $P < 0.001$) and N–water interactions ($F = 25.04$, $P < 0.001$), and the N2 and N3 treatments considerably decreased the specific assimilating shoot length. The only significant change in diameter of assimilating shoots was found in treatments with N addition ($F = 10.99$, $P < 0.001$), which significantly improved for seedlings under water deficit; under well-watered conditions, only N3 improved the diameter of assimilating shoots. N and water interacted to influence the height of seedlings ($F = 8.24$, $P = 0.002$); N-induced increases in seedling height were found in both water treatments. Variation in ground basal diameter was mediated by both N ($F = 85.09$, $P < 0.001$) and water ($F = 114.45$, $P < 0.001$), both of which caused a remarkable increase in ground basal diameter.

The biomass allocation patterns of the seedlings changed according to N addition under watered conditions, and most of the dry mass was allocated to belowground parts (Fig. 4). Under both water regimes, N addition resulted in increases in total biomass, while a lower R/S was found in the N3 treatment (Fig. 4). However, N addition had a non-significant effect on

Fig. 2. Effects of nitrogen deposition (N) and water (W) treatments on potential quantum efficiency of photosystem II (F_v/F_m) in *Calligonum mongolicum* seedlings. N0, control; N1, 3 g-N·m⁻²·year⁻¹; N2, 6 g-N·m⁻²·year⁻¹; N3, 9 g-N·m⁻²·year⁻¹. Bars are mean ± SE (n = 3). Different letters above bars indicate significant differences ($P < 0.05$) among the four N treatments in each of the two water regimes according to the LSD test. Letters below bars indicate significant differences ($P < 0.05$) among all treatments. A summary of the P -values of a two-way ANOVA addressing effects of N and water treatments is given in the figure. * $P < 0.05$, **0.01, ***0.001 indicate significant F -values.



tap root mass fraction and fine root mass fraction. Both N1 and N2 significantly decreased allocation to assimilating shoots of seedlings. Stem mass fraction showed an increasing trend along with N addition gradients, while coarse root mass fraction showed the opposite trend. For well-watered seedlings, the highest assimilating shoots of seedlings were found at the highest N input, N3. N1 significantly improved stem mass fraction, while N2 and N3 resulted in decreases in stem mass fraction compared to N0 plants. Higher tap root mass fractions were found in the N1 and N2 treatments. Additional N caused a decline in coarse root mass fraction. The N2 and N3 treatments did not significantly influence fine root mass fraction, while the lowest fine root mass fraction was found in the N1 treatment.

Nutrient allocation and stoichiometry

The N and P distribution strategy and concentrations among different organs of *C. mongolicum* seedlings changed according to N addition and water treatment (Fig. 5; Table S1). N addition significantly increased total N and P mass of plants in both water regimes, and these values were generally higher in well-watered treatments than under drought conditions.

The distribution of N to assimilating shoots of drought-stressed seedlings was significantly reduced in the N1 and N2 treatments but enhanced in N3. For well-watered seedlings, only N3 increased the N mass in assimilating shoots. Both N2 and N3 significantly improved stem N mass in drought-stressed seedlings. The stem N mass of well-watered seedlings was increased by the N1 treatment but reduced by N3 compared to N0 seedlings. N inputs had non-significant effects on the N mass of drought-stressed tap roots. Compared to well-watered N0 seedlings, N mass in tap roots was higher in plots with N1 and N2 addition but lower in N3 seedlings. N interacted with water resulting in a decreasing trend in N distribution to coarse roots in both water regimes. High N input (N3) resulted in a decrease in N in fine roots under drought stress.

The pattern of P distribution to assimilating shoots was similar to that of N. A higher P allocation to stems was found in

treatments with N addition under water deficit. However, N3 treatment reduced P allocation to stems of well-watered seedlings. There was no significant variation in P distribution to tap roots among treatments under drought. Along with N addition gradients, P distribution to coarse roots first increased and then decreased under drought, while there was a decreasing trend in P distribution to coarse roots of well-watered seedlings. N3 significantly decreased P allocation to fine roots of drought-stressed seedlings.

Nitrogen and water treatments had different effects on N:P ratios among different organs (Fig. 6). There was no significant variation in the ratio for assimilating shoots and stems in any treatment. N and N–water interactions significantly affected the ratio in tap roots. N, water and their interaction influenced N:P ratios of coarse and fine roots.

Plant trait networks

Because many traits tend to be interrelated, traditional correlation analysis cannot explain the precise effect of each trait; hence, we used a plant trait network (PTN) to clarify the relationships among traits and their significance in the present study (Fig. 7; Tables S4 and S5). In the PTN, soluble proteins showed highest degree of response, *i.e.* most edges that linked this focus node trait to other nodes, which can be thought of as general hub trait (Table S5). In contrast, stem N:P (S_N :P) was only connected to stem P (S_P). Coarse root mass fraction, soluble protein, S_P , tap root P (TR_P) and NR activity showed higher betweenness, represented by the shortest paths traversing a hub node; thus, these traits can be viewed as mediators of interactions.

The fast greedy cluster modularity optimization algorithm decomposed the PTN into four different modules. Generally, the N and P content in stem and fine root were grouped with biochemical traits (*e.g.* MDA, soluble sugar, soluble protein, NiR, Chl). Photosynthetic parameters (*e.g.* P_n , E , WUE, F_v/F_m) were clustered with morphological traits, total biomass, CRMF and higher tap root mass fractions. Fine root mass fraction and

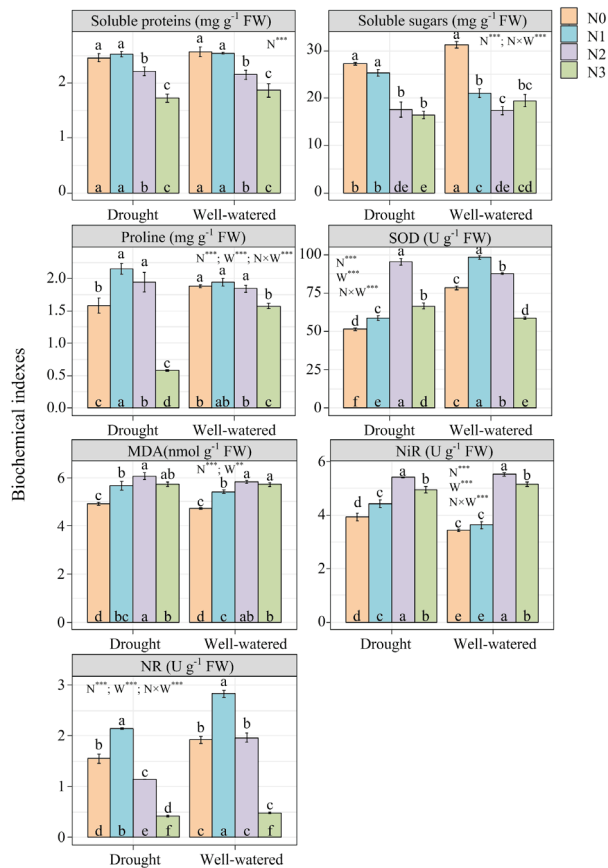


Fig. 3. Effects of nitrogen deposition (N) and water treatments (W) on osmolyte, antioxidant enzyme activity and N assimilating-related enzymes in *Calligonum mongolicum* seedling leaves. N0, control; N1, 3 g-N·m⁻²·year⁻¹; N2, 6 g-N·m⁻²·year⁻¹; N3, 9 g-N·m⁻²·year⁻¹. Bars show mean ± SE (n = 3). Different letters above bars indicate significant differences ($P < 0.05$) among the four N treatments in each of the two water regimes according to the LSD test. Letters below bars indicate significant differences ($P < 0.05$) among all treatments. A summary of the P -values of a two-way ANOVA addressing the effects of N and water treatments is given on the right-hand side. * $P < 0.05$, **0.01, ***0.001 indicate significant F -values.

Table 1. Effects of nitrogen deposition (N) and water (W) treatments on morphological traits of *Calligonum mongolicum* seedlings.

Responses		N	W	N × W	Drought				Well-watered				
					N0	N1	N2	N3	N0	N1	N2	N3	
SRWC	F	14.02	1.15	1.27	Mean	73.24b	85.83a	87.11a	83.22a	77.18b	82.90a	88.27a	87.31a
%	P	<0.001	0.30	0.32	SE	2.10	0.45	0.96	0.47	0.49	0.48	0.15	0.35
SASL	F	164.59	3.33	25.04	Mean	312.5a	284.4a	229.3b	26.4c	357.3a	336.9a	41.2b	34.6b
g·cm ⁻¹	P	<0.001	0.71	<0.001	SE	4.1	6.2	11.5	1.1	12.8	12.1	2.6	2.6
SD	F	10.99	0.78	2.83	Mean	1.19b	1.28a	1.28a	1.29a	1.20b	1.22b	1.23b	1.34a
mm	P	<0.001	0.39	0.07	SE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Height	F	68.34	48.31	8.24	Mean	55.40d	80.03b	88.87a	71.97c	62.90b	92.23a	91.30a	97.20a
cm	P	<0.001	<0.001	0.002	SE	1.00	0.67	0.50	1.10	1.40	1.33	2.00	0.98
GBD	F	85.09	114.45	0.36	Mean	4.43c	6.52b	6.57b	8.26a	6.25c	8.59b	8.13b	10.14a
mm	P	<0.001	<0.001	0.78	SE	0.10	0.11	0.09	0.18	0.16	0.10	0.05	0.14

The results of two-way ANOVA are in left columns of the table. Values in the right columns of the table show mean ± SE (n = 3) of each variable at each N addition rate (N0, N1, N2, N3) in each water treatment level (drought and well-watered). Lowercase letters in the right columns indicate significant differences following N addition ($P < 0.05$, one-way ANOVA). N0, control; N1, 3 g-N·m⁻²·year⁻¹; N2, 6 g-N·m⁻²·year⁻¹; N3, 9 g-N·m⁻²·year⁻¹; SRWC, assimilating shoot relative water content, %; SASL, specific assimilating shoot length; SD, assimilating shoot diameter, mm; GBD, ground basal diameter.

stem mass fraction grouped with R:S and coarse root nutrients. The nutrients in assimilating shoot and tap roots were grouped.

DISCUSSION

Our results displayed a series of desert plant functional strategies matched to the response to varied N and water regimes in this hyper-arid region. Divergent responses by some functional traits were associated with aboveground and belowground resource uptake, maintenance and processing, implying coordination among aboveground and belowground biomass and nutrients in *C. mongolicum*.

Effects of N and water availability on ecophysiological traits

Our results suggest that an interaction between drought and N had a strong influence on gas exchange variables. Drought-stressed N0 *C. mongolicum* seedlings showed reduced P_n (Fig. 1), combined with lower levels of soluble sugars, free Pro, SOD and NR (Fig. 3), indicating an impaired physiological process mediated by drought. The addition of low levels of N can relieve the adverse effects caused by drought stress, reflected in the improved P_n in the N1 treatment. N inputs significantly reduced g_s and E , which increased the WUE of drought-stressed seedlings. However, moderate and high N loads reduced P_n . These results indicate that photosynthetic activity in *C. mongolicum* seedlings is sensitive to N deposition, which raises the issue as to whether further N deposition beyond a critical N threshold could lead to adverse impacts on the seedlings.

Ward *et al.* (2015) proposed that g_s and E can be used as early indicators of physiological changes after N input. In contrast to well-watered seedlings, drought-stressed plants given more N showed a significant reduction in g_s and E (Fig. 1). Interestingly, N addition considerably enhanced WUE in these plants but suppressed it under well-watered conditions (Fig. 1). An elevated WUE is usually associated with improved C allocation to shoots rather than belowground parts, which might increase plant sensitivity to drought stress (Graciano *et al.* 2005). However, in our study, WUE was negatively

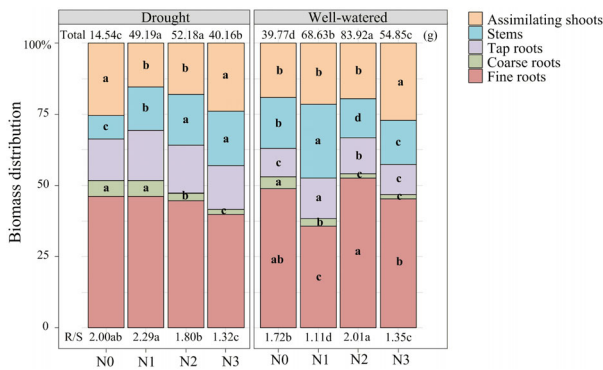


Fig. 4. Effects of nitrogen deposition (N) and water treatments (W) on biomass allocation of *Calligonum mongolicum* seedlings among organs. N0, control; N1, 3 g-N·m⁻²·year⁻¹; N2, 6 g-N·m⁻²·year⁻¹; N3, 9 g-N·m⁻²·year⁻¹. Bars show mean ± SE (n = 3). Numbers followed by letters above bars are total biomass (g-plant⁻¹) for each treatment. Numbers followed by letters below bars are root to shoot biomass (R/S). Different letters indicate significant differences ($P < 0.05$) among the four N addition treatments in each of the two water regimes according to the LSD test, and only those with significant differences are shown. The other information on biomass and a summary of the P -values of a two-way ANOVA addressing effects of N and water treatments are provided in the Figure S1.

correlated with the assimilating shoot mass fraction, and not the stem mass fraction, of drought-stressed seedlings (Figure S3). Chlorophyll fluorescence can be used as a probe to monitor photosynthetic activity and predict plant stress tolerance (Maxwell & Johnson 2000). Our results showed that additional N did not alter the photochemical response (F_v/F_m) of drought-stressed seedlings (Fig. 2), indicating that F_v/F_m may not be a sensitive indicator for drought-stressed *C. mongolicum* seedlings. Inconsistent with the findings of Shen *et al.* (2020) and Wang & Chen (2019) that additional N improves chlorophyll content, we found that N inputs significantly decreased chlorophyll *a* and *a + b* in the assimilating shoots of *C. mongolicum* seedlings (Figure S1). This may be because the influences of N addition on chlorophyll are species-specific (Zhang *et al.* 2017), and the differences in structure and functions between foliage and assimilating shoots might explain these disparate phenomena.

Nitrogen, as a necessary component of proteins, can be directly absorbed by *C. mongolicum* seedlings and readily assimilated into proteins for plant growth. However, the addition of medium and high levels of N reduced soluble proteins, implying that excess N load impairs the metabolic functions of assimilating shoots (Guy 1990). In the current study, increases in SOD caused by N addition may have helped scavenge excess reactive oxygen species and thus improve adaptability to environmental stress (Subrahmanyam *et al.* 2006). However, external N input had a negative effect on plant plasma membranes, leading to increases in cell membrane lipid peroxidation, reflected by elevated MDA levels. Soluble sugars and Pro are essential osmotic adjustment substances that protect plants against oxidative stress (Foyer & Noctor 2005; Morgan 1992). In the present study, drought-stressed N0 seedlings had higher levels of soluble sugars and Pro. The addition of low and medium levels of N further improved Pro content. Regardless of water regime, N addition reduced soluble sugar content in assimilating shoots, indicating that more photosynthates were

used for growth instead of for stress tolerance. We also found that N application changed the activities of N metabolic enzymes in assimilating shoots (Fig. 3). N addition increased the NiR activity of water-stressed seedlings, indicating the promotion of ammonium output. Regardless of water condition, the activity of NR was improved by the addition of low levels of N, but reduced by medium and high N loads, implying that excess N load may impair nitrate reduction.

Responses of biomass allocation to N addition and water availability

It is difficult to understand the physiological mechanism underlying changes in biomass allocation, because it involves a series of C transport processes that are not easily measured, especially at a whole-plant level. However, the overall outcome of these processes can easily be obtained from the current biomass present in all organs (Poorter *et al.* 2012). Divergences in biomass allocation patterns are of essential importance for plants to handle environmental changes, and such patterns are altered by availability of nutrients and water (Poorter *et al.* 2012).

Generally, *C. mongolicum* seedlings mainly allocated biomass to roots, especially fine roots, indicating a limiting resource in belowground parts. Drought led to a considerable reduction in total biomass, while R:S ratios did not significantly differ between drought-stressed seedlings and well-watered plants (Fig. 4; Figure S2). We speculate that the negative influence of drought on the aboveground parts was comparable to that on the belowground parts in the desert environment, indicating that *C. mongolicum* at the seedling stage showed morphological adaptations to drought stress. The interaction between drought and N addition had a negative effect, by decreasing the R:S ratio under high N loads (Fig. 4; Figure S2) because elevated drought sensitivity is mainly associated with a decrease in R:S ratio, which would lead to higher transpiration demand (Meyer-Gruenefeldt *et al.* 2015). This finding may be because fine root biomass was improved by N addition. The allocation of a higher proportion of root biomass to fine roots (Fig. 4), which showed the most nutrient absorption and water acquisition activity, helped *C. mongolicum* seedlings to better adapt to a water deficit environment. However, some N addition treatments, such as N2 and N3 under drought conditions and N1 in the well-watered regime, reduced the fraction of fine root mass, indicating that both nutrient and water limitation were released by N enrichment. However, compared to N0 seedlings, lower ratios of coarse (tap root plus coarse root in our study) to fine roots induced by N2 and N3 in both water regimes could suggest that lower investment in long-lived roots in charge of water and nutrient transport would increase susceptibility to root cavitation compared to fine roots (Chen & Brassard 2013), which was the opposite in the N1 treatment. Under drought conditions, *C. mongolicum* seedlings allocated more dry mass to assimilating shoots in aboveground parts to enhance assimilation capacity. Due to foliage degeneration of assimilating shoots with photosynthetic function as an adaptive strategy in the harsh environment, we used specific assimilating shoot length (Table 1) to estimate dry mass per shoot length. In line with N addition gradients, specific assimilating shoot length showed a decreasing trend under all water conditions (Table 1), implying that N addition caused a lower relative

growth rate and resource use efficiency (Freschet *et al.* 2015; Poorter *et al.* 2009).

The intrinsic physiological mechanisms regulating photoassimilate distribution and final biomass allocation are key to plant development and performance (Poorter *et al.* 2012). During these processes, NR may influence the expression of a variety of proteins (Hermans *et al.* 2006; Scheible *et al.* 1997; Stitt & Feil 1999). In the present study, a significantly positive correlation between P_n -soluble proteins and P_n -NR seem to support this idea (Figure S3). However, which genes or compounds control assimilate partitioning and biomass allocation requires further exploration.

Effects of N addition and water availability on nutrient allocation and stoichiometry

Patterns of N and P distribution among organs of plants may significantly influence ecosystem processes, such as litter decomposition and nutrient cycling (Sardans *et al.* 2017). In this study, we investigated the concentrations and allocation strategies of N and P among different organs (Fig. 5, Table S1). *C. mongolicum* seedlings showed divergent responses to N enrichment and water addition. When plants allocate more nutrients to short-lived organs, rather than to woody organs, it can lead to a larger loss of nutrients (Tsujii *et al.* 2020). In our study, we found that more N and P were allocated to roots under drought stress, especially to fine roots (Fig. 5). However, the addition of N led to significant suppression of N and P to roots, enhancing the levels of those nutrients in aboveground organs (Fig. 5), implying a nutrient shift from roots to aboveground parts. This could be explained as a larger nutrient requirement in the aboveground parts in order to acquire light resources under N-sufficient conditions (Poorter & Nagel 2000), and resorbed nutrients from drought-stressed roots possibly was stored in stems for future use (Zhao *et al.* 2020).

Among all treatments, the N concentrations of assimilating shoots were highest among organs (Table S1), implying a greater requirement for N (Cunningham *et al.* 1998). However, N accumulation in these organs did not vary by N addition gradient; for example, it first decreased and then increased with N enrichment. The specific N deposition rates may have counteracted the natural N accumulation of assimilating shoots. However, the adverse effects of long-term increases in P_n and specific assimilating shoot length, coupled with lower N accumulation in assimilating shoots, could potentially lead to vital ecological feedbacks on primary production, herbivory and litter decomposition processes (Barker *et al.* 2006). As the resource-acquiring organ belowground, fine root N concentrations under drought were lower in the N2 and N3 treatments than in the N0 and N1 treatments. There are some possible explanations for this. First, *C. mongolicum* seedlings may have limited capacity to improve N uptake when N is suddenly abundant due to adaptive limitations to infertile soils (Barker *et al.* 2006; Chapin 1980). Second, the artificial monthly application of N may weaken the effect of N deposition compared to natural N deposition, which theoretically occurs throughout the whole year. Although the latter cannot completely be ruled out, the former is most likely the case because there was no significant effect of N inputs on the accumulation of fine root N in the presence of additional water.

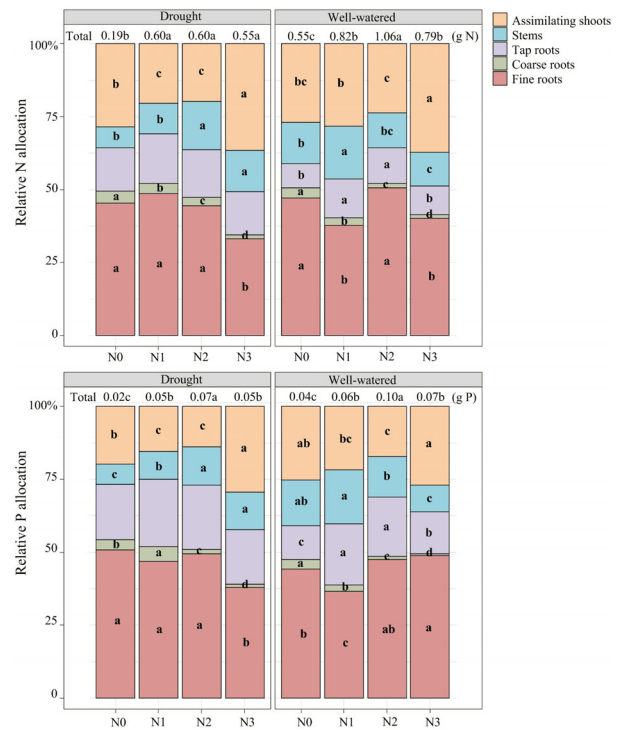


Fig. 5. Effects of nitrogen deposition (N) and water treatments (W) on nitrogen (N) and phosphorus (P) relative content in *Calligonum mongolicum* seedlings. N0, control; N1, 3 g·N·m⁻²·year⁻¹; N2, 6 g·N·m⁻²·year⁻¹; N3, 9 g·N·m⁻²·year⁻¹. Numbers followed by letters above bars are total N and P content (mg·plant⁻¹) for each treatment. Different letters indicate significant differences ($P < 0.05$) among the four treatments in each of the two water regimes according to the LSD test, and only those with significant differences are shown. Other information on biomass and a summary of P -values of a two-way ANOVA addressing the effects of N and water treatments are provided in the Table S2.

Foliar stoichiometry of N:P ratios is a vital tool for gauging nutrient availability and limitation (Carmen Blanes *et al.* 2013; Schreeg *et al.* 2014). In our study, the ratios for all root types were more sensitive to N addition than those for aboveground parts (Fig. 6). Roots (especially absorptive roots, *i.e.* fine roots) respond rapidly to altered soil nutrient availability, whereas foliage (assimilating shoots in this study), being the terminus of nutrient and water transport, responds weakly (Schachtman & Goodger 2008). Therefore, nutrients in roots are more sensitive to changing nutrients in the soil (Huang *et al.* 2015; Kou *et al.* 2018). Another possible explanation is that plants maintain stoichiometric homeostasis to stabilize physiological activities in aboveground parts. Hence, the stoichiometric ratios of aboveground parts (*i.e.* assimilating shoots and stems) might not be dependable markers of N deposition effects in desert shrub seedlings. Drought significantly reduced N:P ratios of roots, indicating limited N assimilation by roots under drought stress. Inconsistent ratios of roots were found under different soil moisture conditions (Fig. 6). Well-watered conditions improved N availability in the soil, and thus may have stimulated the growth of seedlings, leading to a more substantial requirement for N, reflected by decreases in the N:P ratios of fine roots and tap roots.

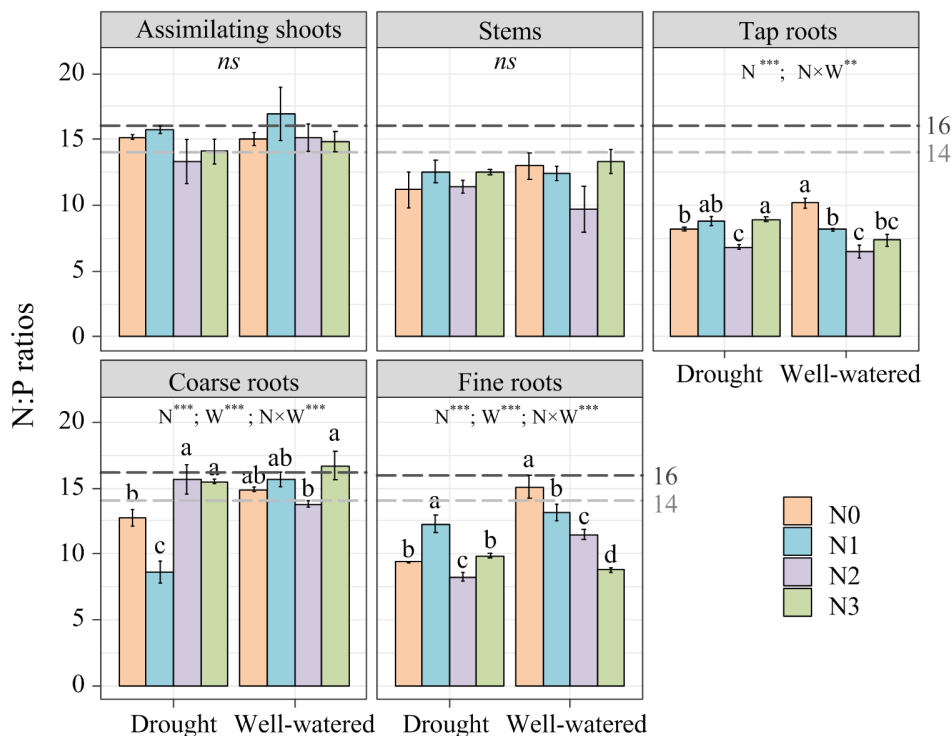


Fig. 6. The effects of nitrogen deposition (N) and water (W) treatments on N:P ratios among different organs of *Calligonum mongolicum* seedlings. N0, control; N1, 3 g-N·m⁻²·year⁻¹; N2, 6 g-N·m⁻²·year⁻¹; N3, 9 g-N·m⁻²·year⁻¹. Bars show mean ± SE (n = 3). Different letters above bars indicate significant differences ($P < 0.05$) among the four N treatments in each of the two water regimes according to the LSD test, and those with significant differences are shown in the figure. * $P < 0.05$, ** 0.01 , *** 0.001 indicate significant F values; ns denotes no significant difference.

Plant trait network

Recent studies have provided a novel perspective toward visualizing the relationships among different plant traits and their significance using network theory, *i.e.* a PTN (Flores-Moreno *et al.* 2019; He *et al.* 2020; Kleyer *et al.* 2019). We observed many trait–trait correlations using the PTN method, supporting the concept of the plant as a harmonized system of biological functions (Fig. 7). Soluble proteins showed high centrality in the network, indicating that they take a hub regulatory position and suggesting that they play vital roles in critical functions. A shift in this central element will influence many other downstream traits, especially those with high betweenness (*e.g.* coarse root mass fraction), which may, in turn, impact subnetworks (Kleyer *et al.* 2019) or have a greater influence on high-level properties (*e.g.* fitness). Indeed, soluble protein content is a crucial trait in photosynthesis, because half of these proteins function as enzymes (Andrews *et al.* 1999). The close associations among total biomass, coarse root biomass and biochemical and photosynthetic properties formed the centre of the PTN, where these nodes dominated most connections. Surprisingly, the nutrient traits of assimilating shoots had a relatively minor effect on other traits, reflected by fewer links. This may reflect weak or time-lagged nutrient transport from soil to aboveground parts. However, these low connectivities do not invalidate the functional relevance, as traits with few connections can facilitate numerous trait combinations and generate multiple plant phenotypes (Messier *et al.* 2017). This implies that different traits or organs have trade-offs. Plant traits in the PTN also

tended to cluster. Four distinct feature modules with a mass of intra-group links and very few links to other traits in the PTN may imply the formation of independent dimensions. For example, the subnetwork of nutrients in fine roots that grouped with biochemical characteristics may imply a direct relationship among these traits. The P_n and chlorophyll content were divided into different subnetworks. These results emphasize the relative role of related traits in the adaptation of *C. mongolicum* seedlings in the face of changeable environments.

CONCLUSION

The current study suggests that N addition and soil moisture can alter ecophysiological performance, nutrient levels and biomass allocation patterns of desert shrub seedlings. N addition can alleviate the impaired growth of seedlings induced by drought stress through physio-biochemical adjustments. However, moderate to high N loads (6.0–9.0 g-N·m⁻²·year⁻¹) have a negative impact on some ecophysiological processes under drought stress by decreasing the net photosynthesis rate, osmolyte accumulation and NR activity. As a coping strategy, stoichiometric homeostasis in aboveground parts of *C. mongolicum* seedlings is more stable than that in belowground parts under N addition and drought; while more biomass and nutrients are allocated to fine roots to enhance the uptake of insufficient resources. We conjecture that soluble proteins may have a vital influence on the interplay among multiple traits of *C. mongolicum* seedlings. Thus, further study of these proteins may provide vital clues on underlying plant growth strategies through future research. Finally, *C. mongolicum*

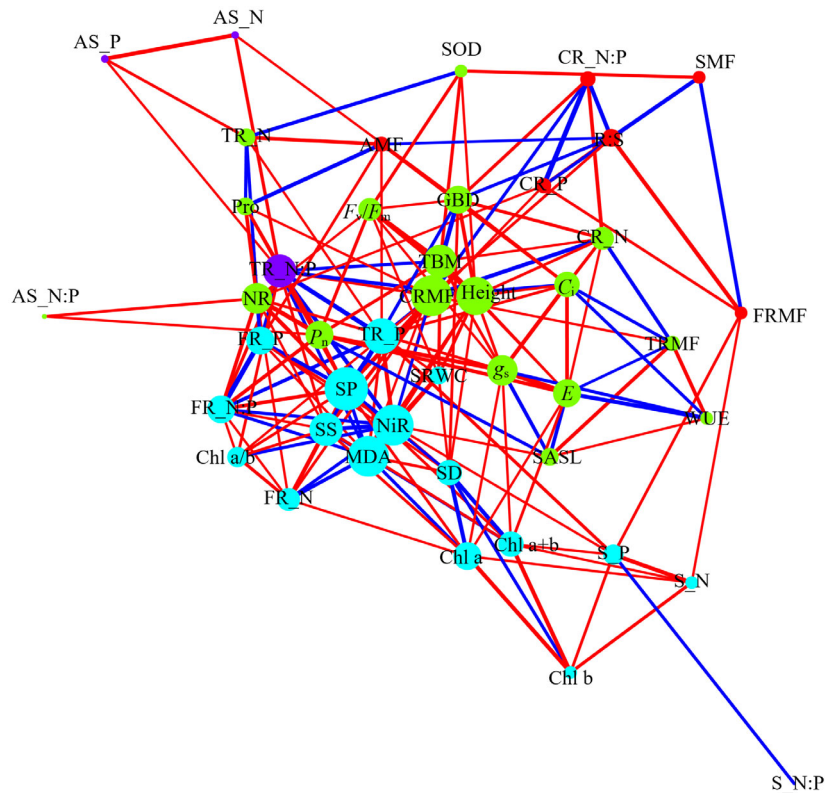


Fig. 7. Plant Trait Network (PTN) for *Calligonum mongolicum* seedlings. Nodes represent different plant traits. PTN is divided into four modules in different colours and node size shows the degree. Red and blue lines represent positive and negative correlations, respectively. The width of the line indicates the strength of the association. AS, assimilating shoots; S, stems; CR, coarse roots; FR, fine roots; TR, tap roots; T, total plant. Biomass allocation: BM, biomass; MF, mass fraction; nutrients: _N, nitrogen; _P, phosphorus. Photosynthetic parameters: P_n , net photosynthesis rate; g_s , stomatal conductance; C_i , intercellular CO_2 concentration; E , transpiration rate; WUE, water use efficiency; F_v/F_m , potential quantum efficiency of photosystem II. Biochemical indices: Pro, free proline; SP, soluble proteins; SRWC, assimilating shoot relative water content; SS, soluble sugars; NR, nitrate reductase; NiR, nitrite reductase. Morphological traits: SASL, specific assimilating shoot length; SD, assimilating shoot diameter. The topological properties of the network and node (plant trait) characteristics are shown in Tables S4 and S5.

seedlings coordinate multiple eco-physiological processes to respond to N deposition and drought, which will provide a reference for vegetation recovery in arid lands.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 41977050), the Key Program of Joint Funds of the National Natural Science Foundation of China and the Government of Xinjiang Uygur Autonomous Region of China (Nos. U1603233, U1903102) and the President's International Fellowship Initiative – Postdoctoral Researchers, Chinese Academy of Sciences (2020PB0002). We would like to thank Guo Pinglin, Yue Zewei, Jing Ruixue, Yu Yang, Xu Mengqi and the concerned staff of the Cele National station for field and lab assistance.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

REFERENCES

- Andrews M., Sprent J.I., Raven J.A., Eady P.E. (1999) Relationships between shoot to root ratio, growth and leaf soluble protein concentration of *Pisum sativum*, *Phaseolus vulgaris* and *Triticum aestivum* under different nutrient deficiencies. *Plant, Cell & Environment*, **22**, 949–958.
- Barker D., Vanier C., Naumburg E., Charlet T., Nielsen K., Newingham B., Smith S. (2006) Enhanced monsoon precipitation and nitrogen deposition affect leaf traits and photosynthesis differently in spring and summer in the desert shrub *Larrea tridentata*. *New Phytologist*, **169**, 799–808.
- Bates L., Waldren R., Teare I. (1973) Rapid determination of free proline for water-stress studies. *Plant and Soil*, **39**, 205–207.
- Benjamini Y., Krieger A.M., Yekutieli D. (2006) Adaptive linear step-up procedures that control the false discovery rate. *Biometrika*, **93**, 491–507.
- Bhattachan A., D'Odorico P., Dintwe K., Okin G.S., Collins S.L. (2014) Resilience and recovery potential of duneland vegetation in the southern Kalahari. *Ecosphere*, **5**, <https://doi.org/10.1890/es13-00268.1>.
- Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of

Table S1. The effects of nitrogen deposition (N) and water (W) treatments on N and phosphorus (P) concentrations among different organs of *Calligonum mongolicum* seedlings.

Table S2. The effects of nitrogen deposition (N) and water (W) treatments on soil properties among different depth.

Table S3. Results of a two-way ANOVAs for soil properties of different depth (D) as dependent in nitrogen addition (N), water addition (W), and their interaction ($N \times W$, $N \times D$, $W \times D$, $N \times W \times D$).

Table S4. Topological properties of plant traits network of *Calligonum mongolicum* seedlings.

Table S5. Node (plant traits) properties of plant traits network of *Calligonum mongolicum* seedlings.

Figure S1. Effects of nitrogen deposition (N) and water (W) treatments on chlorophyll pigment parameters in *Calligonum mongolicum* seedlings.

Figure S2. Effects of nitrogen deposition (N) and water treatments (W) on biomass among different organs, total biomass and R/S of *Calligonum mongolicum* seedlings.

Figure S3. Correlation pattern of soil-plant traits under drought condition.

- protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, **72**, 248–254.
- Bremner J., Mulvaney C. (1982) Nitrogen-total. In: Page A.L. (Ed), *Part 2. Chemical and microbiological properties, method of soil analysis*. American Society of Agronomy, Madison, WI, USA.
- Carmen Blanes M., Vinegla B., Merino J., Carreira J.A. (2013) Nutritional status of *Abies pinsapo* forests along a nitrogen deposition gradient: do C/N/P stoichiometric shifts modify photosynthetic nutrient use efficiency? *Oecologia*, **171**, 797–808.
- Chapin F.S. III (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics*, **11**, 233–260.
- Chen H.Y.H., Brassard B.W. (2013) Intrinsic and Extrinsic Controls of Fine Root Life Span. *Critical Reviews in Plant Sciences*, **32**, 151–161.
- Crowley K.F., McNeil B.E., Lovett G.M., Canham C.D., Driscoll C.T., Rustad L.E., Denny E., Hallett R.A., Arthur M.A., Boggs J.L., Goodale C.L., Kahl J.S., McNulty S.G., Ollinger S.V., Pardo L.H., Schaberg P.G., Stoddard J.L., Weand M.P., Weathers K.C. (2012) Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the Northeastern United States? *Ecosystems*, **15**, 940–957.
- Csardi G., Nepusz T. (2006) *The igraph software package for complex network research. R package version 0.8.4*, 1695. R Foundation for Statistical Computing, Vienna, Austria.
- Cunningham S., Summerhayes B., Westoby M. (1998) Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecology*, **69**, 569–588.
- D’Odorico P., Bhattachan A., Davis K.F., Ravi S., Runyan C.W. (2013) Global desertification: drivers and feedbacks. *Advances in Water Resources*, **51**, 326–344.
- Dye P., Versfeld D. (2007) Managing the hydrological impacts of South African plantation forests: an overview. *Forest Ecology and Management*, **251**, 121–128.
- Fan B., Zhao C., Zhang X., Sun K. (2018) Impacts of sand burial and wind erosion on regeneration and growth of a desert clonal shrub. *Frontiers in Plant Science*, **9**, 1696.
- Fisher F.M., Zak J.C., Cunningham G.L., Whitford W.G. (1988) Water and nitrogen effects on growth and allocation patterns of creosotebush in the northern Chihuahuan desert. *Journal of Range Management*, **41**, 387–391.
- Flores-Moreno H., Fazayeli F., Banerjee A., Datta A., Kattge J., Butler E.E., Atkin O.K., Wythers K., Chen M., Anand M., Bahn M., Byun C., Cornelissen J.H.C., Craine J., Gonzalez-Melo A., Hattingh W.N., Jansen S., Kraft N.J.B., Kramer K., Laughlin D.C., Minden V., Niinemets U., Onipchenko V., Penuelas J., Soudzilovskaia N.A., Dalrymple R.L., Reich P.B. (2019) Robustness of trait connections across environmental gradients and growth forms. *Global Ecology and Biogeography*, **28**, 1806–1826.
- Foyer C.H., Noctor G. (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *The Plant Cell*, **17**, 1866–1875.
- Freschet G.T., Swart E.M., Cornelissen J.H.C. (2015) Integrated plant phenotypic responses to contrasting above- and below-ground resources: key roles of specific leaf area and root mass fraction. *New Phytologist*, **206**, 1247–1260.
- Galloway J.N., Townsend A.R., Erismann J.W., Bekunda M., Cai Z., Freney J.R., Martinelli L.A., Seitzinger S.P., Sutton M.A. (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, **320**, 889–892.
- Giannopolitis C.N., Ries S.K. (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, **59**, 309–314.
- Gonzalez-dugo V., Durand J.L., Gastal F., Bariac T., Poincheval J. (2012) Restricted root-to-shoot translocation and decreased sink size are responsible for limited nitrogen uptake in three grass species under water deficit. *Environmental and Experimental Botany*, **75**, 258–267.
- Graciano C., Guaiamet J.J., Goya J.F. (2005) Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptus grandis* seedlings. *Forest Ecology and Management*, **212**, 40–49.
- Guy C.L. (1990) Cold-acclimation and freezing stress tolerance – role of protein-metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology*, **41**, 187–223.
- He N., Li Y., Liu C., Xu L., Li M., Zhang J., He J., Tang Z., Han X., Ye Q., Xiao C., Yu Q., Liu S., Sun W., Niu S., Li S., Sack L., Yu G. (2020) Plant trait networks: improved resolution of the dimensionality of adaptation. *Trends in Ecology & Evolution*, **35**, 908–918.
- Heath R.L., Packer L. (1965) Effect of light on lipid peroxidation in chloroplasts. *Biochemical and Biophysical Research Communications*, **19**, 716–720.
- Hermans C., Hammond J.P., White P.J., Verbruggen N. (2006) How do plants respond to nutrient shortage by biomass allocation? *Trends in Plant Science*, **11**, 610–617.
- Huang W., Zhou G., Liu J., Zhang D., Liu S., Chu G., Fang X. (2015) Mineral elements of subtropical tree seedlings in response to elevated carbon dioxide and nitrogen addition. *PLoS One*, **10**, e0120190.
- IPCC (2013) Climate change: the physical science basis. In: Stocker T.F., Qin D., Plattner G.-K., Tignor M., Allen S.K., Boschung J., Nauels A., Xia Y., Bex V., Midgley P.M. (Eds), *Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Kassambara A. (2019) *Package ‘ggcorrplot’*. R package version 0.1.3. R Foundation for Statistical Computing, Vienna, Austria.
- Kleyer M., Trinogga J., Cebrian-Piqueras M.A., Trenkamp A., Flojgaard C., Ejrnaes R., Bouma T.J., Minden V., Maier M., Mantilla-Contreras J., Albach D.C., Blasius B. (2019) Trait correlation network analysis identifies biomass allocation traits and stem specific length as hub traits in herbaceous perennial plants. *Journal of Ecology*, **107**, 829–842.
- Kou L., Chen W., Jiang L., Dai X., Fu X., Wang H., Li S. (2018) Simulated nitrogen deposition affects stoichiometry of multiple elements in resource-acquiring plant organs in a seasonally dry subtropical forest. *Science of the Total Environment*, **624**, 611–620.
- Lichtenthaler H.K., Wellburn A.R. (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, **11**, 591–592.
- Liu B., He J., Zeng F., Lei J., Arndt S.K. (2016) Life span and structure of ephemeral root modules of different functional groups from a desert system. *New Phytologist*, **211**, 103–112.
- Liu G., Freschet G.T., Pan X., Cornelissen J.H.C., Li Y., Dong M. (2010) Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. *New Phytologist*, **188**, 543–553.
- Liu X., Zhang Y., Han W., Tang A., Shen J., Cui Z., Vitousek P., Erismann J.W., Goulding K., Christie P., Fangmeier A., Zhang F. (2013) Enhanced nitrogen deposition over China. *Nature*, **494**, 459–462.
- Maxwell K., Johnson G.N. (2000) Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany*, **51**, 659–668.
- Messier J., Lechowicz M.J., McGill B.J., Violle C., Enquist B.J. (2017) Interspecific integration of trait dimensions at local scales: the plant phenotype as an integrated network. *Journal of Ecology*, **105**, 1775–1790.
- Meyer-Gruenefeldt M., Friedrich U., Klotz M., Von Oheimb G., Haerdle W. (2015) Nitrogen deposition and drought events have non-additive effects on plant growth – Evidence from greenhouse experiments. *Plant Biosystems*, **149**, 424–432.
- Morgan J.M. (1992) Osmotic components and properties associated with genotypic differences in osmoregulation in wheat. *Australian Journal of Plant Physiology*, **19**, 67–76.
- Poorter H., Nagel O. (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology*, **27**, 1191.
- Poorter H., Niinemets U., Poorter L., Wright I.J., Villar R. (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, **182**, 565–588.
- Poorter H., Niklas K.J., Reich P.B., Oleksyn J., Poot P., Mommer L. (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytologist*, **193**, 30–50.
- Revelle W. (2018) *Psych: procedures for personality and psychological research*. Northwestern University, Evanston, IL, USA.
- Sardans J., Grau O., Chen H.Y.H., Janssens I.A., Ciais P., Piao S., Penuelas J. (2017) Changes in nutrient concentrations of leaves and roots in response to global change factors. *Global Change Biology*, **23**, 3849–3856.
- Schachtman D.P., Goodger J.Q.D. (2008) Chemical root to shoot signaling under drought. *Trends in Plant Science*, **13**, 281–287.
- Scheible W.R., Lauerer M., Schulze E.D., Caboche M., Stitt M. (1997) Accumulation of nitrate in the shoot acts as a signal to regulate shoot–root allocation in tobacco. *The Plant Journal*, **11**, 671–691.
- Schreeg L., Santiago L., Wright S.J., Turner B.L. (2014) Stem, root, and older leaf N: P ratios are more responsive indicators of soil nutrient availability than new foliage. *Ecology*, **95**, 2062–2068.
- Shen H., Dong S., Li S., Wang W., Xiao J., Yang M., Zhang J., Gao X., Xu Y., Zhi Y., Liu S., Dong Q., Zhou H., Yeomans J.C. (2020) Effects of warming and N deposition on the physiological performances of *Leymus secalinus* in Alpine Meadow of Qinghai-Tibetan Plateau. *Frontiers in Plant Science*, **10**, 1804.
- Stitt M., Feil R. (1999) Lateral root frequency decreases when nitrate accumulates in tobacco transformants with low nitrate reductase activity: consequences for

- the regulation of biomass partitioning between shoots and root. *Plant and Soil*, **215**, 143–153.
- Subrahmanyam D., Subash N., Haris A., Sikka A.K. (2006) Influence of water stress on leaf photosynthetic characteristics in wheat cultivars differing in their susceptibility to drought. *Photosynthetica*, **44**, 125–129.
- Sun J., Yao F., Wu J., Zhang P., Xu W. (2018) Effect of nitrogen levels on photosynthetic parameters, morphological and chemical characters of saplings and trees in a temperate forest. *Journal of Forestry Research*, **29**, 1481–1488.
- Taiyun W., Viliam S. (2017) *R package "corrplot": Visualization of a Correlation Matrix. R package version 0.84*. R Foundation for Statistical Computing, Vienna, Austria.
- Tariq A., Pan K., Olatunji O.A., Graciano C., Li N., Li Z., Song D., Sun F., Justine M.F., Huang D., Gong S., Pandey B., Idrees M., Dakhil M.A. (2019) Role of nitrogen supplementation in alleviating drought-associated growth and metabolic impairments in *Phoebe zhenan* seedlings. *Journal of Plant Nutrition and Soil Science*, **182**, 586–596.
- R Core Team (2013) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Tsujii Y., Aiba S.-I., Kitayama K. (2020) Phosphorus allocation to and resorption from leaves regulate the residence time of phosphorus in above-ground forest biomass on Mount Kinabalu, Borneo. *Functional Ecology*, **34**, 1702–1712.
- Vale C.G., Brito J.C. (2015) Desert-adapted species are vulnerable to climate change: insights from the warmest region on Earth. *Global Ecology and Conservation*, **4**, 369–379.
- Wang G., Yu K., Gou Q. (2019) Effects of sand burial disturbance on establishment of three desert shrub species in the margin of oasis in northwestern China. *Ecological Research*, **34**, 127–135.
- Wang N., Chen H. (2019) Increased nitrogen deposition increased the competitive effects of the invasive plant *Aegilops tauschii* on wheat. *Acta Physiologiae Plantarum*, **41**, 176.
- Ward E.J., Domec J.-C., Lavinier M.A., Fox T.R., Sun G., McNulty S., King J., Noormets A. (2015) Fertilization intensifies drought stress: water use and stomatal conductance of *Pinus taeda* in a midrotation fertilization and throughfall reduction experiment. *Forest Ecology and Management*, **355**, 72–82.
- Wright I.J., Reich P.B., Westoby M. (2003) Least-cost input mixtures of water and nitrogen for photosynthesis. *The American Naturalist*, **161**, 98–111.
- Xu D., Wang W., Gao T., Fang X., Gao X., Li J., Bu H., Mu J. (2017a) Calcium alleviates decreases in photosynthesis under salt stress by enhancing antioxidant metabolism and adjusting solute accumulation in *Calligonum mongolicum*. *Conservation Physiology*, **5**, cox060.
- Xu S., Ji X., Jin B., Zhang J. (2017b) Root distribution of three dominant desert shrubs and their water uptake dynamics. *Journal of Plant Ecology*, **10**, 780–790.
- Yang X., Tang Z., Ji C., Liu H., Ma W., Mohhamot A., Shi Z., Sun W., Wang T., Wang X. (2014) Scaling of nitrogen and phosphorus across plant organs in shrubland biomes across Northern China. *Scientific Reports*, **4**, 1–7.
- Yemm E., Willis A. (1954) The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, **57**, 508.
- Yu K., Wang G. (2018) Long-term impacts of shrub plantations in a desert–oasis ecotone: accumulation of soil nutrients, salinity, and development of herbaceous layer. *Land Degradation & Development*, **29**, 2681–2693.
- Yuan Z., Chen H.Y.H. (2009) Global trends in senesced-leaf nitrogen and phosphorus. *Global Ecology and Biogeography*, **18**, 532–542.
- Yue P., Cui X., Gong Y., Li K., Goulding K., Liu X. (2019) Impact of elevated precipitation, nitrogen deposition and warming on soil respiration in a temperate desert. *Biogeosciences*, **146**, 17–29.
- Zhang R., Wu J., Li Q., Hanninen H., Peng C., Yao H., Song X., Ying Y. (2017) Nitrogen deposition enhances photosynthesis in moso bamboo but increases susceptibility to other stress factors. *Frontiers in Plant Science*, **8**, 1975.
- Zhao Q., Guo J., Shu M., Wang P., Hu S. (2020) Impacts of drought and nitrogen enrichment on leaf nutrient resorption and root nutrient allocation in four Tibetan plant species. *Science of the Total Environment*, **723**, 138106.