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Effects of water and nutrient availability on morphological, physiological, and biochemical traits of one invasive and one native grass of a Neotropical savanna

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Highlights

- The invasive species accumulates biomass by using resources more efficiently
- The invasive species showed higher photon fluxes and lower pigment concentrations
- The invasive species showed a weaker oxidative response to water stress
- The species grow similarly in scenarios of low water and nutrient availability
- Unnatural nutrient inputs and high-water availability benefit the invasive species

ABSTRACT

The cerrado is a Neotropical savanna characterized by a soil and vegetation mosaic where plants endure dystrophic soils and seasonal drought. Dry spells or flooding are the main environmental stress native species face in their growth period. African grasses are common invasive species, jeopardizing the biodiversity by displacing native species and outgrowing them. Invasive species may benefit from human interventions that increase nutrient availability in natural areas and may respond differently than natives to environmental conditions. Therefore, we compared the performance of one native (*Schizachyrium microstachyum*) and one invasive (*Melinis minutiflora*) grass in different conditions of water and nutrient availability simulating possible cerrado scenarios. Five-week-old seedlings were submitted to different irrigation treatments (simulating dry spells, normal rainfall, and flooding) and fertilization treatments (high or low nutrient availability) for four weeks, and were analyzed for morphological (leaf area, length of the shoot, number of tillers, seedling dry weight, and root:shoot ratio) and physiological parameters (chlorophyll fluorescence, pigment concentration, nutrient content, and biochemical assays). There was a trend for the invasive species to show better responses to water stress by growing more profusely, showing an even higher effect when the soil was richer in nutrients. The invasive species may outcompete the native species by using nutrients and water more efficiently, showing a weaker oxidative response to drought and fertilization. The native species would perform at a similar pace to the invasive species in conditions of less water and nutrient

availability, whereas unnatural fertilization inputs and high-water availability would benefit the invasive species.

Keywords: invasive species; water stress; nutrient use; biomarkers; seedling growth; cerrado;

INTRODUCTION

Invasive species are considered a threat to biodiversity worldwide (Gurevitch and Padilla, 2004). In the Neotropical savannas, they have been used as substitutes of native grasses for forage or in interventions for the recovery of degraded areas (Zenni and Ziller, 2011). African grasses adapt well to Neotropical savannas and can easily become invasive species (Pivello et al., 1999; Martins et al., 2011; Zenni and Ziller, 2011). Environmental conditions in the South American savannas are similar to their original habitat, and African grasses have the potential to outcompete native plant species (Baruch et al., 1989; Pivello et al., 1999; Martins et al., 2011; Zenni et al. 2019).

The Brazilian cerrados are Neotropical savannas composed of a mosaic of vegetation and soil types (Ribeiro and Walter, 2008). They have a tropical climate with a well-defined dry season (May to September) and an abundant herbaceous layer represented mainly by grasses (Coutinho, 1990; Ribeiro and Walter, 2008), which comprise over 650 native species (*sensu* Flora do Brasil, 2020 Database). These species show profuse growth during the rainy season, which slows as water availability in the soil decreases towards the dry season (Monasterio and Sarmiento, 1976; Sarmiento, 1992). In general, cerrado soils are acidic dystrophic soils (Reatto et al., 2008). Local conditions of soil humidity and fertility vary not only with season but also along the vegetation and soil gradients according to changes in soil type and to the distance to water sources (Coutinho, 1990; Reatto et al., 2008). Furthermore, anthropogenic activities such as fertilization in agricultural fields and fossil fuel burning in urban areas may become sources of nutrient input to cerrado areas by atmospheric deposition or runoff (Jordan and Weller, 1996; Vitousek et al., 1997). Since the invasive grasses are usually opportunistic, responding more rapidly and efficiently to higher availability of resources (Baruch and Bilbao, 1999; Alpert et al., 2000; Baruch and Jackson, 2005; Silva and Haridasan, 2007), these altered conditions could influence their invasiveness (Alpert et al., 2000).

African grasses can accumulate greater amounts of biomass than native Neotropical grasses (Baruch and Bilbao, 1999; Martins et al., 2011). These species are considered more resilient to

defoliation than cerrado species, as an evolutionary response to the large herbivores present in African (Simoes and Baruch, 1991; Klink, 1994). Therefore, African grasses tend to displace native grasses and form dominant vegetation patches, diminishing biodiversity (Pivello et al., 1999; Baruch and Jackson, 2005; Martins et al., 2011). On the other hand, native grasses are considered resilient to the adverse conditions in the Cerrado, being adapted to fire, low nutrient availability, and seasonal drought (Coutinho, 1990; Sarmiento, 1992). Neotropical savanna plants are considered resilient to drought and fire by evading or tolerating such conditions through resprouting from underground organs and seasonal dormancy (Monasterio and Sarmiento, 1976; Sarmiento, 1992; Pausas et al., 2018).

While fire and drought are the main stress factors during the dry season (Coutinho, 1990), periodic dry spells or local flooding during the rainy season are the main environmental stress native species face in their growth period (Monasterio and Sarmiento, 1976; Sarmiento, 1992; Assad et al., 1993). Dry spells are not uncommon in the cerrado, occurring mainly in the middle of the rainy season and lasting for up to two weeks (Assad et al., 1993). Constraints to water availability during the growth period may affect plant metabolism. For instance, water shortage may arrest photosynthesis and increase water consumption and the use of nutrient reserves, causing changes to morphological traits, shifts in plant biomass investment or an overall reduction in plant growth (Jones et al., 1980; Ludlow, 1980; Wilson et al., 1980; Baruch and Fisher, 1991). Flooding, on the other hand, may occur on poorly drained soils in the cerrado (Ribeiro and Walter, 2008), affecting plant metabolism and plant development by altering root aerobic respiration (Crawford, 1982). Plants survive flooding through strategies such as the development of adventitious rootlets and aerenchyma tissue, which help to tolerate the anoxic situation (Jackson and Drew, 1984)., both conditions may change species response and growth pace, affecting their establishment and competition with other species (Barger et al., 2003).

In this study, we compared the performance of two grass species (one native to the cerrado and one invasive) in different conditions of soil water and nutrient availability simulating possible

cerrado scenarios. The native species *Schizachyrium microstachyum* (Ham.) Roseng. B. R. Arril. & Izag and the invasive species *Melinis minutiflora* P. Beauv. were subjected to treatments that simulate dry spells or flooding during the rainy season, under two possible scenarios of nutrient availability. As environmental stress triggers many responses in plants, from subcellular to structural levels, several morphological, physiological, and biochemical parameters were assessed. Simultaneously studying these responses may help to reveal some underlying mechanisms of their morphological responses. By this comparison, we aimed to answer the following questions: (1) Is the response between species different among water treatments? (2) If so, which conditions most privilege the invasive species over the native? (3) Does the invasive species show adaptations that confer advantages and enhance its invasiveness? Since *M. minutiflora* is an opportunist invasive species, we hypothesized that it would perform better in high nutrient and water availability, showing a better physiological (reduced oxidative stress, increased photosynthetic capacity, increased nutrient content) and morphological (higher biomass and growth) response. As the native species is used to poor well-drained soils, we expect a better physiological response to water shortage in comparison to *M. minutiflora*. We expect a better enzymatic response to anoxic conditions in *M. minutiflora*, favoring its colonization in flooded environments.

MATERIALS AND METHODS

Species and seed collection

The African grass *M. minutiflora* is a widespread species in the cerrado, with a long-term invasion process dating from the 19th century (Zenni and Ziller, 2011). It is an aggressive invasive species, displacing native flora, and forming monodominant vegetation patches (Pivello et al., 1999; Martins et al., 2011). The native grass *S. microstachyum* is a widely distributed species in the cerrado grasslands and savannas, showing a high percentage of fertile seeds, seed viability, and germination, which facilitates manipulation in the laboratory (Carmona et al., 1998; Aires et al., 2014). Mature panicles of *M. minutiflora* and *S. microstachyum* were harvested manually in May and July 2010,

respectively, at the Reserva Ecológica do IBGE (15°56'41"S, 47°53'07"W), 25 km South of Brasília, Brazil. Seeds were collected by walking imaginary transects and harvesting all inflorescences found. Seeds were homogenized and stored in paper bags at room temperature (ca. 25ºC) until use.

Experimental design and sampling

Seeds were germinated in Petri dishes with moistened cotton and filter paper, at 37°C/22°C and 10h/14h photoperiod, simulating field conditions (Castro-Neves and Miranda, 1996; Andrade and Miranda, 2014). One-week-old seedlings were transplanted to 24 clay pots (20 cm height; 20 cm top diameter) filled with 1 kg of soil. Soil was prepared by mixing equal parts of organic matter and mineral soil (adapted from Simoes and Baruch, 1991). Ten seedlings per plot were cultivated for four weeks in a climatic chamber (28°C and 12h of light 156 ± 5.37 W m^{-2}) simulating field conditions in the growing season. The number of seedlings per pot was chosen on pre-trials and based on standardized ecotoxicological lab experiments using grass species (ISO 11269–2:2012). Soil was watered daily until saturation (300 mL of water). After this period, the two smallest individuals of each pot were discarded, ensuring all replicates had similarly healthy and representative individuals (adapted from ISO 11269–2:2012).

The remaining seedlings were subjected to different water treatments, simulating possible cerrado scenarios: watered every day (1d; control group simulating normal rainfall); watered every five days (5d; short dry spells); watered every ten days (10d; long dry spells); overwatered every day (Ow; flooding). Soil was always moistened to saturation, except for Ow in which the soil was watered until a 2 cm layer of water was observed aboveground. Each treatment was applied to a set of six pots (n = 6), from which three were fertilized at the beginning of the water treatment (0.5 g of solid NPK 10–10–10), and the other three were not fertilized. In this four (water treatment) × two (presence of fertilizer) factorial design, each combination had three replicates (n = 3). This number was achieved by pondering effort and statistical analysis. Seedlings were cultivated in these conditions for four weeks and were analyzed for morphological (leaf area, length of the shoot, number of tillers, seedling

dry weight, and root:shoot ratio) and physiological parameters (chlorophyll fluorescence, pigments concentration, nutrient content, and biochemical assays). Each seedling was considered a sub-replicate, and each pot was considered a replicate (n = 3) for all morphological parameters, except the leaf area. For the physiological parameters and leaf area, each seedling was considered as replicates, selected at the moment of harvest.

Before the harvesting for morphological parameters, chlorophyll$_a$ fluorescence was measured on the adaxial side of ten mature leaves. Each leaf belonged to a different seedling and was selected according to apparent general health and advantageous position for the measurement (n = 10; at least two individuals per each replicate/treatment). For this matter, plants were pre-adapted to darkness for 30 min, and minimal fluorescence (F_0; fluorescence intensity with all PS_{II} reaction centers open while the thylakoid membrane is in the non-energized state) was measured. Then, maximal fluorescence (F_m; fluorescence intensity with all PS_{II} reaction centers closed) was measured by applying a saturating pulse of white light (0.7 s, >1 500 mol m^{-2} s^{-1} of white light). All measurements were taken using a pulse-amplitude-modulated fluorimeter (FMS 2, Hansatech Instruments, Norfolk, England). The obtained values were used to calculate the non-photochemical quenching (NPQ) and the optimum quantum yield (F_v/F_m). The formula described by Bilger and Björkman (1990), ($NPQ = (F_m - F_m')/F_m'$), was used to calculate NPQ. For the optimum quantum yield, the factor F_v corresponds to the variable fluorescence in dark-adapted leaves (F_m-F_0; van Kooten and Snel 1990).

After fluorescence measurements, the shoot of each seedling was harvested at soil level, immediately measured, and counted for tillers. To calculate the average leaf area, five mature leaves from different individuals were selected according to their apparent general health (n = 5; at least one individual per each replicate/treatment) and measured using a desk multifunctional printer and image analysis software (ImageJ). All roots were removed carefully and washed. For each experimental replicate, four seedlings were randomly drawn and oven-dried for 48 h at 70 ºC to determine the dry weight and then the concentration of nitrate and phosphate, and the remaining four were snap-frozen and stored at –80ºC for biochemical assays (pigments and MDA concentration; SOD and G-POX

activity). The oven-dried replicates were weighed, and the root:shoot ratio was estimated by dividing the total dry root biomass by the total dry shoot biomass of each pot (n = 3).

The concentration of nitrate and phosphate was determined according to the HACH KIT method (DR/2000 Spectrophotometer). Here, each replicate consisted of a mix of dry leaves from the larger individual that was oven dried (n = 3; one individual per replicate/treatment). The leaves were ground with a mortar and homogenized in distilled water in a proportion of 1:2 (w/v). The plant extract was filtered with activated coal and filter papers (180 μm of thickness and 11 μm pore size for particle retention). The leachate was analyzed according to protocols 8151 (Program 363, 500 ηm) for nitrate and 8183 (Program 510, 890 ηm) for phosphate. Results were presented as the percentage of the dry weight.

For pigment and MDA concentrations as well as enzymatic activity, frozen samples were used. In all cases, each replicate consisted of a mix of leaves from one seedling selected randomly from the frozen individuals (n = 5; at least one individual per each replicate/treatment). In pigment concentration determination, leaves were ground with a mortar with the extraction buffer (a solution of cold acetone and 50 mM Tris buffer, pH 7.8 in a proportion of 80:20, v/v) for pigment extraction. Homogenates were centrifuged at 5,000 g for 10 min. The absorbance of the supernatant was determined at 470, 537, 647, and 663 ηm in microplates. The concentrations were calculated as follows (Sims and Gamon 2002):

$$Chl_a = 0.01373\ A_{663} - 0.000897\ A_{537} - 0.003046\ A_{647}$$

$$Chl_b = 0.02405\ A_{647} - 0.004305\ A_{537} - 0.005507\ A_{663}$$

$$\text{Carotenoids} = ((A_{470} - (17.1 \times (Chl_a + Chl_b) - 9.479 \times \text{Anthocyanins})) / 119{,}26$$

Lipid peroxidation is indicative of oxidative damage to the cell membranes. It was estimated by measuring malondialdehyde (MDA) production (Dhindsa et al., 1981). An amount of 0.5 g of frozen leaves was ground to a powder in a mortar with liquid nitrogen and then homogenized with a solution of TCA (0,1% w/v; g/100 ml). Samples were centrifuged, and an aliquot was mixed with

another solution (TCA 20% w/v containing 0.5% m/v TBA) and left to react for 30 min at 95ºC. MDA concentration was estimated by subtracting the nonspecific absorption at 600 ηm from the absorption at 532 ηm using an absorbance coefficient of extinction (ε), 155 mM^{-1} cm^{-1} (Elkahoui et al., 2005). Absorbance was measured with a Thermo Fisher Scientific (Waltham, USA) spectrophotometer (Genesys 10-uv S).

For assessment of enzymatic activity of the antioxidant system, leaves were ground to a powder in a mortar with liquid nitrogen. Then, they were homogenized with an extraction buffer that had concentrations of 100mM of phosphate buffer (pH 7.5) and 0.5 mM of EDTA. After centrifugation (10,000 g, 20 min; Howcroft et al., 2011), the supernatant (enzyme extract) was used for the determination of guaiacol peroxidase (G-POX) and superoxide dismutase (SOD) activity. For G-POX, the reaction mixture had solute concentrations of 10 mM of phosphate buffer (pH 6.1), 12 mM of hydrogen peroxide, 96 mM of guaiacol, to which 5 µL enzyme extract was added. Absorbance was recorded at 470 ηm for 5 min, and the specific activity was calculated using the 26.6 mM^{-1} cm^{-1} molar extinction coefficient (Castillo et al., 1984). SOD activity was estimated by recording the enzyme-induced decrease in absorbance of formazone formed by the nitro-blue tetrazolium with the superoxide radicals (Dhindsa et al., 1981). The reaction mixture had solute concentrations of 13mM of methionine, 25 mM of nitro-blue tetrazolium chloride (NBT), 0.1 mM of EDTA, 50 mM of phosphate buffer (pH 7.8), 50 mM of calcium carbonate, to which 0.6 µl of enzyme extract was added. The reaction was started by adding a solution of 2 mM of riboflavin and placing the microplates under a 15 W fluorescent lamp for 15 min. The absorbance was then recorded at 560 ηm, and values were calculated based on the curve previously calculated with a standard (Activity = $-2.0789(Abs)^2 + 26.316(Abs) - 1.2766$). Protein concentration was determined in quadruplicate by the Bradford method (Bradford, 1976), at 595 nm, using bovine serum albumin (BSA) as the standard. G-POX and SOD activities were corrected by protein content and fresh weight, respectively. All enzymatic activities protocols were adapted for microplate reader, regarding proportions, and a Labsystem Multiskan EX microplate (Labsystems Inc., Franklin, MA) reader was used.

Data analysis

When the data did not present normal distribution, they were log or arcsine transformed. For each parameter, means were compared with factorial ANOVA using species (*S. microstachyum*, native; *M. minutiflora*, invasive), fertilization (with or without) and water treatment (1d, 5d, 10d, and Ow) as independent variables and multiple comparisons were carried out using the TukeyHSD test. Using a scaled covariance data matrix, a Principal Components Analysis (PCA) was carried out to explore and highlight the relationships and patterns between species and treatments, as well as to assess which were the most important parameters in explaining variation between species, as a response to water and nutrient availability. All data were analyzed using R software (R 3.6.3 for Windows; R Core Team, 2013). Graphs were built using the *ggplot2* (version 3.3.0; Wickham, 2016) and *vegan* packages (version 2.3-3, Oksanen et al., 2013).

RESULTS

Morphological traits showed a decreasing trend as water stress increased, but the over-watered treatment was closer to the control group (Figure 1). Species, water treatment, and fertilization individually affected morphological traits, but their interaction showed no significant effect (Table 1). However, we observed other significant interactions among pairs of variables (Table S1). Overall, the invasive species showed higher dry weight, leaf area, and shoot length than the native species when fertilized (Figure 1), and differences were similar among water treatments. The number of tillers of both species was not affected by water treatment when not fertilized. However, nutrient addition increased the number of tillers, and the effects were heightened if the soil was watered every day (Figure 1; significant interaction of water treatment and fertilization, $p < 0.001$, Table S1). The root:shoot ratio did not differ significantly between species in any treatment.

Results for chlorophyll fluorescence and photosynthetic pigment concentrations are shown in Table 2. The Fv/Fm parameter was affected by the over-watered treatment, with a significant reduction in maximum quantum yield. The native species showed lower Fv/Fm than the invasive

species when under high-stress conditions (10d and Ow). However, these differences were reduced with fertilization. NPQ was higher for the invasive species and was affected by moisture, increasing in 10d and 5d treatments, but unaffected by fertilization (Table 2). Pigments tended to be more concentrated in the native species, when compared to the invasive (Chl_a, $p < 0.001$; Chl_b, $p = 0.008$; Car, $p < 0.001$). Pigment concentrations were not affected by water treatment, and only $chlorophyll_a$ increased with fertilization. $Chlorophyll_{a/b}$ ratio differed among species, with a higher ratio for the native species ($p < 0.001$; Table 1 and 2). Moisture level changed the Chla/b ratio only in non-fertilized groups, showing significantly higher levels in the over-watered treatment if compared to the control group ($p = 0.029$).

Phosphate and nitrate concentrations were higher for the invasive species, especially when fertilized (Figure 2). However, differences among species were reduced when seedlings were not watered every day and were not fertilized.

Results of biomarkers for oxidative stress are summarized in Table 3. The native species showed higher MDA concentrations than the invasive species ($p < 0.001$). When not fertilized, plants from the control group were the only ones showing equivalent MDA concentration between species (Table 3). With fertilization, MDA concentration in the native species was reduced.

SOD activity differed between species in some scenarios ($p = 0.002$; Tables 1 and 3), showing that their oxidative response may be different. When fertilized, the SOD activity was higher for the native species than for the invasive species, and the difference increased with water availability. G-POX activity also differed among species ($p = 0.020$) in some combinations. In the invasive species, G-POX activity was not at all affected by water treatment, while in the native species, values tended to increase with soil water availability. This pattern was intensified when fertilization was added, with significantly higher values of G-POX activity in the Ow treatment in comparison to the same treatment for the invasive species ($p < 0.05$).

The PCA showed a clear separation between species (Figure 3). The axis PC1 explained 46% of the variation, while PC2 explained 24%. In PC1 scores were higher for length, nitrate (positive), and MDA (negative), while in PC2 the variation was due mostly to changes in the leaf pigment concentrations. *M. minutiflora* variables are strongly positively correlated with morphological parameters, F_v/F_m, nitrate, and NPQ, while *S. microstachyum* variables are correlated with higher $chlorophyll_{a/b}$ ratio and MDA content.

DISCUSSION

Dry spells are the most common stress that grasses in the Neotropical savannas endure during the rainy season (Monasterio and Sarmiento, 1976; Sarmiento, 1992; Assad et al., 1993). Flooding may occur on poorly drained soils, which are not always common to the cerrado (Reatto et al., 2008; Ribeiro and Walter, 2008). When poorly watered, both species showed similar growth pace, especially in unfertilized soils. However, in well-watered soils, *M. minutiflora* was able to accumulate higher biomass than *S. microstachyum*, and the native species was more negatively affected by flooding than the invasive species. Furthermore, there was a trend for the invasive species *M. minutiflora* to show better responses to both water stress conditions when the soil was richer in nutrients, growing more and using nutrients more efficiently. The establishment of invasive species may be related to the availability of resources, and plants are more likely to invade habitats where limitations are removed and resources are abundant (Galatowitsch et al., 1999; Alpert et al., 2000). The observed features confer a higher competitive advantage to the invasive species and may explain how *M. minutiflora* can displace native species in the Neotropical savannas (Pivello et al., 1999; Martins et al., 2011).

Biomass was not only greater in *M. minutiflora*, but also differently partitioned. Seedlings of the invasive species grew both higher and wider, showing a trend to produce more tillers and wider leaves, and to higher root investment. Wider leaves and a higher number of tillers could confer a better ability to compete for light and aboveground space, shading other species' seedlings and seeds

(Silva and Castro, 1989; Zenni et al., 2019), or even preventing seed rain from reaching the soil. Together, these attributes could hinder the establishment of other species. *Melinis minutiflora* patches are reported to impair tree regeneration in the cerrado, significantly reducing tree seedling survivorship as a result of light competition (Hoffmann and Haridasan, 2008). Baruch and Jackson (2005) also reported the success of this invasive species in Neotropical savannas, attributing it to a higher growth rate, leaf area, and biomass production. Although these traits may as well reduce the success of the seedlings of the invasive species, *M. minutiflora* shows massive seed production, with high viability and germination rates (Martins et al., 2009; Carmona and Martins, 2010), which may compensate for this intraspecific competition.

In addition, belowground root partitioning should be considered, as the reduction of root competition may enable increases in height and biomass of seedlings under high competition (Holl, 1998; Barger et al., 2003). The overall greater investments in roots showed by the invasive species could confer an advantage when competing for nutrients belowground. Also, the response of *M. minutiflora* to flooding suggests its greater root biomass may have been more efficient to avoid creating an anoxic environment. Since flooding impairs the aerobic respiration of roots, hindering plant metabolism and development (Crawford, 1982), the poor response of *S. microstachyum* suggests this species may not be as well adapted to poorly drained soils as *M. minutiflora*.

Although *S. microstachyum* showed higher concentrations of pigments, the difference between species reduced when fertilized. This difference did not seem to confer higher photosynthetic capacity to the native species, given the lower maximum potential quantum yield (F_v/F_m) values. Furthermore, the data show a higher $chlorophyll_{a/b}$ ratio for the native species, especially in the flood treatment. This response indicates oxidative stress since $chlorophyll_b$ is degraded before $chlorophyll_a$ in such conditions, increasing the ratio (Alberte et al., 1977; Ashraf and Habib-ur-Rehman, 1999; Huang et al., 2004). Furthermore, F_v/F_m decreased with flooding while NPQ increased. A decrease in F_v/F_m with flooding may indicate photodamage (Rengifo et al., 2005; Fernández, 2006), even though the values did not decrease below 0.71, which is considered the threshold for healthy plants (Bolhàr-

Nordenkampf and Öquist, 1993). Higher NPQ values are observed for plants under water stress (Correia et al., 2014), and the higher NPQ values for the invasive species might confer stronger protection against photoinhibition (Li et al., 2002).

When fertilized, pigment content increased for the invasive species, which also showed higher efficiency in allocating nutrients into leaves, given the higher nitrate concentration. This species may reutilize nitrogen more efficiently given its better ability to incorporate nutrients to leaves (Baruch and Jackson, 2005; Silva and Haridasan, 2007). These characteristics favor *M. minutiflora,* especially in areas with higher nitrogen deposition due to anthropogenic activities (Alpert et al., 2000). Furthermore, water stress does not seem to impair nutrient assimilation as fertilization did. However, in the unfertilized group with low water availability, nitrate concentration in the native species reached the levels of the invasive species. Native Neotropical savanna grasses under water deficit conditions tend to accumulate nitrogen in their leaves (Baruch, 1994). Also, the native species may better compete with the invasive species in situations of lower resource availability (Baruch and Fernández, 1993). As observed in the PCA, the points corresponding to the invasive plants in drought treatments without fertilization are closer to the native species group, suggesting that the performance of the invasive species was closer to the native species if under low water and nutrient availability.

Although the native Neotropical savanna grasses are adapted to seasonal drought and are reported to better tolerate drought than invasive species (Sarmiento, 1992; Baruch and Fernández, 1993), water constraints in the growth period were damaging. The oxidative stress parameters indicate that the native species showed a stronger response to water stress and fertilization at a biochemical level. However, this mechanism might not be sufficient to overcome water stress, as indicated by the reduced dry weight values during water stress and in higher oxidative damage during drought. MDA levels suggests elevated lipid peroxidation and damage to the cell membrane for the native species. This damage increased with drought for both species and is in accordance with their lower biomass accumulation. Other studies show an increase of MDA in plants under water deficit (Zhang and Kirkham, 1994; Lima et al., 2002; Correia et al., 2014), and drought-tolerant plants do

not present such high levels in MDA content in these conditions (Arora et al., 2002; Gill and Tuteja, 2010). Therefore, given its smaller MDA values, *M. minutiflora* seems to be more tolerant of water constraints than the native species. Nevertheless, fertilization seemed to diminish lipid peroxidation under drought. When fertilized, the native species was affected only in the driest treatment (10d), while the invasive species was not affected at all.

G-POX and SOD are important antioxidant enzymes. SOD is the first line of defense in the scavenging system of reactive oxygen species. It dismutates superoxide (O_2^-) into hydrogen peroxide, which can then be reduced by G-POX, which consumes H_2O_2 by oxidizing guaiacol. Although it was not possible to measure the activity of these enzymes in the 10d treatment for the native species due to lack of material, both enzymes seemed to have their activity enhanced with the combination of watering and fertilization. Water seems to make the nutrients more available, being diluted in the water, and acting synergistically with fertilization. Other studies showed increases in G-POX and SOD in grass species of rye and wheat exposed to chemical stress (Milone et al., 2003; Khan et al., 2007; Silva et al., 2013). Although fertilization had a positive effect on biomass accumulation, it also seemed to cause oxidative stress in the native species. This response may be a reflection of its adaptation to soils with low nutrient availability.

CONCLUSIONS

Overall, the invasive species may outcompete the native species by accumulating more biomass and growing faster, using nutrients and water more efficiently, and investing in wider leaves and a greater underground biomass. Also, invasive species maintained high photon fluxes despite the lower concentrations of photosynthetic pigments, and showed a weaker oxidative response to drought and fertilization. The native species would perform at a similar pace to the invasive species in conditions of less water and nutrient availability, whereas unnatural fertilization inputs and high-water availability would benefit the invasive species. In the cerrado region, sites that present natural low nutrient availability and well-drained soils could represent a situation where these native and invasive

grasses could compete at a similar pace. On the other hand, disturbed sites with anthropogenc inputs of nutrients, and especially in the rainy season, could favor the advance of *M. minutiflora* invasion.

Author statement

Carolina Musso: Conceptualization and design of methodology, Conduction of experiment, field and laboratory work; Collection, assembly and curation of data; Data analyses, visualization and statistical treatment; Writing and Final approval the version to be submitted.

Hudson G. V. Fontenele: Data visualization and analysis; Writing and critical revision of the article for important intellectual content; Final approval the version to be submitted.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aires SS, Sato MN, Miranda HS (2014) Seed characterization and direct sowing of native grass species as a management tool. Grass Forage Sci 69:470–478. https://doi.org/10.1111/gfs.12077

Alberte RS, Thornber JP, Fiscus EL (1977) Water Stress Effects on the Content and Organization of Chlorophyll in Mesophyll and Bundle Sheath Chloroplasts of Maize. Plant Physiol 59:351–353. https://doi.org/10.1104/pp.59.3.351

Alpert P, Bone E, Holzapfel C (2000) Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. Perspect Plant Ecol Evol Syst 3:52–66. https://doi.org/10.1078/1433-8319-00004

Andrade LAZ, Miranda HS (2014) The dynamics of the soil seed bank after a fire event in a woody savanna in central Brazil. Plant Ecol 215:1199–1209. https://doi.org/10.1007/s11258-014-0378-z

Arora A, Sairam RK, Srivastava GC (2002) Oxidative stress and antioxidative system in plants. Curr Sci 82:1227–1238

Ashraf M, Habib-ur-Rehman (1999) Interactive effects of nitrate and long-term waterlogging on growth, water relations, and gaseous exchange properties of maize (Zea mays L.). Plant Sci 144:35–43. https://doi.org/10.1016/S0168-9452(99)00055-2

Assad ED, Sano EE, Masutomo R, et al (1993) Veranicos na região dos cerrados brasileiros frequência e probabilidade de ocorrência. Pesqui Agropecuária Bras 28:993–1003

Barger NN, D'Antonio CM, Ghneim T, Cuevas E (2003) Constraints to colonization and growth of the African grass, Melinis minutiflora, in a Venezuelan savanna. Plant Ecol 167:31–43. https://doi.org/10.1023/A:1023903901286

Baruch Z (1994) Responses to drought and flooding in tropical forage grasses - II. Leaf water potential, photosynthesis rate and alcohol dehydrogenase activity. Plant Soil 164:97–105. https://doi.org/10.1007/BF00010115

Baruch Z, Bilbao B (1999) Effects of fire and defoliation on the life history of native and invader C4 grasses in a Neotropical savanna. Oecologia 119:510–520. https://doi.org/10.1007/s004420050814

Baruch Z, Fernández DS (1993) Water relations of native and introduced C4 grasses in a neotropical savanna. Oecologia 96:179–185. https://doi.org/10.1007/BF00317730

Baruch Z, Fisher M (1991) Factores climaticos y de competencia que afectan el desarrollo de Insplantulas de las especies forrajeras. In: Lascano CE, Spain J. M (eds) Establecimiento y Renovacion de Pasturas. Centro Internacional de Agricultura Tropical, pp 103–142

Baruch Z, Hernandez AB, y Miguel R, Montilla G (1989) Dinamica del crecimiento, fenologia y reparticion de biomasa gramineas nativas e introducidas de una sabana neotropical. Ecotropicos 2:1–13

Baruch Z, Jackson RB (2005) Responses of tropical native and invader C4 grasses to water stress, clipping and increased atmospheric CO2 concentration. Oecologia 145:522–532. https://doi.org/10.1007/s00442-005-0153-x

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by

measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. Photosynth Res 25:173–185. https://doi.org/10.1007/BF00033159

Bolhàr-Nordenkampf HR, Öquist G (1993) Chlorophyll fluorescence as a tool in photosynthesis research. In: Hall DO, Scurlock JMO, Bolhàr-Nordenkampf HR, et al., (eds) Photosynthesis and Production in a Changing Environment. Springer Netherlands, Dordrecht, pp 193–206

Bradford M (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem 72:248–254. https://doi.org/10.1006/abio.1976.9999

Carmona R, Martins CR (2010) Qualidade física, viabilidade e dormência de sementes recém-colhidas de capim-gordura (Melinis minutiflora P. Beauv.). Rev Bras Sementes 32:77–82. https://doi.org/10.1590/S0101-31222010000100009

Carmona R, Martins CR, Fávero AP (1998) Fatores que afetam a germinação de sementes de gramíneas nativas do cerrado. Rev Bras Sementes 20:16–22. https://doi.org/10.17801/0101-3122/rbs.v20n1p16-22

Castillo FJ, Penel C, Greppin H (1984) Peroxidase release induced by ozone in Sedum album leaves: Involvement of Ca2+. Plant Physiol 74:846–851. https://doi.org/10.1104/pp.74.4.846

Castro-Neves BM de, Miranda HS (1996) Efeitos do fogo no regime térmico do solo de um campo sujo de cerrado. In: Miranda HS, Saito CH, Dias BF de S (eds) Impacto de queimadas em áreas de Cerrado e Restinga. Universidade de Brasília, Brasília, Brazil, pp 20–30

Correia B, Pintó-Marijuan M, Neves L, et al (2014) Water stress and recovery in the performance of two Eucalyptus globulus clones: Physiological and biochemical profiles. Physiol Plant 150:580–592. https://doi.org/10.1111/ppl.12110

Coutinho LM (1990) Fire in the ecology of the Brazilian cerrado. In: Goldammer JG (ed) Fire in the tropical biota. Springer, Berlin, Germany, pp 82–105

Crawford RMM (1982) Physiological Responses to Flooding. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological Plant Ecology II. Springer Berlin Heidelberg, Berlin,

Heidelberg, pp 453–477

Dhindsa RS, Plumb-dhindsa P, Thorpe TA (1981) Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 32:93–101. https://doi.org/10.1093/jxb/32.1.93

Elkahoui S, Hernández JA, Abdelly C, et al (2005) Effects of salt on lipid peroxidation and antioxidant enzyme activities of Catharanthus roseus suspension cells. Plant Sci 168:607–613. https://doi.org/10.1016/j.plantsci.2004.09.006

Fernández MD (2006) Changes in photosynthesis and fluorescence in response to flooding in emerged and submerged leaves of Pouteria orinocoensis. Photosynthetica 44:32–38. https://doi.org/10.1007/s11099-005-0155-2

Flora do Brasil 2020 Database Flora do Brasil 2020. http://floradobrasil.jbrj.gov.br/. Accessed 14 Apr 2020

Galatowitsch SM, Anderson NO, Ascher PD (1999) Invasiveness in wetland plants in temperate North America. Wetlands 19:733–755. https://doi.org/10.1007/BF03161781

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gurevitch J, Padilla DK (2004) Are invasive species a major cause of extinctions? Trends Ecol Evol 19:470–474. https://doi.org/10.1016/j.tree.2004.07.005

Hoffmann WA, Haridasan M (2008) The invasive grass, Melinis minutiflora, inhibits tree regeneration in a Neotropical savanna. Austral Ecol 33:29–36. https://doi.org/10.1111/j.1442-9993.2007.01787.x

Holl KD (1998) Effects of above- and below-ground competition of shrubs and grass on Calophyllum brasiliense (Camb.) seedling growth in abandoned tropical pasture. For Ecol Manage 109:187–195. https://doi.org/10.1016/S0378-1127(98)00248-5

Howcroft CF, Gravato C, Amorim MJB, et al (2011) Biochemical characterization of

cholinesterases in Enchytraeus albidus and assessment of in vivo and in vitro effects of different soil properties, copper and phenmedipham. Ecotoxicology 20:119–130. https://doi.org/10.1007/s10646-010-0562-4

Huang XD, El-Alawi Y, Penrose DM, et al (2004) Responses of three grass species to creosote during phytoremediation. Environ Pollut 130:453–463. https://doi.org/10.1016/j.envpol.2003.12.018

ISO 11269-2 (2012) Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early growth of higher plants. https://www.iso.org/obp/ui/#iso:std:iso:11269:-2:ed-3:v1:en

Jackson MB, Drew MC (1984) Effects of Flooding on Growth and Metabolism of Herbaceous Plants. In: Kozlowski (ed) Flooding and Plant Growth. Elsevier, San Diego, pp 47–128

Jones CA, Pefia D, Gomez CA (1980) Effects of plant water potential, leaf diffusive resistance, rooting density and water use on the dry matter production of several tropical grasses during short periods of drought stress. Trop Agric 57:211–219

Jordan TE, Weller DE (1996) Human Contributions to Terrestrial Nitrogen Flux. Bioscience 46:655–664. https://doi.org/10.2307/1312895

Khan NA, Samiullah, Singh S, Nazar R (2007) Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (Triticum aestivum) cultivars differing in yield potential under cadmium stress. J Agron Crop Sci 193:435–444. https://doi.org/10.1111/j.1439-037X.2007.00272.x

Klink CA (1994) Effects of clipping on size and tillering of native and African grasses of the Brazilian savannas. Oikos 70:365–376. https://doi.org/10.2307/3545774

Li XP, Müller-Moulé P, Gilmore AM, Niyogi KK (2002) PsbS-dependent enhancement of feedback de-excitation protects photosystem II from photoinhibition. Proc Natl Acad Sci U S A 99:15222–15227. https://doi.org/10.1073/pnas.232447699

Lima ALS, DaMatta FM, Pinheiro HA, et al (2002) Photochemical responses and oxidative stress in

two clones of Coffea canephora under water deficit conditions. Environ Exp Bot 47:239–247. https://doi.org/10.1016/S0098-8472(01)00130-7

Ludlow MM (1980) Stress physiology in tropical pasture plants. Trop Grasslands 14:136–145

Martins CR, Hay JDV, Carmona R (2009) Potencial invasor de duas cultivares de Melinis minutiflora no cerrado brasileiro - características de sementes e estabelecimento de plântulas. Rev Árvore 33:713–722. https://doi.org/10.1590/S0100-67622009000400014

Martins CR, Hay JDV, Walter BMT, et al (2011) Impacto da invasão e do manejo do capim-gordura (Melinis minutiflora) sobre a riqueza e biomassa da flora nativa do Cerrado sentido restrito. Rev Bras Botânica 34:73–90. https://doi.org/10.1590/S0100-84042011000100008

Milone MT, Sgherri C, Clijsters H, Navari-Izzo F (2003) Antioxidative responses of wheat treated with realistic concentration of cadmium. Environ Exp Bot 50:265–276. https://doi.org/10.1016/S0098-8472(03)00037-6

Monasterio M, Sarmiento G (1976) Phenological strategies of plant species in the tropical savanna and the semi-deciduous forest of the Venezuelan Llanos. J Biogeogr 3:325–355. https://doi.org/10.2307/3037976

Oksanen J, Blanchet FG, Friendly M, et al (2013) vegan: Community ecology package. https://cran.r-project.org/package=vegan

Pausas JG, Lamont BB, Paula S, et al (2018) Unearthing belowground bud banks in fire-prone ecosystems. New Phytol 217:1435–1448. https://doi.org/10.1111/nph.14982

Pivello VR, Shida CN, Meirelles ST (1999) Alien grasses in Brazilian savannas: A threat to the biodiversity. Biodivers Conserv 8:1281–1294. https://doi.org/10.1023/A:1008933305857

R Core Team (2013) R: A language and environment for statistical computing. https://www.R-project.org

Reatto A, Correia JR, Spera ST, Martins É de S (2008) Solos do bioma Cerrado: aspectos pedológicos. In: Sano SM, Almeida SP, Ribeiro JF (eds) Cerrado: ecologia e flora. Embrapa Cerrados/ Embrapa Informação Tecnológica, Brasília, Brazil, pp 107–150

Rengifo E, Tezara W, Herrera A (2005) Water relations, chlorophyll a fluorescence, and contents of saccharides in tree species of a tropical forest in response to flood. Photosynthetica 43:203–210. https://doi.org/10.1007/s11099-005-0034-x

Ribeiro JF, Walter BMT (2008) As principais fitofisionomias do bioma Cerrado. In: Sano SM, Almeida SP, Ribeiro JF (eds) Cerrado: ecologia e flora. Embrapa Cerrados/ Embrapa Informação Tecnológica, Brasília, Brazil, pp 151–212

Sarmiento G (1992) Adaptive strategies of perennial grasses in South American savannas. J Veg Sci 3:325–336. https://doi.org/10.2307/3235757

Silva JF, Castro F (1989) Fire, growth and survivorship in a Neotropical savanna grass Andropogon semiberbis in Venezuela. J Trop Ecol 5:387–400. https://doi.org/10.1017/S0266467400003849

Silva JSO, Haridasan M (2007) Acúmulo de biomassa aérea e concentração de nutrientes em Melinis minutiflora P. Beauv. e gramíneas nativas do cerrado. Rev Bras Botânica 30:337–344. https://doi.org/10.1590/S0100-84042007000200016

Silva S, Pinto G, Correia B, et al (2013) Rye oxidative stress under long term Al exposure. J Plant Physiol 170:879–889. https://doi.org/10.1016/j.jplph.2013.01.015

Simoes M, Baruch Z (1991) Responses to simulated herbivory and water stress in two tropical C4 grasses. Oecologia 88:173–180. https://doi.org/10.1007/BF00320808

Sims DA, Gamon JA (2002) Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens Environ 81:337–354. https://doi.org/10.1016/S0034-4257(02)00010-X

van Kooten O, Snel JFH (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth Res 25:147–150. https://doi.org/10.1007/BF00033156

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. Science (80-) 277:494–499. https://doi.org/10.1126/science.277.5325.494

Wickham H (2016) ggplot2: Elegant graphics for data analysis. https://ggplot2.tidyverse.org

Wilson J, Ludlow M, Fisher M, Schulze E (1980) Adaptation to Water Stress of the Leaf Water

Relations of Four Tropical Forage Species. Funct Plant Biol 7:207. https://doi.org/10.1071/PP9800207

Zenni RD, Ziller SR (2011) An overview of invasive plants in Brazil. Rev Bras Botânica 34:431–446. https://doi.org/10.1590/S0100-84042011000300016

Zenni, R.D., Sampaio, A.B., Lima, Y.P., Pessoa-Filho, M., Lins, T.C.L., Pivello, V.R., Daehler, C., 2019. Invasive Melinis minutiflora outperforms native species, but the magnitude of the effect is context-dependent. Biol Invasions 21, 657–667. https://doi.org/10.1007/s10530-018-1854-5

Zhang J, Kirkham MB (1994) Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. Plant Cell Physiol 35:785–791. https://doi.org/10.1093/oxfordjournals.pcp.a078658

FIGURE LEGENDS

Figure 1. Morphological traits of the seedlings of one invasive (*Melinis minutiflora*) and one native (*Schizachyrium. microstachyum*) grass cultivated under different water treatments (1d, watered every day; 5d, watered every five days; 10d, watered every ten days; Ow, overwatered every day) with or without the addition of NPK (10–10–10). Interval bars show standard deviation. Asterisks show significant differences among species within treatments; uppercase letters show differences among fertilized and unfertilized treatments; lowercase letters show differences among water treatments. Factorial ANOVA with species, water treatment, and fertilization as independent variables followed by Tukey HSD test for multiple comparisons ($p < 0.05$).

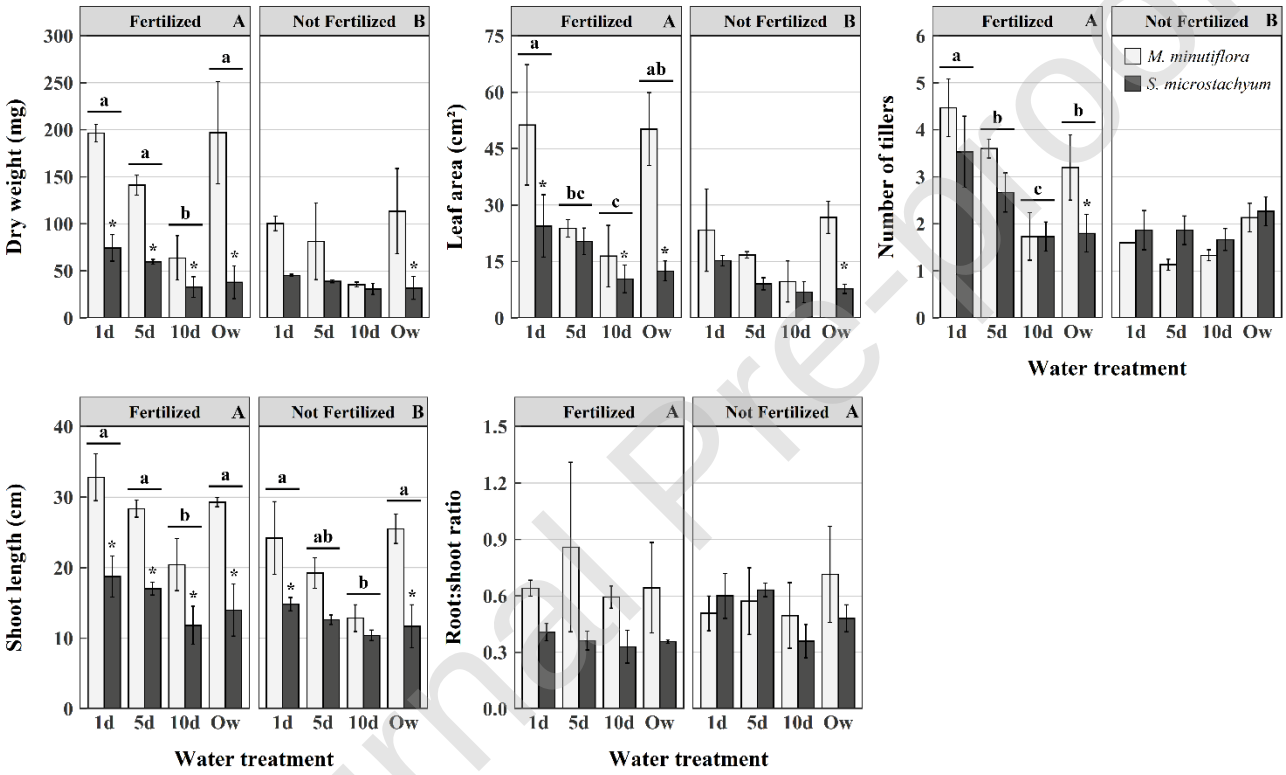

Figure 2. Nitrate and phosphate concentrations in seedlings of one invasive (*Melinis minutiflora*) and one native (*Schizachyrium microstachyum*) grass cultivated under different water treatments (1d, watered every day; 5d, watered every five days; 10d, watered every ten days; Ow, overwatered every day) with or without the addition of NPK (10–10–10). Interval bars show standard deviation. Asterisks show significant differences among species within treatments; uppercase letters show differences among fertilized and unfertilized treatments; lowercase letters show differences among water treatments. Factorial ANOVA with species, water treatment, and fertilization as independent variables followed by Tukey HSD test for multiple comparisons ($p < 0.05$).

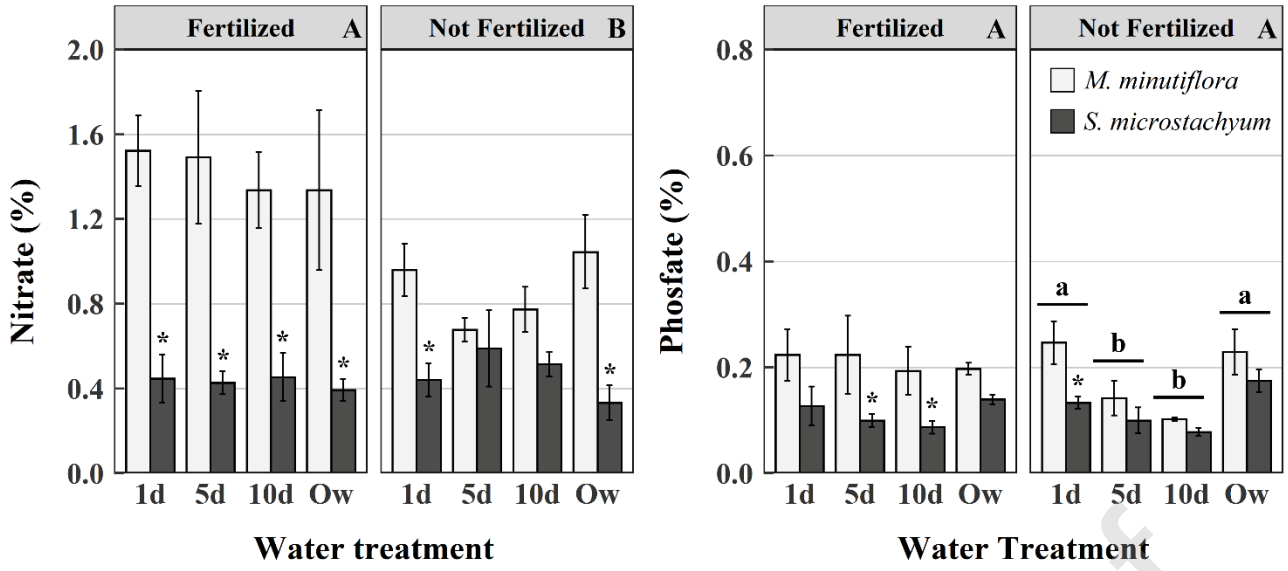

Figure 3. Principal components analysis ordination using dry weight (DW), leaf area (A), length (L), number of tillers (Til), root:shoot ratio (RS), pigment concentration (Chloa, Chlob, Carot), MDA concentration, G-POX and SOD activities, and nitrate concentration (Nit) as variables for one invasive (Mm; *Melinis minutiflora*) and one native (Sm; *Schizachyrium microstachyum*) grass cultivated under different water treatments (CTR, watered every day; 5, watered every five days; 10, watered every ten days; OVW, overwatered every day) and nutrients availability (F, fertilized; C, unfertilized).

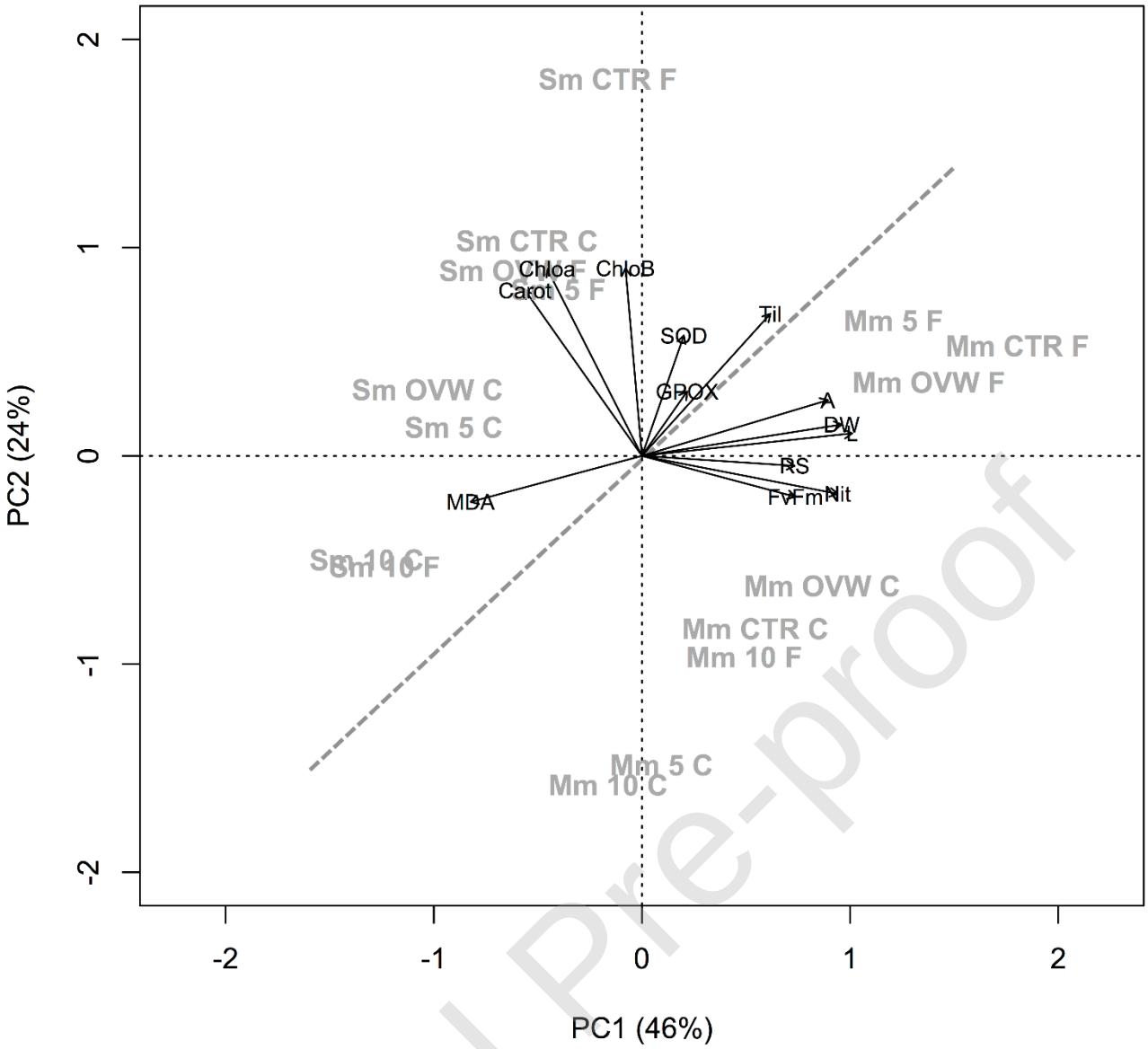

Table 1. Factorial ANOVA scores for each measured variable using species (S; *Melinis minutiflora* or *Schizachyrium microstachyum*), water treatment (watered every day, watered every five days, watered every ten days, or over watered every day), and fertilization (F; with or without) as the independent variables.

Variable	Species		Water treatment		Fertilization		S × W × F	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Carotenoids	48.936	< 0.001	0.952	0.421	1.415	0.239	0.481	0.693
Chlorophyll a	53.632	< 0.001	1.641	0.189	12.054	< 0.001	0.698	0.556
Chlorophyll a/b	204.802	< 0.001	4.265	0.008	22.719	< 0.001	2.221	0.094
Chlorophyll b	7.509	0.008	1.976	0.127	2.540	0.116	0.462	0.709
Dry weight	120.616	< 0.001	18.104	< 0.001	38.327	< 0.001	0.814	0.496
F_v/F_m	59.824	< 0.001	5.100	0.003	11.451	0.001	1.093	0.359
G-POX	6.002	0.020	1.756	0.175	5.169	0.030	1.588	0.220
Leaf area	52.918	< 0.001	16.440	< 0.001	37.554	< 0.001	2.253	0.101
MDA	120.741	< 0.001	35.729	< 0.001	23.535	< 0.001	6.785	< 0.001
Nitrate	209.310	< 0.001	0.472	0.701	29.362	< 0.001	2.552	0.073
NPQ	18.404	< 0.001	4.115	0.010	3.385	0.070	0.315	0.815
Number of tillers	3.627	0.066	18.871	< 0.001	88.005	< 0.001	1.610	0.206
Phosphate	65.787	< 0.001	12.601	< 0.001	1.262	0.270	1.840	0.300
Root:shoot ratio	15.240	< 0.001	1.954	0.140	0.192	0.663	1.387	0.265
Shoot length	186.289	< 0.001	24.438	< 0.001	47.452	< 0.001	0.436	0.729
SOD	53.236	< 0.001	2.236	0.103	0.006	0.937	2.117	0.136

Table 2. Mean values (±SD) of leaf fluorescence pigments in the leaves of one invasive (Melinis minutiflora) and one native (Schizachyrium microstachyum) grass cultivated under different water treatments (1d, watered every day; 5d, watered every five days; 10d, watered every ten days; Ow, overwatered every day) and nutrients availability. The p column shows differences among water treatments; asterisks show a significant difference between species. ns = not significant.

Variable	Fertilized	Water	*p*	*M. minutiflora*	*S. microstachyum*
Chlorophyll a (µmol g FW^{-1})					
	No	1d	ns	0.98 ± 0.06	1.73 ± 0.18 *
	No	5d	ns	0.80 ± 0.02	1.63 ± 0.07 *
	No	10d	ns	0.88 ± 0.09	1.42 ± 0.14
	No	Ow	ns	1.08 ± 0.04	1.70 ± 0.22 *
	Yes	1d	ns	1.22 ± 0.09	2.13 ± 0.18 *
	Yes	5d	ns	1.52 ± 0.13	1.64 ± 0.14
	Yes	10d	ns	1.14 ± 0.42	1.63 ± 0.14
	Yes	Ow	ns	1.30 ± 0.18	1.74 ± 0.15
Chlorophyll b (µmol g FW^{-1})					
	No	1d	a	0.33 ± 0.02	0.60 ± 0.13
	No	5d	a	0.29 ± 0.03	0.49 ± 0.03
	No	10d	b	0.31 ± 0.03	0.41 ± 0.03
	No	Ow	a	0.36 ± 0.01	0.43 ± 0.04
	Yes	1d	ns	0.44 ± 0.04	0.54 ± 0.05
	Yes	5d	ns	0.52 ± 0.05	0.46 ± 0.03
	Yes	10d	ns	0.40 ± 0.15	0.39 ± 0.04
	Yes	Ow	ns	0.44 ± 0.06	0.34 ± 0.06
Carotenoids (µmol g FW^{-1})					
	No	1d	ns	0.63 ± 0.03	1.18 ± 0.18 *
	No	5d	ns	0.58 ± 0.04	1.05 ± 0.05 *
	No	10d	ns	0.59 ± 0.06	1.01 ± 0.07 *
	No	Ow	ns	0.70 ± 0.03	0.98 ± 0.11
	Yes	1d	ns	0.74 ± 0.07	1.13 ± 0.07
	Yes	5d	ns	0.91 ± 0.09	1.02 ± 0.05
	Yes	10d	ns	0.62 ± 0.22	1.01 ± 0.05
	Yes	Ow	ns	0.82 ± 0.11	0.92 ± 0.06
Chlorophyll $_{a/b}$ ratio					
	No	1d	a	2.93 ± 0.04	3.14 ± 0.35
	No	5d	a	2.80 ± 0.23	3.34 ± 0.18
	No	10d	ab	2.83 ± 0.38	3.41 ± 0.15
	No	Ow	b	3.00 ± 0.04	3.98 ± 0.06 *
	Yes	1d	ns	2.82 ± 0.05	3.95 ± 0.07
	Yes	5d	ns	2.91 ± 0.04	3.97 ± 0.12
	Yes	10d	ns	2.87 ± 0.06	4.22 ± 0.12 *

Variable	Fertilized	Water	*p*	*M. minutiflora*	*S. microstachyum*
	Yes	Ow	ns	2.96 ± 0.08	4.04 ± 0.56
F_v/F_m					
	No	1d	a	0.782 ± 0.010	0.771 ± 0.004
	No	5d	a	0.777 ± 0.006	0.749 ± 0.009
	No	10d	a	0.796 ± 0.005	0.723 ± 0.004 *
	No	Ow	b	0.788 ± 0.005	0.719 ± 0.019 *
	Yes	1d	A	0.789 ± 0.004	0.779 ± 0.003
	Yes	5d	A	0.798 ± 0.006	0.775 ± 0.006
	Yes	10d	A	0.805 ± 0.003	0.765 ± 0.013
	Yes	Ow	B	0.774 ± 0.004	0.744 ± 0.016
NPQ					
	No	1d	ns	4.018 ± 0.173	2.549 ± 0.120
	No	5d	ns	3.550 ± 0.598	2.625 ± 0.515
	No	10d	ns	3.120 ± 0.551	2.243 ± 0.207
	No	Ow	ns	3.963 ± 0.005	2.602 ± 0.396
	Yes	1d	AB	3.221 ± 0.053	2.867 ± 0.348
	Yes	5d	AB	2.675 ± 0.118	2.386 ± 0.301
	Yes	10d	A	2.441 ± 0.279	2.441 ± 0.287
	Yes	Ow	B	3.186 ± 0.061	3.023 ± 0.455

Table 3. Mean values (±SD) of MDA and enzymatic activities of one invasive (*Melinis minutiflora*) and one native (*Schizachyrium microstachyum*) grass cultivated under different water treatments (1d, watered every day; 5d, watered every five days; 10d, watered every ten days; Ow, overwatered every day) and nutrients availability. The *p* column shows differences between water treatments; asterisks show significant differences between species. ns = not significant.

Variable	Fertilized	Water	*p*	*M. minutiflora*	*S. microstachyum*
MDA (mmol g FW^{-1})					
	No	1d	b	4.29 ± 0.38	7.08 ± 0.39
	No	5d	a	9.89 ± 2.38	17.00 ± 1.62 *
	No	10d	a	8.11 ± 1.28	15.67 ± 1.26 *
	No	Ow	b	3.08 ± 0.18	11.57 ± 0.81 *
	Yes	1d	B	4.19 ± 0.04	5.73 ± 1.64
	Yes	5d	B	4.01 ± 0.26	7.06 ± 0.29
	Yes	10d	A	5.42 ± 0.34	19.96 ± 1.62 *
	Yes	Ow	B	3.26 ± 0.31	5.95 ± 0.39
GPOx (µmol ml^{-1} $prot^{-1}$ min^{-1})					
	No	1d	ns	1.40 ± 0.16	0.90 ± 0.78
	No	5d	ns	1.56 ± 0.43	0.25 ± 0.13
	No	10d	ns	1.89 ± 0.33	-
	No	Ow	ns	1.47 ± 0.35	2.14 ± 0.10
	Yes	1d	ns	2.17 ± 0.19	2.29 ± 0.39
	Yes	5d	ns	1.63 ± 0.21	1.37 ± 0.15
	Yes	10d	ns	1.40 ± 0.21	-
	Yes	Ow	ns	0.89 ± 0.20	4.31 ± 1.24 *
SOD (mg g FW^{-1} min^{-1})					
	No	1d	a	2.66 ± 0.35	4.16 ± 1.39
	No	5d	b	1.59 ± 0.51	2.74 ± 0.92
	No	10d	ab	0.73 ± 0.19	-
	No	Ow	ab	1.94 ± 0.32	2.57 ± 0.02
	Yes	1d	B	1.67 ± 0.27	3.09 ± 0.53
	Yes	5d	AB	1.35 ± 0.11	4.57 ± 0.51
	Yes	10d	A	0.92 ± 0.20	-
	Yes	Ow	B	1.64 ± 0.22	5.34 ± 1.49 *