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ECOPHYSIOLOGICAL RESPONSES OF *SCHIZACHYRIUM SCOPARIUM* TO WATER AND NITROGEN MANIPULATIONS

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ABSTRACT—Nitrogen is increasing in terrestrial ecosystems as a result of agricultural practices and the burning of fossil fuels. This increase is expected to be accompanied by changes in water availability due to global warming. We examined the effects of nitrogen and water manipulations on *Schizachyrium scoparium,* one of the dominant grasses in the Great Plains. *Schizachyrium scoparium* responded positively to watering, with an increase in photosynthesis, stomatal conductance, water and nitrogen use efficiencies, and water potential. Under watered conditions, fertilization had no significant effect on measured parameters, except for nitrogen-use efficiency. Significant differences appeared between fertilized and nonfertilized plants under moderate drought, with fertilized plants maintaining higher photosynthesis and water-use efficiency than nonfertilized plants. Water potential declined with water stress but did not differ between fertilization treatments, while nitrogen-use efficiency was significantly higher under nonfertilized than fertilized treatment. Differences among fertilization treatments disappeared under severe drought. We conclude that *S. scoparium* will likely respond positively to fertilization under moderate drought in the Great Plains. However, under severe drought, fertilization will not provide any physiological advantages to *S. scoparium.*

Key Words: bluestem, climate change, gas exchange, Great Plains, nutrient availability, water potential

INTRODUCTION

Warm-season (C_4) plant species make up about 18% of global productivity (Ehleringer et al. 1997), dominating ecosystems ranging from wetlands to deserts, tropical to temperate climate, and in terms of productivity, from the least to the most productive ecosystems (Wedin 2004). Their distribution is highly correlated with high summer maximum temperatures (Ehleringer et al. 1997). Little bluestem [*Schizachyrium scoparium* (Michx.) Nash] has a wide distribution in the central Great Plains of North America (Weaver 1954). Its location within the landscape indicates that it is most competitive in resource-poor habitats with moderate environmental stress (Weaver 1954; Tilman 1987; Schacht et al. 2000; Awada et al. 2002; Foster 2002). Little bluestem, like other C_4 species, is physiologically distinguished from cool-season (C_3) plants by concentrating CO_2 at the Rubisco site. This results in the absence of apparent photorespiration, lower CO_2 and light compensation points, and higher light saturation points,

photosynthetic rates, and water and nitrogen use efficiencies (Jones 1992; Wedin 2004).

Water and nitrogen frequently limit primary production in arid and semiarid ecosystems (Knapp and Seastedt 1998; Chen et al. 2005), and the physiology of plants is tightly linked to the availability of these resources. Water deficits have been found to affect net photosynthesis by directly impacting the photosynthetic capacity of the mesophyll or through stomatal closure (Tezara et al. 1998; Awada et al. 2002). Nitrogen is also essential for plant photosynthesis and growth (Chapin 1980; Bowman 1991). Alleviating nitrogen limitation through fertilization resulted in an increase in photosynthesis and biomass production in several natural and managed ecosystems (Chapin 1980; Field and Mooney 1986; Tilman 1987; Lambers et al. 1998; Shangguan et al. 2000). Knapp and Medina (1999) reported that C_4 grasses exhibited contrasting responses to N fertilization. Strong responses were observed in ecosystems where phosphorus in soils was not limited. The C_4 species responses seemed to also depend on photosynthetic subtype. For example, the aspartate-formers subtypes were found to have higher nitrogen requirements and therefore responded better to N fertilization than malate-formers (Knapp and Medina 1999), such as little bluestem.

Nitrogen concentration is on the increase in terrestrial systems because of agricultural practices and fossil-fuel burning (Vitousek et al. 1997; Galloway and Cowling 2002). Changes in nitrogen and water availabilities may indirectly alter species composition, thus changing ecosystem functions through modifying resource use efficiencies of species (Reich et al. 1989; Yuan et al. 2005). Plants respond to changes in water and nitrogen availabilities by altering their phenology, morphology, biochemistry, and relative allocation to roots and shoots (Reich et al. 1989). Species adapted to low nutrient environments, including some C_4 grasses, may be displaced because of increased nutrient availability (Tilman 1987; Vitousek et al. 1997). Knapp and Medina (1999) and Silletti and Knapp (2001) found that the dominant and codominant C_4 species in the tallgrass prairies of Kansas responded differently to water and nitrogen manipulation. Little bluestem covers a large geographic area in the Great Plains and is especially dominant in the Sandhills of Nebraska. It is not known how this species will respond to such manipulation. Ecophysiological properties such as gas exchange, water and nitrogen use efficiencies, and water potential provide a mechanism for predicting the success of species in a community, as they are the first to respond to changes in resource availability. The objectives of this study were to determine the effects that nutrient addition and water stress have independently of one another, as well as in concert, on the ecophysiological responses of little bluestem.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The experiment was conducted in a greenhouse at the University of Nebraska-Lincoln. Forty (40) five-month-old little bluestem (*Schizachyrium scoparium*) plants, originating from seeds collected from natural communities in Nebraska, were obtained from the Nebraska Statewide Arboretum. Each plant was transferred into an 8 L pot filled with Cornell Mix and sand (1:1v). The soil mixture contained 2.4 ppm ammonium nitrate, 1.5 ppm superphosphate, and 0.6 ppm potassium chloride. Plants were randomly distributed into four treatment groups (10 plants per treatment group), with two levels of watering (watered and nonwatered) and two levels of fertilization (fertilized and nonfertilized). Plants in fertilized treatments were provided with 8 ml of Scott's Miracid (NPK 30-10-10). Fertilization was applied twice, at four weeks and at two weeks before the initiation of measurements. Water stress treatment was initiated by withholding water for 15 days, then rewatering on day 16 to determine the responses of plants to drought and recovery after rewatering. Day and night temperatures in the greenhouse were kept at 29°C ± 2°C and 20°C ± 2°C, respectively, and RH was 45% ± 5%. Maximum photosynthetic active radiation in the greenhouse averaged 1800 μmol m^{-2} s^{-1}. Soil moisture was determined in all pots throughout the study (23 days) using Time Domain Reflectometry (TDR technology, TH_2O portable soil moisture meter, Dynamax Inc., Houston, TX). Soil moisture probes (10 cm long) were carefully inserted next to the plants to avoid root damage, and data were integrated throughout the profile.

Measured Parameters

Net photosynthesis (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), and water-use efficiency (WUE = photosynthesis/transpiration, μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) at light saturation (Photosynthetically Active Radiation, PAR 2000 μmol m^{-2} s^{-1}) were followed during water deprivation, and after rewatering, using a portable infrared gas analyzer mounted with a LED light source (LI 6400-2B, LICOR Inc., Lincoln, NE). Measurements were conducted between 1000 and 1200 hr on eight plants

TABLE 1
REPEATED-MEASURE ANALYSIS FOR PHOTOSYNTHESIS (A), STOMATAL CONDUCTANCE (g_s), WATER POTENTIAL (ψ_w), WATER-USE EFFICIENCY (WUE), AND PHOTOSYNTHETIC NITROGEN-USE EFFICIENCY (PNUE) DURING A DRY-DOWN PERIOD IN FERTILIZED AND NONFERTILIZED LITTLE BLUESTEM PLANTS

		F-values				
Source	df	A $n = 224$	g_s $n = 224$	ψ_w $n = 208$	WUE $n = 224$	PNUE $n = 224$
Date (dry-down)	7	61.53**	21.31**	3.04*	1.74^{ns}	61.92**
Fertilization	1	1.16^{ns}	4.20*	0.77^{ns}	6.83*	34.14**
Date x Fertilization	7	0.74^{ns}	3.80*	0.54^{ns}	2.37*	6.27**

*, ** = F-values significant at 0.05 and 0.01, respectively. ^{ns} = not significant at P 0.05.

per treatment, using the uppermost fully expanded leaves. Photosynthetic nitrogen-use efficiency (PNUE = A/N content) was calculated. To determine leaf nitrogen content, several fully expanded leaves were selected from each plant, oven-dried for 72 hr at 75°C, and ground to 20 mm with a Thomas Scientific Wiley Mini-Mill (Swedesboro, NJ). The ground samples were analyzed for carbon and nitrogen content using a Costech ECS 4010 (Valencia, CA). Leaf nitrogen contents averaged 1.24% ± 0.07% and 2.78% ± 0.08% in nonfertilized and fertilized plants, respectively. Water potential (ψ_w, MPa) was determined on all individuals used in gas exchange measurements with a PMS Instruments Pressure Chamber (Albany, OR).

Statistical Analysis

Repeated-measures analysis were used to evaluate plant responses to water and nitrogen over time (23 days). Data were analyzed using the Mixed Models Procedure in SAS (SAS Institute 1998). Means were separated using the pairwise mean comparisons in SAS (the probability of difference, Pdiff statement in SAS, $P < 0.05$) (Steel et al. 1997).

RESULTS

Soil Moisture

Soil moisture content in pots receiving the drought treatment declined from 33% (field capacity) to 5% (15% of field capacity), 15 days after initiation of treatment. Repeated-measure analysis indicated that while soil moisture in pots declined significantly over time, pots within a sampled date did not differ significantly from one another; therefore, values were averaged across all treatments within dates (Fig. 1).

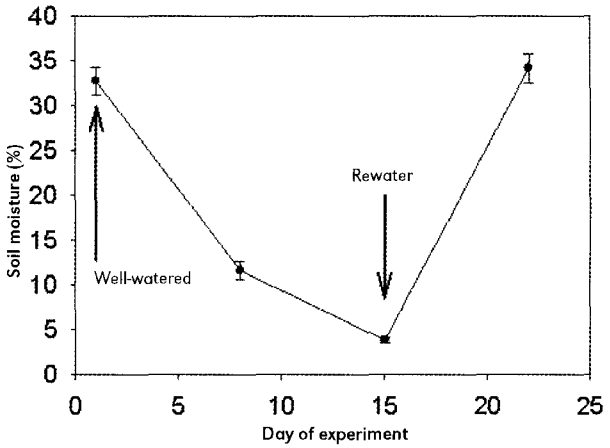

Figure 1. Volumetric soil moisture content (%), with standard errors bars, in fertilized and nonfertilized little bluestem pots (averaged across treatments) during a dry-down period and recovery (rewater) in a greenhouse.

Gas Exchange Measurements

The watered pots, both fertilized and nonfertilized, showed little daily variability in measured parameters throughout the study; therefore, data from each fertilization treatment were pooled across dates and are presented as means of "well-watered" in Figures 1 through 4. Net photosynthesis (A) measured at light saturation did not differ between fertilization treatments under well-watered conditions (average 25 μmol m^{-2} s^{-1}), and declined immediately after the onset of drought. Significant differences in A were observed on day 3 of water deprivation, where

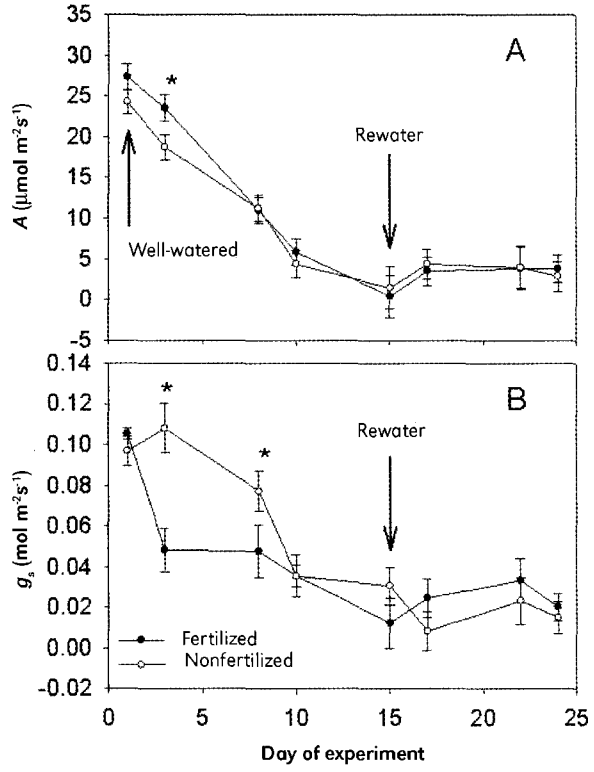

Figure 2. Changes in (A) net photosynthesis (A, μmol m^{-2} s^{-1}), and (B) stomatal conductance (g_s, mol m^{-2} s^{-1}), with standard errors bars, in fertilized and nonfertilized little bluestem plants during a dry-down period and recovery (rewater) in a greenhouse. Asterisk (*) denotes significant differences between treatments within dates at P 0.05 ($n_{(well\text{-}watered)} = 16$; $n_{(dry\text{-}down)} = 8$).

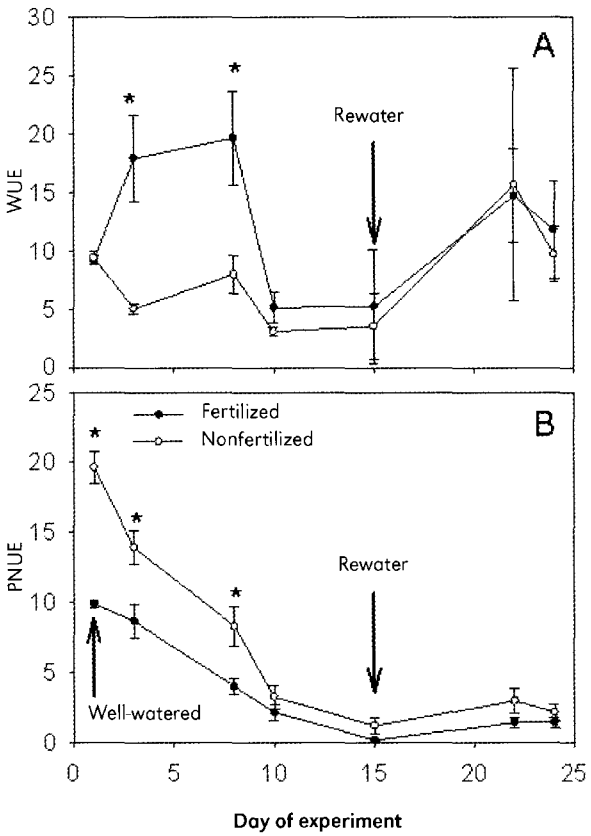

Figure 3. Changes in (A) water-use efficiency (WUE, μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}), and (B) photosynthetic nitrogen-use efficiency (PNUE, μmol CO_2 m^{-2} s^{-1}/%N), with standard errors bars, in fertilized and nonfertilized little bluestem plants during a dry-down period and recovery (rewater) in the greenhouse. Asterisk (*) denotes significant differences between treatments within dates at P 0.05 ($n_{(well\text{-}watered)} = 16$; $n_{(dry\text{-}down)} = 8$).

the nonfertilized treatment displayed a sharper reduction in photosynthetic rates compared to the fertilized treatment (23% and 14%, respectively) (Fig. 2A). Differences between treatments disappeared on day 8, and A values approached zero on day 15 of water deprivation. Recovery of A after rewatering was modest (16%) in both treatments (Fig. 2A).

Fertilized plants showed an immediate reduction in stomatal conductance (g_s) after drought initiation, and g_s continued to decline until rewatering (Fig. 2B). On day 15 of water deprivation, stomata were almost completely shut and rates were close to zero. In contrast, stomata in the nonfertilized plants remained relatively open, with significant differences observed between treatments on days 3 and 8; differences disappeared on day 10 of water deprivation. Similar to photosynthesis, recovery of stomatal conductance after rewatering was modest, and plants showed no significant recovery by day 23 (8 days after rewatering).

Water-use efficiency (WUE) in the fertilized treatment was significantly higher than in the nonfertilized treatment on days 3 and 8 of water deprivation. The WUE declined by 74% and 67% in fertilized and nonfertilized treatments, respectively, in the 15 days of water deprivation. After rewatering, WUE increased threefold by day 23 in both fertilization treatments (Fig. 3A). Photosynthetic nitrogen-use efficiency (PNUE) was significantly higher in the nonfertilized than in the fertilized treatment during the first 8 days of water deprivation (Fig. 3B). These differences disappeared on day 10 and minimum values were reported on day 15. The PNUE showed a slight but insignificant increase in both fertilization treatments after rewatering.

Leaf water potential (Ψ_w) did not vary among fertilization treatments, declining twofold three days after the initiation of drought (Fig. 4). We were unable to record

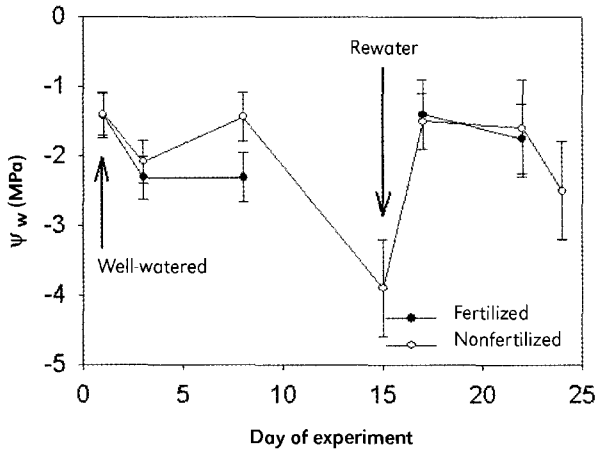

Figure 4. Leaf water potential (ψ_w, MPa), with standard errors bars, in fertilized and nonfertilized little bluestem plants during a dry-down period and recovery (rewater) in a greenhouse. Asterisk (*) denotes significant differences between treatments within dates at P 0.05 ($n_{(well\text{-}watered)} = 16$; $n_{(dry\text{-}down)} = 8$).

Ψ_w for fertilized treatment on day 15 due to the leaves wilting. All plants were able to partially recover from drought stress after rewatering. Water potential improved from -4 to -1.5 MPa two days after the rewatering in both treatments.

DISCUSSION

Fertilization had no positive impact on any of the measured parameters when water was available, possibly because little bluestem often dominates low-nutrient environments and is characterized by high nitrogen-use efficiency. Therefore, the addition of nitrogen did not confer a physiological advantage under well-watered conditions (Foster 2002; Wedin 2004). Similarly, Silletti and Knapp (2001) stated that nitrogen fertilization had no significant effect on stomatal conductance and photosynthesis in the C_4 *Andropogon gerardii* Vitman in the tallgrass prairies in Kansas. Photosynthesis, g_s, ψ_w, and PNUE were high in well-watered plants under both fertilization treatments and declined progressively with the increase in water stress. This positive response to water suggests that little bluestem will be able to take advantage of precipitation changes under the scenario of increased rainfall due to climate change. Similar positive responses were reported in the field for little bluestem (Knapp 1984), *Panicum virgatum* L. (Knapp 1984), and *Sorghastrum nutans* (L.) Nash, but not for *Andropogon gerardii,* which did not respond to the addition of water in the field (Silletti and Knapp 2001). Significant differences appeared between fertilized and nonfertilized pots under moderate drought (between days 3 and 8 of water deprivation). Plants in fertilized pots maintained higher photosynthetic rates, lower stomatal conductance, and consequently a greater WUE relative to nonfertilized plants. Wright et al. (2001) suggested that photosynthetic enzyme production increases with higher N content, permitting the plants to maintain higher rates of *A* at lower g_s, resulting in higher WUE. On the other hand, low WUE and low leaf nitrogen concentration, when associated with high g_s, as was observed in the nonfertilized treatment, result in high PNUE (Vandenboogaard et al. 1995). Field and greenhouse studies on the physiology of warm-season grasses in the Great Plains have shown that little bluestem exhibited higher stomatal conductance (Awada et al. 2002; Eggemeyer 2005), lower water-use efficiency, and higher nitrogen-use efficiency (Tjoelker et al. 2005) than *Andropogon gerardii, Calamovilfa longifolia* (Hook.) Scribn., and *Panicum virgatum* when water was not limiting. Differences between grasses disappeared under drought (Awada et al. 2002; Eggemeyer 2005).

The lower g_s and the slightly more negative Ψ_w in fertilized plants on days 3 and 8 may reflect greater total biomass and transpiring leaf area in fertilized relative to nonfertilized plants (Kalapos et al. 1996; Owensby et al. 1997; Silletti and Knapp 2001). Water potential displayed a moderate decline in the first week of water deprivation, then a sharp drop from day 10 to 15. Similarly, other studies have shown that in the early stages of drought stress, the decline in g_s helps maintain less negative leaf Ψ_w, but a drastic decline in Ψ_w is usually observed afterward (McKersie et al. 1996; Awada et al. 2003, 2004).

Photosynthetic nitrogen-use efficiency (PNUE) is a measure of carbon gain per unit of leaf nitrogen used. This variable has been considered an important leaf trait for characterizing species in relation to their leaf physiology and their resource use strategy (Hikosaka 2004). High PNUE may have important ecological impacts because it can facilitate either higher growth rates under conditions of adequate nitrogen supply, or maintain plant function under N deficiency (Wedin 2004). In comparison to other species, little bluestem had comparable PNUE to the C_4 *Bouteloua gracilis* and significantly higher PNUE than the C_3 *Stipa comata* and *Koeleria cristata* grown in the field in Nebraska (Wedin and Awada unpublished data). Our results have shown that in the first 8 days of the experiment, nonfertilized plants had a significantly higher PNUE than the fertilized plants. Anten et al. (1998) suggested that C_4 grasses growing under very low nutrient availability achieved photosynthetic productivity as high

as others growing under better nutrient conditions due to the high leaf PNUE in those species. Differences in leaf traits disappeared under severe drought stress (between days 10 and 15), and recovery was modest in measured traits after rewatering. Heckathorn and DeLucia (1994) suggested that under severe drought, leaf nitrogen is usually translocated to the roots in order to protect nitrogen from being lost with senescence. This translocation may affect the PNUE and limit the recovery of the photosynthetic system after rewatering.

CONCLUSION

Changes in nutrient availability and precipitation are expected to affect the distribution and competitive ability of C_4 species such as little bluestem. Although commonly used in global change research, greenhouse studies of resource availability and plant response have obvious limitations. Nevertheless, our results have shown that little bluestem will likely respond differently to different climate change scenarios. Little bluestem will likely be able to take advantage of an increase in precipitation; however, the increase in fertilization under this scenario will probably not provide any competitive advantage to this species. Fertilization under moderate drought stress would potentially favor little bluestem, illustrated by the increase in photosynthetic rates and water use efficiencies. We have shown that simple nitrogen enrichment confers no physiological advantage to little bluestem under severe drought stress. This may indicate that little bluestem is susceptible to competitive exclusion under severe drought by (1) the deeper-rooted C_3 shrubs and trees in the Sandhills (Eggemeyer 2005) that are more capable of exploiting the increase in available nitrogen (Wedin 2004) and can better obtain water from deeper in the soil profile relative to grasses (Briggs et al. 2005; Eggemeyer 2005; Lett and Knapp 2005); (2) the deeper-rooted C_4 grasses that have been shown to be less susceptible to drought stress than the relatively shallow-rooted little bluestem (Weaver 1954; Stubbendieck et al. 1985; Wedin 2004); and (3) the C_3 grasses that, in addition to being able to better exploit available nitrogen, emerge earlier in the growing season than the C_4 grasses (Wedin 2004) when water is most available in the Sandhills of Nebraska.

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