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Effect of Aridification on the Replacement of Zonic Species, *Stipa baicalensis* Roshev., by Azonic Species, *Leymus chinensis* Tzvel., in the Steppe of China

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Abstract The perennial grass *Stipa baicalensis* is the zonic species in the steppe of China, but is currently being replaced by the azonic species of *Leymus chinensis*. In this area, aridification is on the increase; therefore, we hypothesize that water competition plays a significant role in this succession. The limit of osmotic adjustment in *S. baicalensis* (13.94%–16.30% PEG) was much lower than that in *L. chinensis* (17.20%–24.50% PEG) in response to drought. The synoptic causal model accounted for most of the variation in the process of physiological regulation as indicated by the small residual effect (0.121–0.161). These results demonstrated that the two species dealt with drought in different ways. Stomata of *L. chinensis* acted more directly on transpiration, and the advantage in water competition resulted from the powerful stomatal control. On the other hand, *S. baicalensis* was more easily affected by non-stomatal limitation which was physiologically inactive in response to drought. Results supported the hypothesis above. *S. baicalensis* was more susceptible to the damage of aridification and was less competitive to water than *L. chinensis*.

Keywords Aridification · Stomata · Feedback regulation · Osmotic adjustment

Stipa baicalensis, as one of the most representative meadow steppe populations, used to be widely distributed in the eastern area of the temperate zone steppe region in central Asia (Wang et al. 2006). However, the serious degradation of *S. baicalensis* is continuing and worsening so that this zonic species has not been found in a continuous large area. Nowadays, it is being replaced by the azonic plant, *Leymus chinensis*, in the steppe of China (Chen et al. 2007). Several studies suggested that the degradation of *S. baicalensis* would result from overgrazing by livestock and intense human activities (Tsuiki et al. 2005). For testing this view, many researches on the primary production (Gao et al. 2000), reproductive strategy (Bai et al. 1999) and grazing management (Tsuiki et al. 2005) of *S. baicalensis* were carrying on. Although these researches arrived at some preliminary conclusions, the immanent cause of species replacement at a large geographical scale was still far from dissolving. Few results helped illuminate the competitive relationships between the two species, so we should offer more insight into the mechanisms of species changes. Researchers gave an overview of the background evolvement in the steppe based on the comparison of plant components in the past century. It revealed that aridification is on the increase in the semi-arid steppe of China in response to global climate change (Qian and Zhu 2001). Based on this finding, we

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have a hypothesis that the water status plays an important role in the replacement of zonic *S. baicalensis* by azonic *L. chinensis*.

Two ways in which plants cope with water deficits are stomatal regulation and osmotic adjustment (Warren et al. 2007). Mechanisms of these methods have been amply described, but it is less quantified and still unknown as to how those underlying physiological processes work (Kramer and Boyer 1995). Understanding these regulation processes in response to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions (Engelbrecht and Kursar 2003). We sought to compare drought resistance by assessing these processes between *S. baicalensis* and *L. chinensis*, and we were also interested in developing a more detailed synoptic causal model that reflects the relationship between the underlying physiological pathways.

Materials and Methods

Experiments were conducted at the Pasture Ecology Research Station of Northeast Normal University, Changling, Jilin Province of China (123°44′E, 44°40′N) in 2005. The seeds used for the experiment were collected from a large wild population near the research station in the autumn of 2004.

The seeds were sown without any physiological or biochemical treatment. All the seeds were watered at 4 days (d) intervals and fertilized at 7 d intervals after planting with no disturbance, competition or nutrient stress. Seedlings were tested at four-leaf stage. Plants selected from a random sample were homogeneous in terms of length, and all subjected to the drought treatments for 24 h. *S. baicalensis* was treated with 0%, 2.5%, 5%, 7.5%, 10%, 12.5% and 15% polyethylene glycol (PEG), which is differ from *L. chinensis* with 0%, 2.5%, 5%, 7.5%, 10%, 15%, 20% and 25% PEG according to their tolerance limit. Each drought treatment was replicated three times, and each replicate was composed of ten individual branchlets of each species.

Gas exchange parameters were measured in a “LI-6400 Portable Photosynthesis System” (LI-COR Company, USA). Values of physiological parameters, including net photosynthetic rate (P_N), transpiration rate (E), vapor pressure deficit (VPD), intercellular CO_2 (C_i) and stomatal conductance (g_s) were logged automatically. Environmental conditions, an air flow of 500 μmol s^{-1}, CO_2 concentration of 370 μmol mol^{-1}, relative humidity of 50% and air temperature of 25°C, were steadily controlled by this instrument. The proline (Pro) content, electrolyte leakage (EL) and leaf water content (LWC) were detected according to the method of Sailerova and Zwiazek (Sailerova and Zwiazek 1993).

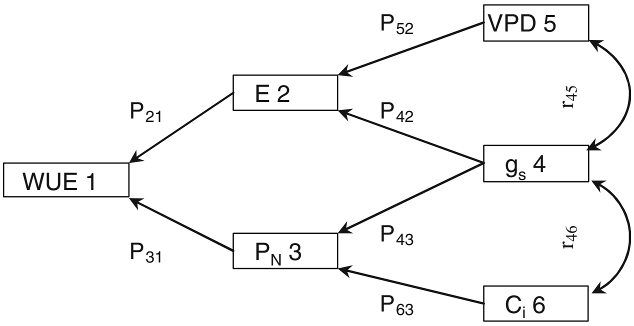


Fig. 1 Path diagram representing the cause-and-effect relationship among the gas exchange parameters

Water use efficiency (WUE) is defined as the ratio of assimilation to water loss as follows:

$$\mathrm{WUE} = \mathrm{P}_N/E \qquad (1)$$

Path analysis can be used to partition the direct and indirect effects among the gas exchange parameters (Hui et al. 2008). The simple recursive path diagrams represent the cause-and-effect relationship (Fig. 1).

The six parameters of WUE, E, P_N, g_s, VPD, C_i were numbered as 1, 2, 3, 4, 5, and 6, respectively. Standardized partial-regression coefficients of the following expressions (2), (3) and (4) were used to estimate path coefficients for E to WUE (P_{21}) and P_N to WUE (P_{31}), VPD to E (P_{52}) and g_s to E (P_{42}), and C_i to P_N (P_{63}) and g_s to P_N (P_{43}).

$$\mathrm{WUE} = b_1 + b_{21}E + b_{31}\mathrm{P}_N \qquad (2)$$

$$E = b_2 + b_{52}\mathrm{VPD} + b_{42}g_s \qquad (3)$$

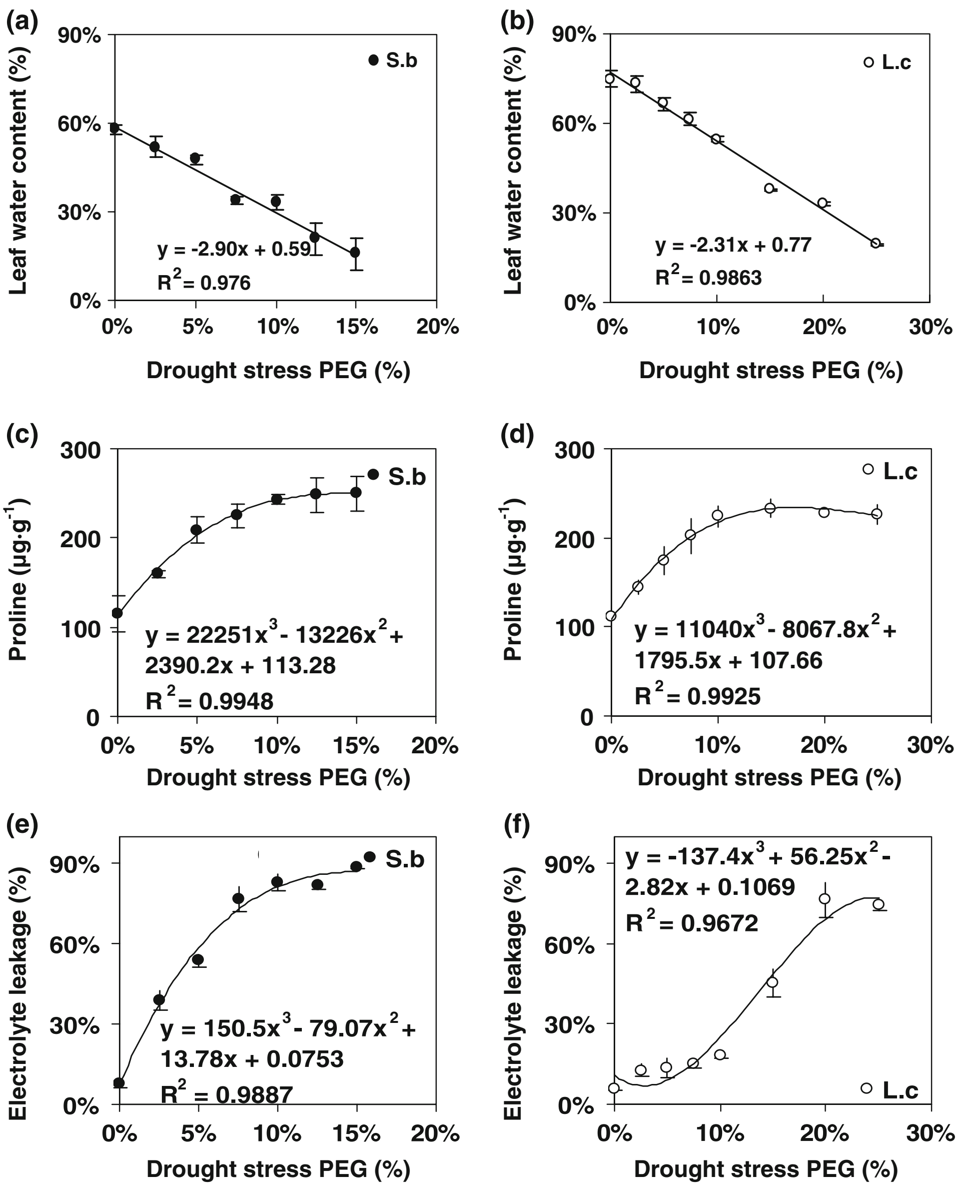
$$\mathrm{P}_N = b_3 + b_{63}\mathrm{C_i} + b_{43}g_s \qquad (4)$$

where b_i is a constant and b_{ij} is a partial-regression coefficient. Indirect effects were determined by multiplying the correlation coefficients (r_{ij}) by their respective path coefficients, $r_{45}P_{52}$ and $r_{46}P_{63}$, representing the indirect effect of g_s to E via VPD and indirect effect of g_s to P_N via C_i. Path coefficients accounted for most of the variation in Water use efficiency as indicated by a small residual effect (0.121–0.161). This suggested that no additional cause parameters were required to explain the effect traits. Fitting of models and path analysis for the two species were performed using statistical software, SPSS 10.0 (SPSS Inc., Chicago).

Results and Discussion

In response to water shortages, leaf water content of *S. baicalensis* decreases linearly from 57.91% to 15.72% as a rate of 2.90% per 1% PEG (Fig. 2a), and leaf water content of *L. chinensis* decreases linearly from 74.81% to 19.41% as a rate of 2.31% per 1% PEG (Fig. 2b). Leaf

Fig. 2 Relationship between leaf water content (**a**, **b**), proline content (**c**, **d**) and electrolyte leakage rate (**e**, **f**) and drought stress in *S. baicalensis* (S.b) and *L. chinensis* (L.c)



water content is less but water lost more rapidly in *S. baicalensis* in contrast to *L. chinensis*. Results indicate that *L. chinensis* has a higher tolerance to drought stress and better ability to keep water than *S. baicalensis*.

Proline is accumulated significantly under drought stress and it is suggested as an indicator of stress response (Vendruscolo et al. 2007). The high levels of proline may protect the plants against the drought-induced damages. The maximum accumulation of proline in *S.baicalensis* (249.74 μg g^{-1} DW) is not significant differ from that in *L. chinensis* (233.98 μg g^{-1} DW), but the limit of proline regulation is much lower in the former (13.94% PEG) than that in the latter (17.20% PEG) (Fig. 2c, d).

A dramatic increase in EL occurs at slight aridity, and it approaches to the maximum at 16.30% PEG treatment in *S. baicalensis* (Fig. 2e). This drought stress is the lethal limitation for *S. baicalensis*, while it is clearly lower than 24.50% PEG concentration for *L. chinensis* (Fig. 2f). In conclusion, both the Pro accumulation and EL of *S. baicalensis* are quantitatively similar to that of *L. chinensis*, but the tolerance limit in drought stress is obviously lower in the former.

The survival of land plants relies on the availability of water and their adaptation under stress in arid or semi-arid environments. Responses of Water use efficiency to increasing drought stress show two distinct phases in the two species. Under mild drought stress, water use efficiency of two species both increase with increasing drought and WUE reach the maximum at 2.5% PEG concentration for *S. baicalensis* and at 5% PEG concentration for *L. chinensis* (Fig. 3). This result is consistent with Anderson's finding that species native to arid or semi-arid

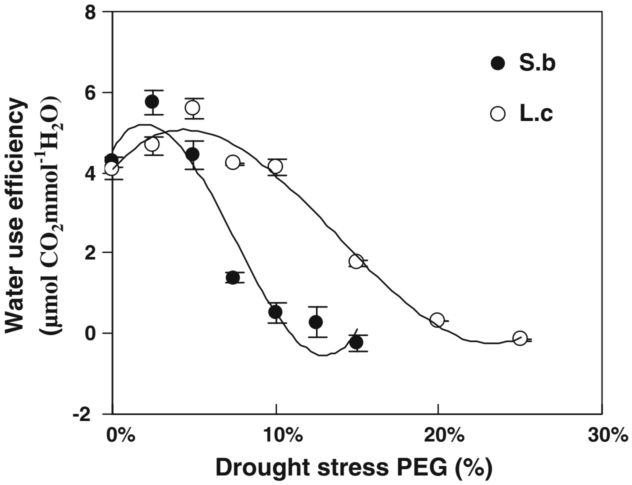


Fig. 3 Relationship between water use efficiency and drought stress in *S. baicalensis* (S.b) and *L. chinensis* (L.c)

environment show no change or an increase in Water use efficiency with decreasing water supply (Anderson et al. 1995). For the two species, efficiency improvement in WUE under mild drought stress is the result of long-term adaptation to arid environment, but the adaptation range is narrower in *S. baicalensis* than that in *L. chinensis*.

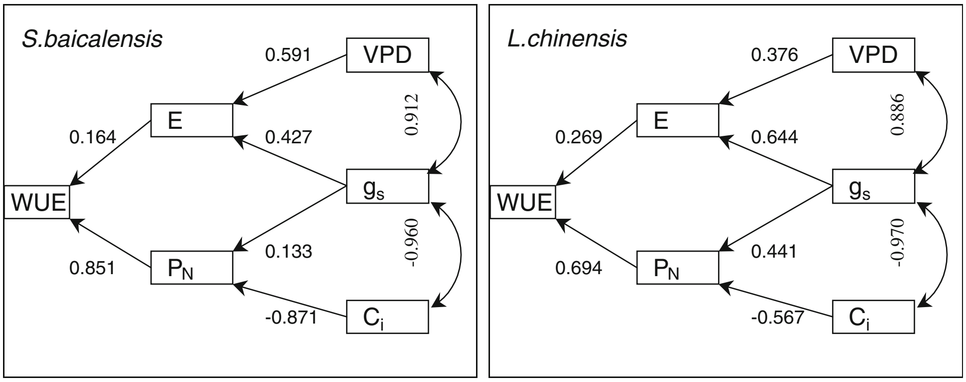
Under severe drought stress, a remarkable drop of Water use efficiency occurs with the increasing drought treatments. The synoptic causal model is developed to reflect the relationships existing in the underlying physiological pathways from 2.5% to 15% PEG in *S. baicalensis* and from 5 to 25% PEG in *L. chinensis* (Fig. 4). Water use efficiency of plants is a trade-off between photosynthesis and stomatal transpiration. Results of path analysis show that the decrease in WUE (*S. baicalensis* and *L. chinensis*) is mainly due to the rapid reduction in P_N (0.851 and 0.694) rather than in *E* (0.164 and 0.269). The reduction of assimilation limits the efficiency of water use.

Stomata play a key role in the regulation of photosynthesis-transpiration compromise. Partial opening or closure of stomata regulates the relative balance between CO_2 absorption and water loss. In response to decreasing water supply stomatal guard cells shrink by losing water, which directly affect the CO_2 and water exchange. In this process, the effect of g_s on *E* (0.427 in *S. baicalensis* and 0.644 in *L. chinensis*) is more powerful than that on P_N (0.133 in *S. baicalensis* and 0.441 in *L. chinensis*).

Apart from the direct effect, stomata also initiate two important negative feedback loops regulating *E* and P_N (Hetherington and Woodward 2003). On the one hand, vapor pressure deficit is the driving force for transpiration and the reduction of VPD, due to stomatal partial closure under drought, weaken the transpiration. The results of path analysis suggest that the direct effect (P_{42}, 0.427) of g_s to *E* is similar to the indirect effect ($r_{45}P_{52}$, 0.539) of g_s to *E* via VPD in *S. baicalensis*. The stomatal conductance of *L. chinensis* is 1.84 times larger than that of *S. baicalensis*. Consequently, stomata of *L. chinensis* are more sensitive to water change, and the direct reduction of stomatal closure to *E* (P_{42}, 0.644) is the dominant effect rather than the indirect effects via VPD ($r_{45}P_{52}$, 0.333) during drought-stress. The larger g_s in *L. chinensis* lead to the more powerful control to transpiration for an effective WUE.

On the other hand, a reduction of C_i, due to stomatal partial closure under drought, will restrict the photosynthetic assimilation. While C_i increases with the increasing drought treatment in the present experiment. According to the theory of Farquhar and Sharkey (Farquhar and Sharkey 1982), the non-stomatal limitation is responsible for the photosynthesis decrease when C_i increases with g_s decrease following. As a result, non-stomatal limitation caused by inactivation of mesophyll cells is the main cause of photosynthesis decrease in the two species under drought treatments. Results of path analysis support this opinion. The direct effect (P_{43}, 0.133) of stomatal closure to P_N decline is obviously less than the indirect effect via C_i ($r_{46}P_{63}$, 0.836) in *S. baicalenis*. But the direct effect (0.441) of stomata to P_N is similar to the indirect effect (0.550) via C_i in *L. chinensis*. Therefore, stomata of *L. chinensis* show more powerful directly control to photosynthesis, but the

Fig. 4 Path diagram representing the cause-and-effect relationship among the water use efficiency (WUE), transpiration rate (*E*), net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 (C_i) and vapour pressure deficit (VPD) from 2.5% to 15% PEG in *S. baicalensis* and from 5% to 25% PEG in *L. chinensis*



decrease of P_N in *S. baicalensis* is primarily due to non-stomatal limitation. Drought induced inactivation of mesophyll cell is a token of physiological damage, so the more non-stomatal limitation means the more damage from drought treating in *S. baicalensis*.

Results in the current study support the hypothesis we propose above. The zonic species of *S. baicalensis* is more susceptible to the damage of aridification than *L. chinensis* in the steppe of China. Differences in water use between the two competitive species result in the gradual degradation of *S. baicalensis* and the replacement by *L. chinensis* in the semi-arid steppe of China. *L. chinensis* has more advantages of water competition in whether the water use efficiency or the drought resistant limit, so it become to the dominant species distributing in an extended region now (Qian and Zhu 2001). Furthermore, it would benefit more from aridification than *S. baicalensis* in the future.

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