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Drought tolerance in tough lovegrass (*Eragrostis plana*): Understanding mechanisms and competitive ability

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Abstract

Eragrostis plana Nees is a perennial grass weed, native to South Africa and an invasive species of dry rangelands across Asia, Australia, and South America. We hypothesise that *E. plana* has evolved mechanisms of acclimation to drought conditions to a greater extent than bahiagrass (*Paspalum notatum*), an important native grass forage species of livestock rangelands of Brazil, leading to a competitive advantage. We measured morphological, physiological, and biochemical response traits in *E. plana* and *P. notatum* to water deficit and competition. Levels of drought tolerance in the two species were greatest at the tillering growth stage, driven by low levels of leaf relative water content in *E. plana* and early stomatal regulation to avoid water loss in *P. notatum*. The mechanisms used to dissipate energy excess under drought are associated with an efficient antioxidant system and the biosynthesis of compatible osmolytes (proline). Despite reductions in fitness, both species survived a series of drought cycles, where *E. plana* showed a competitive advantage over *P. notatum*. These results help to explain the invasion success of *E. plana* in rangelands and demonstrate its potential threat to native biodiversity, particularly under drought conditions.

KEYWORDS

competitiveness, pasture, proline, stomatal regulation, water stress

1 | INTRODUCTION

After habitat loss, the spread of invasive species in natural ecosystems is considered to be one of the main threats to biodiversity (Forner et al., 2022; Wilcove et al., 1998). Evolutionary mechanisms that allow for the success of invasive plant species include purging of genetic load, genotypic and epigenetic plasticity, hybridization, polyploidy and clonal division (Clements & Jones, 2021). Under environmental stress conditions, the likelihood of exotic species becoming invasive and overwhelming native species depends on greater competitive advantage, due to morphological, physiological, and biochemical trait adaptations (Oh et al., 2021).

Water scarcity may affect traits in invasive plants, such as germination, plant size, and seed production and dispersal (Ziska et al., 2011). *Eragrostis plana* Nees (tough lovegrass), a species which is native to South Africa, is considered to be invasive in several regions, including South America (Guido et al., 2016). This grass was accidentally introduced to Brazil in 1957 (Reis Leite, 1993) and has since been a threat to native forage species, due to its greater palatability and associated endozoochoric distribution of the potential 80 000 seeds that are produced per plant in one generation (Caratti, 2019). While the range of *E. plana* in Brazil extends to >10 000 km², its distribution in the north contrasts with that across central regions (Medeiros et al., 2014).

Periods of water deficit are predicted to increase globally under climate change (Nakashima et al., 2014), with associated water stress impacts on plants, due to limited supply of water to roots and/or increases in rates of transpiration (Anjum et al., 2011). Drought response strategies in plants comprise escape (or avoidance), prevention, and tolerance (Volaire, 2018), where escape is the result of rapid development and reproduction before the onset of severe drought conditions, prevention is due to increased water use efficiency, reduced transpiration, limited vegetative growth, and/or increased root growth, and tolerance is adjustment in osmosis and production of molecules, such as enzymes or non-enzymatic oxidants (Li et al., 2013), that stabilize target proteins (Anjum et al., 2011; Ashraf & Foolad, 2007; Volaire, 2018).

Rangeland pastures, such as those of southern Brazil, are characterised by a diversity of grass species with contrasting growth strategies (Marques et al., 2017); for example, the native C4 Paspalum notatum Flügge is used as a high quality forage species in areas of low precipitation in southern Brazil, due to its large and deep fibrous root system (Acuña et al., 2010). These drought avoidance characteristics, combined with high levels of root production (Abichequer et al., 2009), tolerance to low temperatures and high levels of seed production and viability (Medeiros et al., 2014), are shared between E. plana and P. notatum, likely leading to competition; however, a clear understanding of drought responses and associated interspecific competition between E. plana and P. notatum remains lacking. Thus, the aim of this study was to test for competitive differences in tolerance to drought conditions in the invasive species E. plana and the forage species P. notatum, due to variation in morphological, physiological, and biochemical trait responses to contrasting levels of water deficit.

2 MATERIALS AND METHODS

Two experiments were carried out, arranged as a completely randomised design (CRD) and comprising two contrasting water treatments, to test responses of growth and morphological traits and physiological and biochemical traits in E. plana and P. notatum (cv. Pensacola), while a third experiment tested for competitive ability between the two species under water stress. We collected E. plana seeds for the experiments from an area of pasture at the Federal University of Pelotas, Capão do Leão, RS, Brazil (31°80'33.94" S, 52°49'49.78" W) that were then stored at 8°C prior to use.

2.1 Experiment I–Growth and morphological trait responses to drought

In a glasshouse (temperature range: 17.5–26.7°C), E. plana and P. notatum seeds were germinated in 8-kg pots (48 pots species⁻¹) filled with sandy loam soil (Haplosol) containing 1.2% organic matter and 15% clay; germinated seeds were thinned to four plants pot⁻¹ and pots were watered daily to field capacity. The experiment comprised two water treatments of 25 or 100% of water potential (Cw), calculated following Santos dos Brombini et al. (2015), applied to six replicates of four growth stages (5-6 true leaves; tillering; initiation of panicle, E. plana, or spikelet, P. notatum; and, grain filling) of the two grass species (2 \times 6 \times 4 \times 2). Seed sowing was timed so that plants reached the four growth stages concurrently, when the treatments were applied; prior to this, plants were maintained at field capacity.

On 10 March 2016, when the plants had reached the four growth stages, the pots were watered to saturation, drained, and weighed. Then, the pots weighed daily and water lost through evapotranspiration was added (1 ml = 1 g) to maintain experimental levels of C_w (25 or 100%) for the subsequent 30 d, before root volume, root and shoot dry mass (oven dried at 60°C to a constant weight), measurement of relative water content (RWC, %), stomatal conductance (g_s), and, according to growth stage, number of tillers or panicles/spikelets.

Root volume was determined using the water displacement technique, where roots were immersed in a graduated cylinder before the overflow water volume was measured according to Harrington et al. (1994). Fresh and dry leaf mass was determined from 10 cm-long sections of leaf that were weighed (fresh mass) and immediately placed in a plastic box (11 imes 11 imes 3.5 cm) with deionised water for 24 h to achieve water-saturation, before being dried at 60 °C for c. 24 h to a constant weight (dry mass). We calculated RWC as [(fresh mass – dry mass)/(water-saturated mass - dry mass)] \times 100 (Barrs & Weatherley, 1962). Stomatal conductance (g_s) was measured from the last fully expanded leaf between 09:00 and 11:00 hrs using a porometer (Decagon SC-1, Decagon Devices, Pullman, WA).

Experiment II—Physiological and biochemical 2.2 traits of drought tolerance

Based on data from Experiment I, we tested for physiological and biochemical trait responses to slow water-deficit stress at the growth stage when level of tolerance to drought was lowest (panicle initiation in E. plana; spikelet initiation in P. notatum). Plants of the two species were raised as in Experiment I (six replicates of four plants pot^{-1} ; sowing date: 15 December 2016) and maintained at field capacity; when >50% of panicles/spikelets were fully emerged, slow water-deficit stress conditions were induced (Figure S1) and g_s and RWC were measured daily, as described for Experiment I. Irrigation was suspended until a 75% reduction in g_s was achieved in the well-watered treatment (data not shown); then, irrigation resumed until g_s fully recovered to pre-treatment levels. We measured physiological and biochemical indicators of water stress and tolerance when g_s was reduced to <50% (moderate deficit) and <75% (severe deficit) of the well-watered treatment during water-deficit stress and when it had recovered to >50% and 100% during the subsequent rehydration period; at these thresholds for g_s , six leaves were removed per plant for storage at -80° C, prior to biochemical leaf content analyses.

Leaf chlorophyll (Chl) content index was measured from a single, randomly selected location on the adaxial side of one leaf per plant using a chlorophyll meter (Dualex 4 Scientific, FORCE-A, Orsay, France). We measured Chl a transient fluorescence parameters (flux of electron transport per reaction centre, ET_0/RC ; dissipated energy flux per reaction centre, DI_0/RC ; and, maximum yield of primary photochemistry of PSII, Fv/Fm) from the last fully expanded leaf per plant between 09:00 and 11:00 hrs using a portable fluorometer (Handy PEA, Hansatech Instruments, King's Lynn, Norfolk, UK), where leaves were maintained in darkness for 30 min, to allow complete oxidation of the PSII transport system; then, fluorescence emission was induced in a 4-mm-diameter area by exposing leaves to a saturating red actinic light at an intensity of 3000 μ mol m⁻² s⁻¹.

Hydrogen peroxide (H_2O_2) content (µmol g⁻¹ fresh weight, FW) and lipid peroxidation as malondialdehyde content (μ mol g⁻¹ FW) were determined according to Velikova et al. (2000), where 400 mg of leaf material was ground in 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12 000 g and 4° C for 20 min. For analysis of H₂O₂ content, an aliquot of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml 1 M potassium iodide (KI); H₂O₂ content was determined at absorbance of 390 nm using a spectrophotometer and calibrated using a standard curve prepared with known concentrations of H₂O₂. Content of MDA was quantified using the thiobarbituric acid (TBA) test, where an aliquot of the supernatant was added to 1 ml 0.5% (w/v) TBA in 20% TCA and the mixture was incubated in boiling water for 30 min; the reaction was stopped by placing the reaction tubes in an ice bath. The samples were centrifuged at 10 000 g for 5 min; absorbance was read at 532 nm, from which the value for non-specific absorption at 600 nm was subtracted. The amount of the MDA-TBA complex was calculated from the extinction coefficient (ε) as $\varepsilon = 155 \times 10^3$ $M^{-1} cm^{-1}$.

To evaluate protein content, antioxidant-enzyme activity and proline content, 400 mg of leaf material was ground in liquid N₂ and then added to 5% (w/v) polyvinylpolypyrrolidone (PVPP); the mixture was homogenised in 100 mM potassium phosphate buffer (pH 7.8) with 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 20 mM sodium ascorbate and then was centrifuged at 12 000 g and 4 °C for 20 min. The supernatant was used to determine protein content, according to Bradford (1976), using bovine serum albumin as standard; superoxidedismutase activity (SOD; EC 1.15.1.1; µmol mg⁻¹ protein) following Giannopolitis and Ries (1977), by recording inhibition of nitrobluetetrazolium colouration at 560 nm; catalase activity (CAT; EC 1.11.1.6; μ mol H₂O₂ min⁻¹ mg⁻¹ protein), following Azevedo et al. (2006), by monitoring H_2O_2 consumption and measuring declines in absorbance at 240 nm; ascorbate peroxidase activity (APX; EC 1.11.1.11; μ mol min⁻¹ mg⁻¹ protein) by measuring ascorbate oxidation at 290 nm for 3 min (Nakano and Asada, 1981); and, proline content (μ mol g⁻¹ FW) using the ninhydrin method (Bates et al., 1973), that resulted in a two-phase formation, where 1 ml of the upper phase was analysed using a spectrophotometer at absorbance of 520 nm and content was compared against a standard curve.

Starch ($\mu g g^{-1}$), total soluble sugar (mg g⁻¹) and total free amino acid (μ mol g⁻¹) content were extracted by grinding and centrifuging 500 mg of leaf material to obtain an alcoholic extract that was evaporated; distilled water was added to obtain an aqueous phase for determination of total soluble sugars using the anthrone method (Graham and Smydzuk, 1965) and total free amino acid using the ninhydrin method (Yemm and Cocking, 1955); the residue from centrifugation was used to determine starch content using acid hydrolysis (Mccready et al., 1950).

2.3 | Experiment III—Competition between *E. plana* and *P. notatum* under drought conditions

Previous monoculture assays for *E. plana* and *P. notatum* that were conducted under glasshouse conditions (data not shown) informed the experimental density of 32 plants (100% and 50:50 species mix; Figure S2) per box ($18 \times 60 \times 40$ cm) filled with 40 kg of sandy loam soil, as described for Experiment I. The experiment was arranged in a fully randomised 2×2 factorial design, comprising four replicates of two water treatments and two levels of competition.

The water treatments were imposed when plants reached the panicle/spikelet initiation growth stage in February 2017, where a slow water-deficit was created by inducing water stress through four, 50-d cycles of suspension of irrigation from maintenance of field capacity to 5% of volumetric water (measured using a soil moisture sensor; ECHO EC-5, Decagon Devices América Latina Ltda), followed by a recovery period to maximum soil water holding capacity. The four cycles of drought followed by water recovery ended in May 2017. Using methods described in Experiment I, we measured RWC, g_s and transient Chl a fluorescence at grain filling, at the end of the final drought cycle and prior to recovery, while plant height, number of tillers and panicles, and aboveground shoot dry mass (SDM) were measured after the final recovery. Following the removal of shoot material for biomass estimation, the experimental treatment boxes were maintained at full water capacity until February 2018, when the experiment was repeated.

2.4 | Statistical analysis

Data were checked for homoscedasticity and normality using Levene's and Shapiro–Wilk tests, respectively. Main effects of water deficit on morphological (Experiment I) and physiological and biochemical (Experiment II) trait responses were tested using one-way ANOVA and within-growth stage differences in trait responses were tested using Student's *t*-test (P < 0.05); analyses were conducted using SAS v. 9.0 (SAS Institute, Cary, NC, USA). Data are the means of six replications ± SE. The relations between RWC and g_s and RWC and dehydration period were tested using regression analysis with an exponential function, and principal component analysis (PCA) (PC-ORD 4.10 software) was used to visualise growth stage responses to water deficit (Experiment I).

We used two-way ANOVA to test for effects of levels of drought and competition on indicators of drought stress and tolerance in *E. plana* and *P. notatum* (Experiment III) and within-growth stage responses were tested using Student's *t*-test at P < 0.05.

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3 | RESULTS

3.1 | Experiment I—Growth and morphological trait responses to drought

Water deficit led to decreases in RWC and g_s in the two species, independent of growth stage (Figure S3). In *E. plana*, at the 5–6 leaf, initiation of tillering/panicle, and grain filling growth stages, water deficit reduced RWC by 19, 32, 44, and 45% respectively, while in *P. notatum*, there were reductions of 25, 29, 46, and 37% respectively. For both species, the RWC reduction at reproductive stages was more severe compared to vegetative stages (Figure S3).

Water deficit reduced number of tillers at the 5–6 leaf growth stage in *E. plana* by 34%, with no effects on tillers in other growth stages, while in *P. notatum*, there were 38, 20, 32 and 5% reductions in number of tillers at the 5–6 leaf, initiation of tillering and panicles, and grain filling growth stages, respectively (Figure S4A–D).

Water deficit reduced SDM accumulation in both species at all four growth stages and increased root dry mass at the tillering (1.30-fold) and grain filling (1.29-fold) stages in *E. plana* and at tillering (1.34-fold), spikelet initiation (1.62-fold), and grain filling (1.48-fold) in *P. notatum* (Figure S4E–F).

The number of panicles in *E. plana* were reduced by water deficit at panicle initiation and grain filling by 75 and 45%, respectively, and number of spikelets in *P. notatum* were reduced by 73% (Figure S5).

Principal component (PC) axes 1 and 2 accounted for 82.6 and 81.4% of the variation in trait responses to drought in *E. plana* and *P. notatum*, respectively (Figure 1). Variables that contributed most to

discriminate groups (eigenvector values >0.30) comprised SDM, root dry mass and panicle number along PC1 and RWC, g_s and panicle number along PC2 in *E. plana*, and SDM, tiller number and spikelet number along PC1 and RWC, g_s and tiller number along PC2 in *P. notatum*. For both species, there was separation of traits along PC1 and separation of trait responses to drought along PC2 (Figure 1).

3.2 | Experiment II—Physiological and biochemical traits of drought tolerance

As RWC decreased, there were decreases in g_s in the two species where responses of g_s were more sensitive in *P. notatum* than in *E. plana* (Figure 2A). At 7 and 13 d of drought, g_s had reduced by more than 50 and 75%, respectively, in the two species (Figure 2B). Following re-instatement of irrigation, recovery of 50% of g_s occurred in <24 h and 3 d in *E. plana* and *P. notatum*, respectively; moderate water deficit triggered a reduction in leaf Chl in *E. plana*, whereas similar reductions in Chl a in *P. notatum* were triggered by severe water deficits (Figure 3).

Severe water deficit increased Dl₀/RC in *E. plana* and *P. notatum* by c. 3.4 and 2.6-fold, respectively, with no changes under moderate water stress, while under well-watered conditions, Dl₀/RC in *E. plana* was higher than in plants recovering from water deficit, with no differences in *P. notatum* (Figure S6). Severe water deficit led to reduced Fv/Fm in the two species that remained below levels under the well-watered treatment during mid-recovery (50%) and reached 100% of the maximum yield of primary photochemistry at full recovery (Figure S6).



FIGURE 1 Principal component analysis (PCA) of growth stage responses to contrasting levels of soil water in *Eragrostis plana* and *Paspalum notatum*. Five-six leaf (\langle / \rangle), panicle initiation (∇ / ∇), tillering initiation (\langle / \rangle), grain filling (\square / \square). Open and filled symbols represent 25% and 100% of water replacement respectively. Proportion of variation (%) explained by each principal component (PC1 and PC2) is shown.



FIGURE 2 Responses of stomatal conductance (g_s) to level of relative water content (RWC) (A) and responses of RWC to dehydration period (B) in *Eragrostis plana* and *Paspalum notatum*. Data were recorded daily from the fifth day of irrigation suspension.



FIGURE 3 Effects of water deficit on leaf chlorophyll index under contrasting levels of stomatal conductance (g_s) during periods of dehydration and rehydration in *Eragrostis plana* (A) and *Paspalum notatum* (B). Asterisks indicate treatment differences (Student's t-test) at P < 0.05. Error bars are SE.

Severe water deficit (<75% g_s) led to two-fold increase in accumulation of H₂O₂ in *E. plana* (Figure 4A), and 1.8 and 3.7-fold increases in *P. notatum* under moderate (<50% g_s) and severe (<75% g_s) water deficits, respectively (Figure 4B). Under severe water deficit (<75%), there were 2 and 1.5-fold increases in MDA-TBA content in *E. plana* (Figure 4C) and *P. notatum* (Figure 4D), respectively.

Activity of CAT increased in *E. plana* under moderate and severe water deficit and remained higher than under well-watered conditions during mid-recovery (Figure 5A), while in *P. notatum*, CAT activity was lower in water deficit plants during recovery (Figure 5B). Activity of APX in *E. plana* did not vary under moderate and severe water deficit and was lower in water

deficit plants during mid-recovery (Figure 5C), while levels in *P. notatum* were higher under moderate levels of water deficit (Figure 5D). Activity of SOD in *E. plana* did not vary under water deficit and was lower during recovery (Figure 5E), while in *P. notatum*, activity was greater under water deficit, with no effects of water deficit during recovery (Figure 5F).

Content of proline in *E. plana* increased in response to moderate and severe levels of water deficit and until mid-recovery (>50% g_s) (Figure 6A), while content in *P. notatum* increased under severe water deficit until full recovery (Figure 6B).

Accumulation of amino acids in *E. plana* increased under moderate water deficit and at full recovery (Figure 6C), while content in



FIGURE 4 Effects of water deficit on hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) production under contrasting levels of stomatal conductance (g_s) in *Eragrostis plana* and *Paspalum notatum*. Asterisks indicate treatment differences (Student's *t*-test) at *P* < 0.05. Error bars are SE.

P. notatum was greater under moderate and severe water deficit and during recovery (Figure 6D).

Soluble sugar content increased in *E. plana* under water deficit and during recovery (Figure 6E), while in *P. notatum* content was greater under water deficit and lower at full recovery (Figure 6F).

Accumulation of starch in *E. plana* was greater under water deficit and at mid-recovery (Figure 6G), while in *P. notatum* content was greater under severe water deficit and at full recovery, and lower at mid-recovery (Figure 6H).

3.3 | Experiment III—Competition between E. plana and P. notatum under drought conditions

Responses of photosynthesis indicators to water deficit did not vary with competition in both study years (P > 0.05) and there were some effects of competition and water deficit on the indicators (Table 1). In Year 1 for *P. notatum*, Dl₀/RC was greater in plants grown in competition with *E. plana* than under monoculture and ET₀/RC was greater under water deficit. In Year 2, ET₀/RC was greater in *E. plana* under water deficit and greater in *P. notatum* grown in monocuture (Table 1).

In *E. plana*, water deficit led to a reduction in *g*_s, RWC and number of panicles in Years 1 and 2, lower SDM in Year 2, while competition

with *P. notatum* reduced plant height in Year 1, increased number of panicles in Years 1 and 2, and increased number of tillers in Year 2 (Table 2). In *P. notatum*, water deficit led to a reduction in g_s , RWC in Years 1 and 2, lower numbers of tillers and spikelets, and lower SDM in Year 1, and lower plant height in Year 2, while competition with *E. plana* led to lower plant height and number of spikelets in Years 1 and 2, a reduction in g_s and number of tillers in Year 1, and lower RWC and SDM in Year 2 (Table 2).

4 | DISCUSSION

4.1 | Growth and morphological trait responses to drought tolerance

There was a greater reduction in shoot production in *P. notatum* than in *E. plana* in the early growth stage (5–6 true leaves) under water deficit stress, and responses of the number of tillers in this native forage species were consistent across growth stages; in contrast, responses to water deficit in *E. plana* were recorded only during the early growth stage. These results for *P. notatum* are in agreement with those found for the forage grass *Urochloa brizantha* that reduced biomass production as a conservative strategy to avoid drought stress (Santos Menezes et al., 2013). Decreasing aerial biomass due to leaf senescence and leaf



FIGURE 5 Effects of water deficit on enzymatic activity of catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) under contrasting levels of stomatal conductance (g_s) in *Eragrostis plana* and *Paspalum notatum*. Asterisks indicate treatment differences (Student's t-test) at P < 0.05. Error bars are SE.

shedding is a vital strategy in plant for the redution of water losses and to ensure cell turgor (Volaire, 2018). Similarly, decreases in number of tillers under water deficit is likely to have been caused by a reduction in cell division in the meristematic zone (Pezzopane et al., 2017).

We observed an increase in root dry mass allocation under water deficit stress, reflecting the key drought avoidance strategy of biomass partition that allows roots to explore water reserves from deep-soil layers (Alou et al., 2018). Indeed, *E. plana* is characterized by a deep root system, as evidenced by its higher root biomass than native species (including *P. notatum*), at 0–40 cm soil depth (Abichequer et al., 2009), indicating its competitive advantage under water stress.

The PCA showed that panicle initiation was the most sensitive growth stage to drought stress conditions in *E. plana*, driven by responses of RWC, g_s , and panicle number; in contrast, identification of the most sensitive growth stage to water stress in *P. notatum* using PCA was less clear. Based on the assumption that maintenance of growth is reflected by aboveground biomass, the 5–6 leaf growth stage was the most sensitive to drought in both species, as indicated by reduced SDM and tiller number, whereas the most tolerant growth stage in the two species was the tillering stage, as indicated by the lack of effects on growth and reproductive parameters, likely due to maintenance of RWC above critical values.



FIGURE 6 Effects of water deficit on proline content, soluble amino acids, soluble sugar, and starch under contrasting levels of stomatal conductance (g_s) in *Eragrostis plana* and *Paspalum notatum*. Asterisks indicate treatment differences (Student's *t*-test) at *P* < 0.05. Error bars are SE.

	Plant proportion		Water condition			
Fluorescence parameters	100%	50:50	Well-watered	Water deficit	CV (%	
Eragrostis plana						
1st year (2016–2017)						
Fv/Fm	0.7016	0.6803	0.6863	0.6955	5.59	
DI ₀ /RC	0.8497	0.9797	0.9573	0.8721	29.49	
ET ₀ /RC	10.347	10.234	10.332	10.248	7.80	
2nd year (2017-2018)						
Fv/Fm	0.6780	0.6793	0.6611	0.6961	4.85	
DI ₀ /RC	0.6975	0.7105	0.7679	0.6409	21.28	
ET ₀ /RC	0.9411	0.8667	0.8256	0.9422*	7.66	
Paspalum notatum						
1st year (2016–2017)						
Fv/Fm	0.7513	0.7367	0.7400	0.7479	3.15	
DI ₀ /RC	0.7006	0.8488*	0.8179	0.7315	20.12	
ET ₀ /RC	12.115	12.129	1.2907*	11.337	7.63	
2nd year (2017-2018)						
Fv/Fm	0.7716	0.7477	0.7597	0.7596	3.12	
DI ₀ /RC	0.5070	0.4376	0.5042	0.4403	19.58	
ET ₀ /RC	0.9924*	0.8459	0.9657	0.8726	9.72	

Note: Fv/Fm: maximum yield of primary photochemistry of PSII; Dl₀/RC: dissipated energy flux per reaction centre; and, ET₀/RC: flux of electron transport from Q_{A^-} to PQ per reaction centre. Asterisks indicate differences (Student's *t*-test) at P < 0.05.

4.2 | Physiological and biochemical traits of drought tolerance

The reduction in RWC in *E. plana* under moderate water deficit conditions probably induced the activation of the antioxidant system, as indicated by greater activity of CAT, which together with APX in the ASA-GSH enzyme cycle, is responsible for the decomposition of H_2O_2 that is produced under oxidative stress in different cellular organelles (Ren et al., 2016).

Paspalum notatum showed a slight reduction in RWC (80%) under moderate water stress (<50% g_s), where the maintenance of leaf water content by early stomatal closure probably avoided water losses, and effects of the simultaneous accumulation of H₂O₂ may have been offset by accumulation of osmoprotectants (amino acid and sugar) that contribute to membrane stabilisation and reduction of oxidative stress damage.

When the highest stress was observed (75% reduction in g_s and RWC of c. 30 and 29% in *E. plana* and *P. notatum*, respectively), there was a reduction in ET₀/RC and Fv/Fm and an increase in CAT activity in the invasive species *E. plana*, whereas in *P. notatum*, there was no change in ET₀/RC, but a large decrease in Fv/Fm and increase in APX activity. Although effects of these antioxidant enzymes are similar, their characteristics differ, such as subcellular location, that result in contrasting mechanisms of ROS accumulation control (Das & Roychoudhury, 2014).

When the total recovery of g_s was reached, there were simultaneously high concentrations of amino acids and soluble sugars in

E. plana and amino acids and proline in *P. notatum*. These osmolytes play an essential role in the tolerance of plants to water stress, by accelerating damage repair and recovery time (Bandurska et al., 2007). Total recovery of g_s (full recovery time) was reached at 4 and 6 d after water re-establishment for *E. plana* and *P. notatum*, respectively, indicating a potential competitive advantage of, and drought tolerance trait in *E. plana*. The full recovery of g_s included the re-establishment of the PSII machinery and chlorophyll content in the two species, that allowed plants to reduce the activity of antioxidant enzymes.

4.3 | Competition between *E. plana* and *P. notatum* under drought conditions

Water deficit led to physiological and morphological changes in both species, where more rapid responses in the invasive *E. plana* indicate a potential competitive advantage over the native *P. notatum* under water stress conditions.

The inherently taller *E. plana* forms a canopy above *P. notatum* and intercepts greater amounts of light, while under grazing pressure, where herbivores show a preference for the native *P. notatum* and reduce photoassimilate accumulation and partition to its rhizomes and stolons, the deeper root system of *E. plana* allows greater access to water and nutrient lower in the soil profile than species, such as *P. notatum* and *Axonopus afinis* (Medeiros & Fochet, 2007). Thus, under limited water availability, *E. plana* is likely to

TABLE 1Effects of levels ofhydration and competition on chlorophyla fluorescence parameters in *Eragrostis*plana and Paspalum notatum

Plant proportions/treatments	<i>g₅</i> (mmol m ^{−2} s ^{−1})	RWC (%)	Plant height (cm)	Number of tillers	Number of panicles/spikelets	SDM (g)
Eragrostis plana						
1st year (2016–2017)						
100:00	193.0	77.1	106.4*	11.9	2.6	9.5
50:50	185.3	74.2	101.7	10.3	3.7*	12.8*
Well-watered	228.7 *	88.1*	109.2*	11.0	3.4*	11.8
Water stress	149.6	63.1	98.8	11.1	2.8	10.5
CV (%)	9.55	11.08	3.23	25.28	11.28	12.18
2nd year (2017–2018)						
100:00	91.1	59.8	134.0	15.0	6.9	14.7
50:50	97.9	70.7	133.4	21.9*	10.4*	21.7*
Well-watered	130.2*	79.1*	136.1	18.9	9.8*	20.5*
Water stress	58.7	51.5	131.2	17.9	7.4	15.8
CV (%)	13.36	15.42	5.92	15.17	14.80	10.89
Paspalum notatum						
1st year (2016–2017)						
100:00	210.9*	73.3	84.0*	7.3*	1.6 *	5.3
50:50	151.7	67.5	72.9	4.5	0.9	3.2
Well-watered	212.4*	80.7*	81.9	6.2*	1.5*	5.0*
Water stress	150.3	60.2	75.1	5.6	1.0	3.7
CV (%)	15.28	11.54	8.29	8.44	15.36	8.36
2nd year (2017–2018)						
100:00	88.0	69.7*	107.1*	7.6	1.2*	8.3*
50:50	102.3	60.1	93.6	7.1	0.5	4.0
Well-watered	131.5*	83.2*	102.6*	8.6	0.9	6.6
Water stress	58.7	46.5	98.0	5.9	0.8	5.7
CV (%)	18.60	11.81	3.75	37.17	33.96	25.18

TABLE 2 Effects of competition and water deficit on plant height, shoot dry mass (SDM), number of tillers and panicles/spikelets, relative water content (RWC), and stomatal conductance (g_s) in *Eragrostis plana* and *Paspalum notatum*

*Treatment differences (Student's t-test) at P < 0.05.

outcompete *P. notatum*, due to the greater efficiency of its growth and drought tolerance traits.

5 | CONCLUSIONS

Knowledge of water stress response mechanisms in *E. plana* explains the persistence and resilience of this species after long periods of drought in rangelands and highlights the need to limit its spread. The high levels of root mass production by *E. plana* drive its persistence in drought conditions and increase its competitive advantage over native species that are important to managed rangelands. We suggest that rangeland managers and farmers in southern Brazil limit seed production in *E. plana* and reduce potential endozoochoric distribution by restricting the movement of grazing livestock.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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