

# Investigation of responses of plant mixture to different water stress regimes in a pot experiment

Zhen Wang<sup>1,2</sup> Michael P. Schellenberg<sup>1\*</sup> Mengli Zhao<sup>2</sup> Guodong Han<sup>2</sup>

<sup>1</sup>Semiarid Prairie Agricultural Research Center (SPARC), AAFC-AAC

<sup>2</sup>Department of Grassland Science, College of Ecological and Environmental Science, Inner Mongolia Agricultural University. 306 Zhao Wu Da Road. Hohhot, Inner Mongolia

Keywords: soil moisture level, plant mixture, above-ground biomass

## Abstract

When drought occurs, plants often exhibit a tradeoff between productivity and survival. Here we report water deficit effects on biomass production of perennial plants in a pot experiment. We grew experimental plant communities containing monocultures (legume, grasses and shrub) and mixtures (legume+grasses, grasses+shrub and legume+grasses, grasses+shrub). Results from the experiment showed that: 1) compared to well-watered treatment, two water treatments (85 and 70% of field capacity) significantly decreased above-ground biomass of both monoculture and mixture ( $P < 0.0001$ ); 2) The mixture of alfalfa and blue grama had the highest biomass production; and 3) a significant positive ( $P < 0.0001$ ) linear relationship was found between the three water treatments and above-ground biomass.

## Introduction

As a negative factor of climate change, drought has become a worldwide concern in recent years, particularly due to its impact on plant productivity (Knapp et al., 2001; Reynold et al., 2004). Conservative climate change models predict that global average temperature will increase 1.8 °C to 4.0 °C by the end of this century (IPCC, 2007). This temperature increase will result in reduced water storage in part due to increased evapotranspiration (ET) (Saleska et al., 1999).

Plants have evolved a number of methods for dealing with drought. The tolerance of plants to drought stress can be facilitated by increasing water uptake or decreasing water loss (Kozlowski et al., 2002). In addition to changed morphological structures, plants also avoid drought stress by adjusting physiological and biochemical characteristics (Wang et al., 2007).

The pot study was designed to examine drought effects on four different plant types; cool season grasses, warm season grass, shrub and nitrogen fixing legumes grown in mixtures or monoculture. The questions to answer were:

- 1) Does a mixture of plant species have higher net primary productivity than monoculture of a single species under conditions of water deficit?
- 2) Which forage species has the best growth under conditions of water deficit?

A large number of plants are found in the semiarid grasslands of the world with adaptations to drought. We were not able to test all species thus we chose a few key species to test. They include: alfalfa (*Medicago sativa*) an important legume, widely grown in Canada and globally, with good palatability and high forage value; and purple prairie clover (*Dalea purpurea*) a native legume also with good palatability and high forage value, which appears to have more drought tolerance than alfalfa. Some scientists consider purple prairie clover may have an important role in the future. Crested wheatgrass (*Agropyron cristatum*), an introduced grass to North America from Eurasia, known for its ability to grow under drought conditions and was widely used to stop soil erosion in the 1930's dust bowl years in Canada, was one of the grasses. However, the plant is known to invade native prairie. Blue grama (*Bouteloua gracilis*), the second grass selected is a naturally occurring dominant warm season grass of more xeric sites of the dry mixed grass prairie. Winterfat (*Krascheninnikovia lanata*), a shrub of high nutritional value with a range from Mexico to the Yukon Territory, was also selected for testing.

## **Materials and Methods**

The study was conducted at the Semiarid Agricultural Research Centre (SPARC) -Agriculture and Agri-Food Canada (AAFC) located in Swift Current, Saskatchewan (50°17'N, 117°53'E, 825 m a.s.l). The long-term mean annual precipitation is approximately 350 mm for the region.

## **Experiment materials**

Five plant species were selected for testing: two legume plants (alfalfa: ALF and purple prairie clover: PPC), a cool season grass (crested wheatgrass: CWG) and a warm season grass (blue grama: BG) and one shrub (winterfat:WF).

Soil used for the study was an Orthic brown chernozem.

### **Experimental Design**

The experiment was a randomized complete block design with two factors (species mixtures and water treatments). Mixtures included each species as a monoculture and every binary mixture except WF with ALF, and PPC with ALF; these having been examined earlier (Schellenberg and Banerjee 2002). In addition to mono- and binary mixtures a PPC with BG with WF was also tested. Each mixture was allocated randomly to three different watering treatments: a well-watered treatment (100% of field capacity) and two water-stressed treatments (85 and 70% of field capacity). The experiment had three replications, each with 42 pots. The soil water content was maintained at 100, 85 and 70% by replacing the lost water daily.

All experimental pots were placed in the growth chamber, and the daily maximum photosynthetic photon flux density was  $700 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the growth chamber with lights being on for 12 hours. The day/night temperature was 25/18°C respectively. The relative humidity was maintained at 30%.

Plants were harvested after attaining their peak biomass in the growth chamber (14 weeks). Each plant species was separated during harvest. For above-ground biomass, plants were cut at the soil surface. The above-ground biomass was separated, oven-dried at 70 °C for 48 h, and weighed.

### **Statistical analyses**

All data analyses were conducted with SAS 9.0 statistical software (SAS, 9.0, USA). The effects of three water treatment on plant species above-ground biomass was analyzed using ANOVA with Proc MIXED in SAS 9.0. For the three water treatments, we used one-way analysis of variance (ANOVA) to compare different plant species mixtures and monoculture above-ground biomass. The significance level of  $\alpha = 0.05$  was used for all analyses. Multiple linear regressions were used to examine relationships of water treatment and above-ground biomass.

## Results

### Above-ground biomass of different mixtures

Thirteen species combinations above-ground biomass were higher in the well-watered treatment compared to the other two lower water treatments (Table1), the exception was WF. WF had a higher biomass in the 85% field capacity treatment (0.85g, table1) than in the field capacity treatment (0.81g table1) ( $P<0.05$ ).

Compared to other plant treatments, the mixture of ALF and BG had the highest aboveground biomass in the field capacity treatment (1.57g, table1). Water treatment had no effect on the above-ground biomass of PPC, WF, PPC×BG, CWG×BG and CWG×WF (table 1,  $P>0.05$ ).

Statistically significant differences were found for the above-ground biomass of ALF and ALF×PPC and between in field capacity and 85% /70% field capacity. The 70% field capacity water treatment significantly decreased the above-ground biomass of CWG and BG×WF (table1). When compared with the other two water treatments (capacity water treatment and 85%water treatment), 70% capacity had significant effect on the above-ground biomass of PPC×BG×WF.

A positive linear correlation was also found between plant above-ground biomass and the three water treatments ( $R^2=0.6156$   $P<0.0001$  Fig.2). This indicates for this study the water treatments explained 62% of the variation.

### Plant functional group above- and below-ground biomass

#### *Legume*

The two plant species (ALF and PPC) had significantly different above-ground biomass ( $P<0.0001$ , Table 2). A significant difference was also observed in the different water treatments ( $P<0.0001$ , Table 2). The two species both had the highest above-biomass (ALF 1.39g, PPC 0.14g, Fig.1) in the well-watered treatment, and had the lowest above- ground biomass (ALF 0.86g, PPC 0.07g Fig.1) in the 70% water-stressed treatment. However, the mixture of the two species didn't result in increased above-ground biomass for any of the water treatments (Fig.1). The two water-stressed treatments had a larger effect on ALF above-biomass than PPC (Fig.1).

### *Gasses*

Significant differences were observed for above-ground biomass of two species in the water treatment ( $P=0.003$ , table 2), but no difference was detected for two species between BG and CWG ( $P=0.1879$ , Table 2) or the interaction with water ( $P=0.3966$  Table 2). The highest above-ground biomass (BG 0.77g, CWG 0.55g) were obtained in the field capacity treatment, and the lowest aboveground biomass in the 70% field capacity (BG 0.50g, CWG 0.44g). The two water stressed treatments had a greater impact on the BG above-ground than the CWG. Compared to the field capacity impact on BG, the above-ground biomass was reduced by 36.4% in the 85% field capacity and 51.9% in the 70% field capacity. The above-ground biomass of CWG was reduced 20% and 38.2% in two water-stressed treatments (85% and 70% field capacity), respectively (Fig.1).

### *Shrub*

No significant differences were observed in the aboveground biomass for the three water treatments ( $P = 0.2263$ , Table 2).

## **Discussion**

### **Effect of monoculture and mixture plant species on above-ground biomass**

It has been proposed that high-diversity grasslands could have higher yields than monocultures (Tilman et al., 2006). For example, Schellenberg (2002) reported that mixture treatments (legume-shrub) had higher plant biomass than monoculture (legume, shrub) in a pot study. However, in our greenhouse experiment, 6 plant species were planted in every pot. If two species were used to up the mixture, every plant species had 3 seedlings in the pot. If three species were mixture, every plant species had 2 seedlings in the pot. The mixture of legume  $\times$  legume (ALF $\times$ PPC), legume  $\times$  grass (ALF $\times$ CWG), legume $\times$ shrub (BG $\times$ WF) and legume  $\times$  grass  $\times$  shrub (PPC $\times$ BG $\times$ WF) always had a higher biomass than monoculture legume or grass, whereas the mixture of grass $\times$  grass (CWG $\times$ BG ) or grass $\times$ shrub (CWG $\times$ WF and BG $\times$ WF) biomass was lower than monoculture grass or shrub for the three water treatments (Table 1). This suggests selection of species in the combinations is important.

The legume (ALF) did fix N and thus likely supplied N for enhanced growth of BG leading to the higher biomass of ALF × BG in the field capacity water treatment. For other mixtures, some species had slower growth, and greater competition for resources between species, which may have caused lower biomass for these species. Appropriate species combinations for mixtures would therefore appear to be important.

### **Effect of different water on above-ground biomass**

Some climate change research has shown that precipitation is occurring less frequently in late summer while evaporation is increasing, which leads to drought becoming more common and longer, particularly in arid and semi-arid environment (Ludlow et al., 1989; Mostajeran et al., 2009). This future climate scenario would impact biomass productivity.

Accordingly our greenhouse experiment, confirmed whatever the monoculture or mixture of plants we used, the above-ground biomass of field capacity treatment was always higher than other two water-stressed treatments. This supports the statement water is an important limiting factor to plant productivity globally and drought will reduce aboveground biomass (Lambers et al., 1998) for most species. The legumes appear more sensitive to water decreases than grasses or WF. Alfalfa is known to be a heavy water user. To survive, plants have to reduce growth if the supply is lower than 85% field capacity.

### **Conclusion**

Water is an important limiting factor for plant productivity globally. Increasing potential for drought will reduce aboveground biomass. The mixtures of legume with other functional group (grass or shrub) above-ground biomass were always higher than monoculture for all water treatments. Compared with the other mixtures under drought condition, the mixture of ALF × BG had the highest biomass under the two water-stressed treatments. The mixture of grass × grass or grass × shrubs is lower than monoculture of grass or shrub. The biomass of monoculture trends for all water treatments were ALF > WF > BG > CWG > PPC, as the plants mature one would expect this ranking to change but this ranking provides a possible indication of relative competitiveness.

## References

Knapp, A. K. and Smith, M. D. (2001). Variation among biomes in temporal dynamics of aboveground primary production, *Science* 291: 481-484.

Kozłowski, T.T., Pallardy, S.G.: Acclimation and adaptive responses of woody plants to environmental stresses. -*Bot. Rev.* 68: 270-334, 2002.

Lambers, H., Chapin, F.S., and Pons, T.L.(1998). *Plant physiological ecology*, Springer, New York, NY, USA.

Ludlow MM (1989) Strategies of response to water stress. In 'Structural and functional responses to environmental stresses'. (Eds KH Kreeb, H Richter and TM Hinckley) pp. 269-281. (SPB Academic: The Hague)

Mostajeran A, Rahimi-Eichi V. (2009). Effects of drought stress on growth and yield of rice (*Oryza sativa* L.) cultivars and accumulation of praline and soluble sugars in Sheath and Blades of their different ages leaves. *American-Eurasian J. Agric. & Environ. Sci.*, 5(2): 264-272.

Reynolds JF, Kemp PR, Ogle K, Fernandez RJ. ( 2004). Modifying the 'pulse-reserve' paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. *Oecologia* 141: 194-210.

Schellenberg, M.P. ,Banerjee, M.R. (2002). The potential of Legume-shrub Mixtures for Optimum Forage Production: A Greenhouse Study. *Canadian Journal of Plant Science* 82:357-363.

Saleska SR, Harte J, Tron MS (1999). The effect of experimental ecosystem warming on CO<sub>2</sub> fluxes in montane meadow, *Global Change Biology* 5: 125-141.

Tilman D., Hill J. and Lehman C (2006). Carbon-Negative Biofuels from Low-Input High-Diversity Grassland Biomass. *Science* 314:1598-1600.

Wang, T., Zhang, X., C.(2007). Growth, abscisic acid content, and carbon istope composition in wheat cultivars grown under different soil moisture. *Biology Plant.* 51: 181-184.

**Table 1.** Species of monoculture and mixtures below-ground biomass (unit: g) for three water treatments.

water treatment	100%	85%	70%	<i>P</i>	SEM
Above-ground biomass					
ALF	1.39ab(A)	0.86a(B)	0.69a(B)	<0.001	0.01
PPC	0.12g(A)	0.11e(A)	0.07f(A)	0.26	0.02
CWG	0.55ef(A)	0.44d(AB)	0.34de(B)	0.06	0.05
BG	0.77cde(A)	0.50d(B)	0.37cde(B)	0.02	0.07
WF	0.85cd(A)	0.81ab(A)	0.53abc(A)	0.23	0.1
ALF×PPC	1.30b(A)	0.82a(B)	0.47bcd(B)	0.01	0.1
ALF×CWG	1.53ab(A)	0.92a(B)	0.59ab(C)	<0.001	0.07
ALF×BG	1.57a(A)	0.88a(B)	0.52abc(C)	<0.001	0.05
PPC×CWG	0.49f(A)	0.40d(A)	0.40cde(A)	0.42	0.05
PPC×BG	0.60def(A)	0.53cd(A)	0.25e(B)	0.04	0.08
CWG×BG	0.54ef(A)	0.46d(A)	0.36cde(A)	0.36	0.08
CWG×WF	0.72cdef(A)	0.56cd(A)	0.45bcd(A)	0.12	0.08
BG×WF	0.82cd(A)	0.68bc(AB)	0.50bcd(B)	<0.05	0.07
PPC×BG×WF	0.90c(A)	0.69bc(A)	0.45bcd(B)	<0.01	0.06
<i>P</i>	<0.001	<0.001	<0.001		
SEM	0.09	0.06	0.06		

Note: different lower-case letter indicates significant difference of species; Capital letter is the significant difference of water treatment.  $\alpha = 0.05$

**Table 2.** *P* values from two-way ANOVA for the effects of water treatment, species and their interactions of plant functional group above-ground biomass (Legume and Grasses). *P* values from One-way ANOVA on the effects of water treatment on the Above-ground biomass (shrub).

Functional groups	Water	Species	Water×Species
Aboveground Biomass			
Legume	<0.0001	<0.0001	0.0003
Grasses	0.003	0.1879	0.3966
Shrub	0.2263		

Fig.1 Above-ground biomass of the different plant functional groups

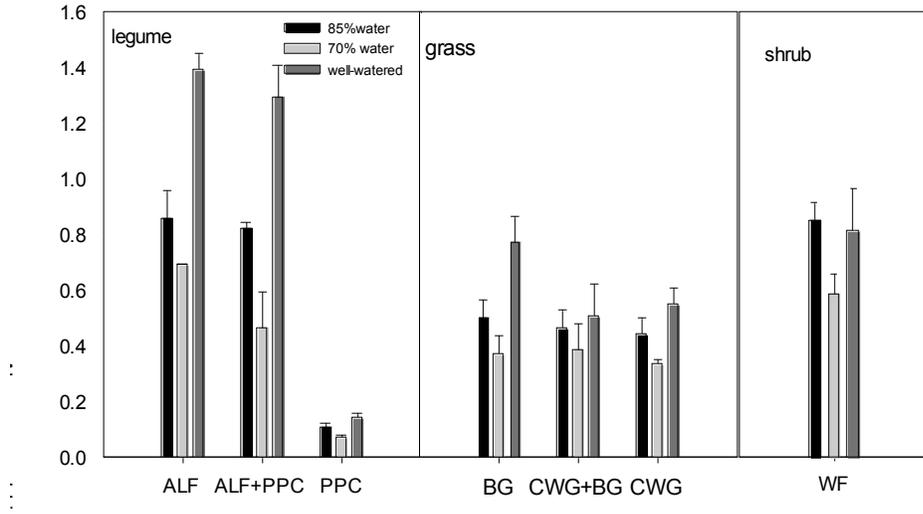


Fig.2 Spatial dependence of average supplied water on above-ground biomass respectively across the 126 pots.

