Combined effects of nitrogen deposition and water stress on growth and physiological responses of two annual desert plants in northwestern China

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ABSTRACT

Two annual desert plants, *Malcolmia africana* (L.) R.Br. (Brassicaceae) and *Bassia hyssopifolia* (Pall.) Kuntz (Chenopodiaceae) were selected to determine the combined effects of nitrogen deposition and water stress on their growth and physiological responses. Nitrogen addition and water stress significantly affected growth of both species. Root weight, leaf number, average leaf area, total biomass, and the shoot/root ratio increased with N addition. For both species, increasing N levels were correlated with higher concentrations of chlorophyll and soluble proteins, higher net photosynthetic rates, and lower content of soluble sugars and proline. *M. africana* was more sensitive to water stress than *B. hyssopifolia*, but few differences were observed between the species in their response to N addition. The negative effects of water stress on growth and physiological responses were partly compensated by increased N supply. Overall, the results suggest that N deposition could lead to an increase in annual plant growth in the Gurbantunggut Desert in northwestern China.

Contents

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1. Introduction

In recent years, considerable debate has focused on the ecological effects of nitrogen (N) deposition, intensive human activities, and global climate change (Phoenix et al., 2006; Zeglin et al., 2007; Pregitzer et al., 2008). Elevated available N input can affect plant growth, reduce biodiversity, and alter ecosystem functioning (Zavaleta et al., 2003; Compton et al., 2004; Schwinning et al., 2005). N deposition tends to increase biomass allocation to the shoot, and thus leaf mass and leaf area ratios are often higher than those achieved by CO_2 enrichment (Hattenschwiler and Korner, 1998). However, increasing N supply can have different, even opposite, ecological effects on biomass production. For example, high N levels significantly increased woody biomass in hardwoods (Berger and Glatzel, 2001), but reduced woody biomass in pine trees (Magill et al., 2000).

Desert ecosystems tend to have low N availability. The addition of water to experimental plots in the Chihuahuan Desert significantly increased the biomass production of annual plants for the first year, but not in the second year due to limited N availability (Gutierrez and Whitford, 1987). However, N deposition is increasing in some desert ecosystems as a result of urbanization, by-products of agriculture and animal production (Fenn et al., 2003), and to a lesser extent dust deposition (Littmann, 1997). In N-limited ecosystems, relatively small increases in N may cause large changes in plant communities, since the increase in N may benefit some plants more than others (Aber et al., 1989). In the Mojave Desert, for example, Brooks (2003) observed that increases in soil N increased the density and biomass of alien annual plants, but decreased the density, biomass and species richness of native species. These results indicated that higher levels of soil N from atmospheric deposition or other sources could increase the dominance of alien annual plants and possibly promote the invasion of exotic species in desert regions (Brooks, 2003).

In arid and semi-arid ecosystems, primary productivity is limited by water availability and to a lesser extent by N supply (Zhang and Zak, 1998). In the Chihuahuan Desert, one study reported that N input increased shoot growth (Fisher et al., 1988), while others reported that N addition had no effect on primary production due to water limitation (Lajtha and Schlesinger, 1986). Wu et al. (2008) found that both water and N influenced seedling growth of the shrubby legume Sophora davidii. Seedlings of S. davidi exhibited a strong positive response to N addition, but drought stress dramatically decreased seedling height, leaf area, root length, and overall biomass. Similarly, both N and drought stress affected carbohydrate pools and the morphology of Loblolly Pine seedlings (Green et al., 1994).

Water and N are essential requirements for plant growth and survival. The photosynthetic rate, chlorophyll content, and concentrations of sugars, proline, and soluble proteins vary in plants subject to different moisture conditions and nutritional status. Nitrogen input increased the net photosynthetic rate (NPR) and whole plant dry mass of Cryptomeria japonica seedlings but reduced those of Pinus densiflora seedlings (Nakaji et al., 2001). Addition of high levels of N enhanced the maximum photosynthetic rate of Leymus chinensis, whereas the photosynthetic rate of Stipa grandis responded only to low levels of N addition (Chen et al., 2005). Plants grown with additional N and water during the summer displayed higher photosynthetic rates than controls and plants that only received additional N (Barker et al., 2006). Increasing N supply either had no effect or significantly reduced the chlorophyll and soluble protein levels of C. japonica and P. densiflora, respectively (Nakaji et al., 2001). Drought stress can increase organic compounds required for osmotic adjustment, such as soluble sugars and proline (Barathi et al., 2001; Garg et al., 2001). Long-term increases in photosynthetic rate, together with changes in chlorophyll, solu-ble proteins, soluble sugars, and proline have potentially important implications for primary productivity, herbivory and other ecological processes (Throop, 2005).

Few studies have analyzed the combined effects of soil water and N input on growth and physiological responses of annual plants in desert ecosystems. Annual plants play an important role in some desert ecosystems; their occurrence affects both the vegetative productivity of deserts and the stability of sand dunes (Wang et al., 2006). Different responses of annual plants to N deposition and water have the potential to change the biodiversity of plant communities within a short period of time and also increase the dominance of alien, annual plants (Brooks, 2003).

The Gurbantunggut Desert is the largest fixed and semi-fixed desert in China. In spring, most annual plants, including short vegetative period annual plants (ephemerals) and long vegetative period annual plants (annuals), germinate during snow thaw and after rain. Patches of farmland adjoin the desert and in recent years, the total nitrogenous fertilizer applied to farms has rapidly increased from 7.4×10^7 kg yr^{-1} in 1980 to 4.9×10^8 kg yr^{-1} in 2004 (Ma et al., 2006). This is a potential source of a significant amount of N that could disperse to adjacent desert ecosystems. N emitted from nearby cities has also increased substantially in recent years (Xu et al., 2008), and this may also increase N input into the desert. In spring, melting snow and rain bring pulses of N and water to the desert ecosystem affecting the growth of annual desert plants. Ephemeral and annual plants may respond differently to inputs of N and water. Malcolmia africana and Bassia hyssopifolia are two desert plants native to northwestern China; both species are common in Central Asian desert ecosystems. M. africana is considered to be an ephemeral, since its life cycle is completed within two to three months (short vegetative period). B. hyssopifolia an annual, lives approximately one to two months longer than M. africana (long vegetative period). Both germinate in spring, after which there is a rapid increase in shoot system biomass. M. africana and B. hyssopifolia were chosen to: (1) evaluate the effects of N addition and water stress on their growth and physiological responses; (2) determine whether increased N alleviates the effects of water stress; (3) test the hypothesis that ephemerals (annuals with short vegetative periods) are more sensitive to N supply and water stress than annuals (those with longer vegetative periods).

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of M. africana and B. hyssopifolia were obtained from the Turpan Eremophytes Botanical Garden, Xinjiang Institute of Ecology and Geography, Chinese Academy of Science (CAS), Xinjiang, China. Soil samples were collected from the Gurbantunggut Desert and were thoroughly mixed. Soil characteristics are described in Table 1. Plastic pots (7.5 L) were each filled with 5.5 kg of sandy soil. Ninety pots were prepared for each species and 15 seeds were sowed in each pot on June 5, 2008. The 180 pots were placed under shade cloth which allowed 75% solar radiation to reach the plants. The experiment was conducted at the CAS Fukang Desert Ecology Station, located in the southern part of the Gurbantunggut Desert (44°30′N, 87°45′E, 460 m a.s.l.). From June 5, 2008 to August 12, 2008, the average day/night conditions were 30/23 °C and 24/36% relative humidity.

2.2. Experimental design

The experiment consisted of a completely randomized factorial combination of three levels of N addition (0, 0.18, 0.72 g N m^{-2} wk^{-1}) and three soil watering regimes (60–70%,

Table 1
Chemical characteristics of soil used in this experiment.

	pH	Organic C (g kg^{-1})	Total (g kg^{-1})			Available (mg kg^{-1})		
			N	P	K	N	P	K
Mean	8.36	1.69	0.19	0.40	10.94	36.31	6.31	167.75
Standard deviation	0.16	0.55	0.09	0.04	0.78	9.44	1.53	20.72

30–40% and 10–20% of field capacity). Each treatment combination was replicated 10 times. After sowing, N was added weekly at concentrations of 0, 18, and 72 mM N (represented as standard N [i.e., no added N], moderately increased N and high N) in the form of NH_4NO_3 solutions. This equated to about 27 ml solution per pot at each application. The N added to the soil was equivalent to 0, 0.18, and 0.72 g N m^{-2} wk^{-1} based on soil surface area. The moderately increased N treatment was roughly equivalent to current N deposition rates in the study area (1 g N m^{-2} yr^{-1}) (Zhang et al., 2008) and the high N was roughly equivalent to that of the Mojave Desert (3.23 g N m^{-2} yr^{-1}) (Brooks, 2003). Total deposition was divided into 5 weekly aliquots applied throughout the growing season as an aqueous solution with a backpack sprayer as described by Throop (2005). Before the induction of water deficits, the pots were irrigated with distilled water to maintain soil moisture at field capacity. Once the plants were established, soil water treatments were started 20 and 22 days after sowing for *M. africana* and *B. hyssopifolia*, respectively. One third of the pots were maintained at a soil relative water content (the ratio of soil water to water content at field capacity) of 60–70% (well-watered), one third at 30–40% (water-stressed) and the others at 10–20% (severely water-stressed) (Xu et al., 2007). Each pot was weighed every 24 h to estimate the water lost daily in order to maintain the required relative water content. This amount of water was then added to the pot to restore it to its initial weight (Wu et al., 2008). All pots were placed on bricks and rotated weekly to limit positional effects under the shade cloth. Once the watering treatments commenced, seedlings were thinned to 4 per pot to minimize competition for nutrients. Ultimately, only the well-watered and water-stressed treatments were analyzed, because most of plants in the severely water-stressed treatment did not survive the experiment.

2.3. Growth characteristics and biomass measurement

Shortly before flowering, for each species, five plants were harvested from each treatment. For *M. africana* and *B. hyssopifolia*, this occurred at about 55 and 77 days after sowing, respectively. Following harvesting, the length of the longest root was measured and the number of leaves recorded. The leaves were also scanned (Uniscan B800, Tsinghua Unisplendour Corp., Beijing, China) and leaf areas estimated from the digital images using CI-400 CIAS (CID, Corp., USA) software. For biomass determination, each plant was divided into leaves, stem, and roots and oven-dried at 70 °C to constant weight. Total plant biomass represents the sum of the weight of roots, stem and leaves; the shoot/root ratio (S/R) was calculated as the combined stem and leaf weight divided by the root weight.

2.4. Gas exchange and chlorophyll measurement

Gas exchange was measured prior to harvesting the plants, using a Li-6400 open gas exchange system (Li-Cor, Lincoln, NE, USA), 46 and 66 days after sowing for *M. africana* and *B. hyssopifolia*, respectively. Measurements in fully expanded leaves were taken between 7:30 and 10:30 am to avoid potential stomatal closure during the middle of the day. A 20 mm × 30 mm leaf chamber was used and maintained at 30 °C. All measurements were carried out using a red blue 6400-02B light source under saturat-

ing photosynthetic photon flux density (1600 μmol m^{-2} s^{-1}); the saturating photon flux density was previously determined based on light response curves. Either one leaf of *M. africana* or 2–3 leaves of *B. hyssopifolia* were positioned across the chamber, taking precautions to avoid self-shading. After the measurements, the leaves were scanned and the leaf areas determined. Immediately after the gas exchange measurements, leaves from the same nodes were collected for chlorophyll analyses. Chlorophyll was extracted from samples taken from the centre of fresh leaves, using 95% (v/v) ethanol. Absorption of the filtrated extract was measured at 665 nm, 649 nm and 470 nm, and chlorophyll content calculated according to the Lichtenthaler formula (Lichtenthaler and Wellburn, 1983).

2.5. Determination of soluble protein, soluble sugar and proline content

The concentration of soluble proteins was determined in extracts obtained from fresh leaves using Coomassie brilliant blue G-250 with bovine serum albumin as the standard (Bradford, 1976). Similarly, soluble sugars were estimated by the anthrone method with glucose as the standard (Yemm and Willis, 1954). Free proline was extracted in aqueous sulphosalicylic acid and measured using ninhydrin according to Bates et al. (1973). The concentrations of soluble proteins, soluble sugars, and proline were calculated on a dry weight basis (mg g^{-1}).

2.6. Data analysis

For each species, the effects of soil water content, N supply and their interaction were analyzed using factorial analysis of variance ($P = 0.05$). Differences between treatments were compared using Duncan's multiple range test at 0.05 level. All the statistical analyses were performed using SAS software (Version 8.0, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Growth response

N supply, water regimes and their interaction significantly influenced root weight, leaf number, average leaf area, and biomass of both species (Table 2, $P < 0.01$). In the well-watered treatment, root weight, leaf number, average leaf area, and biomass of the two species increased with N addition (Figs. 1A–F, 2A and B). Under high N treatment, the biomass of *M. africana* and *B. hyssopifolia* was 62% and 79% higher, respectively, than that of the moderately increased N treatment (Fig. 2A and B). The difference in biomass was even greater between high N and standard N. In the water-stressed treatment, N addition increased root weight, average leaf area, and total biomass, but the increase was smaller than that of the well-watered treatment. Biomass of *M. africana* decreased 46%, 40% and 57%, and that of *B. hyssopifolia* 6%, 6% and 34% in standard N, moderately increased N and high N under water-stressed conditions (Fig. 2A and B). N addition increased the S/R ratio, an effect that was more pronounced in the well-watered treatment (Fig. 2C and D).

Table 2
The effects of water regime, N supply and the interaction between water regime and N supply on growth characteristics using factorial analysis of variance.

	Treatment effects	Root weight	Leaf number	ALA	Biomass	S:R
M. africana	F_{water}	485.71**	22.26**	32.60**	2158.61**	9.01*
	F_N	62.19**	7.43**	77.35**	804.31**	20.79**
	$F_{water \times N}$	11.77**	5.29**	14.46**	235.69**	5.36*
B. hyssopifolia	F_{water}	272.14**	19.19**	21.13**	7319.02**	1.36
	F_N	544.34**	54.98**	4.05*	24217.89**	12.81**
	$F_{water \times N}$	21.99**	31.87**	0.27	4463.67**	3.56

Note: ALA = average leaf area, S:R = shoot/root ratio. Treatment effects are: F_{water} = comparing well-watered and water-stressed; F_N = comparing standard N (i.e., no added N), moderately increased N, and high N supply levels; $F_{water \times N}$ = comparisons of two watering regimes and three N levels.
* $P < 0.05$.
** $P < 0.01$.

3.2. Physiological responses

3.2.1. Gas exchange and chlorophyll content

Net photosynthetic rate (NPR) and chlorophyll content of *M. africana* were significantly influenced by N supply and water treatment. In contrast, in *B. hyssopifolia*, only N supply had a significant effect on NPR and chlorophyll content (Table 3). Under both well-watered and water-stressed conditions, the NPR of both *M. africana* and *B. hyssopifolia* increased with N addition (Fig. 3A and B). At the same level of N supply, NPR was higher in well-watered than water-stressed plants. N addition also increased leaf chlorophyll content in both species. For *B. hyssopifolia*, the chlorophyll content in high N treatment was 56% and 86% higher in the well-watered and the water-stressed regime, respectively, than in the moderately increased N treatment. In contrast, *M. africana* showed no differences in chlorophyll content between high N and moder-

ately increased N treatments, although addition of N resulted in higher chlorophyll content than standard N treatments, and the chlorophyll content was not significantly influenced by water stress (Fig. 3C and D, $P > 0.05$).

3.2.2. Variations in the concentration of soluble proteins, soluble sugars, and proline

The concentration of soluble proteins increased with N addition, albeit to a smaller extent in *B. hyssopifolia* than *M. africana*, most noticeably under well-watered conditions. Water stress had a somewhat negative effect on the levels of soluble proteins in *M. africana* in high N treatment ($P > 0.05$), but increased the protein content of *B. hyssopifolia* in moderately increased N and high N treatments (Fig. 4A and B). In contrast, the concentration of soluble sugars decreased with N addition, particularly for *M. africana*, in which the content of soluble sugars of high N treatment was close
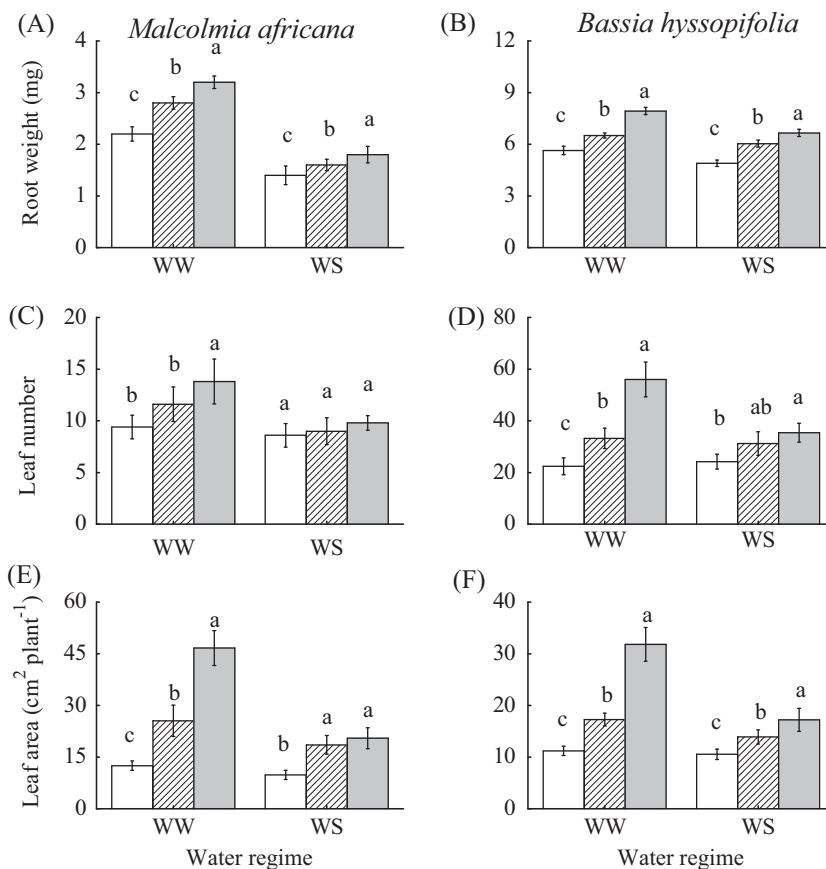

Fig. 1. Root dry weight, leaf number, average leaf area of *M. africana* (A, C, E) and *B. hyssopifolia* (B, D, F) under well-watered (WW) or water-stressed (WS) conditions in combination with either standard, i.e., no added N, (open), moderately increased N (hatched), or high (solid) N-supply levels. Bars represent means of 5 replications ± standard deviations. Within each water regime, values accompanied by different letters differ significantly at $P = 0.05$.
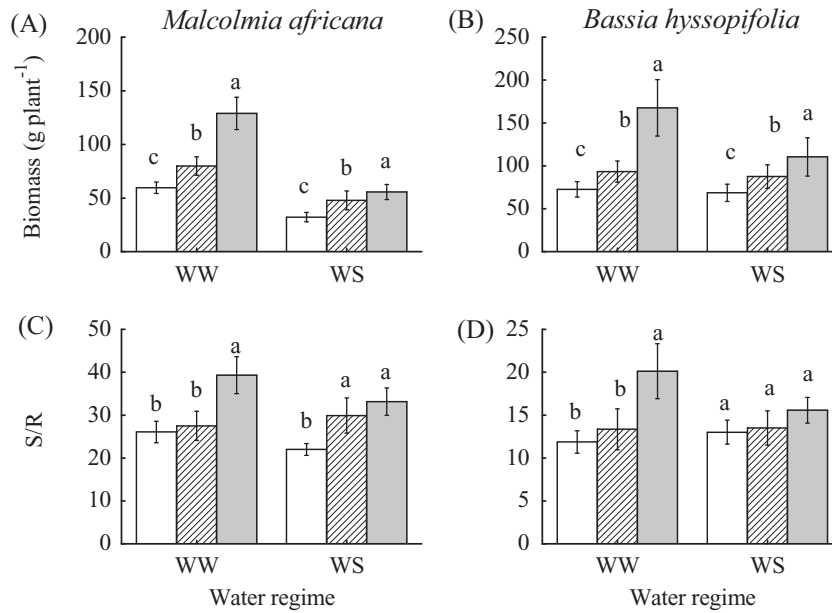
Fig. 2. Biomass and S/R (shoot/root ratio) of *M. africana* (A, C) and *B. hyssopifolia* (B, D) under well-watered (WW) or water-stressed (WS) conditions in combination with either standard, i.e., no added N (open), moderately increased N (hatched), or high (solid) N-supply levels. Bars represent means of 5 replications ± standard deviations. Within each water regime, values accompanied by different letters differ significantly at $P = 0.05$.

Table 3
The effects of water regime, N supply and the interaction between water regime and N supply on physiology using factorial analysis of variance.

	Treatment	NPR	Chlorophyll	Soluble protein	Soluble sugar	Proline
M. africana	F_{water}	148.18**	17.76**	6.85*	1.42	6.34*
	F_N	69.02**	59.05**	51.36*	113.26**	3.89*
	$F_{water \times N}$	1.33	15.24**	0.13	0.53	0.68
B. hyssopifolia	F_{water}	1.97	2.54	84.34**	3.26	43.37**
	F_N	23.38**	201.16**	60.16**	22.47**	19.58**
	$F_{water \times N}$	2.35	4.92**	13.67**	0.22	6.95**

Note: NPR = net photosynthetic rate. Treatment effects are: F_{water} = comparing well-watered and water-stressed; F_N = comparing standard N (i.e., no added N), moderately increased N, and high N supply levels; $F_{water \times N}$ = comparisons of two watering regimes and three N levels.
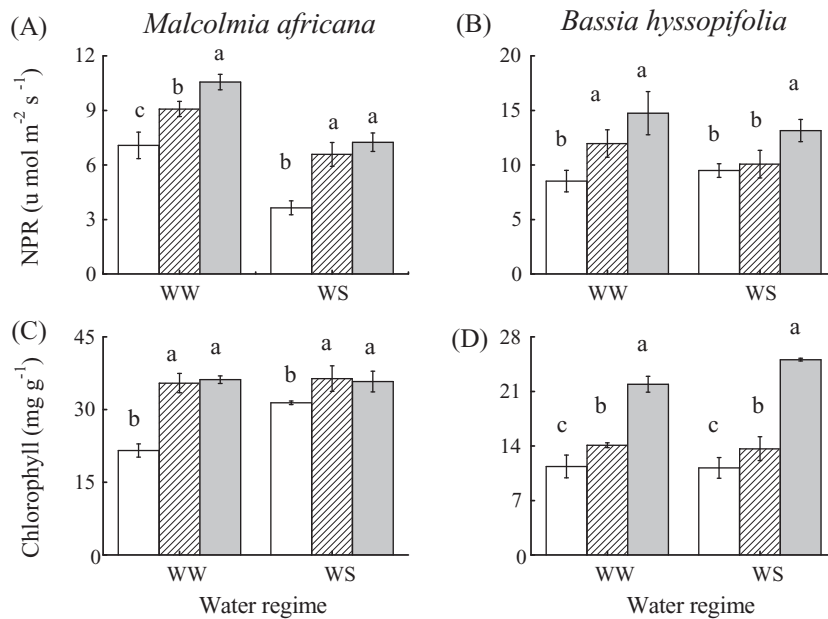
* $P < 0.05$.
** $P < 0.01$.

Fig. 3. NPR (net photosynthetic rate) and chlorophyll content of *M. africana* (A, C) and *B. hyssopifolia* (B, D) under well-watered (WW) or water-stressed (WS) conditions in combination with either standard, i.e., no added N (open), moderately increased N (hatched), or high (solid) N-supply levels. Bars represent means of 5 replications ± standard deviations. Within each water regime, values accompanied by different letters differ significantly at $P = 0.05$.
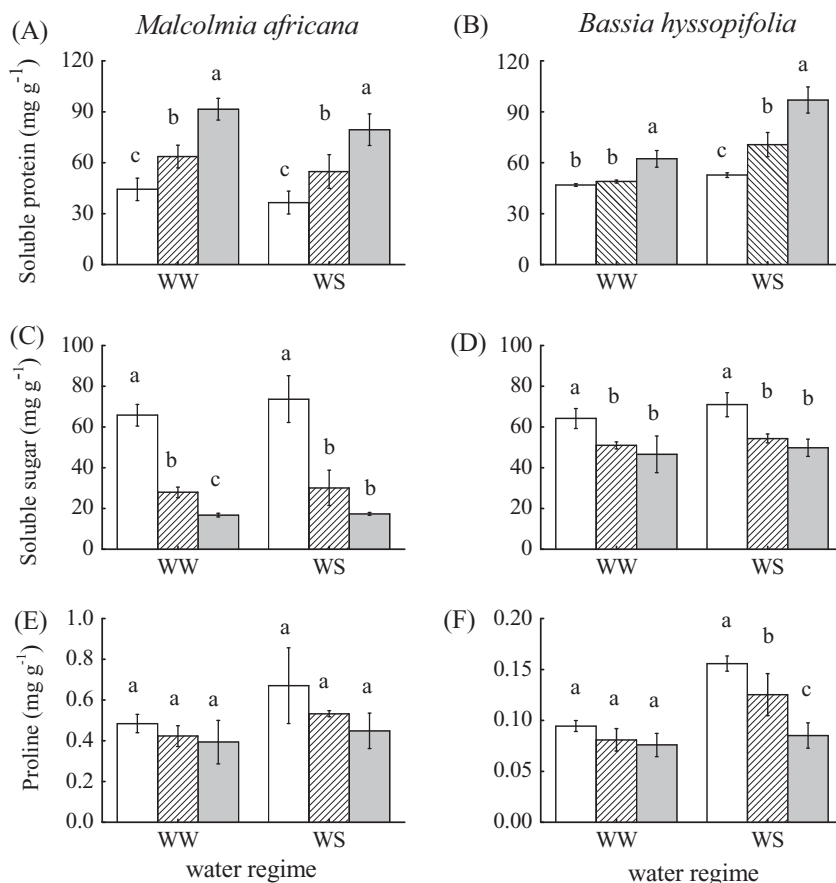
Fig. 4. Soluble protein, soluble sugar and proline content of *M. africana* (A, C, E) and *B. hyssopifolia* (B, D, F) under well-watered (WW) or water-stressed (WS) conditions in combination with either standard, i.e., no added N (open), moderately increased N (hatched), or high (solid) N-supply levels. Bars represent means of 5 replications ± standard deviations. Within each water regime, values accompanied by different letters differ significantly at $P = 0.05$.

to 25% that of the standard N treatment. Water stress did not have a significant effect on the concentration of soluble sugars (Table 3, Fig. 4C and D). Conversely, water stress led to an increase in proline levels, particularly in the standard N treatment. The addition of N tended to reduce the positive effect of water stress on proline content (Fig. 4E and F).

4. Discussion

4.1. Growth responses to N supply and water stress

The strong responses of *M. africana* and *B. hyssopifolia* to N addition support the notion that N deposition can increase annual plant growth in the Gurbantunggut Desert. This is more likely to occur in early spring when melting snow and rain produce high soil moisture content and growth is limited by N availability. Annual plants are prevalent and widely distributed in the Gurbantunggut Desert where increased N deposition has the potential to significantly increase biomass production. Studies in other arid ecosystems have reported similar effects of N addition (Gutierrez and Whitford, 1987; Fisher et al., 1988; Hooper and Johnson, 1999; Wu et al., 2008). Furthermore, some simulations of semi-arid systems have shown that productivity would increase following N input if sufficient water were available (Asner et al., 2001).

As expected, plant growth parameters such as root weight, leaf number, average leaf area, and biomass were negatively affected by water stress. This tendency, however, was partially diminished by N addition. A similar response was observed in *S. davidii* (Wu et al., 2008). These findings suggest that N deposition might alleviate mild water stress. Moreover, the increased N supply may amplify

the positive effects of elevated atmospheric CO_2 on plant biomass (Reich et al., 2006), thus partially compensating for the reduction in biomass caused by water deficits. Under this scenario, N deposition may be important in maintaining productivity not only when water is plentiful but also during periods of mild water deficit.

Drought and nutrient limitations were found to increase carbon translocation from the leaves to the roots, thereby decreasing the S/R ratio (Andrews, 1993; Poorter and Nagel, 1999). Our results were consistent with these findings, as the S/R ratio increased with increasing N supply regardless of the soil moisture condition (Fig. 2C and D). The increase in N alleviated the growth limitations owing to N shortage and more carbon was allocated for aboveground growth. This increase in the S/R ratio may have, however, negative effects later in the season. As water stress develops, the higher S/R ratio would result in a higher demand for water in relation to the root length available for water uptake.

4.2. Physiological responses to N supply and water stress

Physiological responses of plants to N availability have been well documented (Egli and Schmid, 1999; Shangguan et al., 2000; DaMatta et al., 2002). In the present study, N supply significantly increased leaf chlorophyll content, the concentration of soluble proteins, and NPR. The plants showed positive responses to N supply because they were growing in a nutrient-poor habitat and the physiological responses were sensitive to small increase in N. Nitrogen is one of the components of chlorophyll and enzymes; more than half of the enzymes are present in the form of soluble proteins, and many of them are involved in photosynthesis (Evans, 1989; Anderson et al., 1997; Andrews et al., 1999). Therefore, an

increase in soluble proteins caused by N addition is likely to have effects on photosynthetic activity. In our study, the concentration of soluble proteins was significantly correlated with the chlorophyll content and NPR ($P < 0.01$), a result similar to that found in a study of the perennial grass *L. chinensis* by Xu and Zhou (2006).

Soluble sugars and proline play an important role in osmotic adjustment and may protect plants against oxidative stress (Morgan, 1992; Foyer and Noctor, 2005; Molinari et al., 2007). In the present study, the concentration of soluble sugars and proline decreased with N addition, suggesting that N input altered organic carbon allocation with more photosynthetic output destined for growth rather than for the development of stress tolerance. Water stress can increase the soluble sugar and proline contents. For example, water stress increased total soluble sugar and free proline contents in Moth Bean and Mulberry plants (Garg et al., 2001; Barathi et al., 2001). In our study, water-stressed plants showed higher proline content than well-watered plants, particularly under standard N and moderately increased N treatments. In contrast, there were no noticeable differences between the two watering regimes in the concentration of soluble sugars.

Overall, the positive effects of N addition on leaf area and NPR most likely led to biomass increase. On the other hand, N addition had a negative effect on the accumulation of soluble sugars and proline, two metabolic processes, often associated with the development of water stress tolerance. Notwithstanding these effects, N addition promoted photosynthesis and growth in the two water regimes tested. The situation may be different under more severe water stress conditions, when the effect of nitrogen on decreasing both the root/shoot ratio and the concentration of soluble sugars and proline, could lessen the ability of the plant to cope with water deficits. The overall effect of N addition on water stress tolerance remains, however, unclear. Various metabolic processes not considered in this study, such as the accumulation of quaternary ammonium compounds, polyhydric alcohols, and reactive oxygen species-scavenging enzymes, can contribute to water stress tolerance (Bray, 2002). The effect of N addition on these processes remains to be investigated.

4.3. Different responses between the two species

Different species may have different responses to N supply and water stress. Net photosynthetic rate and whole-plant dry mass of *C. japonica* seedlings were increased by N addition, while those of *P. densiflora* seedlings were significantly reduced by this treatment, indicating different N responses between the two species (Nakaji et al., 2001). Addition of N to the soil increased the density and biomass of alien annual plants, but decreased density, biomass and species richness of native species (Brooks, 2003). We found that *M. africana* and *B. hyssopifolia* showed quantitative differences in their response to the water treatments. There was a greater decrease in both biomass and NPR in *M. africana* resulting from water stress than in *B. hyssopifolia* (Fig. 2A and B), possibly because of their different life cycles and difference in the root structure. The short lived ephemeral *M. africana* tends to be more sensitive to water stress than the longer lived *B. hyssopifolia* which appears to be less sensitive to water deficits. Another explanation for dissimilar responses to water stress is that the roots of *B. hyssopifolia* are often longer than those of *M. africana*, thus enabling extraction of moisture from a much greater volume of soil. These characteristics may allow *B. hyssopifolia* to perform better during the summer when water deficits become severe.

Differences in the species response to N addition were not so clear. Nitrogen addition resulted in a greater increase in leaf area in *M. africana* than in *B. hyssopifolia*, while leaf number showed an opposite pattern with a larger increase in *B. hyssopifolia* than *M. africana*. In contrast, most other parameters including biomass, S/R

ratio, NPR, and proline accumulation showed quantitatively similar responses to N addition. The two species showed little difference in sensitivity to N supply. Thus, the results were not in agreement with our initial hypothesis that ephemerals (annuals with short vegetative periods) are more sensitive to N addition than annuals with long vegetative periods.

Acknowledgements

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