Effects of Water and Nutrient Addition on Growth, Physiological Characteristics, and Sensitivity of Stipa glareosa in the Inner Mongolia Desert Steppe (Postprint)

Authors: Hu Ya, Zuo Xiao' an

Date: 2021-04-23T00:00:00+00:00

Abstract

To investigate the response characteristics and adaptation patterns of dominant plants in desert steppe to water and nutrient inputs, we established 2 water levels (natural rainfall, water addition) and 3 nutrient levels (no nutrient addition, N addition, NPK addition), constituting 6 treatments, to examine the effects of water and nutrient addition on growth, physiology, and sensitivity of the desert steppe dominant plant *Stipa glareosa*. Two-way ANOVA results demonstrated that the main effect of water, main effect of nutrient, and their interaction significantly influenced the growth and physiological traits of *Stipa glareosa* ($P<0.05$). Water addition significantly increased leaf fresh weight, dry weight, plant height, and relative electrical conductivity, while decreasing SOD activity; nutrient addition increased leaf area, N addition increased malondialdehyde content, and NPK addition decreased chlorophyll a/b ratio (Ca/Cb) and SOD activity; the water-nutrient interaction significantly affected leaf area, leaf thickness, chlorophyll a (Ca), proline, protein content, and SOD activity. Sensitivity analysis revealed that leaf area and Ca content were more sensitive to combined water and NPK addition treatment, whereas Ca/Cb was more sensitive to water addition treatment. In summary, *Stipa glareosa* can adapt to water and nutrient variations by modulating specific growth and physiological characteristics, which is crucial for elucidating the response mechanisms of desert steppe plants to global change.

Full Text

Effects of Water and Nutrient Addition on the Growth and Physiology of *Stipa glareosa* in a Desert Steppe in Inner Mongolia

HU Ya[12], GUO Xinxin[12], YUE Ping[1], LI Xiangyun[12], ZHAO Shenglong[12], GUO Aixia[12], ZUO Xiao' an[13]

[1]Urat Desert Grassland Research Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000, Gansu, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
[3]Naiman Desertification Research Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000, Gansu, China

Abstract

To investigate the response characteristics and adaptation mechanisms of dominant plants in desert steppe ecosystems to water and nutrient inputs, we conducted a field experiment with two water levels (natural rainfall and water addition) and three nutrient levels (no nutrient addition, N addition, and NPK addition), totaling six treatments. We examined the effects of water and nutrient addition on the growth, physiological traits, and sensitivity of *Stipa glareosa*, a dominant species in desert steppe communities. Two-way ANOVA results indicated that the main effects of water, nutrients, and their interaction significantly influenced the growth and physiological characteristics of *S. glareosa* ($P < 0.05$). Water addition significantly increased leaf fresh weight, dry weight, plant height, and relative electrical conductivity, while decreasing superoxide dismutase (SOD) enzyme activity. Nutrient addition increased leaf area; N addition increased malondialdehyde content, whereas NPK addition decreased chlorophyll a/b ratio and SOD activity. The water × nutrient interaction significantly affected leaf area, leaf thickness, chlorophyll a, proline and protein contents, and SOD activity. Sensitivity analysis revealed that leaf area and chlorophyll a content were most sensitive to combined water and NPK addition, while chlorophyll a/b ratio was more sensitive to water addition alone. In conclusion, *S. glareosa* can adapt to variations in water and nutrient availability by altering specific growth and physiological traits, which is crucial for elucidating the response mechanisms of desert steppe plants to global change.

Keywords: nutrient addition; water addition; nitrogen; sensitivity; desert steppe

Introduction

Since the Industrial Revolution, increasing atmospheric CO_2 concentrations have contributed to global temperature rise, affecting atmospheric water content and intensifying the hydrological cycle, which is projected to alter precipitation patterns [2]. Regional precipitation in China is expected to show large fluctuations, with precipitation increasing to some extent in Northwest China and Inner Mongolia [3]. Increased precipitation during the growing season may play an important role in regulating vegetation growth, enhancing photosynthesis, promoting plant development, and increasing species richness and community coverage [4]. However, increased extreme precipitation also exacerbates flood risks, and prolonged waterlogging can cause plant anaerobic respiration to intensify, leading to accumulation of harmful substances, blocked photosynthesis, reduced biomass, individual mortality, and altered vegetation structure [5,6].

Water and nutrients interactively affect plant growth and development. Water deficiency can limit nutrient availability and affect nutrient transformation, absorption, and utilization in soil, while excessive water can cause nutrient leaching and loss [7]. Desert steppe represents a transitional ecosystem between grassland and desert, characterized by low annual rainfall, large seasonal and diurnal temperature variations, relatively simple plant community composition, simple vegetation structure, low coverage, harsh environmental conditions, and high sensitivity to natural and anthropogenic disturbances [8,9]. Understanding changes in plant growth and physiology in desert steppe under global change is essential for recognizing plant adaptation strategies and mitigating potential losses.

Previous studies have shown that grassland vegetation responds sensitively to water and nutrients, and their synergistic effects significantly influence grassland community composition, structure, and function [10,11,15]. While short-term or low-level N addition can significantly promote plant growth, excessive single-nutrient input can lead to ecosystem degradation [12,13]. Research on the responses of desert steppe to water and nutrient addition has made substantial progress at the community level, but understanding remains limited regarding impacts at the species level. Most existing studies have focused on stoichiometric characteristics of dominant plants [11,15]. Investigating plant growth and physiological traits helps explain species-specific responses to environmental conditions and provides a foundation for understanding plant variability along natural environmental gradients and under climate change [18].

Using the global change multi-factor field control experiment platform at the Urat Desert Grassland Research Station, Chinese Academy of Sciences, we examined the responses of *S. glareosa*, a dominant species in the Urat Desert Grassland of Inner Mongolia, to water, nutrients, and their coupled effects. This study aims to reveal the growth and physiological response mechanisms of dominant desert steppe species to environmental changes, which is important for describing vegetation growth under global change, evaluating grassland

ecosystem functions, and managing grassland resources.

1. Materials and Methods

1.1 Study Area The study area is located in Urat Rear Banner, western Inner Mongolia Autonomous Region (106°58 E, 41°25 N; elevation 1650 m). The region has a continental climate with cold, long winters and cool, short summers. Precipitation is scarce, with strong winds, an average annual temperature of 3.9°C, average annual precipitation of 180 mm, average wind speed of 5 $m \cdot s^{-1}$, and a frost-free period of approximately 130 days. The experimental site is situated at the Urat Desert Grassland Research Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences. Dominant plant species include *Stipa glareosa*, *Peganum harmala*, and *Allium polyrhizum*. Soil types are brown calcic soil and gray desert soil.

1.2 Experimental Design The experiment utilized the global change multi-factor field control platform at the Urat Desert Grassland Research Station, based on protocols from the Nutrient Network and Drought Net. Multiple water and nutrient treatments were established in 2016 and have been maintained since. The experiment included two water levels: natural rainfall (W_0) and water addition (W_1), and three nutrient levels: no nutrient addition (N_0), N addition (N_1), and NPK addition (N_2). A randomized block design was employed with the following treatments: (1) W_0N_0 (control: natural rainfall, no nutrients), (2) W_1N_0 (water addition, no nutrients), (3) W_0N_1 (natural rainfall, N addition), (4) W_1N_1 (water addition, N addition), (5) W_0N_2 (natural rainfall, NPK addition), and (6) W_1N_2 (water addition, NPK addition). Each treatment had four replicates, totaling 24 experimental plots. Each plot measured 6 m × 6 m, with trenches dug around the perimeter, plastic film and iron sheets installed to prevent lateral water and nutrient movement, and a 1 m buffer zone between plots. Water addition treatments received supplemental irrigation equivalent to 50% of natural rainfall after each precipitation event. In 2017, the growing season natural precipitation was 247 mm. Fertilizers were applied once in early July. Nutrient addition rates were 10 $g \cdot m^{-2}$ for N (as urea containing 46% N), 10 $g \cdot m^{-2}$ for P (as triple superphosphate containing 46% P_2O_5), and 10 $g \cdot m^{-2}$ for K (as resin-coated potassium sulfate containing 54% K_2O).

1.3 Measurements In mid-July 2017 (peak growing season), 30 *S. glareosa* individuals were collected from each plot in the morning, placed in ziplock bags, and stored in a mobile refrigerator for transport to the laboratory. In the laboratory, plants were removed from bags, petioles were trimmed, and mature, healthy leaves were selected for measurements. Plant height, leaf area, leaf fresh weight, leaf dry weight, and leaf thickness were measured following standard protocols [20]. Chlorophyll a (Ca), chlorophyll b (Cb), and carotenoid contents were determined using the acetone extraction method [21]. Proline content was measured using the ninhydrin colorimetric method [22]. Protein content was determined using the Coomassie brilliant blue G-250 method [23].

Malondialdehyde (MDA) content was measured using the thiobarbituric acid method [24]. Relative electrical conductivity (REC) was measured using a conductivity meter (SanXin SX823). Superoxide dismutase (SOD) and peroxidase (POD) activities were measured using the nitroblue tetrazolium method and guaiacol method, respectively [25,26].

1.4 Sensitivity Analysis To assess the sensitivity of *S. glareosa* to different water and nutrient treatments, we conducted sensitivity analysis on growth and physiological traits that showed significant responses. Higher sensitivity values indicate greater responsiveness to treatments. The sensitivity index was calculated as:

$$\text{Impact} = \frac{T - CK}{CK}$$

where T represents the trait value under experimental treatment, and CK represents the trait value under control conditions (natural rainfall, no nutrient addition).

1.5 Statistical Analysis All data are presented as means ± standard error. Two-way ANOVA was used to test the effects of nutrients, water, and their interactions on growth and physiological traits of *S. glareosa*. One-way ANOVA was used to test the effects of individual factors and the sensitivity of different treatments, followed by least significant difference (LSD) tests for multiple comparisons ($P < 0.05$). Statistical analyses and figure preparation were performed using SPSS 22.0 and SigmaPlot 12.5 software.

2. Results

2.1 Effects of Water and Nutrient Addition on *S. glareosa* Growth and Physiology: ANOVA Results The growth and physiological characteristics of *S. glareosa* were significantly affected by water and nutrient addition (Table 1). Leaf fresh weight, leaf dry weight, plant height, and SOD activity were significantly affected by the main effect of water. The main effect of nutrients significantly influenced leaf area, chlorophyll a/b ratio, and SOD activity. The interaction between water and nutrients significantly affected leaf area, leaf thickness, chlorophyll a, proline and protein contents, and SOD activity.

2.2 Main Effects of Water and Nutrient Addition on *S. glareosa* Traits Based on ANOVA results, we analyzed traits significantly affected by main effects (Fig. 1). Water addition significantly increased leaf fresh weight (by 34.17%), leaf dry weight (by 50.00%), and plant height (by 22.32%), while decreasing SOD activity by 55.01%. Nutrient addition increased leaf area by 20.36% compared to the control. N addition increased malondialdehyde content by 26.71% and decreased chlorophyll a/b ratio by 18.97%. NPK addition had

no significant effect on chlorophyll a/b ratio, but significantly increased proline content by 7.75% compared to the control, with proline content under NPK addition being 31.76% higher than under N addition.

[Figure 1: see original paper]

2.3 Interactive Effects of Water and Nutrient Addition Based on ANOVA results, we analyzed traits significantly affected by the water × nutrient interaction (Fig. 2). The W_1N_2 treatment significantly increased leaf area and leaf thickness, with significant differences between water treatments under nutrient addition conditions. However, leaf area and thickness under W_1N_1 did not differ significantly from the control. The W_1N_2 treatment significantly increased chlorophyll a content and decreased SOD activity, with significant differences between water conditions under NPK addition. Protein content under W_1N_2 was significantly higher than under W_0N_2, and the sensitivity of protein content to W_1N_2 was significantly higher than to W_0N_2. The chlorophyll a/b ratio was most sensitive to the W_1N_0 treatment. Proline content under W_0N_2 was significantly higher than under W_0N_1, with significant differences between water conditions under NPK addition.

[Figure 2: see original paper]

2.4 Sensitivity of *S. glareosa* Growth and Physiological Traits Sensitivity analysis revealed differential responses among growth and physiological traits (Fig. 3). Leaf area showed highest sensitivity to the W_1N_2 treatment. Leaf dry weight was most sensitive to the W_0N_1 treatment, while leaf thickness was most sensitive to W_1N_1. Plant height sensitivity did not differ significantly among treatments. The W_1N_2 treatment significantly reduced SOD activity sensitivity compared to W_0N_2. Protein content sensitivity to W_1N_2 was significantly higher than to W_0N_2. Chlorophyll a/b ratio was more sensitive to water addition treatments, particularly W_1N_0. Proline sensitivity was significantly higher under W_0N_2 than W_0N_1, with significant differences between water conditions under NPK addition.

[Figure 3: see original paper]

3. Discussion

Precipitation is the primary limiting factor for plant growth and vegetation development in arid and semi-arid grasslands. Increased precipitation can enhance soil nutrient and water availability, accelerating plant growth [27]. In this study, water addition significantly promoted the growth of *S. glareosa* in the desert steppe, increasing leaf fresh weight, leaf dry weight, and plant height. Similar results indicate that herbaceous plants under low water conditions exhibit reduced height and biomass to cope with drought stress [28]. Antioxidant enzymes such as SOD and POD can mitigate reactive oxygen species damage, inhibit decomposition of unsaturated fatty acids in membranes, and maintain

cell membrane permeability within a stable range [29]. During drought stress, plants can increase antioxidant enzyme activity to scavenge reactive oxygen species and reduce osmotic potential to maintain cell turgor. Water addition alleviated soil drought stress, thereby reducing SOD activity and MDA content in *S. glareosa*.

Nutrients are essential for normal plant growth and development. Nutrient addition can enhance photosynthesis and promote plant growth [30]. Some studies suggest that grassland ecosystems may shift from N limitation to co-limitation by multiple elements under climate change and human disturbance [31]. In this experiment, *S. glareosa* responded positively to nutrient addition by increasing leaf area. Research has shown that nutrient addition can increase individual leaf area and total photosynthetic benefit [32]. N addition significantly decreased SOD activity, reflecting reduced photosynthetic activity [30] and weakened antioxidant capacity. NPK addition significantly increased proline content in *S. glareosa*, suggesting that nutrient addition may create a drought stress environment. This could be because rapid plant growth under nutrient enrichment leads to tender tissues, large and thin leaves, and increased water consumption [33].

Water and nutrient addition improved soil resource availability but may also intensified interspecific competition. Under resource-rich conditions, competition can be more intense than under stressful conditions [34]. In this study, protein content under W_1N_2 treatment was significantly lower than under W_0N_2, possibly because higher leaf water content diluted cellular contents, reducing substance concentration per unit mass. The W_1N_2 treatment significantly increased leaf area and decreased SOD activity. Desert steppe is a nutrient-poor ecosystem where, in addition to N limitation, other elements are also deficient; thus, multi-nutrient addition is more beneficial for plant growth and development [31].

Sensitivity analysis showed that different traits exhibited varying sensitivities to treatments. Leaf area was most sensitive to W_1N_2 treatment, indicating that multi-element addition caused substantial changes in leaf area. Leaf dry weight was most sensitive to W_0N_1 treatment, while leaf thickness was most sensitive to W_1N_1 treatment, suggesting that N has substantial effects on leaf dry weight and thickness. Chlorophyll a/b ratio was sensitive to water addition treatments, indicating that in arid and semi-arid regions, plant photosynthetic activity is more sensitive to water availability. Plant physiological responses can be contradictory; while pot experiments often yield consistent results, field experiments reveal more complex patterns. However, pot experiment results may deviate from field conditions, making field studies crucial for exploring plant response mechanisms to environmental changes.

4. Conclusion

Water addition alleviated soil drought stress and significantly promoted the growth of *S. glareosa*, increasing leaf biomass and plant height. Nutrient addition significantly increased leaf area but may have increased water consumption, exacerbating drought stress and significantly affecting physiological traits. The coupling of water and nutrient addition enriched soil resources but may also enhance interspecific competition, resulting in complex responses of *S. glareosa* growth and physiological traits. In arid and semi-arid regions, plant photosynthetic activity is more sensitive to water addition than to nutrient addition. Investigating the growth and physiological responses of *S. glareosa* to water and nutrient addition helps explain plant-specific reactions to climate change and is important for evaluating grassland ecosystem functions and managing grassland resources.

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