Response of Grass Seedlings to Smoke-Water and Smoke-Derived Butenolide in the Absence of Macronutrients (Nitrogen, Phosphorus, and Potassium)

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Abstract

Compositional transformation of South African semiarid grasslands and savannas owing to changes in soil nutrient status and fire-linked attributes is often reported. However, mechanisms of change are not fully understood. Currently, plant-derived smoke has attracted much attention as a fire-related cue responsible for stimulating germination and seedling growth. However, there is very little documentation on how these fire-linked factors, such as smoke, and soil macronutrients, such as nitrogen (N), phosphorus (P), and potassium (K), interact to effect seedling growth of grasses. In this study, smoke-responsive (*Themeda triandra*) and less smoke-responsive species (*Eragrostis curvula* and *Panicum maximum*) were tested with different concentrations and combinations of smoke-water and smoke-isolated butenolide with or without added N, P, or K under greenhouse conditions. In the absence of N, P, or K, smoke-water and butenolide treatments enhanced a number of seedling growth parameters of *T. triandra*. In contrast, exclusion of N from the nutrient solution significantly reduced shoot length, seedling weight, root volume, and vigor index of *E. curvula* at all tested concentrations of smoke-water and butenolide solutions compared to the control. In the presence of N, P, and K, smoke-water and butenolide suppressed seedling growth of *P. maximum*, whereas the absence of one of these macronutrients had a small promotory effect on some parameters. This study may assist in understanding the postfire seedling dynamics of grasses.

Resumen

La transformación de la composición de los pastizales y sabanas de la zona semiárida de Sud-África debido a cambios en el estado de nutrientes del suelo y atributos vinculados con el fuego, son reportados frecuentemente. Sin embargo, no se comprende plenamente los mecanismos de este cambio. Actualmente, el humo derivado de plantas ha atraído mucho la atención como un factor relacionado con el fuego siendo este responsable de estimular el crecimiento y germinación de las plántulas. Sin embargo, hay poca documentación en cómo estos factores relacionados con el fuego, tales como humo, y los macro-nutrientes del suelo, como el nitrógeno (N), el fósforo (P) y el potasio (K) interactúan afectando el crecimiento de las plántulas de las gramíneas. En este estudio, especies como (*Themeda triandra*) que responde al humo y especies con una respuesta menor al humo como (*Eragrostis curvula* y *Panicum maximum*) se probaron con diferentes concentraciones y combinaciones de agua-humo y humo con butenolide con o sin N, P, o K bajo condiciones de invernadero. En la ausencia de N, P, o K los tratamientos de agua-humo y butenolide mejoran un gran número de parámetros del crecimiento de las plántulas de *T. triandra*. En cambio, la exclusión del N de la solución de nutrientes redujo significativamente la longitud del tallo, peso de la plántula, volumen de la raíz y el índice de vigor de *E. curvula* en todas las concentraciones probadas de las soluciones de agua-humo y butenolide comparadas con el control. En presencia de N, P y K, el humo-agua y el butenolide suprimieron el crecimiento de las plántulas de *P. maximum*, mientras que la ausencia de uno de estos macro-nutrientes tiene un pequeño efecto promotor en algunos parámetros. Este estudio puede ayudar al entendimiento de la dinámica de las plántulas después del fuego en las gramíneas.

Key Words: competition, growth, nutrients, Poaceae, smoke constituents

INTRODUCTION

Grassland and savanna communities are the major components of the vegetation in the semiarid regions of South Africa, which support large numbers of herbivores and prevent soil erosion (Smithen and Schulze 1982; Milton and Dean 1995). Plant community dynamics are mostly governed by rainfall variation (O'Connor and Roux 1995; O'Connor et al. 2001), ungulate grazing (O'Connor 1991), and land use systems (Parsons et al. 1997). Fire is generally regarded as one of the major driving forces of grasslands if not the major factor. It is more important than just influencing species composition (O'Connor 1991; Snyman 2003). Fire frequency causes contrasting shifts in species composition and diversity (Everson and Tainton 1984). For instance, the persistence of populations of the economically important bunchgrass *Themeda triandra* in the grassland depends on the reoccurrence of fire at frequent intervals (Everson and Tainton 1984; Morgan and Lunt 1999). In the absence of fire for longer periods, the short native species that predominantly grow well in nutrient-poor habitats decline in vigor and abundance giving space to relatively taller species that normally dominate fertile habitats (e.g., *Eragrostis curvula*; Everson and Tainton 1984).

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Another major compositional transformation of the grassland is often reported when nutrient status of the grassland is modified due to fertilization or fire (Fynn et al. 2003; Fynn and O'Connor 2005; Fynn et al. 2005). With increases in soil nitrogen (N) resources, certain species (e.g., *E. curvula*) come into dominance displacing *T. triandra* (Fynn 2004). Hence, soil substrate (edaphic) differences between burnt and unburnt sites (Fynn et al. 2003), compounded with high deposits of fire-associated cues of smoke and temperature (Ghebrehiwot et al. 2009), may differently influence the recruitment and seedling growth of species.

In recent years the role of fire and plant-derived smoke on germination and regeneration ecology of species has attracted the attention of biologists (Van Staden et al. 2000). Accordingly, gaseous and aqueous solutions of plant-derived smoke have been shown to influence both the germination and seedling growth of many species differently (Baxter et al. 1994; Van Staden et al. 2000). A compound isolated from plant-derived smoke (Van Staden et al. 2004) known as butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) is responsible for enhancing seed germination of many plant species. These smoke solutions exhibit hormone-like responses in many species and interact with gibberellins, cytokinins, abscisic acid, and ethylene in photoblastic and thermodormant seeds (Van Staden et al. 2000; Jain et al. 2008). Many studies have examined the effect of bio-regulators and/or fertilizers (nitrogen, phosphorus, and potassium [NPK]), but no studies have yet examined the combined effect of smoke solutions and NPK simultaneously. Hence, the effect of plant-derived smoke as a new factor influencing plant growth (Van Staden et al. 2000), its interaction with major soil macronutrients (NPK), as a fundamental theme in plant physiology and ecology needs to be understood (Materechera et al. 1998). This is important because factors affecting seed germination and initial seedling growth are among the primary determinants of the distribution of fully grown plant communities in nature.

In the semiarid grassland and savanna ecosystems of southern Africa, the existence of competitive displacement trends among species in response to fire-induced nutrient dynamics such as nitrogen (N), phosphorus (P), and potassium (K) is often reported (Fynn et al. 2003). Frequent fire causes losses of large amounts of carbon, N, and sulfur present in the herbage through volatilization and erosion of ash, which subsequently results in significant losses of organic matter content from the soil surface (Ojima et al. 1994; Fynn et al. 2003). As a result, N becomes more deficient to plants due to a decrease in the rate of its mineralization. Postfire semiarid grasslands are also typified by rapid leaching of K and P, and as a consequence such elements become less available to plants thereby limiting growth (Chapman 1967). This indicates that grass species that dominate postfire habitats thrive more on nutrient-poor soils. Therefore, soil nutrient gradients between burnt and unburnt grasslands may play a significant role in competitive displacement of the species involved (Ghebrehiwot et al. 2006). On the other hand, NH_4^+ is a direct product of biomass burning (disturbance), and significant increases in plant available NH_4^+ and NO_3^- can also occur following fire. Thus, fire can increase the rate of N mineralization whereby immediately after fire, there can be an accumulation of mineralized NH_4^+, which is then converted to NO_3^- by nitrifying bacteria and can increase the availability of mineral N in both canopy and interspace areas for several months following fire. The literature shows that the effect of fire on nutrient availability, especially N, is less predictable (Debano and Conrad 1978; Grogan et al. 2000; Fynn et al. 2003). Studies have also reported the interaction of fire with soil nutrients and its influence on grasslands (White et al. 2006; Harris et al. 2008). The nutrient availability to soil is not always consistent and mainly depends on the intensity and frequency of fires (Richards et al. 2011).

The effect of fire (burning) on grassland nutrient dynamics and its subsequent effect on the growth, diversity, and abundance of grassland species is one of the best studied subjects (Fynn and O'Connor 2005). These studies have contributed much towards the promotion of predictive understanding of community change in response to disturbance. Though much is known of how trends in species composition alter in response to fire, the underlying mechanisms responsible for this strong fire–species relationship syndrome is not fully understood. Particularly, how the inverse relationship between postfire increases in fire-associated cues of smoke and fire-induced changes in the availability of major soil macronutrients (NPK) interact in influencing the initial seedling growth of the component grass species is still unknown. South African perennial grassland species are poorly represented in fire-stimulated research and there is very little or no information on the response of species to smoke and its interaction with soil nutrients. The specific objective was to determine the effect of plant-derived smoke-water and a smoke-isolated butenolide compound, at different concentrations in absence of macronutrients, on various growth parameters of seedlings of three grass species from South African semiarid grasslands. The hypothesis of this study is that smoke-responsive grass species promote seedling growth in absence of nutrients when treated with smoke solutions.

MATERIALS AND METHODS

Seed Collection and Germination

Grass species *E. curvula* Nees (Weeping lovegrass), *T. triandra* Forssk. (Red grass), and *Panicum maximum* Jacq. (Guinea grass) were selected on the basis of their response to fire frequency (Everson and Tainton 1984; Fynn 2004). The seeds of these species were collected in 2008 from the naturally occurring populations around Pietermaritzburg (lat 30°24′S, long 29°40′E), KwaZulu-Natal, South Africa. The seeds were cleaned and stored at ambient laboratory conditions (25°C) until they were used. Subsequently, these seeds were germinated in 90-mm Petri dishes on two layers of Whatman No. 1 filter paper wetted with 4-mL distilled water. Petri dishes were incubated in a plant growth chamber set at 25°C with 16/8 h day/night photoperiod (102 $\mu mol \cdot m^{-2} \cdot s^{-1}$). After 1 wk, germinants of all three species were transplanted into pots for growth experiments.

Nutrient, Smoke-Water, and Butenolide Solutions

For the preparation of smoke extract, dry *T. triandra* Forssk (Poaceae) leaf material (5 kg) was burnt in a 20-L metal drum, and the smoke generated from it was passed through a glass

Table 1. Effect of nutrient solution (50% Hoagland's) with or without N, P, or K on seedling growth parameters of grass species grown under greenhouse conditions ($n = 3$).

Treatment	Shoot length (mm)	Root length (mm)	Leaves (no)	Roots (no)	Seedling weight (g)	Root volume (cm^3)[1]
Themeda triandra						
Control (HS)[2]	127 ± 3.14 b	93 ± 2.60 b	7.8 ± 0.83 a	3.6 ± 0.46 a	0.270 ± 0.01 b	0.325 ± 0.019 b
No N	109 ± 0.26 c	90 ± 0.66 b	4.9 ± 0.66 b	3.0 ± 0.00 a	0.122 ± 0.002 c	0.270 ± 0.006 c
No P	106 ± 0.20 c	111 ± 0.13 a	4.8 ± 0.13 b	3.0 ± 0.06 a	0.259 ± 0.001 b	0.403 ± 0.003 b
No K	161 ± 0.00 a	112 ± 0.00 a	5.1 ± 0.13 b	3.1 ± 0.13 a	0.338 ± 0.001 a	0.520 ± 0.000 a
Eragrostis curvula						
HS	216 ± 1.56 a	160 ± 13.98 ab	7.3 ± 0.63 a	7.4 ± 0.11 a	0.307 ± 0.002 a	0.392 ± 0.025 ab
No N	82 ± 2.31 b	139 ± 12.28 b	4.0 ± 0.00 b	4.4 ± 0.14 b	0.303 ± 0.051 a	0.615 ± 0.159 a
No P	27 ± 1.31 c	159 ± 16.79 ab	3.0 ± 0.11 b	2.5 ± 0.17 b	0.057 ± 0.004 b	0.105 ± 0.014 b
No K	59 ± 20.69 b	219 ± 38.61 a	3.4 ± 0.11 b	2.9 ± 0.40 b	0.134 ± 0.054 b	0.379 ± 0.171 ab
Panicum maximum						
HS	307 ± 23.36 a	208 ± 29.04 a	5.0 ± 0.17 b	4.4 ± 0.06 a	1.399 ± 0.215 a	1.214 ± 0.176 a
No N	52 ± 0.06 c	83 ± 0.00 d	2.1 ± 0.13 d	2.0 ± 0.06 d	0.032 ± 0.002 c	0.050 ± 0.010 c
No P	74 ± 2.77 c	120 ± 5.83 c	3.1 ± 0.24 c	2.8 ± 0.06 c	0.053 ± 0.007 c	0.080 ± 0.006 c
No K	181 ± 0.06 b	172 ± 0.00 b	6.0 ± 0.06 a	3.8 ± 0.13 b	0.481 ± 0.001 b	0.730 ± 0.000 b

[1]Mean values (± SE) of each species in column with different letters are significantly different by Tukey's test ($P < 0.05$).
[2]HS indicates Hoagland's nutrient solution; N, nitrogen; P, phosphorus; and K, potassium.

column containing 500 mL of tap water for 45 min (Baxter et al. 1994). The three different concentrations of smoke-water used in this study were prepared by diluting 1 mL of smoke-extract with 250, 500, and 1 000 mL distilled water (1:250, 1:500, and 1:1000 v/v). A pure butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) used in this experiment was isolated from plant-derived smoke-water as described by Van Staden et al. (2004). This compound is now termed karrikinolide (Flematti et al. 2009). The concentrations of the butenolide tested were 10^{-7}, 10^{-8}, and 10^{-9} M. In this trial half-strength (50%) Hoagland's nutrient solution (HS; Hoagland and Snyder 1933) was used for seedling growth. The effects of three macronutrients N, P, and K with or without different concentrations of test solutions (smoke-water and butenolide) were studied by eliminating each one of these from half-strength HS. Each treatment was then represented as -N, -P, and -K.

Plant Growth Experiments

The pot experiments were conducted in a greenhouse with a relative humidity of 50%. The seedlings were grown in 20.4-cm diameter pots of 4 L filled with sterile quartz sand moistened with HS, -N, -P, and -K (controls) solutions. One hundred milliliters of these solutions were added to respective pots once weekly. Each pot consisted of five seedlings with three replicates per treatment. Subsequently (after 3 d), once weekly a similar volume of treatment solutions and distilled water (control) were added to these pots. Since smoke solutions were prepared using distilled water, the control pots were supplied with distilled water. The pots were arranged randomly in the greenhouse for each species with a midday photosynthetic photon flux density of $405 \pm 7.5\ \mu mol \cdot m^{-2} \cdot s^{-1}$ at $22 \pm 2.5°C$. The seedlings of *P. maximum*, *T. triandra*, and *E. curvula* were harvested respectively at 45, 46, and 88 d after transplanting, and various seedling growth parameters were recorded. Root volume was determined according to the method of Burdett (1979). A seedling vigor index formula as reported by Abdul-Baki and Anderson (1973) was modified and calculated as: seedling vigor index = [mean shoot length (mm) + mean root length (mm)] × mean seedling weight (g).

Statistical Analysis

The data showed normal distribution according to the Shapiro-Wilk test and therefore were not transformed before analysis. Data obtained from the different seedling growth parameters were subjected to one-way analysis of variance (ANOVA), and means were separated using Tukey's test at a 5% level of significance ($P < 0.05$). A general ANOVA was conducted to evaluate the main effects and their interactions (GenStat 2008).

RESULTS

The nutrient deficiency showed inhibitory effects on seedling growth of studied grass species. There was only one exception for seedlings of *T. triandra* that show a significant increase in shoot and root length, weight, and root volume in the absence of K (Table 1). Most of the growth parameters examined for *E. curvula* and *P. maximum* seedlings without N, P, or K showed significantly lower values than the control (Table 1).

Seedlings of *T. triandra* did not show significant results for most of the growth parameters when grown with all nutrients and treated with different concentrations of smoke-water and butenolide solutions (Fig. 1A and Table 2). However, the seedling vigor index was significantly greater than the control at a smoke-water concentration of 1:250 (Fig. 2A). Smoke-water and butenolide treatments under deficiency of N-, P-, or K-promoted seedling growth parameters, and in many cases these parameters were significantly different from the control (Fig. 1A and Table 1). Smoke-water and butenolide-treated seedlings at all dilutions showed significantly higher vigor indices than untreated seedlings when there was no supply of N (Fig. 2A). With the elimination of either P or K, some dilutions

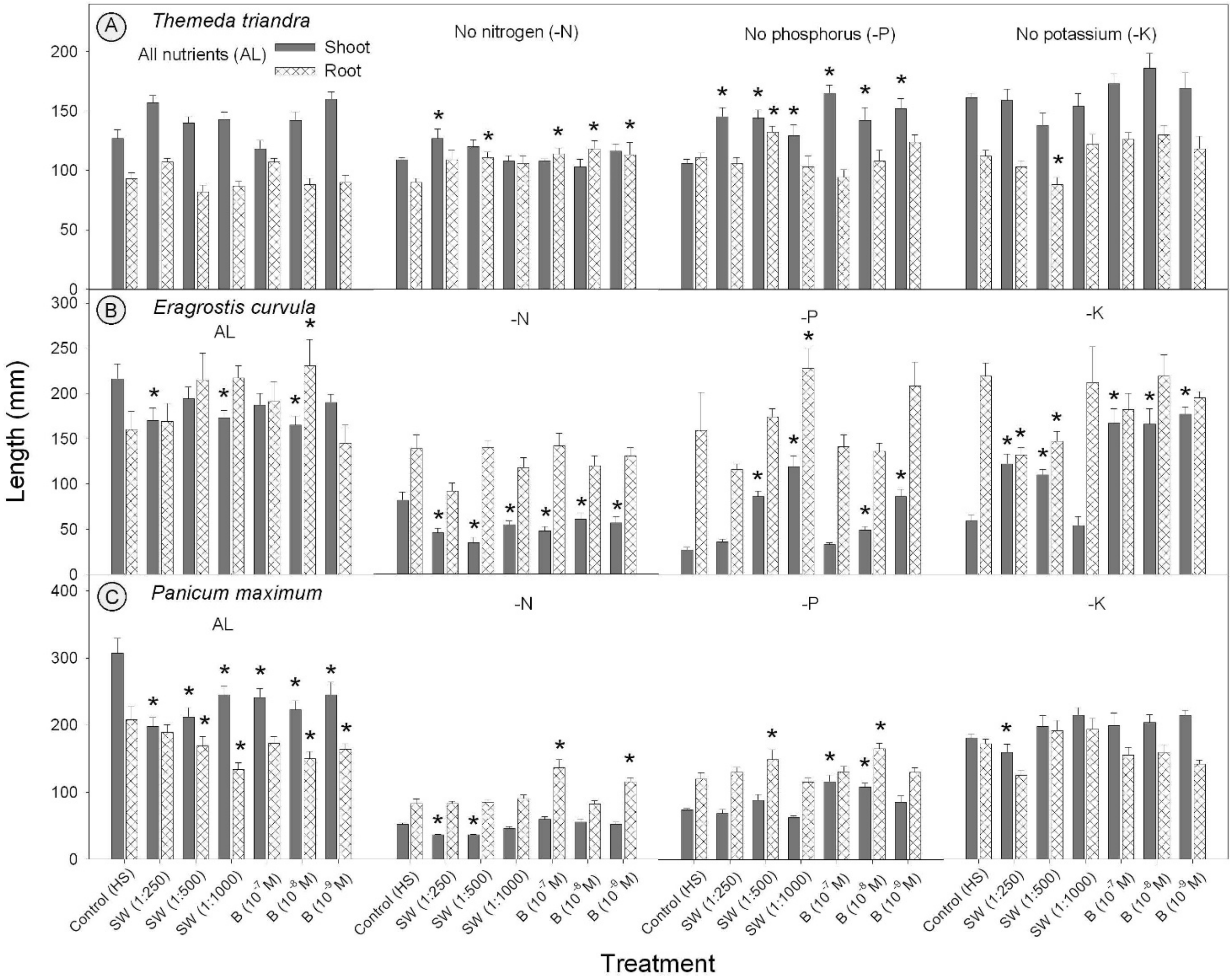

Figure 1. Effect of different concentrations of smoke-water (SW) and butenolide (B) on shoot and root growth of grass species in the absence of N, P, or K under greenhouse conditions. Bar (± SE) with asterisk symbol is significantly different to the respective control by Tukey's test ($P < 0.05$; $n = 3$).

of these treatments significantly increased seedling vigor index (Fig. 2A).

The seedlings of *E. curvula* grown with all nutrients and treated with smoke-water (1:250 and 1:1 000) and butenolide solution (10^{-8} M) showed a significant reduction in shoot length compared to the control (Fig. 1B). In treated seedlings, the number of leaves was less with a significant decrease in the number of roots (data not shown). However, the seedling weight, root volume, and vigor index were all highest at a smoke-water concentration of 1:1 000 (Table 2 and Fig. 2B). Exclusion of N from the nutrient solution significantly suppressed shoot length, seedling weight, root volume, and vigor index at all tested concentrations of smoke-water and butenolide (Figs. 1B and 2B; Table 2). Some concentrations of these treatments significantly increased shoot length, other seedling growth parameters, and vigor indices in the absence of either P or K (Figs. 1B and 2B; Table 2).

Smoke-water and butenolide-treated seedlings of *P. maximum* grown with all concentrations and nutrients showed a significant reduction in shoot length, seedling weight, and vigor index compared to nontreated seedlings (Figs. 1C and 2C; Table 2). The removal of N, P, or K generally enhanced most of the seedling growth parameters with smoke-water and butenolide solutions, and at some concentrations these results were significantly different from the control (Figs. 1C and 2C; Table 2). The results of ANOVA show that the probability levels of treatment, nutrient, concentration and their interactions were highly significant for *T. triandra* and *P. maximum* (Table 3).

DISCUSSION

The seedling growth parameters of *T. triandra* studied were generally better in the absence of K. This finding suggests that this grass may not perform well in soils with high or even low levels of K. When these seedlings were grown without N or P and treated with smoke-water and butenolide solutions, in

Table 2. Effect of different concentrations of smoke-water (SW) and butenolide (B) in absence of nitrogen (N), phosphorus (P), or potassium (K) on seedling growth parameters of grass species under greenhouse conditions ($n = 3$).

	Themeda triandra		*Eragrostis curvula*		*Panicum maximum*	
Treatment	Seedling wt. (g)[1]	Root vol. (cm^3)[2]	Seedling wt. (g)	Root vol. (cm^3)	Seedling wt. (g)	Root vol. (cm^3)
HS						
(Control)	0.27 ± 0.01 ab	0.32 ± 0.03 ab	0.30 ± 0.00 bcd	0.39 ± 0.02 d	1.39 ± 0.21 a	1.21 ± 0.17 a
SW (1:250)	0.41 ± 0.09 a	0.50 ± 0.09 a	0.20 ± 0.00 d	0.49 ± 0.02 cd	0.62 ± 0.07 c	0.81 ± 0.06 ab
SW (1:500)	0.32 ± 0.11 ab	0.34 ± 0.09 ab	0.27 ± 0.00 cd	0.59 ± 0.01 bcd	0.61 ± 0.07 c	0.67 ± 0.13 b
SW (1:1 000)	0.31 ± 0.04 ab	0.40 ± 0.01 ab	0.45 ± 0.03 a	0.90 ± 0.07 a	0.78 ± 0.13 bc	0.68 ± 0.14 b
B (10^{-7} M)	0.25 ± 0.04 b	0.24 ± 0.07 b	0.40 ± 0.01 ab	0.77 ± 0.00 ab	0.90 ± 0.07 bc	0.97 ± 0.09 ab
B (10^{-8} M)	0.22 ± 0.02 b	0.25 ± 0.00 b	0.27 ± 0.00 cd	0.51 ± 0.04 cd	0.75 ± 0.12 bc	0.77 ± 0.18 b
B (10^{-9} M)	0.28 ± 0.06 ab	0.34 ± 0.07 ab	0.33 ± 0.05 bc	0.69 ± 0.13 abc	0.99 ± 0.08 b	0.92 ± 0.11 ab
No N						
(Control)	0.12 ± 0.00 b	0.27 ± 0.00 b	0.30 ± 0.05 a	0.61 ± 0.15 a	0.03 ± 0.00 d	0.05 ± 0.01 c
SW (1:250)	0.17 ± 0.02 ab	0.30 ± 0.04 b	0.02 ± 0.00 b	0.05 ± 0.01 b	0.03 ± 0.00 cd	0.08 ± 0.01 bc
SW (1:500)	0.20 ± 0.02 a	0.31 ± 0.06 b	0.03 ± 0.00 b	0.09 ± 0.00 b	0.03 ± 0.00 cd	0.08 ± 0.00 bc
SW (1:1 000)	0.18 ± 0.03 a	0.46 ± 0.07 a	0.03 ± 0.03 b	0.05 ± 0.01 b	0.05 ± 0.00 bc	0.12 ± 0.02 b
B (10^{-7} M)	0.20 ± 0.02 a	0.32 ± 0.06 b	0.05 ± 0.00 b	0.09 ± 0.01 b	0.08 ± 0.01 a	0.20 ± 0.05 a
B (10^{-8} M)	0.21 ± 0.02 a	0.29 ± 0.04 b	0.04 ± 0.01 b	0.12 ± 0.03 b	0.08 ± 0.01 a	0.18 ± 0.04 a
B (10^{-9} M)	0.16 ± 0.03 ab	0.26 ± 0.07 b	0.12 ± 0.02 b	0.15 ± 0.02 b	0.08 ± 0.01 ab	0.18 ± 0.02 a
No P						
(Control)	0.25 ± 0.00 c	0.40 ± 0.00 bc	0.05 ± 0.00 c	0.10 ± 0.01 c	0.05 ± 0.00 d	0.08 ± 0.00 d
SW (1:250)	0.32 ± 0.03 abc	0.42 ± 0.13 bc	0.02 ± 0.00 c	0.11 ± 0.02 c	0.12 ± 0.04 bcd	0.34 ± 0.06 ab
SW (1:500)	0.40 ± 0.04 a	0.67 ± 0.03 a	0.12 ± 0.00 b	0.29 ± 0.00 b	0.24 ± 0.12 a	0.43 ± 0.18 a
SW (1:1 000)	0.21 ± 0.03 c	0.35 ± 0.07 c	0.19 ± 0.01 a	0.43 ± 0.01 a	0.11 ± 0.01 cd	0.16 ± 0.04 cd
B (10^{-7} M)	0.31 ± 0.03 abc	0.36 ± 0.05 bc	0.03 ± 0.00 c	0.09 ± 0.00 c	0.23 ± 0.08 ab	0.37 ± 0.07 ab
B (10^{-8} M)	0.28 ± 0.04 bc	0.41 ± 0.06 bc	0.05 ± 0.00 c	0.12 ± 0.02 c	0.23 ± 0.00 ab	0.30 ± 0.05 abc
B (10^{-9} M)	0.38 ± 0.07 ab	0.55 ± 0.07 ab	0.10 ± 0.00 b	0.21 ± 0.02 b	0.20 ± 0.05 abc	0.24 ± 0.06 bc
No K						
(Control)	0.33 ± 0.00 bc	0.52 ± 0.00 ab	0.13 ± 0.05 abc	0.37 ± 0.17 ab	0.48 ± 0.00 b	0.73 ± 0.00 ab
SW (1:250)	0.29 ± 0.02 c	0.31 ± 0.05 b	0.12 ± 0.01 c	0.33 ± 0.02 ab	0.64 ± 0.08 ab	0.81 ± 0.05 ab
SW (1:500)	0.28 ± 0.06 c	0.44 ± 0.09 ab	0.12 ± 0.00 bc	0.31 ± 0.01 ab	0.85 ± 0.31 a	1.01 ± 0.30 a
SW (1:1 000)	0.35 ± 0.07 bc	0.60 ± 0.07 a	0.13 ± 0.03 abc	0.22 ± 0.04 b	0.84 ± 0.20 a	1.04 ± 0.11 a
B (10^{-7} M)	0.44 ± 0.07 ab	0.55 ± 0.10 a	0.21 ± 0.02 a	0.48 ± 0.04 a	0.50 ± 0.13 b	0.57 ± 0.13 b
B (10^{-8} M)	0.51 ± 0.07 a	0.60 ± 0.08 a	0.21 ± 0.00 abc	0.44 ± 0.02 a	0.68 ± 0.15 ab	0.72 ± 0.24 ab
B (10^{-9} M)	0.43 ± 0.03 ab	0.50 ± 0.08 ab	0.21 ± 0.02 ab	0.48 ± 0.02 a	0.83 ± 0.03 a	0.89 ± 0.09 ab

[1]wt. indicates weight; vol., volume; and HS, Hoagland's nutrient solution.
[2]Mean values (± SE) of each species and nutrient condition in column with different letters are significantly different by Tukey's test ($P < 0.05$).

many cases they exhibited longer shoots and roots than control-treated seedlings. Under these conditions the other growth parameters such as number of leaves and roots, seedling weight, root volume, and vigor index were significantly enhanced. Interestingly, all smoke treatments and concentrations without N demonstrated the best seedling vigor index (Fig. 2A). These findings clearly indicate that seedlings of *T. triandra* can perform well after burns under nutrient-deficient soil conditions (Ghebrehiwot et al. 2006; Fynn and Naiken 2009). This could be an advantage for many fire-climax grasses to compete with other species such as *E. curvula*, which requires high nutrients. Everson and Tainton (1984) reported that irrespective of soil nutrients, *T. triandra* requires regular burning to sustain its dominance in grasslands. The productivity of *T. triandra* declined when there was a long interval between fires (Morgan and Lunt 1999). In South Africa, restoration of native *T. triandra* is highly preferred to improve productivity for subsistence and commercial agricultural practices and for the conservation of biodiversity (Everson et al. 2009).

E. curvula seedlings responded better when supplied with all nutrients (Ghebrehiwot et al. 2006; Fynn and Naiken 2009). The elimination of N, P, or K negatively affected growth indicating the necessity of soil nutrients. The shoot growth and number of roots were significantly suppressed when they were grown with all nutrients and treated with smoke solutions. When N was removed, all the smoke treatments significantly inhibited shoot growth, seedling weight, root volume, and seedling vigor. These results suggest that deficiency of N in postburn soils will not promote growth of *E. curvula* seedlings. Studies have shown that addition of N to the grassland resulted in the replacement of *T. triandra* by *E. curvula* (Fynn and O'Connor 2005), which indicates that N is important for the growth of *E. curvula*. The presence of both P and K

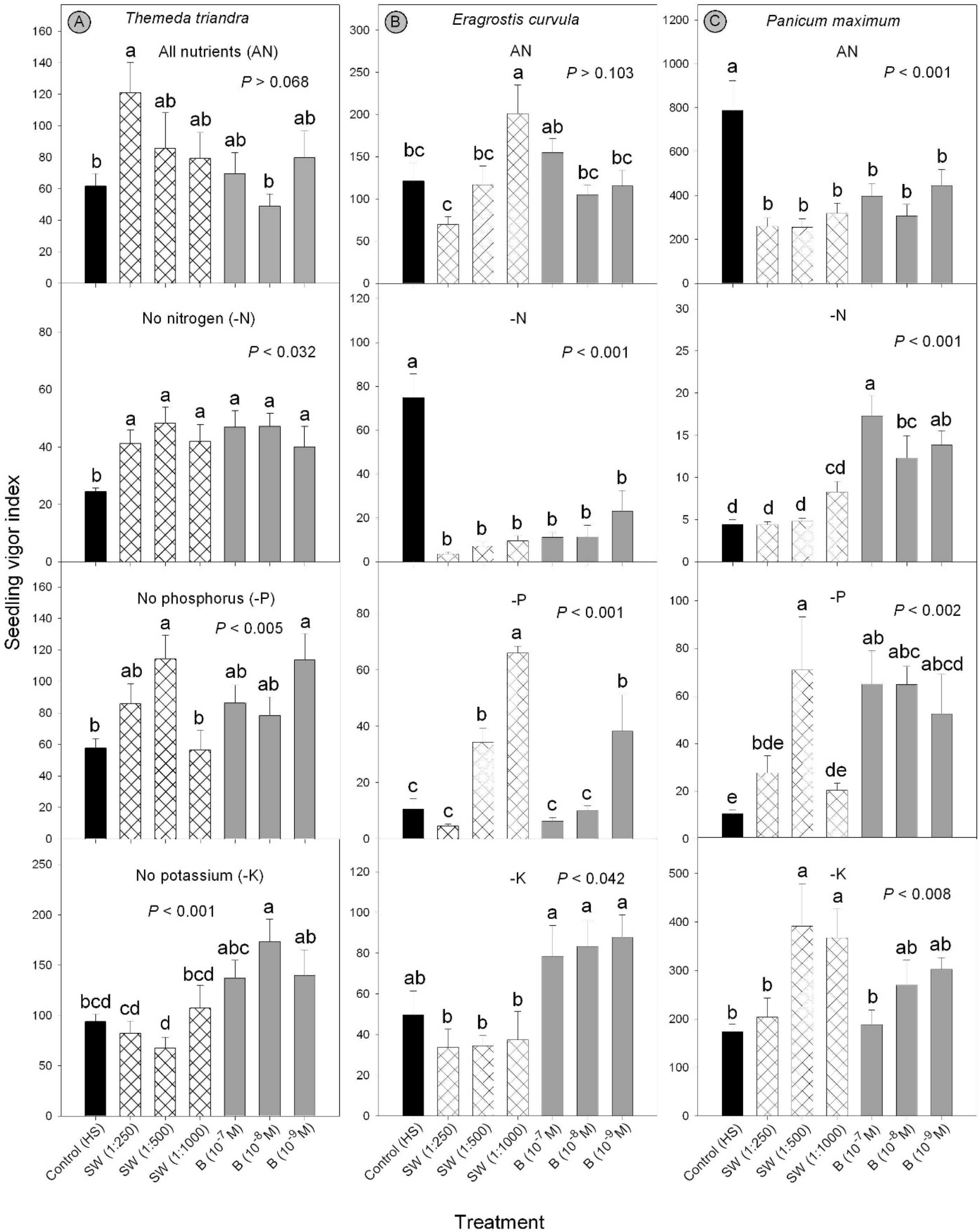

Figure 2. Effect of different concentrations of smoke-water (SW) and butenolide (B) on seedling vigor index of grass species in the absence of N, P, or K under greenhouse conditions. Bar (± SE) with different letters is significantly different by Tukey's test ($P < 0.05$; $n = 3$).

Table 3. General ANOVA with main effects and their interactions for seedling length (shoot + root length) of three mesic grassland species.

Source	df[1]	Sum of squares	Mean squares	*F* value	*P* level[2]
Themeda triandra					
T[3]	2	38 855	19 427	8.08	< 0.001
N	3	57 454	19 151	7.97	< 0.001
C	6	73 741	12 290	5.11	< 0.001
T × N	6	42 047	7 008	2.92	0.009
N × C	18	139 760	7 764	3.23	< 0.001
Residual	384	922 997	2 404		
Total	419	1 274 853			
Eragrostis curvula					
T	2	33 633	16 816	0.68	0.508
N	3	72 334	24 111	0.97	0.406
C	6	321 247	53 541	2.16	0.046
T × N	6	260 698	43 450	1.75	0.108
N × C	18	818 054	45 447	1.83	0.020
Residual	384	9 523 304	24 800		
Total	419	11 029 270			
Panicum maximum					
T	2	69 040	34 520	6.78	< 0.001
N	3	926 544	308 848	60.67	< 0.001
C	6	641 681	106 947	21.01	< 0.001
T × N	6	818 637	136 440	26.80	< 0.001
N × C	18	2 769 687	153 871	30.23	< 0.001
Residual	384	1 954 779	5 091		
Total	419	7 180 368			

[1]Degrees of freedom.
[2]Probability level.
[3]T indicates treatment; N, nutrient; and C, concentration.

(-N treatment) in the soils after fire therefore can be responsible for suppressing the growth of *E. curvula* seedlings (Fig. 2B). Snyman (2004) showed that *E. curvula* seedling establishment decreased heavily as a result of fire. The butenolide treatment significantly ($P < 0.042$) improved seedling vigor of *E. curvula* over smoke-water without K (Fig. 2B). This can be attributed to pure butenolide, which can effectively promote the growth of seedlings in the absence of K. On the other hand, smoke-water contains several other compounds and a newly discovered inhibitor 3,4,5-trimethylfuran-2(*5H*)-one, which is related to butenolide. This molecule inhibits germination and has an ability to reduce the promotory effect of butenolide (Light et al. 2010). The presence of this inhibitory molecule in smoke-water may interact with micro- or macronutrients in the soils, generating suppressive effects. However, this needs further investigation.

P. maximum seedlings grew better in the presence of all nutrients. This result suggests that *P. maximum* can compete well with other species in highly fertile soils. The performance of *P. maximum* was superior to *T. triandra* and *E. curvula* in high-fertility treatments (Fynn 2004; Ghebrehiwot et al. 2006). In contrast, when these seedlings were exposed to smoke-water and butenolide solutions, they showed inhibitory effects on most of the seedling growth parameters examined. However, the response of *P. maximum* seedlings to smoke treatments was better in the absence of N, P, or K. These findings suggest that *P. maximum* can show better performance after fires with less macronutrients in soils. In this study, *T. triandra* and *P. maximum* seedlings responded positively to all treatments (Table 3).

MANAGEMENT IMPLICATIONS

Fire (burning) is an integral part and an efficient tool in the management and maintenance of South African mesic grasslands, which determines the course of species turnover (Tainton et al. 1978; Westoby et al. 1989). Hence an understanding of plant responses to fire and fire-induced attributes in the environment is of paramount significance. Furthermore, knowledge of the action and interaction of the fire-associated factors (e.g., smoke, nutrients, and hydrological characteristics) in influencing grassland species is essential when making rangeland fire decisions in fertile and infertile areas in South Africa.

Our findings demonstrate that though nutrients had an overriding effect on seedling growth, the interaction of the smoke solutions with macronutrients also appeared to be important. Seedling growth performance of bunch grasses *T. triandra*, *E. curvula*, and *P. maximum* responded differently to the interactive effects of nutrient levels (Fynn 2004) and the smoke solutions (Ghebrehiwot et al. 2009). These results further suggest that *T. triandra* can be more competitively advantaged over the other two species when there is frequent burning, which causes significant decline in soil nutrients and simultaneously deposits the smoke cue in the habitat. On the other hand, such a situation of low nutrient (N, P, and K) levels caused by frequent fires can significantly limit the growth of *E. curvula* (Fynn and Naiken 2009). Frequent burning or an application of smoke-water, smoke-isolated karrikinolides, and inhibitors present in smoke can be a useful tool in encouraging the germination and seedling growth of *T. triandra*, with equivalent curbing effects of the germination and growth of *E. curvula* (Ghebrehiwot et al. 2009; Fynn and Naiken 2009). These findings have further implications for understanding the response of perennial grassland species to fire, grazing, and grassland fertilization, which significantly shift the resource base.

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