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Does chronic nitrogen deposition have effects on grass physiology of natural habitats?

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ABSTRACT

Anthropogenic activities increase nitrogen deposition, which can have major consequences for biodiversity and ecosystem function. We experimentally investigated how a community of grass species would respond to three levels of nitrogen enrichment, considering their biomass and efficiency of photosynthesis. The experiments were conducted in an open savanna area with sparse trees (cerrado *sensu stricto*). Fifteen plots were divided considering high nitrogen supplementation, low nitrogen supplementation, and control (without nitrogen application) as treatments. The nitrogen dosages applied in each treatment were based on deposition estimates for 2050 in the Cerrado biome. Three native species had their photosynthetic metabolism evaluated 45 days after the last supplementation. Except for *Echinolaena inflexa*, all the 16 species found in the area expressed C_4 metabolism. *Echinolaena inflexa* and *Loudetiopsis chrysothrix* showed an increase in leaf nitrogen with supplementation, with differences in chloroplastidic pigment contents. Differently from L. *chrysothrix* which increased its chlorophyll content, *E. inflexa* showed a decrease in chlorophyll and carotenoid contents under N supplementation. *E. inflexa* is a small species and its leaf expansion under higher contents of nitrogen suggests that this species was affected by shading caused by other community species that increased their biomass. Supplemental nitrogen did not affect the efficiency of photosynthesis of these species or severely change the grass community biomass. However, we noted only a tendency for higher biomass and biomass per tiller for *Urochloa decumbens*, which suggests a concern about exotic species' performance under conditions of greater availability of nitrogen.

1. Introduction

Anthropogenic activities have become the main cause of global changes, since they are responsible for the increase in the atmospheric CO_2 concentration, changes in precipitation patterns, the higher global average temperature, and rates of nitrogen (N) deposition (Galloway et al., 2004; IPCC 2013). Among these factors, the N deposition rate increases annually and has been illustrated by models that jointly evaluate their deposition and global emissions (Bobbink et al., 2010). According to the model proposed by Dentener et al. (2006), NH_x (a reactive nitrogen) deposition in 2030 is expected to be 5–20 % higher in South America. This amount is five times lower than that presented by the model by Galloway et al. (2004) for some areas of the planet, like Asia, and half of that predicted for South America. These models have considered current legislation and show a rapid increase in N deposition rates in natural areas, a factor considered by researchers as an important driver of change in species composition in different ecosystems and their ecological processes (Bobbink et al., 2010).

Nitrogen is an essential mineral for the photosynthetic process, both in C_3 and C_4 species, as it is a primary constituent of chlorophylls and proteins (Maathuis 2009). However, studies suggest that native C_4 grass species in ecosystems with this nutrient restriction stand out in relation to C_3 species because they are more efficient in nitrogen use (Niu et al., 2008), especially by exotic species (Kolar and Lodge 2001; Ferreira et al., 2016). It is believed that this occurs mostly by the efficient CO_2 concentrator mechanism in the C_4 vascular sheath cells, leading to the saturation of CO_2 by the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), decreasing photorespiration and increasing nitrogen use efficiency (Oaks 1994; Sage et al., 2012). Consequently, a smaller amount of RuBisCO is required to maintain high rates of photosynthesis, growth, and leaf expansion per unit of N in C_4 in relation to C_3 species (Sage and Pearcy 1987).

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Nitrogen deposition in natural areas, especially in regions with low N availability, affects many ecological processes. For instance, an increase in N availability may alter soil pH, change the availability of ions (including Al^{3+} toxic and other metals), or severely reduce nitrification, nitrate availability, and increase ammonium deposition (Bobbink and Hicks 2014). It could also change plant responses to disturbances or make them more susceptible to attack by pathogens and herbivores (Bobbink and Hicks 2014). How individuals of different plant species respond to these changes will ultimately be reflected in the local biomass of each species. However, nitrogen enrichment could still lead to declines in species richness in cases where all species respond positively and increase in biomass (Suding et al., 2005; Bobbink et al., 2010). The "random loss of biomass" hypothesis predicts that higher productivity results in increased competition, resulting in the death of small individuals of all species. Because of the small size of their populations, however, the rare species are at much greater risk of extirpation (Suding et al., 2005). There may also be disparities in extinction probability due to other factors; for instance, native species may be more susceptible to local extinction than invasive species (Suding et al., 2005).

In general, terrestrial ecosystems are strongly influenced by increases in N deposition rates, mainly in ecosystems of acidic and nutrient-poor soils (Bobbink et al., 2010), as is the case in the Brazilian Cerrado savannas (Haridasan 2001). An increase in N rates maximizes productivity and favors the establishment of invasive grasses, often to the detriment of native species (Knochel and Seastedt 2010). Considering the predictions regarding the acceleration of nitrogen deposition in natural areas, the present study has the following goals: (1) to determine the impact of an increase in N deposition on aspects of C_3 and C_4 native grass photosynthetic metabolism, verifying whether C_4 species will be favored to the detriment of C_3 species; (2) to determine the impact of N deposition on exotic and native grasses species, trying to understand whether the exotic species in a Brazilian Cerrado area will be favored to the detriment of native species, and; (3) to test the random loss of biomass hypothesis in species of a Brazilian Cerrado area with sparse trees (*campo sujo*), to determine whether some species will be favored within the community while others will decrease their biomass, and may even disappear from the system. It is expected that nitrogen deposition will lead to favoring of dominant, exotic, and/or C_4 species. Within the community studied, native species, C3 species, and species with low abundance are most at risk of decreased biomass and extirpation."

2. Materials and methods

2.1. Study area and experimental design

The experiment was established at a savanna reserve (Reserva Ecológica do Panga - REP), located 30 km from Uberlândia (19°11'40" S, 48°19'06" W), in southeastern Brazil (Fig. 1). The experiments were conducted in an open area with sparse trees (locally known as cerrado *sensu stricto*) (Gonçalves et al., 2021). The local climate has two well-defined seasons: one hot and humid (October to March) and the other cold and dry (April to September) (Franco 2002). According to the Köppen system, the climate is classified as tropical, Cwa (Alvares et al., 2013), with high temperatures, above 35 °C during summer (Costa and Araújo 2001).

Fifteen 10 × 10 m (100 m^2) plots were demarcated and subdivided into four subplots of equal size (Fig. 1a). High nitrogen supplementation (50 kg N ha^{-1}) (1), low nitrogen supplementation (20 kg N ha^{-1}) (2) and control (without nitrogen application) (3) were used as treatments. The nitrogen dosages applied in each treatment were calculated based on deposition estimates for 2050 in the Cerrado biome (Galloway et al., 2004), which indicate an increase between 1 and 50 kg N ha^{-1} per year until 2050 (the low supplementation represents an optimistic forecast, and the higher supplementation represents a pessimistic forecast). The experiment was established using encapsulated urea (Polyon ®, Koch Agronomic Services, USA) with an NPK formulation of 43–0–0. The experiment was implemented in 2007, with nitrogen applications gradually 6 times throughout the year, and repeated annually until 2015. However, it was observed that the nitrogen applied only in the rainy season had the same effect as the split application, probably because the nitrogen is only available in the presence of soil moisture, decreasing losses by volatilization (according to the manufacturer). In this way, the applications have been conducted during the rainy season since 2015. In order to check the best period for N application, soil moisture was monitored over a year in the experimental area. For this purpose, a soil sample at the center of each subplot (20 samples per treatment) was collected at 5 cm depth. Samples were collected between 8:00 and 10:00 a.m., and the soil was sealed in a plastic bag to prevent the loss of water during transfer to the laboratory. Fresh samples were weighed on an analytical scale (BEL Engineering MARK M 503, Italy), and taken to a forced-air circulation kiln at 60 °C for 24 h for drying and obtaining dry mass. Moisture was determined by the difference between dry and fresh masses. After that, the best moment for fertilizer application and gradual release of the nitrogen was defined as February (Fig. 1).

2.2. Photosynthetic metabolism, chloroplastidic pigment content, and chlorophyll fluorescence

After species identification, samples were submitted to isotopic carbon discrimination (Smith and Epstein 1971) for photosynthetic metabolism (C_4 or C_3) determination. The C_4 photosynthetic pathway was attributed to plants that showed $\delta^{13}C$ values between −7 and −15 ‰, while plants with C_3 metabolism expressed $\delta^{13}C$ values between −20 and −35 ‰ (Ehleringer and Osmond 1989). For this procedure, leaf fragments of three individuals of each species (n = 3) were collected and dried as fast as possible at 60 °C for further maceration and cryogenic grinding. About 50 μg of the samples were stored in tin capsules and taken to an isotopic ratio mass spectrometer (IRMS) (Flash 2000 – Delta V, Thermo Scientific) (Ehleringer and Osmond 1989) in the Stable Isotopes Center, UNESP-Botucatu.

Three native grass species (one C_3 and two C_4) with representative biomass within sampled plots were selected for physiological evaluations. Samples were collected at the end of the rainy season, 45 days after nitrogen application in February 2016. To determine chlorophyll and carotenoid contents, leaf fragments of 10 individuals (n = 10) of *Echinolaena inflexa* (C_3-metabolism), *Tristachya leiostachya* (C_4-metabolism), and *Loudetiopsis chrysothrix* (C_4-metabolism) were taken from each treatment, and their respective areas were measured using ImageJ software. Leaf fragments were weighed and stored for 24 h in 80 % acetone. Afterward, they were macerated, centrifuged, and subjected to spectrophotometric reading (Biospectro SP-220, Brazil), and the chlorophyll *a, b,* and carotenoid contents were calculated according to Lichtenthaler and Wallburn (1983).

Chlorophyll *a* fluorescence was evaluated in five individuals of each species (n = 5) using a modulated fluorimeter (MINI-PAM, Waltz). The values of maximum quantum yield of the photosystem II (F_v/F_m) were determined after dark adaptation for 30 min, where F_m is the maximum fluorescence adapted to the dark and F_v is the variable fluorescence between F_m and minimum dark fluorescence (F_0). The effective quantum yield ($\Delta F/F'_m$) of the photosystem II and the apparent rate of electron transport (ETR) were also determined. $\Delta F/F'_m$ was calculated considering $\Delta F/F'_m = (F'_m - F)/F'_m$, where F is the chlorophyll fluorescence in the sample adapted to light and F'_m is the sample maximum fluorescence adapted to light, with a saturating pulse (Genty et al., 1989). ETR was determined as $ETR = 0.5 \times 0.84 \times (\Delta F/F'm) \times PPFD$, where *0.5* is a factor that considers the luminous excitation of the two photosystems, *0.84* is the

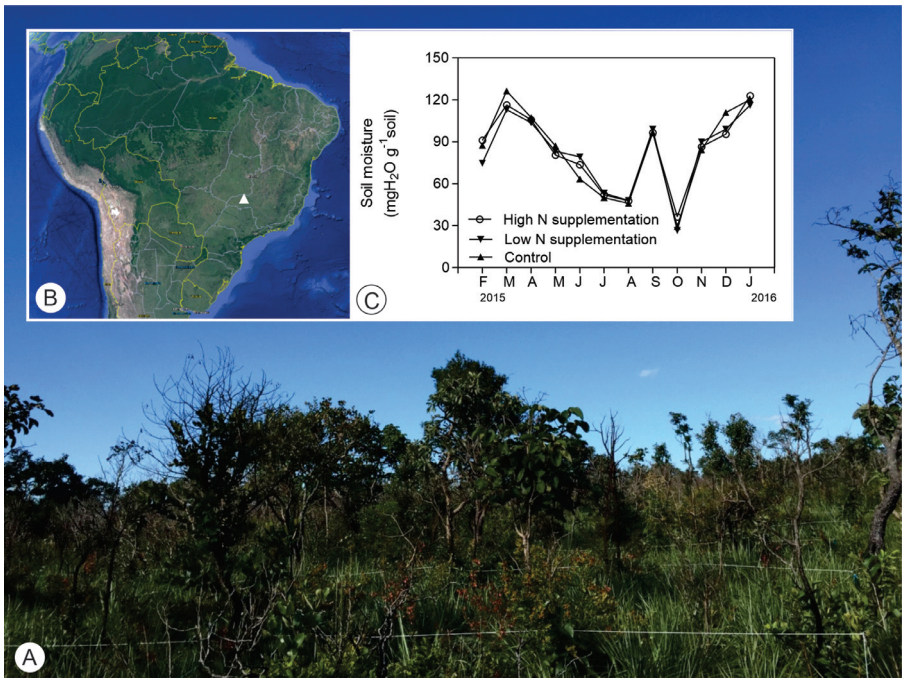

Fig. 1. Experimental area in Estação Ecológica do Panga, southeast of Brazil. **A** – Overview of the experimental unit implemented in a Cerrado area with sparse trees (*campo sujo*). Fifteen plots were established with three N treatments: no N supplementation (control), low N supplementation (20 kg N ha^{-1} per year) and high N supplementation (50 kg N ha^{-1} per year). The nitrogen supplementations were defined according to optimistic and pessimistic predictions of natural deposition in 2050 (Galloway et al., 2004). **B** – Location of the Reserve in the southeast of Brazil. **C** – Soil moisture (%) evaluated throughout the year of experiment (2016).

incident light fraction absorbed and *PPFD* corresponds to the photosynthetically photon flux density (Schreiber et al., 1995).

2.3. Specific leaf area and relative water content

Specific leaf area (SLA) and relative water content (RWC) were obtained from leaf fragments (1 cm^2, except for *E. inflexa*, for which whole leaves were used and their areas were determined using ImageJ software) taken from 15 individuals of each species (n = 15). For RWC determination, after obtaining fresh mass (FM), fragments were immersed in distilled water for 24 h and weighed on an analytical scale (Shimadzu AUY 220, Japan) to obtain the turgid mass (TM). Finally, samples were dried in a forced-air circulation kiln at 60 °C until reaching constant weight, determining the dry mass (DM). From these data, SLA was calculated as *SLA=A/DM* (Witkowski and Lamont 1991), while the RWC was obtained as $RWC = [(FM - DM)/(TM - DM)] \times 100$ (Turner 1981).

2.4. Carbohydrate and nitrogen contents

To determine carbohydrate contents, leaf and stem samples of 10 individuals of each species (n = 10) were oven-dried to constant mass. After grinding for material homogenization, 0.2 g of the samples were centrifuged in methanol:chloroform:water (12:5:3) solution to extract water-soluble sugars. Water-soluble polysaccharide content determination was obtained by resuspension of the pellet in 10 % ethanol. The residue was resuspended in 5 % perchloric acid for starch extraction. All the extracts were submitted to a phenol-sulphuric acid reaction to obtain carbohydrate content, using glucose as standard. The extracts were read on a spectrophotometer (Biospectro SP-220, Brazil) at wavelength ok 490 nm for carbohydrate content determination (Chow and Landhaüsser 2004).

To determine nitrogen content, samples were dried, and 0.2 g of material was stored in tin capsules to be analyzed in a CNH Elemental Analyzer (Eurovector, EA3 3000, Italy) placed in the Laboratory of Multiusers, UFU-Uberlândia.

2.5. The impact of N deposition on grass community (aboveground biomass and species identification)

Aboveground biomass evaluation was performed for the grasses 90 days after fertilizer application, in areas of 1 m^2 drawn within each subplot (4 m^2 per plot), excluding plot borders. Tussocks of the species contained within the limits of this square were collected for identification, counting of tillers, and biomass evaluation. Species identification was performed by comparison with herbal material deposited in the HUFU Herbarium or by consulting specialists. Aboveground and living biomass was determined after drying the material in a forced air circulation kiln at 60 °C until reaching constant weight and weighing on an analytical scale.

2.6. Statistical analysis

All data obtained from physiological analyses performed with the species *Echinolaena inflexa, Tristachya leiostachya,* and *Loudetiopsis chrysothrix* were tested for normality and homoscedasticity and compared among treatments (without and with two nitrogen supplementations). Parametric data were submitted to analysis of variance (ANOVA), and non-parametric data were compared using Kruskal-Wallis, with $p < 0.05$ of significance. Subsequently, a Tukey test was applied to compare means. All statistical tests were performed using Systat software, version 10.2.

3. Results

3.1. The impact of N deposition on C_3 and C_4 native grass photosynthetic metabolism

3.1.1. Pigment content and chlorophyll *a* fluorescence

Among the identified species, all showed C_4 photosynthetic metabolism, with isotopic carbon ratios between −13.86 and −15.20 ‰, except *E. inflexa*, classified metabolically as C_3 ($\delta^{13}C$ of −29.8 ‰) (Table 1).

Table 1
– Isotopic ratio of carbon (δ13C), photosynthetic pathway (C_3 or C_4), and aboveground biomass of grasses under three distinct treatments of nitrogen supplementation: no N supplementation (control), low N supplementation (20 Kg N ha^{-1} per year) and high N supplementation (50 Kg N ha^{-1} per year). The nitrogen supplementations were defined according to optimist and pessimist predictions of natural deposition in 2050 (Galloway et al., 2004). The data show the total biomass of each species followed by the number of plots in which the species appears (between parenthesis). The experimental area was established in a *campo sujo* area (Brazilian savanna), in southeast Brazil.

Species	$\delta^{13}C$	C_3 / C_4	Aerial biomass found in the 5 plots of each treatments (g of dry mass)			Total aerial biomass per species (g m^2)
			Control	Low N	High N	
Aristidoideae						
Aristida riparia	−14.4	C_4	1.67 (2)	0.84 (3)	no	2.51
Panicoideae						
Axonopus aureus	−14.7	C_4	0.15 (1)	No	no	0.15
Axonopus barbigerus	−15.2	C_4	7.99 (4)	6.14 (5)	0.54 (1)	14.67
Echinolaena inflexa	−29.8	C_3	6.97 (2)	2.85 (2)	7.51 (3)	17.33
Elionurus muticus	−14.0	C_4	3.84 (3)	5.66 (3)	0.54 (3)	10.04
Loudetiopsis chrysothrix	−14.8	C_4	10.82 (4)	18.73 (5)	4.43(2)	33.98
Panicum olyroides	−14.1	C_4	0.70 (2)	43.22 (2)	11.58 (2)	55.50
Paspalum lineare	−13.8	C_4	0.62 (1)	No	2.76 (2)	3.38
Paspalum geminiflorum	−14.0	C_4	No	0.42 (1)	0.25 (1)	0.67
Paspalum hyalinum	−14.4	C_4	2.61 (3)	0.32 (1)	No	2.93
Paspalum sp.	−14.1	C_4	2.11 (2)	0.47 (2)	4.28 (3)	6.86
Paspalum sp.1	−13.9	C_4	21.57 (5)	18.22 (5)	11.34 (4)	45.07
Schizachyrium glaziovii	−14.3	C_4	2.58 (5)	5.62 (4)	4.38 (5)	12.58
Tristachya leiostachya	−14,5	C_4	131.9 (5)	115.11(5)	131.9 (5)	378.91
Urochloa decumbens	−14.7	C_4	1.21 (2)	1.89 (3)	35.21 (5)	38.31
No identified (6 plants)	–	–	0.27	2.56	0.17	3.00

Echinolaena inflexa showed lower values of total chlorophyll content under the high N supplementation treatment (Fig. 2), and lower carotenoid contents under both the low and high N supplementation treatments. Despite low values for both chlorophyll and carotenoid, only plants under the high nitrogen supplementation treatment showed differences in the chlorophyll/carotenoid ratio, with values higher than those under low N supplementation and control treatments. *Loudetiopsis chrysothrix* had higher chlorophyll content for plants under the high N supplementation treatment (Fig. 2). However, the ratios between chlorophyll *a* and *b*, and chlorophyll and carotenoids were not different.

Maximum ($\Delta F/F'_m$) and effective (F_v/F_m) quantum yields, and relative electron transport rate (ETR) did not show differences among treatments for any of the evaluated species (Fig. 3).

3.1.2. Relative water content and specific leaf area determination

Only the C_3 species, *E. inflexa*, showed differences in relative water content (RWC) and specific leaf area (SLA) values among treatments (Fig. 2e and f). For RWC, plants under low and high N supplementation treatments expressed means approximately 37 % higher than those under the control treatment. Regarding SLA, the values in plants under the high N supplementation treatment were 46 % higher than those found for plants under the control treatment.

3.1.3. Determination of carbohydrate and nitrogen contents

For carbohydrate quantification, the three native grass species did not show statistical differences among treatments considering the values of total soluble sugar content (TSS), for both leaves and stems (Fig. 4a and d, respectively). However, *E. inflexa* had higher levels of TSS in stems (mean among treatments of 255.8 $\mu g\ g^{-1}$ DM for leaves and 3681.7 $\mu g\ g^{-1}$ DM for stems), while *T. leiotaschya* showed the inverse strategy, with higher storage of TSS in leaves (mean among treatments of 1037.1 $\mu g\ g^{-1}$ DM for leaves and 124.6 $\mu g\ g^{-1}$ DM for stems). *Tristachya leiostachya* and L. *chrysothrix* also did not show differences in water-soluble polysaccharides (WSP) and starch contents, both in leaves and stems (Fig. 4b, c, e, and f). However, the C_3 species, *E. inflexa*, showed lower WSP values in both leaves and stems when N supplementation was applied (Fig. 4b and e), and, in general, had higher starch accumulation in stems of individuals under the low N supplementation treatment.

High N supplementation increased nitrogen contents in *E. inflexa* and L. *chrysothrix* leaves (36.3 % and 25.9 % higher in relation to the control treatment, respectively). Nitrogen supplementation did not alter this nutrient level in *T. leiostachya* leaves (Fig. 5).

3.2. The impact of N deposition on grass community

A total of 21 species of grasses were found, of which 15 were identified: *Aristida riparia* Trin., *Axonopus aureus* P. Beauv., *Axonopus barbigerus* Hitchc., *Echinolaena inflexa* (Poir.) Chase, *Elionurus muticus* (Spreng.) Kuntze, *Loudetiopsis chrysothrix* (Nees) Conert, *Panicum olyroides* Kunth, *Paspalum lineare* Trin, *Paspalum hyalinum* Nees ex Trin., *Paspalum* L. sp., *Paspalum geminiflorum* Steud., *Paspalum* L. sp.1, *Schizachyrium glaziovii* Peichoto, *Tristachya leiostachya* Nees and *Urochloa decumbens* (Stapf.) RD Webster. (only exotic species found in the area), and six other species were not identified due to a lack of reproductive material (Table 1).

Paspalum geminiflorum was not found in plots under the control treatment, *A. aureus* and *P. lineare* were not present in plots under the low nitrogen supplementation treatment, and *A. riparia, A. aureus,* and *P. hyalinum* were not present in plots under the high nitrogen supplementation treatment (Table 1). Despite the absence of these species in plots under specific treatments, there was no statistical difference in the number of species found among treatments (Table 2). Altogether, species that were not found in plots under all treatments represented less than 2 % of the total aboveground biomass of all species.

The average aboveground biomass obtained for all species under the high nitrogen supplementation treatment was 212.6 ± 65.3 g m^{-2}, under the low nitrogen supplementation treatment was 222.1 ± 48.2 g m^{-2}, and in the control was 195.1 ± 13.6 g m^{-2} (Table 2). No statistical differences were detected in the aboveground biomasses among the treatments. *Tristachya leiostachya* had the highest aboveground biomass, representing 61 % of the total biomass. *Loudetiopsis chrysothrix, P. olyroides, Paspalum* sp.1, and *U. decumbens* accounted for 28 % of the total biomass, and the other species completed the remaining 11 %. The C_3 grass, *E. inflexa*, contributed only 3 % of the total biomass (Table 1). The experiment was conducted in a natural area characterized by high species diversity, with a non-uniform distribution of species. In this way, certain species are more prevalent in some plots than others. To prevent

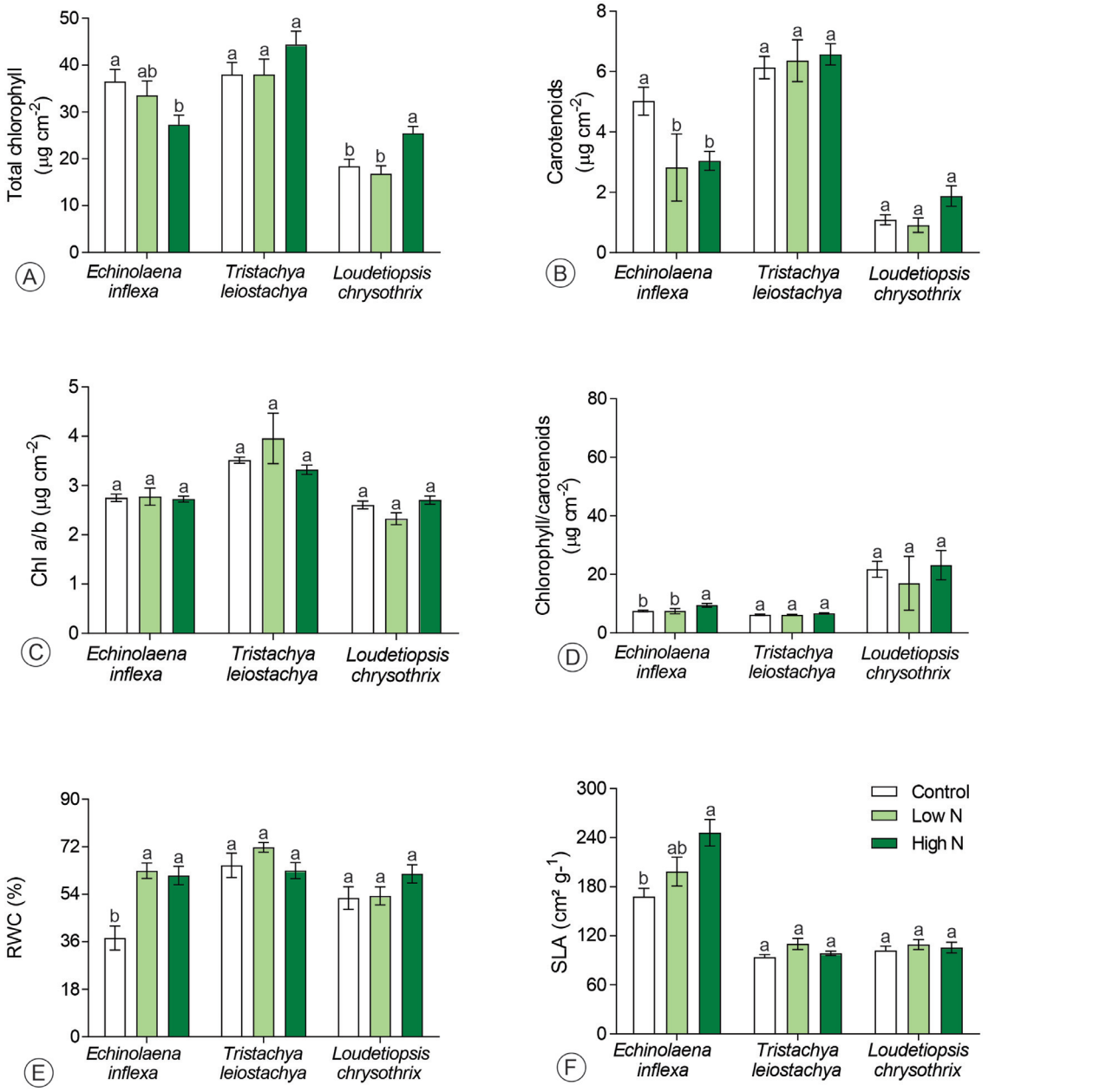

Fig. 2. Content of chloroplast pigments, relative water content (RWC) and specific leaf area (SLA) in leaves of *Echinolaena inflexa* (C_3), *Tristachya leiostachya* (C_4) and *Loudetiopsis chrysothrix* (C_4) under three distinct treatments of nitrogen supplementation: no N supplementation (control), low N supplementation (20 kg N ha^{-1} per year) and high N supplementation (50 kg N ha^{-1} per year). **A** – Total chlorophyll; **B** – Carotenoids; **C** – Chlorophyll *a*/*b* rate; **D** – Chlorophyll/carotenoid rate; **E** – RWC and **D** – SLA. Values represent means ± standard deviation (n = 10). Different letters indicate statistical differences among treatments at 5 % of probability.

misinterpretation, the data presented in Table 1 were expressed considering the total biomass of each species across all plots in which they appear.

Aboveground biomass distribution appears to be different in the three nitrogen treatments (Table 1), however, all species were not found in the five plots of each treatment, which made statistical analysis difficult. Some species had lower values of biomass in nitrogen treatments than the plants on control treatment, such as *A. barbigerus* under both treatments (23 % lower under low N and 93 % lower under high N supplementation); *P. hyalinum* (87 %), *Paspalum* sp. (77 %), *E. inflexa* (59 %) and *A. riparia* (50 %), under low N supplementation; and *E. muticus* (86 %), L. *chrysothrix* (59 %) and *Paspalum* sp.1 (49 %) under high N supplementation. Some species had higher values of biomass in N treatments, such as *P. olyroides* (61 and 16 times higher) and *S. glaziovii* (2.17 and 1.69 times higher) under low N supplementation and high N supplementation treatments, respectively; *E. muticus* (1.4 times) and L. *chrysothrix* (1.7 times) showed higher values under low supplementation; and *P. lineare* (4.5 times), *Paspalum* sp. (2 times) and *U. decumbens* (29 times) showed higher values under high N supplementation.

The average of biomass per tiller of each species did not change among treatments, except for *P. olyroides* ($p \leq 0.01$, $F = 83.9$), which showed 1.26 g per tiller under control and 4.5 g per tiller under high N supplementation treatment (Table 3). Although some species did not have significant differences in this parameter among treatments (probably due to the low number of plots in which they appeared), some species showed lower values of biomass by tiller in the higher nitrogen supplementation treatment, e.g. *A. riparia* showed 37 % lower values of biomass under control treatment in relation to low N supplementation. L. *chrysothrix* and *P. hyalinum* showed not only

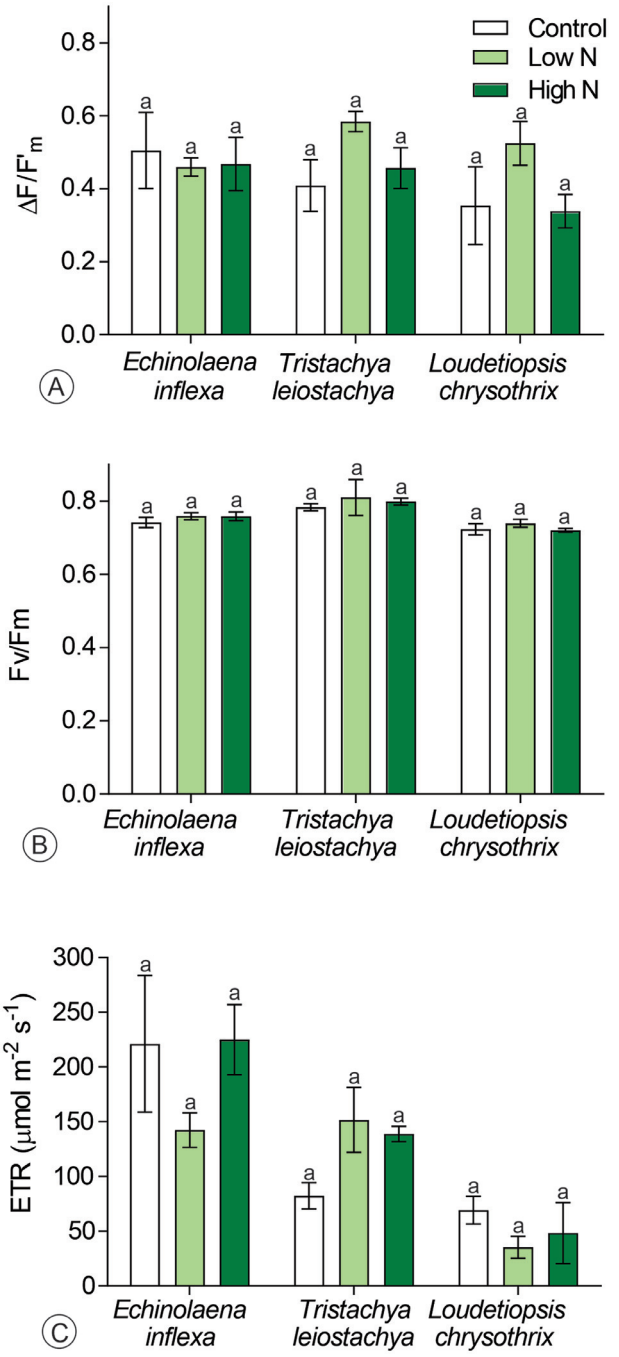

Fig. 3. Evaluation of chlorophyll *a* fluorescence in leaves of *Echinolaena inflexa* (C_3), *Tristachya leiostachya* (C_4) and *Loudetiopsis chrysothrix* (C_4) under three distinct treatments of nitrogen supplementation: no N supplementation (control), low N supplementation (20 kg N ha^{-1} per year) and high N supplementation (50 kg N ha^{-1} per year). **A** – Effective quantum yield ($\Delta F/F'_m$) in light adapted leaves; **B** – Maximum quantum yield (F_v/F_m) in dark adapted leaves; **C** – Relative electron transport rate (ETR). Values represent means ± standard deviation (n = 5). No statistical differences among treatments were found at 5 % of probability.

lower values of aboveground biomass, but also the number of plots in which they were present, and *A. barbigerus* appeared in four plots under the control treatment and in only one plot under high N supplementation treatment. This latter species, along with *Paspalum* sp. and *U. decumbens*, had higher values of biomass by tiller under high N supplementation, but also appeared in a low number of plots.

4. Discussion

4.1. Nitrogen effects on C_3 and C_4 native grass metabolism

The higher N content in *E. inflexa* reflects the lower investment in RuBisCO in C_4 relative to C_3 species. Although *T. leiostachya* leaves did not show differences in the nitrogen contents even after N supplementation, both *E. inflexa* and L. *chrysothrix* showed an increase in leaf nitrogen when high N supplementation was performed. In C_3 species, RuBisCO can represent 50 % of the soluble proteins, whereas in C_4 species it varies between 10 and 25 % (Gunasekera and Berkowitz 1993). The C_4 species may forward the high nitrogen availability to the synthesis of other nitrogenous compounds, which may include complex proteins and photosynthetic pigments. In this case, nitrogen supply may have a greater impact on the photochemical step of C_4 plants and corroborates the increase of chlorophyll content in L. *chrysothrix*. However, it is worth remembering that the nitrogen partition within the whole plant is dynamic and can be modified through time and according to limiting and stressful environmental factors. For example, on sunny days, most of the nitrogen in C_3 plants leaves is destined for RuBisCO production to increase carbon fixation, whereas on days with low luminosity, nitrogen can be translocated to chlorophyll synthesis, increasing light absorption (Kimura et al., 1998). *Echinolaena inflexa* showed a decrease in chlorophyll and carotenoid contents under N supplementation, but these changes were not enough to produce an effect on the efficiency of photosynthesis. Indeed, the low chloroplastidic pigment contents under N supplementation may reflect the increase in SLA values and some leaf expansion.

The entire photosynthetic process begins with energy absorption and transfer by photosynthetic pigments found in photosystems' antenna complexes. Chlorophylls and carotenoids are, then, responsible for sunlight transformation into chemical energy, and are considered excellent indicators of light stress (Ballottari et al., 2012; Jahns and Holzwarth 2012; Ruban et al., 2012). The imbalance in chlorophyll/carotenoid ratios found here may reflect environmental conditions that are unfavorable to plant growth and development, such as greater water deficit and excess of light (Hendry and Prince 1993). Carotenoids are considered photoprotective, since energy excess in photosystems leads to de-epoxidation of violaxanthin to anteraxanthin and zeaxanthin, the reverse process being responsible for regulating energy dissipation. Energy is released mainly as heat, increasing the values of non-photochemical quenching (Demmig-Adams et al., 1996; Jahns and Holzwarth 2012; Domonkos et al., 2013). However, the negative impact of nitrogen supplementation on chloroplastidic pigment content in *E. inflexa* was general and increased the chlorophyll/carotenoid ratio. This change indicates that high light is not a stressful resource for *E. inflexa*, which remains shaded by other grasses and shows low investment in energy-dissipating pigments. Despite the changes in chloroplastidic pigment content and balance, no effect was detected directly on photochemical yield in N supplementation treatments. Maximum quantum yield was similar for all the three species in all treatments (values close to 0.8), which also indicates that these plants were not photoinhibited (Lüttge et al., 1998). When environmental conditions are advantageous for plant growth, F_v/F_m is kept in a stable range, but it decreases when environmental conditions are adverse for plant growth (Allen and Ort 2001).

Carbohydrates produced by grasses during the rainy season are primarily intended for immediate requirements and secondarily for storage (Jordan 1983). Total soluble sugars such as glucose, fructose, and sucrose are carbohydrates used for transport and osmoregulation in cells, while polysaccharides are mainly structural carbohydrates (cell wall); starch, as an insoluble carbohydrate, acts as a reserve

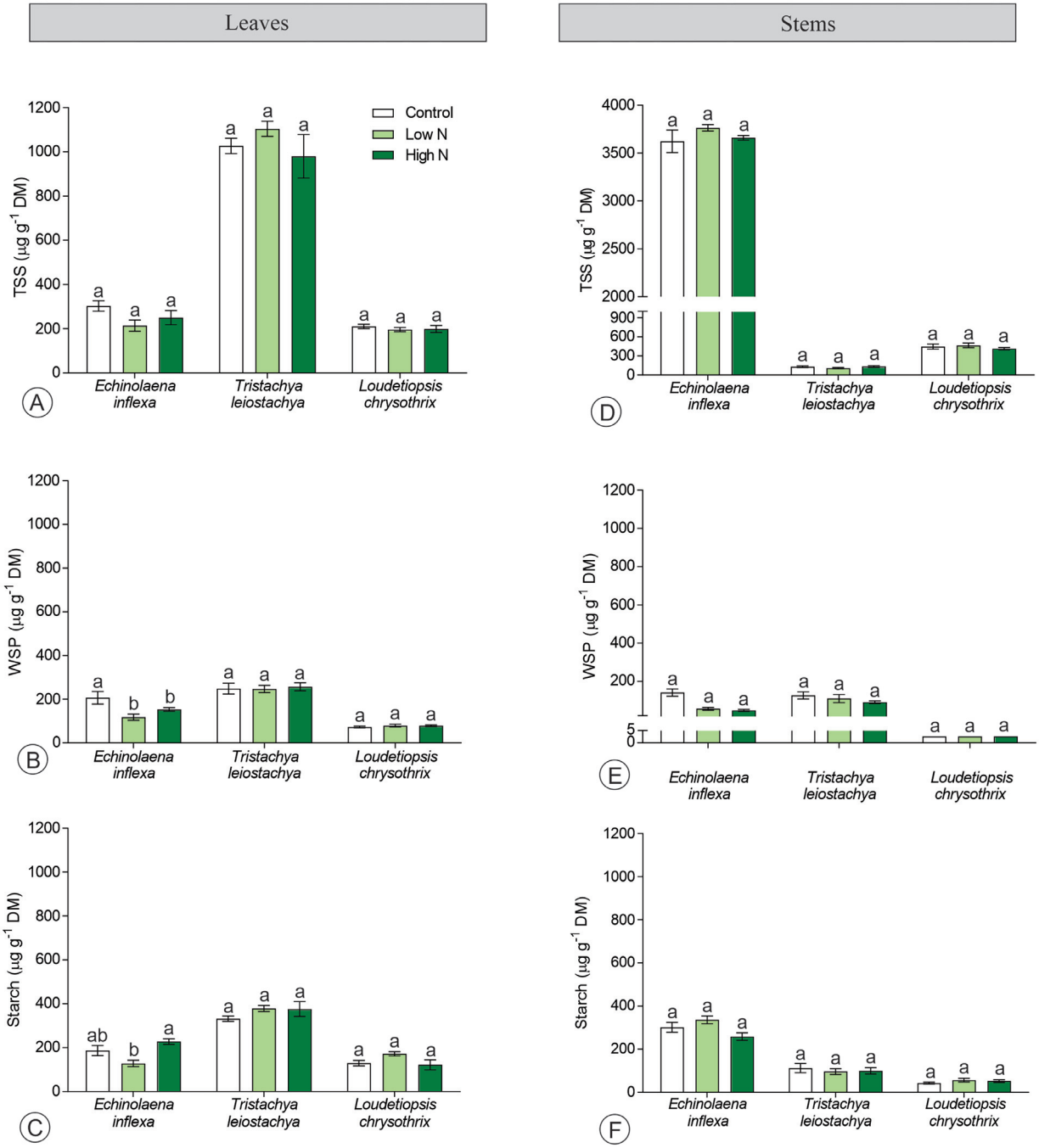

Fig. 4. Content of carbohydrates in leaves and stems of *Echinolaena inflexa* (C_3), *Tristachya leiostachya* (C_4), and *Loudetiopsis chrysothrix* (C_4) under three distinct treatments of nitrogen supplementation: no N supplementation (control), low N supplementation (20 kg N ha^{-1} per year) and high N supplementation (50 kg N ha^{-1} per year). **A**–**C** – Leaves. **(A)** Total soluble sugars (TTS); **(B)** Water soluble polysaccharides (WSP) and **(C)** Starch content. **D**–**F** – Stem. **(D)** Total soluble sugars (TTS); **(E)** Water soluble polysaccharides (WSP) and **(F)** Starch content. Values represent means ± standard deviation (n = 10). Different letters indicate statistical differences among treatments at 5 % of probability.

(Preiss 1988). The higher specific leaf area and relative water content for *E. inflexa* leaves under high nitrogen concentrations suggests cellular and leaf expansion and reflects the lower contents of water-soluble polysaccharides. The foliar expansion at this moment may be a compensatory mechanism of shading caused by other benefited species within the community by the nitrogen supply. *Echinolaena inflexa* has a small size, simple architecture, and small leaves. Despite the lower tissue density, the impact of nitrogen supply on it cannot be seen as negative, as there was no biomass loss of these plants under high N supplementation in relation to those under the control treatment. The low starch content in *E. inflexa* stems under high N supplementation may be a result of re-distribution due to the mobilization of these carbohydrates to other plant organs, which could increase biomass; this has previously been reported in other studies with nitrogen supplementation (Pettit and Fagan 1974).

Tristachya leiostachya appears to be less affected by nitrogen supplementation. The absence of metabolic or biomass differences indicates the adaptation of some grasses to Cerrado soils (Humphreys 1991; Barbosa et al., 2014) and their competence in nitrogen use (Yuan et al., 2007). This efficiency reflects its dominance under all treatments, representing 61 % of the total biomass in relation to the other species identified in the area. Its competence regarding nitrogen use can be noted by the higher contents of leaf nitrogen, shown by plants under the high N supplementation treatment, only

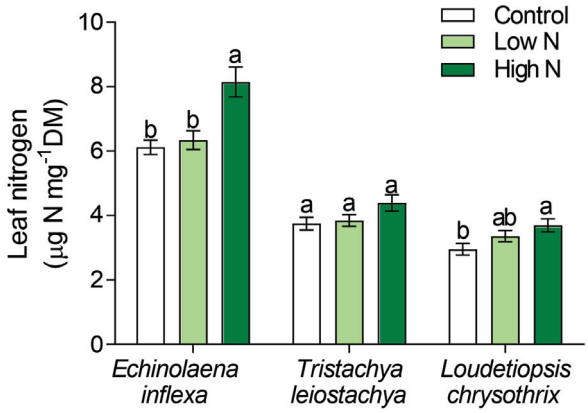

Fig. 5. Content of nitrogen in leaves of *Echinolaena inflexa* (C_3), *Tristachya leiostachya* (C_4) and *Loudetiopsis chrysothrix* (C_4) under three distinct treatments of nitrogen supplementation: no N supplementation (control), low N supplementation (20 kg N ha^{-1} per year) and high N supplementation (50 kg N ha^{-1} per year). **A** – Chlorophyll *a*; **B** – Chlorophyll *b*; **C** – Total chlorophyll; **D** – Carotenoids; **E** – Chlorophyll *a*/*b* rate; **F** – Chlorophyll/carotenoid rate. Values represent means ± standard deviation (n = 10). Different letters indicate statistical differences among treatments at 5 % of probability.

influencing the increase in total chlorophyll content, not demonstrating a large-scale effect for such species (such as total biomass increases or change in carbohydrate contents). In a way, the same can be said for the other C_4 species studied, L. *chrysothrix*, which, despite the lower representation when compared to *T. leiostachya*, was still among the six species with the highest biomass and homogeneous distribution (occurred in a high number of plots).

4.2. Consequences of nitrogen increase in community structure and in exotic species propagation

The results show that the nitrogen supplementation, with optimist and pessimist predictions of N increasing in 2050, does not severely change the studied grass community. Besides our analysis showed some differences in plant metabolism, the experiment has been performed since 2007, and small differences were found for plant biomass or biomass of tillers for each species. The study was carried out in a conservation area (Panga), where the *U. decumbens* is an invasive species and is spreading in some places. Although its occurrence is incipient and heterogeneous, the higher absolute values of biomass and biomass per tiller suggest a concern about its performance under conditions of greater availability of nitrogen. The growth and competitive ability of invasive plants under N supplementation have been proven to be superior to those of native plants (Sun et al., 2023). However, few studies have tested competitive interactions between invasive aliens and multiple native species, particularly in response to N deposition in natural areas (Guo et al., 2023).

Anthropogenic activities have contributed to the increase of nitrogen deposition rates in the world (Nybakken et al., 2009; Fowler et al., 2013), which in turn facilitates the invasion, permanence, and expansion of exotic grass species (Vilà and Weiner 2004; Concilio et al., 2017; Eller and Oliveira 2017). The International Union for Conservation of Nature (IUCN) and Brazilian authorities have identified exotic species as the third greatest threat to biodiversity in Brazil after habitat loss and direct actions resulting from interactions among native species (Alho et al., 2011). Several African Poaceae species have become very well adapted to Brazil's climatic conditions, with one of them being *U. decumbens*, also found in the study area. *Urochloa decumbens* stands out for its high growth rate, high photosynthetic performance efficiency, high reproductive and regeneration capacity, effective use of nutrients, as well as high tolerance to defoliation and herbivory, factors that together guarantee its high efficiency in open area colonization (Kolar and Lodge 2001; Ferreira et al., 2016). As well as other exotic C_4 species, the rapid development of *U. decumbens* makes it quite efficient in nitrogen uptake (Asner and Beatty 1996; He et al., 2011; Fonseca et al., 2014). N deposition may enhance the growth of invasive species, increasing the total leaf

Table 2
– Aboveground biomass and number of tillers of grasses under three distinct treatments of nitrogen supplementation: no N supplementation (control), low N supplementation (20 Kg N ha^{-1} per year), and high N supplementation (50 Kg N ha^{-1} per year). The data show means ± standard deviation.

	Treatments with different N supplementation		
	Control	Low N	High N
Number of tillers (tillers m^{-2})*	179.3 ± 45.5	218.7 ± 90.3	141.6 ± 46.1
Aboveground biomass per treatment (g m^{-2})*	195.1 ± 13.6	222.1 ± 48.2	212.6 ± 65.3
Number of species per plot	8.2 ± 1.3	8.6 ± 2.4	7.2 ± 0.8

* Data of no-identified species were included in the number of tillers and aboveground biomass comparisons.

Table 3
– Average of biomass per tiller (g DM tiller-1) of grasses under three distinct treatments of nitrogen supplementation: no N supplementation (control), low N supplementation (20 Kg N ha^{-1} per year), and high N supplementation (50 Kg N ha^{-1} per year). The data show means ± standard deviation followed by the number of plots in which the species appears (between parenthesis). No statistical differences among treatments were found according to ANOVA at 5 % of probability.

Species	Average of tillers biomass (g DM per tiller $^{-1}$) under three treatments of nitrogen supplementation		
	Control	Low N	High N
Aristida riparia	0.45 ± 0.48 (2)	0.28 ± 0.14 (3)	0.00
Axonopus aureus	0.77 (1)	0.00	0.00
Axonopus barbigerus	0.68 ± 0.55 (4)	1.08 ± 0.93 (5)	2.17 (1)
Echinolaena inflexa	0.54 ± 0.18 (2)	0.60 ± 0.05 (2)	0.73 ± 0.2 (3)
Elionurus muticus	0.25 ± 0.10 (3)	0.20 ± 0.11 (3)	0.26 ± 0.1 (3)
Loudetiopsis chrysothrix	0.49 ± 0.25 (4)	0.35 ± 0.09 (5)	0.31 ± 0.01 (2)
Panicum olyroides	1.26 (2)	2.11 ± 0.31 (2)	4.49 ± 0.1 (2)
Paspalum lineare	0.27 (1)	0.00	0.30 ± 0.16 (2)
Paspalum geminiflorum	0.00	1.39 (1)	1.68 (1)
Paspalum hyalinum	0.89 ± 0.19 (3)	0.59 (1)	0.00
Paspalum sp.	0.74 ± 0.26 (2)	0.97 ± 0.90 (2)	1.43 ± 0.76 (3)
Paspalum sp.1	0.89 ± 0.19 (5)	0.65 ± 0.23 (5)	0.77 ± 0.2 (4)
Schizachyrium glaziovii	0.13 ± 0.3 (5)	0.26 ± 0.33 (4)	0.17 ± 0.67 (5)
Tristachya leiostachya	2.52 ± 0.34 (5)	2.28 ± 0.40 (5)	2.65 ± 0.39 (5)
Urochloa decumbens	0.70 ± 0.63 (2)	0.75 ± 0.57 (3)	2.50 ± 0.68 (3)

area, plant size, and biomass accumulation (Guo et al., 2023; Sun et al., 2023).

Exotic species invasion occurs accidentally or by a predetermined introduction (Grace et al., 2002; Hulme 2017) and has caused damage to biodiversity by the great competitive ability related to native grass species (Pivello et al., 1999a,b; Knochel et al., 2010; Rossi et al., 2014; Ferreira et al., 2016) and interference in fire regime (Rossi et al., 2014), which may lead to native species extinction (Mooney and Cleland 2001; Rossi et al., 2014; Theoharides and Jeffrey 2007). A study that deals with the effect of *Melinis minutiflora* proliferation, another exotic grass, in a transition area between the Cerrado and the Atlantic Forest, showed a negative correlation between the biomass of native monocotyledons and the exotic grass (Rossi et al., 2014). Species composition and productivity of natural environments are influenced by soil nutrient status. When resource availability is high, competition is expected to increase with the predicted exclusion of species of lower competitive ability (Tsuvuura and Kirkman 2013). In addition, the increased availability of exotic grasses aboveground biomass can drastically decrease light incidence on the soil surface, which impairs germination and recruitment of native species in the seed bank (Ferreira et al., 2016). This higher biomass becomes combustible material during the drought season, increasing the intensity, frequency, and duration of fires (D'Antonio and Vitousek 1992; Medeiros and Miranda 2005).

In conclusion, the high nitrogen supplementation applied in our experiment, representing a pessimistic scenario of N deposition, did not affect C_4 native grasses (two dominant species were studied), but improved aspects of the metabolism of *Echinolaena inflexa*, the only C_3 grass species in the area. The leaf expansion of *E. inflexa* (a structurally small species with small leaves) under higher contents of nitrogen suggests that this species can be affected by shading caused by other community species that increased their biomass. However, the changes observed in *E. inflexa* metabolism were not sufficient to modify its photosynthetic performance or to cause a significant increase in its biomass within the community. Actually, small differences were found in plant biomass or biomass of tillers for each species. Our data, with higher absolute values of biomass and biomass per tiller in *U. decumbens* under N supplementation, suggest some concern about its performance and spread under conditions of greater availability of nitrogen.

Declaration of Competing Interest

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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Author contributions

AMF: Conceptualization, Writing, Editing, APM: Data curation, Writing, Editing. HLV and EMB: Original draft preparation, Reviewing. VEC: Isotopic analysis. ASFPM: Conceptualization, Writing, draft preparation, Editing, and Reviewing.

References

Alho, C.J.R., Mamede, S., Bitencourt, K., Benites, M., 2011. Introduced species in the Pantanal: implications for conservation. Braz. J. Biol. 71, 321–325.
Allen, J., Ort, D.R., 2001. Impacts of chilling temperatures on photosynthesis in warm-climate plants. Trends Plant Sci. 6, 39–42.
Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M., 2013. Modeling monthly mean air temperature for Brazil. Theor. Appl. Climatol. 113, 407–427. https://doi.org/10.1007/s00704-012-0796-6.
Asner, G.P., Beatty, S.W., 1996. Effects of an African grass invasion on Hawaiian shrubland nitrogen biogeochemistry. Plant Soil 186, 205–211.
Ballottari, M., Girardon, J., Dall'Osto, L., Bassi, R., 2012. Evolution and functional properties of Photosystem II light harvesting complexes in eukaryotes. Biochim. Biophys. Acta 1817, 143–157.
Barbosa, E.R.M., Tomlinson, K.W., Carvalheiro, L.G., Kirkman, K., de Bie, S., Prins, H.H.T., van Langevelde, F., 2014. Short-term effect of nutrient availability and rainfall distribution on biomass production and leaf nutrient content of savanna tree species. PLoS One 9, e92619. https://doi.org/10.1371/journal.pone.0092619.
Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmentt, B., Erisman, J.W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol. Appl. 20, 30–59.
Bobbink, R., Hicks, K., 2014. Factors affecting N deposition impacts on biodiversity: an overview. In: Sutton, MA, Mason, KE, Sheppard, LJ, Sverdrup, H, Haeuber, R, Hicks, WK (Eds.), Nitrogen deposition, Critical Loads and Biodiversity. Springer, Berlin, pp. 127–138.
Chow, P.S., Landhäusser, S.M., 2004. A method for routine measurements of total and starch content in woody plant tissue. Tree Physiol. 24, 1129–1136.
Concilio, A.L., Seastedt, T.R., Nippert, J.B., 2017. Changing edaphic conditions and exploitation of an expanded phenological niche allows for increased exotic (introduced) plant species dominance. Plant Soil 415, 299–315.
Costa, A.A., Araújo, G.M., 2001. Comparação da vegetação arbórea de cerradão e cerrado na Reserva do Panga, Uberlândia, Minas Gerais. Acta Bot. Bras. 15, 63–72.
D'antonio, C.M., Vitousek, P.M., 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annu. Rev. Ecol. Syst. 23, 63–87.
Demmig-Adams, B., Gilmore, A.M., Adams, III, W.W., 1996. Carotenoids 3: in vivo function of carotenoids in higher plants. FASEB J. 10, 403–412.
Dentener, F., Drevet, J., Lamarque, J.F., Bey, I., Eickhout, B., Fiore, A.M., Hauglustaine, D., Horowitz, L.W., Krol, M., Kulshrestha, U.C., Lawrence, M., Galy-Lacaux, C., Rast, S., Shindell, D., Stevenson, D., Van Noije, T., Atherton, C., Bell, N., Bergman, D., Butler, T., Cofala, J., Collins, B., Doherty, R., Ellingsen, K., Galloway, J., Gauss, M., Montanaro, V., Muller, J.F., Pitari, G., Rodriguez, J., Sanderson, M., Solmon, F., Strahan, S., Schultz, M., Sudo, K., Szopa, S., Wild, O., 2006. Nitrogen and sulfur deposition on regional and global scales: a multimodel evaluation. Global Biogeochem. Cy. 20. https://doi.org/10.1029/2005GB002672.
Domonkos, I., Kis, M., Gombos, Z., Ughy, B., 2013. Carotenoids, versatile components of oxygenic photosynthesis. Prog. Lipid Res. 52, 539–561.
Ehleringer, J.R., Osmond, C.B., 1989. Stable isotopes. In: Pearcy, RW, Mooney, HA, Rundel, PW (Eds.), Plant Physiological Ecology - field methods and Instrumentation. Chapman and Hall, London, pp. 281–300.
Eller, C.B., Oliveira, R.S., 2017. Effects of nitrogen availability on the competitive interactions between an invasive and a native grass from Brazilian cerrado. Plant Soil 410, 63–72.
Ferreira, L., Parolin, P., Matos, D.C.L., Cunha, D.A., Chaves, P.P., Neckel, S.O., 2016. The effect of exotic grasses *Urochloa decumbens* (Stapf) R.D. Webster (Poaceae) in the reduction of species richness and change of floristic composition of natural regeneration in the Floresta Nacional de Carajás, Brazil. An. Acad. Bras. Cienc. 88, 589–597.
Fonseca, M.B., Carolino, M.M.S.S.L., Dias, T., Cruz, C., França, M.G.C., 2014. Early growth of Brazilian tree *Dimorphandra wilsonii* is also threatened by African grass *Urochloa decumbens*. J. Plant Interact. 9, 92–99. https://doi.org/10.1080/17429145.2013.770085.
Fowler, D., Pyle, J.A., Raven, J.A., Sutton, M.A., 2013. The global nitrogen cycle in the twenty-first century: introduction. Philos. Trans. R. Soc. B 368, 20130165. https://doi.org/10.1098/rstb.2013.0165.
Franco, A.C., 2002. Ecophysiology of woody plants. In: Oliveira, PS, Marquis, RJ (Eds.), The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna. Columbia University Press, New York, pp. 178–197.
Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Voosmarty, C.J., 2004. Nitrogen cycles: past, present, and future. Biogeochem 70, 153–226.
Genty, B., Briantais, J.M., Baker, N., 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990, 87–92.
Gonçalves, R.V.S., Cardoso, J.C.F., Oliveira, P.E.A.M., Oliveira, D.C., 2021. Changes in the Cerrado vegetation structure: insights from more than three decades of ecological succession. Web Ecol. 21, 55–64.
Grace J.B., Smith M.D., Grace S.L., Collins S.L., Stohlgren T.J. (2002) Interactions between fire and invasive plants in temperate grasslands of North America. In: Galley K, Wilson T (eds.) Proceedings of the Invasive Species workshop: the Role of Fire in the Control and Spread of Invasive species. Fire conference 2000: the First National Congress on Fire Ecology, Prevention, and Management. Miscellaneous Publication. Tallahassee, Tall Timbers Research Station 11:40–65

Gunasekera, D., Berkowitz, G.A., 1993. Use of transgenic plants with Ribulose-1,5-Bisphosphate carboxylase/oxygenase antisense DNA to evaluate the rate limitation of photosynthesis under water stress. Plant Physiol. 103, 629–635.
Guo, X., Hu, Y., Ma, J.Y., Wang, H., Wang, K.L., Wang, T., Jiang, S.Y., Jiao, J.B., Sun, Y.K., Jiang, X.L., Li, M.Y., 2023. Nitrogen deposition effects on invasive and native plant competition: implications for future invasions. Ecotoxicol. Environ. Saf. 259, 115029.
Haridasan, M., 2001. Nutrient cycling as a function of landscape and biotic characteristics in the cerrados of central Brazil. In: Mcclain, ME, Victoria, RL, Richey, JE (Eds.), Biogeochemistry of the Amazon basin and its Role in a Changing World. Oxoford University Press, New York, pp. 68–83.
He, W.M., Yu, G.L., Sun, Z.K., 2011. Nitrogen deposition enhances *Bromus tectorum* invasion: biogeographic differences in growth and competitive ability between China and North America. Ecograph 34, 1059–1066.
Hendry, G.A.F., Price, A.H., 1993. Stress indicators: chlorophylls and carotenoids. In: Hendry, GAF, Grime, JP (Eds.), Methods in Comparative Plant Ecology. Chapman and Hall. London, pp. 148–152.
Hulme, P.E., 2017. Climate change and biological invasions: evidence, expectations, and response options. Biolog. Rev. 92, 1297–1313.
Humphreys, L.R., 1991. Tropical Pasture Utilization. Cambridge University Press, Cambridge.
IPCC, 2013. Summary for Policymakers. Qin, D, Plattner, GK, Tignor, M, Allen, SK, Boschung, J, Nauels, A (Eds.), 2013. Summary for Policymakers. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel On Climate Change Stocker TF 1–30.
Jahns, P., Holzwarth, A.R., 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. Biochim. Biophys. Acta 1817, 182–193.
Jordan, W.R., 1983. Whole plant response to water deficits: an overview. In: Taylor, HM, Jordan, WR, Sinclair, TR (Eds.), Limitations to Efficient Water Use in Crop Production. American Society of Agronomy, Madison, pp. 289–317.
Knochel, D.G., Flagg, C., Seastedt, T.R., 2010. Effects of plant competition, seed predation, and nutrient limitation on seedling survivorship of spotted knapweed (*Centaurea stoebe*). Biolog. Invas. 12, 3771–3784. https://doi.org/10.1007/s10530-010-9769-9.
Knochel, D.G., Seastedt, T.R., 2010. Reconciling contradictory findings of herbivore impacts on spotted knapweed (*Centaurea stoebe*) growth and reproduction. Ecol. Appl. 20, 1903–1912.
Kimura, K., Ishida, A., Uemura, A., Matsumoto, Y., Terashima, I., 1998. Effects of current-year and previous-year PPFDs on shoot gross morphology and leaf properties in *Fagus japonica*. Tree Physiol. 18, 459–466.
Kolar, C.S., Lodge, D.M., 2001. Progress in invasion biology: predicting invaders. Tree 16, 199–205.
Lichtenthaler, H.K., Wallburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.
Lüttge, U., Hariclasan, M., Fernandes, G.W., Mattos, E.A., Trimborn, P., Franco, A.S., Caldas, L.S., Ziegler, H., 1998. Photosynthesis of mistletoes in relation to their hosts at various sites in tropical Brazil. Trees 12, 167–174.
Maathuis, F.J.M., 2009. Physiological functions of mineral macronutrients. Curr. Opin. Plant Biol. 12, 250–258.
Medeiros, M.B., Miranda, H.S., 2005. Mortalidade pós-fogo em espécies lenhosas de campo sujo submetido a três queimadas prescritas anuais. Acta Bot. Bras. 19, 493–500.
Mooney, H.A., Cleland, E.E., 2001. The evolutionary impact of invasive species. Proc. Natl Acad. Sci. 98, 5446–5451.
Niu, S.L., Liu, W.X., Wan, S.Q., 2008. Different growth responses of C_3 and C_4 grasses to seasonal water and nitrogen regimes and competition in a pot experiment. J. Exp. Bot. 59, 1431–1439.
Nybakken, L., Johansson, O., Palmqvist, K., 2009. Defensive compound concentration in boreal lichens in response to simulated nitrogen deposition. Glob. Change Biol. 15, 2247–2260.
Oaks, A., 1994. Efficiency of nitrogen utilization in C_3 and C_4 cereals. Plant Physiol. 106, 407–414.
Pettit, R.D., Fagan, R.E., 1974. Influence of nitrogen and irrigation on carbohydrate reserves of buffalo grass. J. Range Manage. 27, 279–282.
Pivello, V.R., Shida, C.N., Meirelles, S.T., 1999a. Alien grasses in Brazilian savannas: a threat to the biodiversity. Biodiv. Conserv. 8, 1281–1294.
Pivello, V.R., Carvalho, V.M.C., Lopes, P.F., Peccinini, A.A., Rosso, S., 1999b. Abundance and distribution of native and alien grasses in a “Cerrado” (Brazilian Savanna). Biotropica 31, 71–82.
Preiss, J., 1988. The Biochemistry of plants: Carbohydrates. Academic Press, San Diego.
Rossi, R.D., Martins, C.R., Viana, P.L., Rodrigues, E.L., Fiqueira, J.E.C., 2014. Impact of invasion by molasses grass (*Melinis minutiflora* P. Beauv.) on native species and on fires in areas of campo-cerrado in Brazil. Acta Bot. Bras. 28, 631–637.
Ruban, A.V., Johnson, M.P., Duffy, C.D.P., 2012. The photoprotective molecular switch in the photosystem II antenna. Biochim. Biophys. Acta 1817, 167–181.
Sage, R.F., Pearcy, R.W., 1987. The nitrogen use efficiency of C_3 and C_4 plants. Vol. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* L. and *Amaranthus retroflexus*. Plant Physiol. 84, 959–963.
Sage, R.F., Sage, T.L., Kocacinar, F., 2012. Photorespiration and the evolution of C_4 photosynthesis. Ann. Rev. Plant Biol. 63, 19–47.
Schreiber, U., Bilger, W., Neubauer, C., 1995. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment in vivo photosynthesis. In: Schulze, ED, Caldwell, CW (Eds.), Ecophysiology of Photosynthesis. Springer, Berlin, pp. 49–70.
Smith, B.N., Epstein, S., 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiol. 47, 380–384.
Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., Milchunas, D.G., Pennings, S., 2005. Functional and abundance-based mechanisms explain biodiversity loss due to N fertilization. Proc. Natl Acad. Sci. 102, 4387–4392.
Sun, J.K., Liu, M.C., Tang, K.Q., Tang, E.X., Cong, J.M., Lu, X.R., Liu, Z.X., Feng, Y.L., 2023. Advantages of growth and competitive ability of the invasive plant *Solanum rostratum* over two co-occurring natives and the effects of nitrogen levels and forms. Front. Plant Sci. 14, 1169317.
Theoharides, K.A., Jeffrey, S.D., 2007. Plant invasion across space and time: factors affecting non-indigenous species success during four stages of invasion. New Phytol. 176, 256–273.
Tsuvuura, Z., Kirkman, K.P., 2013. Yield and species composition of a mesic grassland savanna in South Africa are influenced by long-term nutrient addition. Austral. Ecol. 38, 959–970.
Turner, N.C., 1981. Techniques and experimental approaches for measurement of plant water status. Plant Soil 58, 339–366.
Vilá, M., Weiner, J., 2004. Are invasive plant species better competitors than native plant species? Evidence from pair-wise experiments. Oikos 105, 229–238.
Witkowski, E.T.F., Lamont, B.B., 1991. Leaf specific mass confounds leaf density and thickness. Oecologia 88, 486–493.
Yuan, Z.Y., Liu, W.X., Niu, S.L., Wan, S.Q., 2007. Plant nitrogen dynamics and nitrogen-use strategies under altered nitrogen seasonality and competition. Ann. Bot. 100, 821–830.