SÃO PAULO STATE UNIVERSITY – UNESP
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WARMING AND WATER DEFICIT IMPACT THE NUTRITIONAL PERFORMANCE OF A C4 AND C3 TROPICAL GRASS

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DADOS CURRICULARES DO AUTOR

Dilier Olivera Viciedo nasceu em 16 de junho de 1984 na cidade de Sancti Spíritus, Cuba. Ingressou no curso de Engenharia Agronômica na Universidad Central “Martha Abreu” de las Villas (UCLV), Cuba em 2003 onde se tornou Engenheiro Agrônomo em julho de 2008. Em setembro de 2008 início como professor na Universidad de Sancti Spíritus “José Martí Pérez” (UNISS), Cuba obtendo o título de Mestre em Ciências Agrícolas em 2011 nesta própria instituição de educação superior. Durante o período de graduação e, mais recentemente, em estudos de pós-graduação desenvolveu experimentos na área da Ciência do Solo, Nutrição de Plantas, Vermicomposto e Biofertilizantes. Tem recebido vários prêmios durante sua carreira e é Autor/coautor de diversos artigos em periódicos científicos internacionais, além de revisor e editor de revistas científicas. Participou de diversos projetos nacionais e internacionais em países como Cuba, Espanha, Canadá e Brasil. Nestes dois últimos países, obteve bolsas de estudos para intercambio e pesquisa concedido pela Canadian International Development Agency (CIDA) and the Association of Universities and Colleges of Canada (AUCC) in Kwantlen Polytechnic University, British Columbia, Canada in 2013. Em 2014 obteve uma bolsa de pesquisa concedida pela World Academy of Sciences (TWAS) e o Conselho Brasileiro de Desenvolvimento Científico e Tecnológico (CNPq), Brasil. Em 2016 ingressou no curso de pós-graduação em Agronomia (Produção Vegetal), nível Doutorado da UNESP - Câmpus de Jaboticabal, com bolsa da CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). Fez parte do Grupo de Estudos em Nutrição de Plantas da Unesp (Genplant) e Ecofisiologia de Plantas Tropicais da USP - Câmpus de Ribeirão Preto, onde desenvolveu os experimentos correspondentes com a tese de doutorado.

“La gratitud, como ciertas flores,
no se da en la altura, y mejor reverdece
en la tierra buena de los humildes”
José Martí

De todas as coisas que a sabedoria fornece
para nos fazer completamente felizes,
o maior é a posse de amizade.
Epicurus

There are many injustices in the world,
but there is one of which no one speaks,
which is that of climate.
Albert Camus

Dedicatória

Aos meus avós Edolfina Viciedo e Edelberto Olivera
que são o meu modelo a seguir

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Muito Obrigado!

TOPICS

WARMING AND WATER DEFICIT IMPACT THE NUTRITIONAL PERFORMANCE OF A C4 AND C3 TROPICAL GRASS

ABSTRACT- Global warming is predicted to increase the intensity and duration of extreme weather events, such as droughts, heat waves, and floods, especially in tropical regions. Climate change affect growth of forage species. However, information regarding the effects of global climate change on the nutritional performance of tropical pastures is lacking, especially under field conditions. We, thus, conducted two field experiment with *Panicum maximum* and *Stylosanthes capitata* using a temperature free-air controlled enhancement system and evaluated the effects of two temperature conditions, ambient temperature and moderate warming (2°C above ambient canopy temperature), and two levels of water availability, irrigated and non-irrigated, on nutrients accumulation, nutrient use efficiency (NUE), the stoichiometric patterns of C:N:P and leaf biomass production. Both experiments was conducted using a randomized complete block design in a factorial arrangement. Our findings revealed in plants of *P. maximum* (C4- grass) that the N and P leaf concentration greatly decreased under water-stressed, which increased the C:N and C:P ratios, while warming increased the N:P ratio. Leaf biomass production was impaired by up to 16% under water stress and ambient temperature conditions, but the biomass production was improved by 20% under warming and irrigated conditions. Our results also showed that homeostatic instability under rainfed conditions resulted in decreased leaf biomass production, and it was concluded that warming is only beneficial for plant growth under well-irrigated conditions. Meanwhile, drought decreased NUE of K, Ca, and B and the leaves dry biomass, while root growth was stimulated. However, under combined warming and irrigation increased the leaves dry biomass, root dry biomass, root length density, root surface area and the NUE of N, P, K, Ca, Mg, Cu, Mn, and Zn. On the other hand, in the second experiment with plants of S. *capitata* (C3-pasture), our results showed that drought conditions decreased phosphorous (P) concentrations and increased N:P ratio in different plant organs. We found that aboveground biomass production declined significantly under drought and high temperature conditions (wSaT and wSeT); we observed a decrease in nitrogen (N) content, a decrease in leaf/inflorescence and leaf/stem ratios, and an increase in C:N ratio in vegetative organs. However, under warm and well-irrigated conditions, the leaf biomass increased approximately 33%. We have concluded that warm and well-watered conditions without restrictions in soil nutrient availability can enhance plant production, presumably due to a higher level of stoichiometric homeostasis.

Key words: Tropical pasture, nutrients uptake, combined stresses, climate change, stoichiometric homeostasis, field assessment

CHAPTER 1- General considerations

1. Introduction

Human activity is currently changing our environment rapidly, with predicted temperature increases of 1–5°C over the coming century (IPCC, 2018). Global change is characterized by increased CO_2 concentration in the atmosphere, increasing average temperature and more frequent extreme events including drought periods, heat waves and flooding (Feller, 2016). Nowadays, the adverse consequences of global warming, expressed as global climate change, are among the main actual environmental concerns faced by human beings (Kerr, 2005; IPCC, 2018).

Global climate change is already occurring and will become increasingly evident, where plants, whether native or cultivated, will be confronted with limited options to avoid loss of habitat or extinction: adapting, migrating, or dying. Ecosystems such as forestry, agriculture and tropical pasture are particularly vulnerable (Martinez et al., 2015). Therefore, it is crucial to understand the mechanisms involved in plant responses to climate change and the possible consequences for biodiversity and human and animal feeding (Olivera Viciedo et al., 2019).

According to the Food and Agriculture Organization (FAO) reports that permanent meadows and pastures cover 3.4 billion ha or 69 % of the world's agricultural area (O'Mara, 2012). Only Brazil has an area of more than 196 Mha of pastures (23% of the country) (FAO, 2013). Thus, tropical grasslands represent an important resource for the Brazilian cattle industry, which is heavily dependent on grazed pastures (Faría et al., 2013). Approximately half of the world's bovine meat is produced in tropical or subtropical areas (FAOSTAT, 2011). Pastures serve as the basis for beef production in Brazil and occupy an area of 101.4 million hectares (IBGE, 2011), which, when considered together with natural pastures, is similar to the area occupied by crops and forests (Jank et al., 2011).

In tropical region, forages such as *Panicum maximum* and crops such as maize and sorghum are the most important C4 plants (Ghannoum et al., 2000). In addition, the C3 legume *Stylosanthes capitata* Vogel, widely used in tropical and subtropical regions with a wide distribution in Brazil (Martinez et al., 2014); da Silva Chaves et al., 2016). This species is often rainfed and when is mixed with other tropical grasses such as *Panicum maximum* could increase productivity and improved nutritional quality. However, sown pasture grasses in Brazil differ in growth rate, nutrient requirements, and the sensitivity to warming and water stress.

For plant nutrition the knowledge of nutrients accumulated at each growth stage provides very important information that may aid in the crop fertilization program. However, they reflect what the plant needs and not how much should be applied, because the NUE and stoichiometric ratio must be considered, which is variable and responds according to the climatic conditions. Understanding these complex relationships requires a detailed study will simulate the changes that could happen in the coming years due the increase of temperature and drought.

Nowadays there are few works studying interaction between warming and drought particularly in field conditions and especially in *Panicum maximum* and *Stylosanthes capitata* pasture and this information may change or even reverse our actual knowledge of biomass production and survival of both plants. This presupposes that the behavior of plants in adverse situations is dependent (at least in part) on their adaptation and tolerance to isolated or combined stress conditions. However, evaluations of these additive effects are rare; therefore, it is important that multifactorial experiments are designed when evaluating plant responses to climate change variables (Olivera Viciedo et al., 2019).

A perusal of literature reveals that information on comparative responses of C3 and C4 plant types to water stress and warming, especially at nutritional performance is meager. Hence, evaluation of these plants for their response (s) to water stressand elevated temperature, especially at nutritional performance would be worthwhile due to both academic and practical reasons.

In this study, we tested three main hypotheses: i) warming under well-watered conditions and nutrients promote a higher stoichiometric homeostasis and increase the plant growth and biomass production; ii) water stressed plants will decrease the accumulation of nutrients, stoichiometric homeostasis and NUE, resulting in a reduced biomass production; iii) warming will exacerbate the negative impacts of drought.

2. Literature review

Plant responses to climate change have been extensively studied using systems such as growth chambers, gradient tunnels, open top chambers and FACE (Free Air Carbon Enrichment) (Ainsworth and Long, 2004). However, the great majority of the studies were conducted with species of temperate climate, with little information regarding the effects of air temperature on tropical agroecosystems, including foraging pastures (Gonzalez-Meler et al., 2017). Especially the impacts of drought and warming on accumulation of nutrients, stoichiometric homeostasis, *NUE* and biomass production of two tropical grass are considered in this review.

2.1. Global Warming

Global warming are characterized by increased CO_2 concentration in the atmosphere, increasing average temperature and more frequent extreme events including drought periods, heat waves and flooding (Feller, 2016). This change have direct influence on natural and agrosystem functioning (Lal, 2002).

Modifications in hydrologic regimes and atmosphere temperature due to anthropogenic greenhouse effect provoke variations in plant productivity and therefore, affect food production (IPCC, 2007, 2018). Crop simulation models, driven by future climate scenarios from global circulation models, suggest that the reduction in agricultural production would be more severe in tropical regions, where there is still a shortage of food production (Cerri et al., 2007).

Only Brazil did undergone an increase of minimum temperatures in 1.4 ˚C every 10 years from 1951 to 2002, meanwhile, the maximum temperatures increased in 0.4-0.6 ˚C (Orsini 2007). In addition, São Paulo state presented a maximum temperature of 37.2 ˚C in 2014 (Marengo, 2014). The elevated temperature will bring variations in the precipitation of different regions and seasons (Trenberth et al., 2014), for instance, according to INPE (2016), Brazil will have a reduction of the precipitation, and an increase of temperature, causing a detrimental effect on the agricultural activity (Marengo et al., 2017). Uncertainties about the future behavior of the climate are especially significant as regards what will happen to the hydrological cycle, especially in the southern hemisphere. In relation to rainfall, however, there are uncertainties, with studies showing different trends for some regions, although in others, there is great reliability, as for the Southern Region of Brazil and the Prata basin, where rains will increase, however in the east of the amazon and the northeast will be a reduction (Marengo, 2014).

2.3. Water stress

Drought is defined as insufficiency of rainfall over an extended period causing adverse impacts on living organisms: vegetation, animals and people (Habermann et al. 2019). Low water availability is a primary environmental factor limiting the growth of numerous plant species (Peterson et al., 1992), via reductions in leaf appearance and extension rates (Volaire et al., 1998). The inadequate water availability causes water stress for plant and reduced productivity (Habermann et al., 2019; Olivera Viciedo et al., 2019). Plant responses to water stress involve molecular, biochemical, morphological mechanisms (Basu et al., 2016) and stoichiometric (Olivera Viciedo et al., 2019) and may differ between C3 and C4 species.

During water deficit, the photosynthetic capacity of C3 plant leaves is limited by reduced CO_2 diffusion from the atmosphere to the carboxylation site due to stomatal closure and reduced mesophyll conductance (Flexas et al., 2006). Photosynthesis in C4 plants is equally, or even more, sensitive to water deficit than C3 plants, despite the greater capacity and water-use efficiency (WUE) of their photosynthetic pathway (Lee et al., 2013).

2.4. C3 and C4 plants

Grasslands cover a greater fraction of Earth's land surface than any other plant type (Williams et al., 1968). In northern latitudes and higher elevations, grasses (Poaceae) with the C3 photosynthetic pathway are more abundant, while grasses with the C4 photosynthetic pathway are more abundant in hotter climates (Teeri and Stowe, 1976). C3 and C4 grasses are also often separated in time, as the terms ‘cool-season’ (C3) and ‘warm-season’ (C4) grasses suggest. The adaptation of C4 grasses to high-temperature and high-light environments has produced anatomical and biochemical features that impact their nutritional quality for herbivores. The leaves of C4 grasses have narrowly spaced veins, each of which is composed of a vascular bundle surrounded by concentric layers of bundle sheath cells and mesophyll cells (Laetsch, 1974). As a result of their greater photosynthetic efficiency, C4 grasses frequently have lower levels of photosynthetic enzymes and lower protein levels overall compared with C3 grasses. Caswell et al. (1973) hypothesized that C4 grasses are nutritionally inferior to C3 grasses (Barbehenn et al. 2004). However, a more recent comparison found no significant differences between protein or water levels in C3 and C4 grasses (Scheirs et al., 2001).

Water deficit stress has profound impact on ecological and agricultural systems (Rochefort and Woodward, 1992). The ecosystem function may be altered due to change in relative abundance of plant functional groups (e.g., C3 versus C4) in natural systems (Epstein et al., 1998). Moreover, the production potential of crops having either C3 (e.g., *S capitata*) or C4 (e.g., *P. maximum*) is greatly reduced due to frequent droughts causing marked yield loss (Habermann et al., 2019; Olivera Viciedo et al., 2019).

The reactions of the plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Chaves et al., 2003). C3 and C4 plants have evolved in different climates and consequently differ from each other both structurally and functionally as well as for their climatic requirements (Ward et al., 1999). While C3 plants are believed to have a temperate origin, C4 plants have evolved in tropical and arid environments (Ward et al., 1999).

2.5. Stoichiometric homeostasis

Stoichiometric homeostasis is the ability of plants remaining their element composition relatively stable regardless of changes in nutrient availability, via various physiological mechanisms (Peng et al., 2016). Nutrient absorption is one of such key mechanisms, however how nitrogen and phosphorus homeostasis and resorption in plants would change with growth-stages under different soil water availabilities and warming on one C3 and C4 pasture grass was unclear.

Organisms with high homeostatic capacities can maintain a relatively constant content of different elements and their ratios in changing environments (Sterner and Elser, 2002). However, it is widely known that primary producers exhibit a wide range of homeostatic capacities among species, each with their own stoichiometric ratio and survival strategy (Elser et al., 2000). Over the past few decades, plant growth and stoichiometric ratios of plant species in tropical regions have been changing in response to climate change, mainly owing to higher air temperatures, heat waves, and water stress conditions (Prado and Silva, 2017; Olivera Viciedo et al., 2019).

2.6. *Panicum maximum*

Brazilian commercial beef cattle herds are the largest in the world, and Brazil is the leader in global bovine meat exports (FAOSTAT, 2011). Bovine meat production in Brazil is performed exclusively on pasture, according to international market concerns regarding food security (Jank et al., 2011). Guinea grass (*Panicum maximum* Jacq or *Megathyrsus maximus* Jacq.) as it is known interchangeably, is a forage grass that is native to Eastern and Southern Africa and is found in tropical and subtropical regions used to feed beef cattle, which is an important economic activity in Brazil. African forage grasses evolved in the presence of large mammals, so they are able to tolerate intense grazing and are very productive, vigorous and robust (Jank et al., 2011).

Panicum maximum is important for pasture and for green silage and production in the tropical Americas because of its high yield and nutritional content. This specie is adaptable to diverse ecosystems and is grown in several countries (Savidan 1981). It is the most productive seed-propagated tropical forage grass and the second most cultivated forage grass in Brazil (Toledo-Silva et al., 2013).

2.7. *Stylosanthes capitata*

Stylosanthes capitata Vogel is an herbaceous native plant of areas and savanna formations of center of Brazil and Venezuela. Species of *Stylosanthes* are among the most important tropical pasture legumes for large tracts of acid and infertile soils in semiarid and arid environments (Edye and Stace, 1984). When used in association with suitable grass species or as cut and carry fodder, *Stylosanthes* can increase meat and milk production in tropical and subtropical regions of the world where animals often suffer from a low protein diet (Chakraborty et al., 2002).

This legume is used as a forage in a consortium with native and exotic grasses with good adaptation to the tropical conditions (Fernandes et al., 2005). Besides presenting high indices of dry matter weight, nutritional quality and seed productivity (Miles, 1983). Only in Brazil, about 30 species are found, mainly in the cerrado (Brandão and Costa, 1979). *Stylosanthes* can be easily integrated in subsistence agriculture as an alternative to expensive protein supplements.

2.8. Temperature Free-Air Controlled Enhancement (T-FACE)

Temperature Free-Air Controlled Enhancement (T-FACE) can be used in field experiments to simulate global warming conditions (Figure. 1). This type of experiment applies a heating treatment to open-field plant canopies, simulating models of future warmer conditions. The degree of warming is maintained using the proportional-integrative-derivative control system for controlling the rise in temperatures (Kimball, 2005). Apogee Infrared Radiometers sense the canopy temperatures of the heated plots as well as unheated reference plots which are used by dataloggers to modulate the output of the heaters. Regarding to the use of T-FACE there are many reports displaying is a useful this system for field experiments (Olivera Viciedo et al., 2019; Habermann et al., 2019; Martinez et al., 2015). Today T-FACE is using in different country and in different crops such as wheat (White et al., 2012), rice (Hasegawa et al., 2013) and soybean (Prior et al., 2006).

Figure 1. Temperature Free-Air Controlled Enhancement (T-FACE) Systems in guinea grass. Photo: Dilier Olivera Viciedo

2.9. Pasture nutritive value

Predicted climate changes will also alter the nutritive value of pasture and crop plants. Greater temperatures and a higher incidence of extreme events such as drought may reduce nutritive value, at least of C3 plants. High temperatures increase the fiber content and reduce the WSC content and digestibility of C3 plants (Roche et al., 2009). Meanwhile, in a recent study with *P. maximum* plants exposed to moderately warm conditions, the plants' biomass production and fiber content increased, while crude protein content decreased, leading to a reduction of forage digestibility (Habermann et al., 2019)

Increased temperatures in the future will also bring forward reproductive development due to the required number of growing degree days being achieved more quickly (Tait et al., 2012). Earlier reproductive development and more rapid senescence are likely to penalize nutritive value (i.e. increase lignin content) of dairy pastures, with the normal reduction in summer pasture nutritive value occurring sooner.

One of the flow-on effects of greater lignification/indigestible neutral detergent fiber (NDF) content in C3 plants is reduced rate of passage through the rumen, which reduces voluntary DM intakes and makes it more difficult to achieve high per cow milk production on farms where stocking rates may be reduced as a strategic adaptation to climate change (Clark and Wilson, 1993).

These plant-level responses also need to be considered at the pasture ecosystem level, again an area upon which there is little information. Increasing contribution from subtropical C4 grasses and weeds will likely reduce nutritive value of the pasture as a whole as these species have greater indigestible fiber content/reduced digestibility and lower CP and WSC content than C3 species (Barbehenn et al., 2004).

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CHAPTER 2- Short-term warming and water stress affect *Panicum maximum* Jacq. stoichiometric homeostasis and biomass production

Abstract

Climate changes affect the growth of forage species. However, information regarding the effects of global climate change on the stoichiometry of tropical pastures is lacking, especially under field conditions. Such information is crucial to understand how temperature conditions and water availability states are likely to affect the stoichiometric homeostasis and biomass production of *Panicum maximum*, an important C4 tropical forage species, under future climate change scenarios. We, thus, conducted a field experiment using a temperature free-air controlled enhancement system and evaluated the effects of two temperature conditions, ambient temperature and moderate warming (2°C above ambient canopy temperature), and two levels of water availability, irrigated and non-irrigated, on the stoichiometric patterns of C:N:P and leaf biomass production. The experiment was conducted using a randomized complete block design in a factorial arrangement with four replications over 3 weeks. Our findings revealed that the N and P leaf concentration greatly decreased in water-stressed plants, which increased the C:N and C:P ratios, while warming increased the N:P ratio. Leaf biomass production was impaired by up to 16% under water stress and ambient temperature conditions, but the biomass production was improved by 20% under warming and irrigated conditions. Our findings showed that homeostatic instability under rainfed conditions resulted in decreased leaf biomass production, and it was concluded that warming is only beneficial for plant growth (i.e., a high homeostatic capacity was maintained) under well-irrigated conditions.

Key words: Ecological stoichiometry, temperature free-air controlled enhancement (T-FACE), tropical grass, global climate change, drought.

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1. Introduction

Ecosystems worldwide are experiencing stresses due to rapid anthropogenic climate change. Over the past few decades, anthropogenic activities have significantly altered the global climate pattern as a result of changes to the carbon cycle and increased emissions of greenhouse gases (IPCC 2018). These changes have led to an atmospheric carbon dioxide concentration [CO_2] that is presently higher than it has been in the past 800,000 years (Lüthi et al. 2008) and an average global temperature increase of 0.85°C compared to pre-industrial values (IPCC 2018).

Climate change is causing extreme weather events with predictions of increased frequency, intensity, and duration of heat waves, floods, and droughts (IPCC 2018). Therefore, it is crucial to understand the mechanisms involved in plant responses to climate change and the possible consequences for biodiversity and human and animal feeding.

Plants use several different strategies to obtain nutrients from environmental sources to sustain metabolic and growth processes. Plants are able to adjust their nutrient demands to maintain internal nutrient homeostasis. Stoichiometric homeostasis is defined as the degree with which a living organism can maintain a constant ratio of elements in their tissues despite changes in the prevalence of these compounds in the environment (Yu et al. 2015). Ecological stoichiometric studies, therefore, aim to clarify the balance between C and other elements, such as N and P, and how these ratios are affected by environmental changes (Sterner and Elser 2002).

In general, primary producers often show low stoichiometric homeostatic capacities, i.e., weak abilities to maintain a constant ratio of internal elements under external environmental changes, and therefore, the chemical compositions of plants are strongly influenced by the concentrations of those compounds in the environment. Organisms with high homeostatic capacities can maintain a relatively constant content of different elements and their ratios in changing environments (Sterner and Elser 2002). However, it is widely known that primary producers exhibit a wide range of homeostatic capacities among species (Elser et al. 2000), each with their own stoichiometric ratio and survival strategy.

N and P are often considered to be the most limiting elements for plant growth. Abiotic conditions such as water stress, temperature, and soil nutrient availability affect growth rates and modify the internal C:N:P ratio. Plants with fast growth rates tend to have higher C:N ratios. N is present in RNA (in low concentrations), and its presence, thus, provides a reasonable indicator of the proteins that must be synthesized to maintain balanced growth (Niklas and Cobb 2005). However, rapid growth rates require an increased rate of photosynthesis and, thus, require a higher demand for N to produce more chlorophyll molecules (Felsemburgh et al. 2006). Thus, to maintain higher growth rates, it is important that the N leaf concentration and ratio of C:N do not change. Some environmental conditions may impair soil nutrient uptake and, thus, decrease the N and P content in plant tissues and increase the N:P ratio (Wang et al. 2018). This ratio is often used as an indicator for the changes of nutrient availabilities and the adaptation of plant to habitats (Wright and Westoby 2003; Zhang et al. 2018). Ratios are especially important because the concentration of an element in plant tissues is affected by the biomass production. Therefore, sometimes a compound has a low concentration not because the plant is suffering from nutrient restrictions, but because of its higher biomass production. The use of elemental ratios neutralizes this biomass effect.

Over the past few decades, plant growth and stoichiometric ratios of plant species in tropical regions have been changing in response to climate change, mainly owing to higher air temperatures, heat waves, and water stress conditions (Prado and Silva 2017). However, only limited information is available on how plant stoichiometry is affected by the combination of warming and reduced soil water availability. In tropical and subtropical climate zones, *Panicum maximum* Jacq. (a C4 plant) is an important grass forage species that is widely used in cattle production. This species is adapted to tropical regions with an optimum growth temperature range of 30–40°C (Yamori et al. 2014). A moderate warming of 2°C was shown to increase the aboveground biomass production of *P. maximum* by 22% under adequate conditions of soil moisture and nutrient availability (Prado et al. 2016), which was a result of the regulation of starch remobilization in the source–sink relationship (Habermann et al. 2019a). However, under rainfed conditions, this warming only improved leaf biomass production under a CO_2-enriched atmosphere (Habermann et al. 2019b). Water stress conditions have been shown to decrease biomass production in *P. maximum* by up to

44% (Bade et al. 1985), mainly owing to the stomatal and metabolic limitations of photosynthesis (Habermann et al. 2019a).

Water stress often decreases soil nutrient uptake by roots in forage species (Dias Filho et al. 1992). This reduces the N and P content in plant tissues, which results in an increase in the C:N and C:P ratios and a decrease in biomass production. However, to our knowledge, the stoichiometric response of *P. maximum* to the combination of water stress and warming has not been investigated. Therefore, it is uncertain how climate change variables may potentially modify the biomass production and stoichiometry of *P. maximum*. It is critically important to clarify this response, because although this species is commercially developed to obtain higher productivity in pastures, the improvements made are dependent on high soil nutrient content.

In the present study, we tested two main hypotheses: i) warming under adequate conditions of soil moisture will increase biomass production and maintain a stable stoichiometric homeostasis, and ii) non-irrigated plants will exhibit reduced homeostatic capacities, higher C:P and C:N ratios, and consequently reduced biomass production. Therefore, the objective of this study was to evaluate the combined and isolated effects of short-term warming (2°C above ambient temperature) and soil water availability on the stoichiometry (C:N:P ratios) and leaf biomass production of *P. maximum* under field conditions.

2. Material and methods

2.1. Study site and climate conditions

The experiment was conducted at the Temperature Free-Air Controlled Enhancement (T-FACE) facility at the University of São Paulo, Ribeirao Preto, Brazil (21°10′8″S, 47°51′48″W). According to the classification of Köppen-Geiger, the climate of the experimental area is tropical with dry winters and rainy summers (Alvares et al. 2013). The experimental area is characterized by an annual average temperature of 22°C, an average yearly precipitation of 1508 mm (Climate-Data 2016), and Rhodic Ferralsol soil (IUSS - WRB 2014).

2.2. Experimental design

The experiment was conducted using four randomized blocks in a factorial arrangement (2 × 2), with two factors, soil water availability (W) and canopy temperature (T). Experiments were each replicated four times, i.e., n = 16 experimental plots. The experiment involved two levels of canopy temperature, ambient temperature (aT) and elevated temperature (eT, 2°C above ambient canopy temperature), and two conditions of soil water availability, irrigated (sW) and non-irrigated (wS).

2.3. Canopy warming

The plant canopies of the plots sWeT and wSeT were warmed under field conditions using the T-FACE system (Kimball et al. 2008; Martinez et al. 2014; Habermann et al. 2019a). Six infrared (IR|) heaters (Salamander TFE 750-240), mounted on Salamander ALEX-F (Mor Electric Heating, MI, USA) reflectors, were arranged in a hexagonal pattern 0.8 m above the plant canopy. The angle and height of the heaters were adjusted according to plant growth to maintain the 2°C set point of elevated temperature. Canopy temperature in each plot was monitored throughout the entire experiment (Fig. 1) using IR radiometers (SI-1H1-L20, Apogee Instruments, UT, USA). The temperature of each heater was controlled using a proportional-integrative-derivative control system installed in a datalogger (CR1000) with multiplexors AM25T (Kimball et al. 2008). Dummy reflectors were mounted in the non-warmed plots (sWaT and wSaT) to produce the same shade in all plots. Canopy temperature was monitored every 15 min, and the data were stored using Logger net software (Campbell Scientific, USA).

Despite the experimental set-point being 2°C above the ambient canopy temperature, there was variation in the actual warming during the diurnal period due to wind and plant transpiration, which resulted in an average warming of 1.60 ± 0.30°C (Fig. 1). The average night-time warming was 1.95 ± 0.20°C. In addition, soil water content influenced leaf temperatures during the diurnal period because stomatal closure was induced by drought, especially during the hottest hours of the day (Habermann et al. 2019a). In the wSaT treatment, the average canopy temperature was observed to be 0.18°C warmer than that in the sWaT treatment.

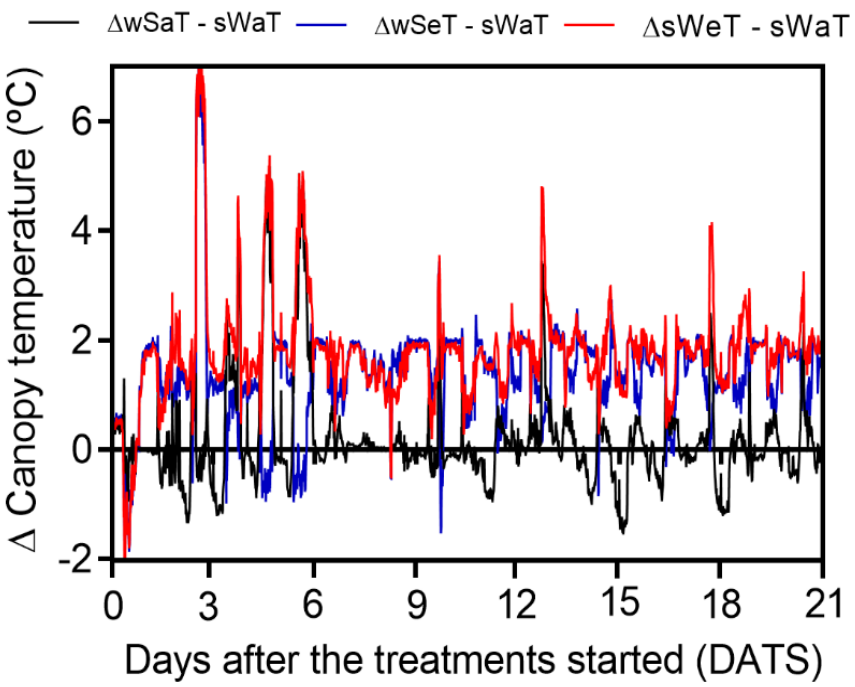


Fig. 1. Difference (Δ) in the canopy temperatures among treatments and ambient conditions. The T-FACE set-point was 2°C above ambient canopy temperature. Treatments: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT).

2.4. Climatic parameters

Throughout the entire growing season, air temperature and relative air humidity were measured each hour by using an automatic microclimate station WS-PH1 connected to a DL2e datalogger (Delta-T Devices Ltd., Cambridge, UK) located in the center of the T-FACE facility. Precipitation data were obtained from a weather station located near the experimental area. The average air temperature during the experimental period was 23.5°C, with a maximum and minimum of 33.8°C and 12.7°C, respectively (Fig. 2A). The average relative air humidity was 81% with a minimum value of 28% during the diurnal period (Fig. 2A). Throughout the experimental period, the total accumulated rainfall was 197 mm, with the most intense precipitation occurring between 7 and 8 days after the start of the experiment.

2.5. Irrigation management

During the experimental period, the sW plots were irrigated with reference to the soil moisture registered by the sensors to maintain the soil moisture at 80% of soil field capacity, i.e., 0.45 m^3 m^{-3} of volumetric soil water content (SWC). Irrigation was performed using sprinklers installed 0.8 m above the plant canopy. Irrigation was suspended in the wS plot until the end of the experiment.

The SWC and soil temperature (Fig. 2B, C) were monitored hourly in the center of each plot at a depth of 10 cm by using a Theta Probe ML2xe and ST2 sensors, respectively. Data were stored in a datalogger (DL2e, Delta –T Devices, UK).

The average SWCs of the sWaT and sWeT plots were ~0.50 m^3 m^{-3} and 0.47 m^3 m^{-3}, respectively (Fig. 2B). This slight decrease in SWC in the sWeT treatment, presumably associated with an increased vapor pressure deficit (VPD) as a result of the IR warming (Habermann et al. 2019a), indicated that experimental warming had almost no effect on the leaf gas exchange and did not alter the photosynthetic performance of *P. maximum* in the irrigated plots. In addition, the SWC of non-irrigated plots (wSaT and wSeT treatments) were not affected by the warming level, and both treatments showed average SWCs of 0.38 m^3 m^{-3}, with minimum SWC values of 0.25 m^3 m^{-3} (Fig. 2B). Soil temperature remained 0.7°C higher in warmed plots compared to non-warmed plots (Fig. 2C).

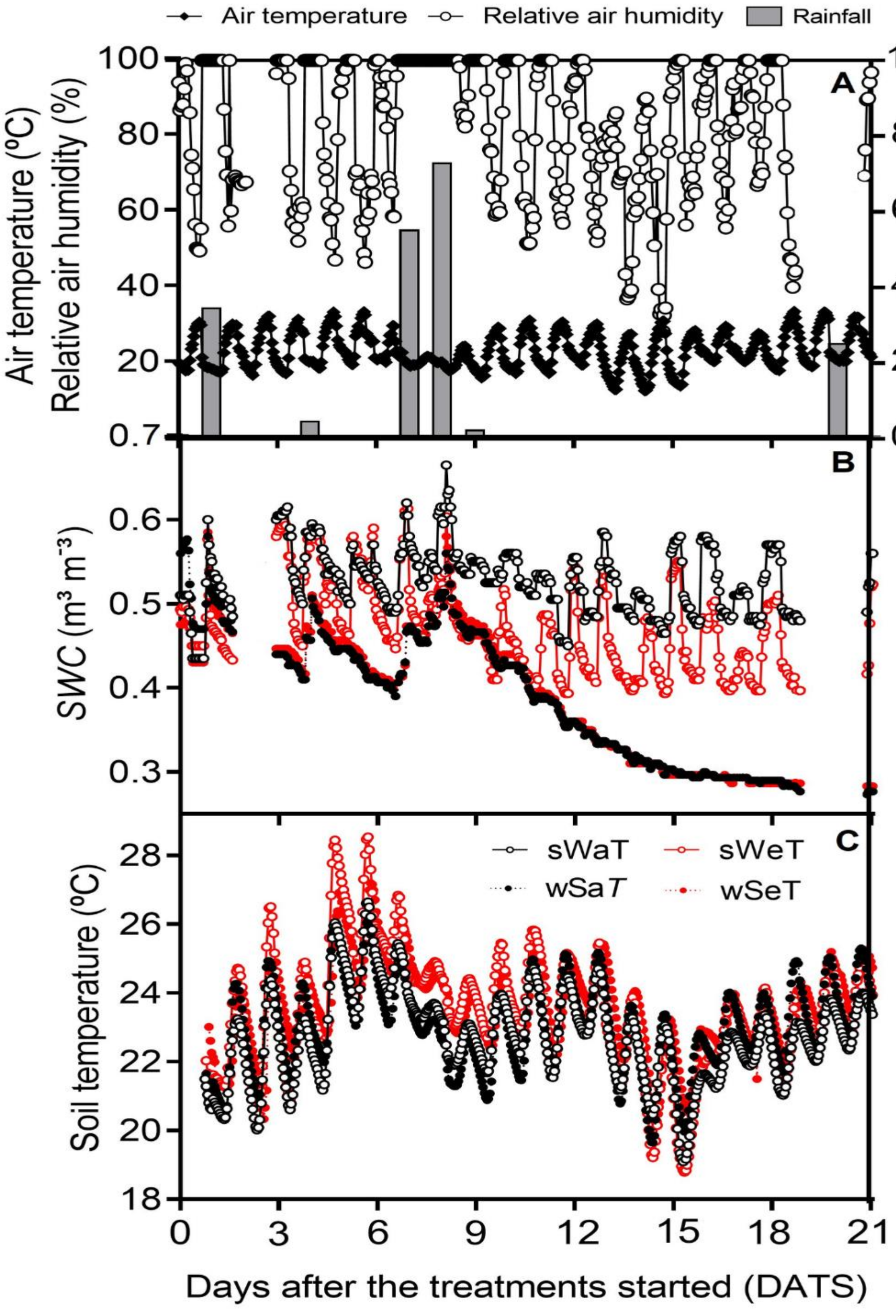
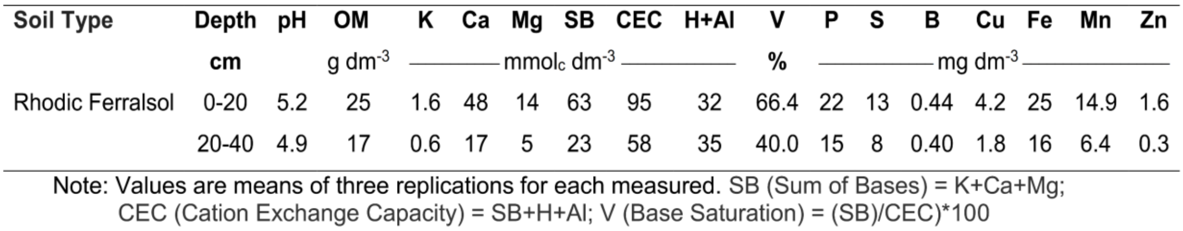


Fig. 2. Meteorological conditions measured throughout the experimental period at the T-FACE facility. (A) Daily average relative humidity and air temperature and total daily precipitation. (B) Daily average volumetric soil water content (SWC). (C) Daily average soil temperature. Treatments: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT).

2.6. Soil chemical analysis and pasture management

The soil chemical analysis before sowing is presented in Table 1. Soil samples were collected from depths of 0–20 cm and 20–40 cm at three randomly selected locations in each experimental plot. The soil samples were combined and air dried to create a composite soil sample for each plot. Soil liming was performed to adjust the pH to 5.5. We determined the following from the soil samples: pH in $CaCl_2$ (0.01 mol L^{-1}); organic matter (OM) content by using the Walkley–Black method; available P content by using spectrophotometry after anion exchange resin extraction; extracted potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) content by using ion exchange resin; extracted sulfur ($S\text{-}SO_4^{2-}$) content by using tricalcium phosphate ($Ca_3(PO_4)_2$; and other available micronutrients. Soil chemical analyses were performed according to the methods described by (Raij et al. 2001). To maintain homogenous soil mineral composition throughout the experimental area. Doses of N, P_2O_5, K_2O and Zn were 40, 100, 60 and 3 kg ha^{-1}, respectively. Each plot was differentially fertilized according to the recommendations by (Raij et al. 1997). These data were used to differentially fertilize each plot to maintain a homogeneous soil mineral composition throughout the experimental area.

Table 1. Soil chemical analysis of samples from two soil depths (0–20 cm and 20–40 cm) before seeding.



Soil Type	Depth	pH	OM	K	Ca	Mg	SB	CEC	H+Al	V	P	S	B	Cu	Fe	Mn	Zn
	cm		g dm^{-3}	———	———	mmol$_c$ dm^{-3}	———	———	———	%	———	———	———	mg dm^{-3}	———	———	———
Rhodic Ferralsol	0-20	5.2	25	1.6	48	14	63	95	32	66.4	22	13	0.44	4.2	25	14.9	1.6
	20-40	4.9	17	0.6	17	5	23	58	35	40.0	15	8	0.40	1.8	16	6.4	0.3

Note: Values are means of three replications for each measured. SB (Sum of Bases) = K+Ca+Mg; CEC (Cation Exchange Capacity) = SB+H+Al; V (Base Saturation) = (SB)/CEC)*100

Seeds of *P. maximum* Jacq. 'Mombaça' were sowed in each of the 16 plots (4 × 4 m). Supplemental irrigation was provided during seedling growth. After seeding and pasture establishment (~3 months), plants were trimmed to 30 cm above the ground to simulate grazing, as per the recommendations of Embrapa (the Brazilian Agricultural Research Corporation) (Santos and Corrêa 2009). After trimming, the treatments were initiated and maintained for 21 days. During this experimental period, we performed three evaluations (once per week) for each analyzed parameter. Plants were trimmed each week to simulate continued cattle grazing.

2.7. Biomass production

Leaf biomass production was estimated per week using a quadrat of 0.04 m^2 (0.20 × 0.20 m) at 30 cm aboveground in three randomly selected locations in each plot. Three subsamples were collected per plot per week, and the average weekly estimates were compared. Clipping was performed in different areas every week and, therefore, the samples collected each time was one-week older than the previously collected sample. Leaves were oven-dried at 60 ± 5°C to a constant weight. Then, were weighted to quantify in the representative area, and powdered using an electric mill (Willey type).

2.8. Carbon, nitrogen, and phosphorus analysis

Dried biomass was used to measure total C and N concentration by dry combustion (1000°C) using an elemental analyzer (LECO Truspec CHNS) calibrated with the pattern LECO 502-278 of wheat (C = 45.00% and N = 2.68%). Total P concentrations were measured using the molybdenum antimony colorimetric method and an ultraviolet spectrophotometer subsystem (model SP-1105) after digestion following the methods described by (Bataglia et al. 1983).

2.9. Statistical analysis

Data were subjected to a two-way analysis of variance (ANOVA) after checking for homogeneity of variances. The Shapiro-Wilks W-test was used to test the normality of the data. We used a factorial analysis to test the main effects of the two levels of soil water availability (W) and two canopy temperatures (T) and their interactions (T × W). The mean values were compared using the least significant difference (LSD) Fisher test with a significance level of $p < 0.05$. All statistical analyses were conducted using the SAS program, version 8.02 (SAS, System 1999).

3. Results

3.1. Effects of temperature and soil water availability on the concentration of N, P and C in leaves

The ANOVA revealed a significant interaction ($p \leq 0.01$) between W and T during the first week of the experimental period. Elevated temperatures were, therefore, shown to exacerbate the decrease in leaf N concentration ([N]) promoted by drought under the wSeT treatment (Fig. 3A). In the second week, both the drought and warming conditions decreased leaf [N] (with a significant interaction), which also indicated an exacerbating effect (Fig. 3B). However, in the final week of the experimental period, the leaf [N] decreased by ~16% in the non-irrigated plots compared to the irrigated plots (Fig. 3C).

Similarly, leaf P concentration ([P]) decreased in wS plots during the first and second weeks and showed a significant difference ($p \leq 0.01$) compared to that in sW plots, regardless of the temperature effect, which also aggravated the effect of drought (Fig. 3D, 3E). During the final week of the experiment, [P] decreased in the wS plots regardless of the temperature treatment (Fig. 3F). Meanwhile leaf C concentration ([C]) remained stable during the first week, without significant changes in response to the different treatments (Fig. 3G). However, in the second and third weeks, the [C] increased in the wS plots independent of the temperature treatments (Fig. 3H, 3I), for none evaluations interactions between the factors were observed (W × T).

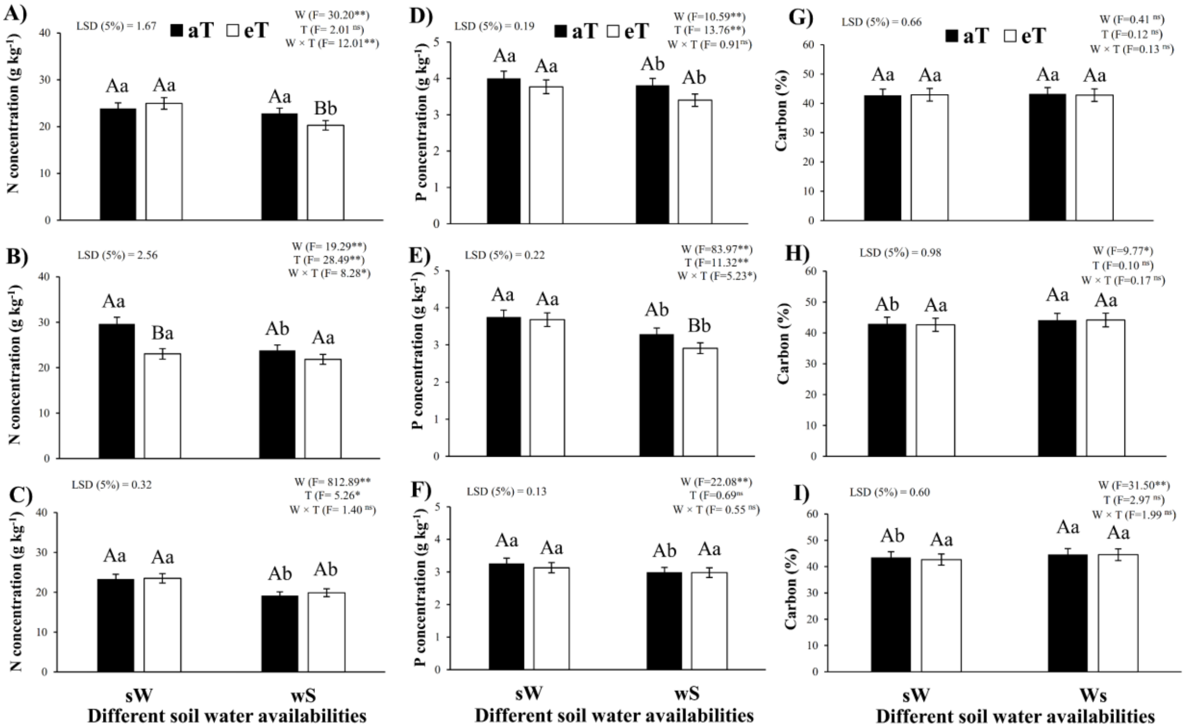


Fig. 3. Changes in elemental concentrations in the leaves of *Panicum maximum* grown under different canopy temperature and soil water availability treatments: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT). Nitrogen concentration during the first (A), second (B), and third (C) weeks of the experiment. Phosphorus concentration during the first (D), second (E), and third (F) weeks. Carbon concentration during the first (G), second (H), and third (I) weeks. Different uppercase letters indicate significant differences between the same soil water availabilities at the different temperatures, and lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; ^{ns}, not significant; W × T, water–temperature interaction; LSD, least significant difference. Stack bars show the standard error based on the average of four replicates.

3.2. Stoichiometric ratios of C:N, C:P, and N:P

The C:N ratio during the first week in the wSeT treatment, increased by ~19% compared to the sWeT plots ($p \leq 0.01$), with warming exacerbating the C:N ratio increase that is promoted by drought (Fig. 4A). However, in the last two weeks, the wS treatment increased the C:N ratio by ~20%, regardless of the temperature level ($p \leq 0.01$) (Fig. 4B, 4C). During the first week, the C:P ratio increased ($p \leq 0.05$) as a result of the wS and eT treatments, with no interaction. However, in the combined wSeT treatment, the C:P ratio was higher than that observed in response to the isolated effects (Fig. 4D). However, in the final two weeks of the experiment, the C:P ratio only increased in non-irrigation plots, regardless of the temperature level (Fig. 4E, 4F). No effects of the interactions were found in any of the evaluations.

Meanwhile, the N:P ratio increase in the first week as a result of irrigation and the elevated canopy temperature (sWeT), compared to wSeT and sWaT, showed a significant W × T interaction ($p \leq 0.01$) (Fig. 4G). In the second week, no significant differences were observed between any factors (Fig. 4H). In the final week, a drought effect (independent of temperature level) was observed (Fig. 4I).

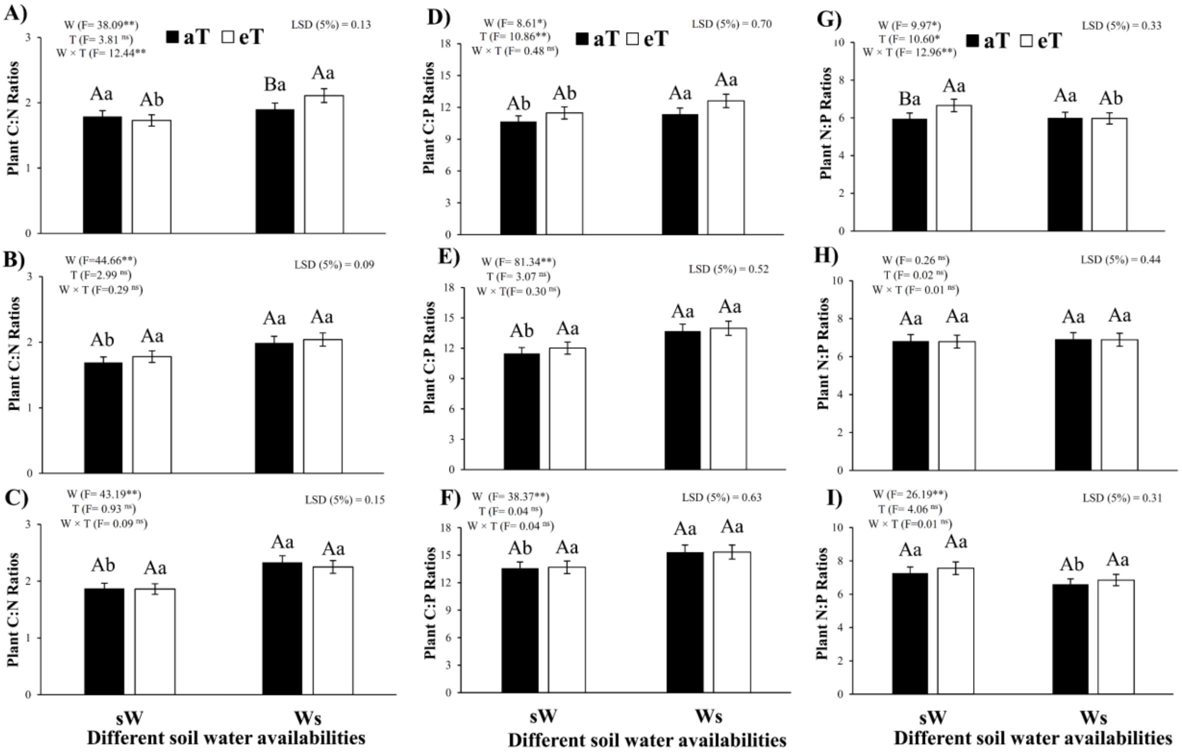


Fig. 4. Changes in elemental ratios in the leaves of *Panicum maximum* grown under different canopy temperature and soil water availability treatments: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT). Plant C:N ratios during the first (A), second (B), and third (C) weeks of the experiment. Plant C:P ratios during the first (D), second (E), and third (F) weeks. Plant N:P ratios during the first (G), second (H), and third (I) weeks. Different uppercase letters indicate significant differences between the same soil water availabilities at the different temperatures, and lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. $^*p \leq 0.05$; $^{**}p \leq 0.01$; ^{ns}, not significant; W × T, water–temperature interaction; LSD, least significant difference. Stack bars show the standard error based on the average of four replicates.

Leaf biomass production decreased by 10%, 12%, and 16% in the first, second, and third weeks, respectively (Fig. 5A, 5B, 5C) in the wSaT treatment compared to the sWaT treatment. However, under the wSeT treatment, warming partially or fully offset the drought effect, especially when soil water availability was highly limited (Fig. 2B), as was also observed in the final week (Fig. 5C). In the final week, our results indicated that there was a significant interaction ($p \leq 0.05$) between the different soil water availabilities and canopy temperatures (W × T). Our findings highlighted the positive effect of elevated canopy temperatures (sWeT) in each week, which had a positive effect on the forage production and, thus, contributed to a higher yield, while drought had a negative effect in all weeks. When combined, warming partially mitigated the drought effect in two of three regrowth cycles, but in the final week, warming fully mitigated the wS effect, which increased dry mass. Therefore, only in the final week of the experiment did we observe increases of ~20%, 16%, and 33% in the sWeT treatment in relation to the sWaT, wSeT, and wSaT treatments, respectively (Fig. 5C).

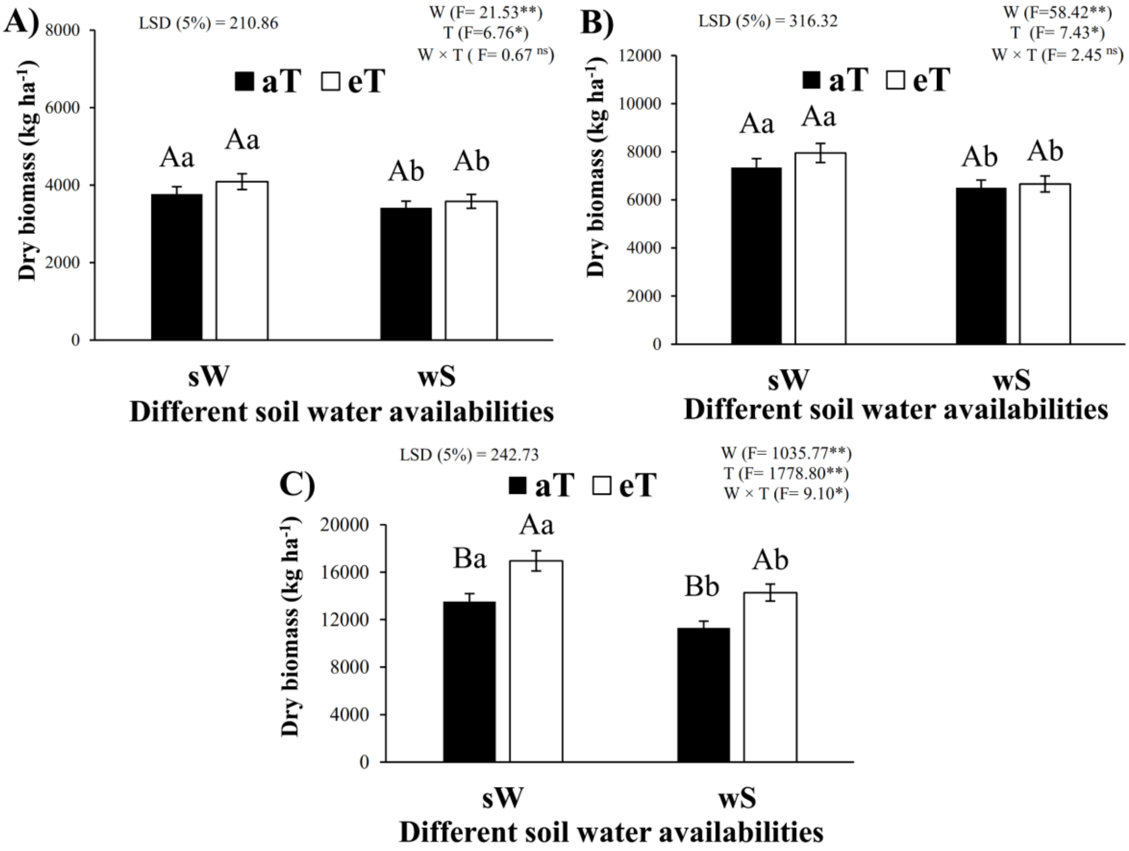


Fig. 5. Leaf dry matter of *Panicum maximum* under different canopy temperature and soil water availability treatments: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT). Leaf dry matter during the first (A), second (B), and third (C) weeks of the experiment. Different uppercase letters indicate significant differences between the same soil water availabilities at the different temperatures, and lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. $^*p \le 0.05$; $^{**}\ p \le 0.01$; ^{ns}, not significant; W × T, water–temperature interaction; LSD, least significant difference. Stack bars show the standard error based on the average of four replicates.

4. Discussion

Climatic changes can cause considerable losses in plant yield as a result of drought conditions associated with increases in temperature (Wiebe et al. 2015; IPCC 2018). Recent studies have indicated that the behaviors and vulnerability of plants to different isolated stresses are different from those in response to a combination of the stresses (Zhou et al. 2017; Borjas-Ventura et al. 2019). This presupposes that the behavior of plants in adverse situations is dependent (at least in part) on their adaptation and tolerance to isolated or combined stress conditions. However, evaluations of these additive effects are rare; therefore, it is important that multifactorial experiments are designed when evaluating plant responses to climate change variables

In addition, many studies aiming to evaluate the effect of abiotic factors on plant stoichiometry have been conducted in controlled environments, such as open top chambers, pots, greenhouses, or growth chambers. However, these artificial conditions may exacerbate plant responses to climate change variables. Therefore, plant responses may not reflect the same magnitude or acclimation mechanisms that would be present in the natural environment. To our knowledge, the present study is the first to provide experimental evidence of *P. maximum* stoichiometry in a field-grown pasture. The observed set of characteristics will enable more precise predictions of the responses of this important crop species through climate and productivity models and will assist decision-making in future pasture management.

In the present field study, soil water stress was observed to affect the N and P leaf concentration in *P. maximum* (Fig. 3A-3C). To our knowledge only one previous study has shown a decrease in the N and P concentrations in *P. maximum* plants; however, the study was conducted using pot experiments (Dias Filho et al. 1992). One possible explanation for the reported findings is that soil water deficit decreases the absorption of N and P because of the reduced contact between the roots and soil (Barber 1995; Hu et al. 2007; Rouphael et al. 2012) and the reduced water potential at the root surface (Kano-Nakata et al. 2011). The reduced availability of these nutrients in the soil could resulted in an adjustment in the demand for nutrients by the plants

(Chapin 1980; Lee 1982). Often, plant N and P concentrations influence each other during the growing season, especially in the leaf tissue (Kang et al. 2011; Yuan and Chen 2012; Wang et al. 2018). This N and P coupling behavior is particularly important in the regulation of nutrient limitation, as well as in the nutrient acquisition strategies of plants in changing environments (Sterner and Elser 2002; Niklas and Cobb 2005; Wang et al. 2018).

We also observed a significant increase in the concentrations of N and P in the leaves of *P. maximum* in response to elevated canopy temperature and irrigation (sWeT). This behavior indicates that this C4 forage species is correlated with and responds very well to elevated temperature, but without water and nutrient limitations, which is corroborated by a higher biomass production during the three evaluations (Fig. 5A-C), Such findings have also been demonstrated in other recent studies (Prado et al. 2016; Habermann et al. 2019a). Therefore, while warming increased foliar N and P concentration, drought increased foliar C concentrations (Fig. 3A-3I). An enhanced growth of this species under the warmer and well-watered condition is consistent with the findings of (Prado et al., 2016) and were coupled with increased leaf N and P concentration. Increased [N] is often related to a higher rate of photosynthesis owing to the Rubisco enzyme content (Suzuki et al. 2010). However, studies have not shown evidence of a positive effect of warming on the photosynthesis of *P. maximum*, including a 3°C heating under ambient temperature and 700 ppm of atmospheric [CO_2] (Faria et al. 2018) and 2°C heating under ambient temperature (Habermann et al. 2019a, b) and 600 ppm of atmospheric [CO_2] (Habermann et al. 2019b), whereas the capacity to resist drought is related to high amounts of structural compounds rich in C (Sardans et al. 2006).

In contrast, under the combined stresses of warming and drought, leaf N and P concentrations were reduced throughout most of the experimental period, which resulted in alterations in the stoichiometry and, thus, impaired plant growth (Fig. 5A-5C). This result is consistent with that of a previous study on *Leymus chinensis* (Trin.) Tzvel. plants, in which the combination of warming and water stress caused more significant reductions in leaf nutrient concentrations than that by the factors independently (Xu and Zhou 2006). Furthermore, these authors suggested that high temperature, combined with severe soil drought, can weaken the N pool and transfer function, as well as osmotic adjustment and reduce the accumulation of free amino acids. According to Ghannoum et al. (2000), under most field and controlled environment conditions (e.g., high midday radiation and high temperatures), C4 plants may experience substantial evaporative demands that lead to the development of transient shoot water stress with adverse effects on leaf growth.

Warming can increase, decrease, or have no effect on the C:N ratios of plants depending on the type of plant and the climate (Sardans et al. 2012). Our results showed that warming and drought increased the C:N and C:P ratios of *P. maximum* in a tropical ecosystem, probably because of decreased leaf N and P concentration (Reich and Oleksyn 2004; Wang et al. 2019) or increased plant resistance under the combined effects of warming and drought (Luo et al. 2009). In another study of the leaves of Mediterranean shrubs and trees, drought increased the C:N and C:P ratios as a result of protection mechanisms (Sardans et al. 2008a; Matías et al. 2011). Thus, evidence suggests that drought (i.e., soil water restriction) tends to increase C:N and C:P ratios. In the present study, a similar effect was observed with the N:P ratio; however, elevated temperature had no direct effect among the different soil water levels, except in the first week of the experimental period. This result suggests that an offset effect exists between warming and drought.

In contrast, under the sWeT treatment, *P. maximum* plants showed a higher degree of stoichiometric homeostasis. This response is often found in species with fast growth rates, which is consistent with our results and previous findings (Elser et al. 2000), wherein low C:N:P ratios were observed. The higher degree of stoichiometric homeostasis under the sWeT treatment may help to explain the enhanced biomass production (Fig. 5A-5C). A similar response has already been reported for another closely related species, *Panicum coloratum* (Poaceae) under a daytime temperature of 35°C and a nighttime temperature of 30°C (Dwyer et al. 2007). Another two studies using similar experimental conditions showed that *P. maximum* biomass production increased by 22%–25% when exposed to a moderate warming of 2°C with adequate soil moisture conditions and nutrient availability than that under ambient temperature conditions (Prado et al., 2016; Habermann et al., 2019a).

There is some evidence that this increased growth under warming and well-watered conditions is related to a different pattern of sugar allocation between organs (Faria et al. 2018; Habermann et al. 2019 a, b), enhanced antioxidant defenses (Borjas-Ventura et al. 2019), and leaf anatomical acclimation mechanisms (Habermann et al. 2019b). Recently, Wedow et al. (2019) reported increased amino acids derived from oxaloacetate and pyruvate (many of which are related to stress defenses) under eT conditions. Therefore, our study further contributes to the understanding of the improved *P. maximum* growth under eT, which suggests that a higher homeostatic capacity plays an important role in plant acclimation to future climate conditions. However, this response was shown not to persist under warmer and rainfed conditions (Habermann et al. 2019a) and may occur only under the combined conditions of warming and elevated [CO_2] (Habermann et al. 2019b).

5. Conclusions

A higher degree of stoichiometric homeostasis was observed in the sWeT plots and was associated with higher biomass production. The non-irrigated plots showed a lower degree of biomass production, and this reduction can be exacerbated under the combined conditions of warming and drought. Our study contributes to the current understanding of acclimation mechanisms of tropical grasses to climate change variables. The results of this study suggest that short-term warming and drought, both as isolated or combined factors, can increase the C:N and C:P ratios in *P. maximum* Jacq. in tropical ecosystems.

Declarations of interest: **none**

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CHAPTER 3- Changes in C:N:P ratios impact biomass production in *Stylosanthes capitata* Vogel under drought and high temperatures

Abstract

Global warming is predicted to increase the intensity and duration of extreme weather events, such as droughts, heat waves, and floods, especially in tropical regions. Therefore, global climate change can negatively affect the productivity of crops and livestock feed due to changes in the composition of forage species. Although pastures cover extensive areas of tropical and subtropical countries, little is known regarding the effects of climate change variables on the stoichiometric homeostasis and biomass production of tropical forage species. In this study, we investigated the isolated and combined effects of two temperatures: ambient (aT) and elevated temperature (eT, 2 °C above ambient temperature), as well as two levels of soil water availability: irrigated plants (sW) and non-irrigated plants (wS). Our results showed that drought conditions decreased phosphorous (P) concentrations and increased N:P ratio in different plant organs. We found that aboveground biomass production declined significantly under drought and high temperature conditions (wSaT and wSeT); we observed a decrease in nitrogen (N) content, a decrease in leaf/inflorescence and leaf/stem ratios, and an increase in C:N ratio in vegetative organs. However, under warm and well-irrigated conditions, the leaf biomass increased approximately 33%. We have concluded that warm and well-watered conditions without restrictions in soil nutrient availability can enhance plant production, presumably due to a higher level of stoichiometric homeostasis.

Keywords: Tropical pasture; Leaves; Inflorescence; Stem; Stoichiometric homeostasis; Drought.

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1. Introduction

Global warming is occurring owing to the increased emissions of greenhouse gases, such as carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). Because of these compounds, a part of the infrared radiation stays in the atmosphere, heating the Earth’s surface and increasing atmospheric temperature. Recent models have predicted an expected rise of 1.1 °C to 6.4 °C in average air temperature during this century, compared to pre-industrial average temperature (IPCC 2007). As a consequence, climate patterns around the world are changing; it has been predicted that in the following decades, the intensity and duration of extreme weather events, such heat waves, floods, and droughts spells will increase (Schimel et al. 2001; Dukes et al. 2005; IPCC 2018).Therefore, the impact of human-induced climate change may have considerable negative effects on natural and managed ecosystems, such as tropical pastures. Multiple lines of evidence indicate that the temperature and the level of drought are the main abiotic factors controlling the CO_2 flux in an ecosystem, with several studies evaluating the impact of climate change factors on plants and pastures of temperate regions (Davidson and Janssens 2006; Hopkins et al. 2013; Gonzalez-Meler et al. 2017). However, only a few studies conducted in open field conditions and on tropical plants can be found in literature, and vulnerabilities of tropical species are still poorly understood (Gonzalez-Meler et al. 2017)

In a terrestrial ecosystem, nitrogen (N) and phosphorous (P) are the most important elements that influence the metabolism and growth of plants (Marschner 2011). However, effect of global warming on carbon (C) accumulation is still debatable (Cao and Woodward 1998; Davidson and Janssens 2006). Some predictions indicate that changes in worldwide C, N and P biogeochemical cycles are to be expected as a result of climate change (Yuan and Chen 2015). While some evidence suggests that elevated temperature can increase soil microbial activity, it may also induce droughts, which can have a negative effect on photosynthesis and plant stoichiometry (Huang et al. 2018).

The concept of stoichiometric homeostasis describes the degree to which a living organism can maintain stable stoichiometric ratios regardless of the changes in nutrient availability by using a wide range of biochemical, anatomical, and physiological mechanisms (Peng et al. 2016). States of low stoichiometric homeostasis may negatively impact survival, nutrition, growth and production of plants (Peng et al. 2016; Olivera Viciedo et al. 2019). Several studies have predicted that climate change will disrupt the stoichiometric ratios in soils and plants (Huang et al. 2018). Disruption of the biogeochemical cycles may decrease plant nutrient content and growth rate (Olivera Viciedo et al. 2019), consequently influencing all trophic levels (Peñuelas et al. 2012, 2013). In addition, climate change can impact different plant tissues in different ways and alter growth rate and biomass allocation between organs (Basu et al. 2016; Yang et al. 2018).

Although tropical regions play an important role in global C budget (Raich et al. 2006), there is little knowledge about the effects of global warming on tropical ecosystems (Schimel et al. 2001; Wood et al. 2012; Gonzalez-Meler et al. 2017). Tropical pastures cover large areas in many tropical countries, but only a few studies have attempted to assess the vulnerabilities of tropical grasslands to the impacts of climate change, the most prominent being higher temperatures (Webb et al. 2012) and higher levels of water stress (Habermann et al. 2019b). Some analyses have indicated that global warming is responsible for changes in forage production of many ecosystems (Dukes et al. 2005), and that higher temperatures combined with well-watered conditions can improve aboveground biomass production (Habermann et al. 2019b).

Stylosanthes capitata Vogel (*Fabaceae*; C_3 plant) is a tropical forage legume widely used in tropical and subtropical regions (Quecini et al. 2002; Martinez et al. 2014), with a wide distribution in Brazil (da Silva Chaves et al. 2016). This species grows in rainfed conditions on pastures along with different C_4 tropical grasses. *S. capitata* has high leaf protein content and is very resistant to drought. This species has the potential to fix nitrogen, improving the amount of this element in the soil. This is especially important because soil nitrogen content is the most important limiting factor for pasture production in the tropics (Prado 2008). Recent studies have indicated that a moderate increase of 2 °C under well-watered or rainfed conditions can improve the electron transport rate and photochemistry and reduce heat and fluorescence dissipation of plants, enhancing the photosynthetic performance under the conditions of presumed future atmospheric temperatures (Martinez et al. 2014; Habermann et al.

2019a). Under rainfed conditions, stomatal conductance and xylem water potential of *S. capitata* were not affected by a 2 °C temperature raise at anthesis stage (Habermann et al. 2019a). In addition, elevated CO_2 levels greatly improved water use efficiency, even under the conditions of low soil water content and lower temperatures, indicating that this species may be a suitable crop in presumed future climate conditions (Habermann et al. 2019a). There is also some evidence that well-irrigated *S. capitata* plants in high temperature conditions show an the increase in leaf N content, mainly due to the improved N-fixation process, resulting in enhanced aboveground biomass production and larger leaf area (Gonzalez-Meler et al. 2017). However, the nature of the interaction between high temperatures and drought is still unknown, and acquiring this information could have a big impact and improve our knowledge of biomass production and survival of *S. capitata*.

In this study, we tested three main hypotheses: i) higher temperatures combined with well-watered soil conditions will increase biomass production as well as the leaf/stem ratio, and promote a high level of stoichiometric homeostasis; ii) in water-stressed plants, the level of stoichiometric homeostasis will decrease with higher C:P and N:P values, resulting in reduced biomass production, and iii) higher temperatures will exacerbate the negative effects of drought. Therefore, we aim to evaluate the isolated and combined effects of high temperatures and drought on leaf, stem, and inflorescence C, N, and P concentrations, C:N:P stoichiometric ratios, and the aboveground biomass production of *S. capitata* under field conditions.

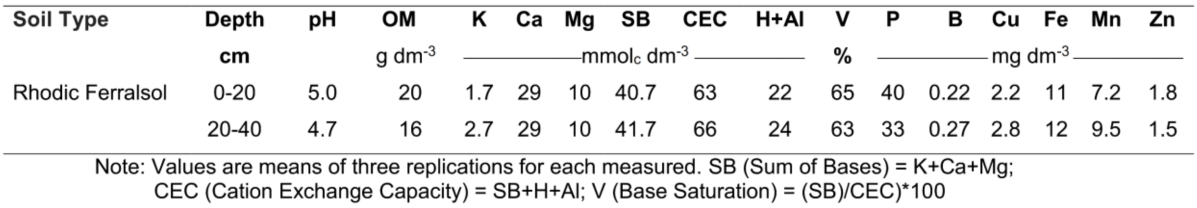
2. Materials and methods

The experiment was conducted at the T-FACE facility located in the University of São Paulo, city of Ribeirão Preto, Brazil (21°10′8″S, 47°51′48″W), using *Stylosanthes capitata* plants planted in Rhodic Ferralsol with clay texture according to IUSS - WRB (2014). The climate in the facility area is tropical with dry winters and rainy summers (Alvares et al. 2013). The average annual temperature is 22 °C, with average annual precipitation of 1508 mm (Climate-Data 2016).

2.1. Soil chemical analysis and pasture management

Before seeding, we collected soil samples from two depths, 0–20 cm and 20–40 cm, on three randomly selected locations in each plot. We performed soil liming to increase the pH from 4.0 to 5.5. Considering the results of soil chemical analysis (Table 1), we applied N, P_2O_5, K_2O, and Zn in doses of 40, 100, 60, and 3 kg ha^{-1}, respectively, to maintain homogenous soil mineral composition throughout the experiment (Raij et al. 1997).

Table 1. The results of soil chemical analysis of samples from two depths (0–20 cm and 20–40 cm) before seeding.



Soil Type	Depth	pH	OM	K	Ca	Mg	SB	CEC	H+Al	V	P	B	Cu	Fe	Mn	Zn
	cm		g dm^{-3}	mmol$_c$ dm^{-3}						%	mg dm^{-3}					
Rhodic Ferralsol	0-20	5.0	20	1.7	29	10	40.7	63	22	65	40	0.22	2.2	11	7.2	1.8
	20-40	4.7	16	2.7	29	10	41.7	66	24	63	33	0.27	2.8	12	9.5	1.5

Note: Values are means of three replications for each measured. SB (Sum of Bases) = K+Ca+Mg;
CEC (Cation Exchange Capacity) = SB+H+Al; V (Base Saturation) = (SB)/CEC)*100

After soil homogenization and pH correction, the seeds of *S. capitata* were sowed in lines 30 cm apart in 12 plots of 16 m². Higher temperature treatment was applied from the beginning of the germination process until the end of the experiment. Supplementary irrigation was performed during the seedling growth and was continued until pasture establishment, which occurred approximately four months after sowing. Afterwards, the plants were submitted to a water stress cycle for 30 days.

2.2. Experimental design

We used three randomized block designs in a factorial arrangement (2×2), with two changing factors, canopy temperature (T) and soil water availability (W). The experiment was conducted in triplicate (n = 12) in the experimental plots. We tested two levels of canopy temperature (ambient temperature, aT, and elevated temperature, eT, 2 °C above ambient temperature), and two conditions of soil water availability (irrigated, sW, and non-irrigated, wS).

The temperature was warmed 2 °C above ambient temperature using the T-FACE system (Kimball et al. 2008; Martinez et al. 2014; Habermann et al. 2019a; Olivera Viciedo et al. 2019). In each warmed plot, six infrared (IR) heaters (Salamander TFE 750-240), mounted on Salamander ALEX-F reflectors (Mor Electric Heating, MI, USA), arranged in a hexagonal pattern 0.8 m above the plant canopy, warmed the air around the plant canopy. We adjusted the height and angle of the heaters during each phase of plant growth. We monitored canopy temperature in each plot using IR radiometers (SI-1H1-L20, Apogee Instruments, UT, USA). IR heaters were controlled using a proportional-integrative-derivative control system installed in a datalogger (CR1000) with multiplexors AM25T (Kimball et al. 2008). We used dummy reflectors in non-warmed plots (sWaT and wSaT) to have the same amount of shade between plots. Canopy temperature was recorded every 15 min, and the T-FACE system worked 24 hours a day. Besides being influenced by the level of plant transpiration during the hottest hours, canopy temperature during daytime was influenced by soil water content (SWC) and wind, despite the experimental set-point temperature value being 2 °C above the ambient temperature. In addition, we observed an increase in plant canopy temperature in the plots subjected to water stress and temperature of 0.54 °C above that of the irrigated plots (sWaT). Soil water content influenced leaf temperatures in this C3 legume, probably as a result of stomatal closure due to drought, especially during the hottest hours where the temperature reached the highest level (Habermann et al. 2019b). There was variation in the actual warming level during the diurnal period, which resulted in an average warming temperature of 1.23 ± 0.72 °C. Meanwhile, the average night-time warming temperature was 1.94 ± 0.18 °C (Fig. 1C).

We monitored soil water content (SWC) and soil temperature (Fig. 1A, 1B) hourly during the entire experiment using Theta Probe ML2xe and ST2 sensors, respectively. The sensors were located in the center of each plot at a depth of 0-10 cm. SWC and soil temperature data were stored in a datalogger (DL2e, Delta –T Devices, UK). Soil temperature remained 0.32 °C higher in the non-irrigated plots (wSaT) compared to the irrigated plots (sWaT), while in the warmed plots (sWeT and wSeT), the soil temperature was 1.78 °C and 2.27 °C higher compared to the irrigated plots (sWaT), respectively (Fig. 1B). At 0 days after the treatment has started (DATS), irrigation was suspended in wS plots, and SWC started to decrease gradually until the end of the experiment. The average SWC of the irrigated plots was 0.51 m^3 m^{-3} under sWaT conditions, and 0.55 m^3 m^{-3} under sWeT conditions, while in the non-irrigated plots, the average SWC was 0.38 m^3 m^{-3} under wSaT conditions and 0.40 m^3 m^{-3} under wSeT conditions (Fig. 1B).

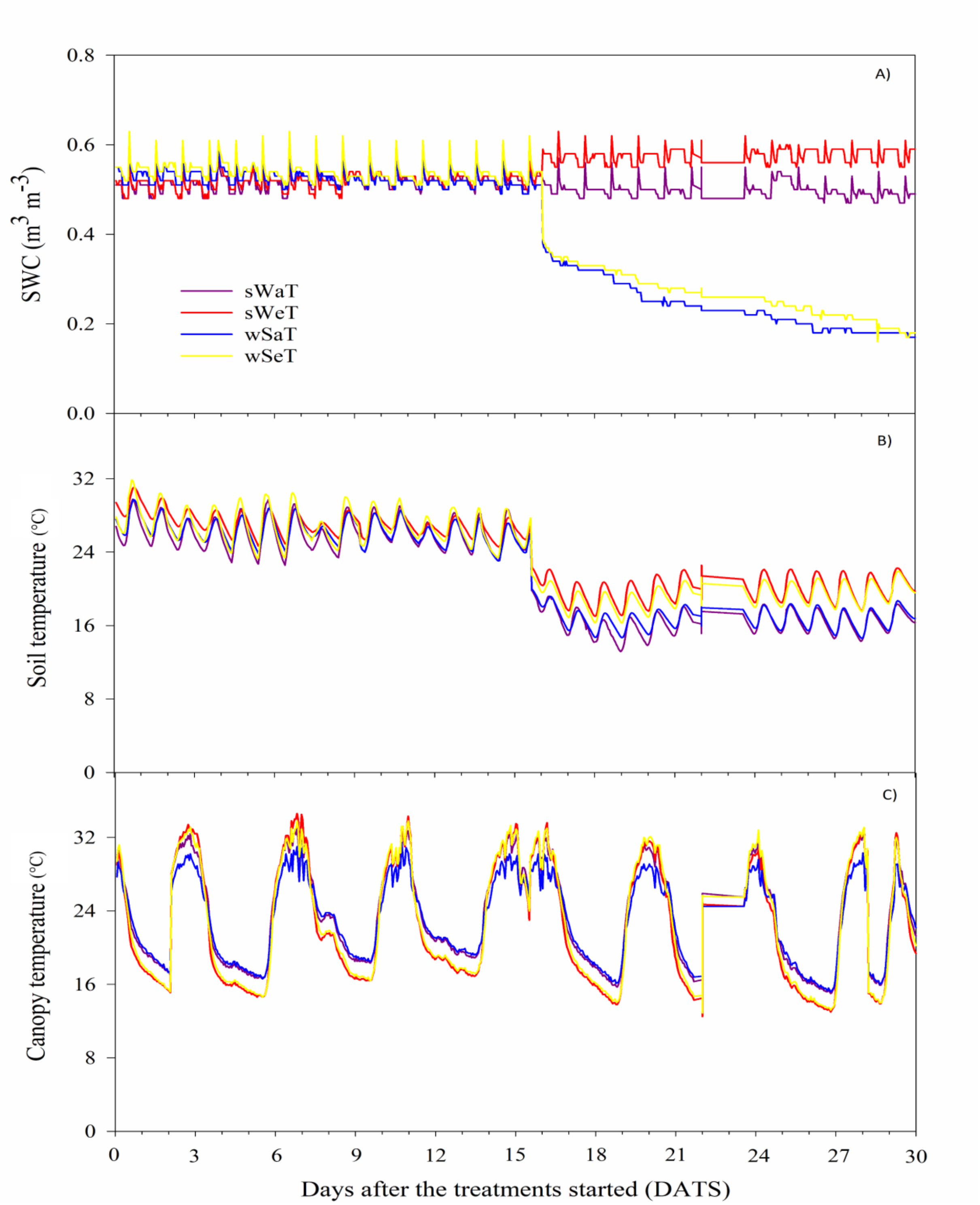


Fig. 1. Soil water content (A), soil temperature (B) and canopy temperature (C) among treatments and in ambient temperature conditions. Treatments are marked as follows: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT).

2.3. Irrigation management

During the experimental period, the sW plots were irrigated periodically to maintain soil moisture at 80% of soil field capacity. Irrigation was performed using drip irrigation system and was suspended in the wS plots until the end of the experiment, regardless of the temperature at 0 DATS.

2.4. Biomass production and morphological composition

We estimated the production of leaves, inflorescences and stems 30 cm above ground using a quadrat of 0.04 m^2 (0.2×0.2 m) in three randomly selected locations in each plot. We harvested all plant material inside of the quadrat and separated the leaves, inflorescences, and stems. All plant material was oven-dried at 60 °C until they reached constant weight. Each plant part was weighed to obtain the dry mass. We calculated the percentage of leaves, inflorescences, and stems, and estimated the leaf/inflorescence and leaf/stem ratios. Afterwards, all plant material was powdered using an electric mill (Willey type).

2.5. Carbon, nitrogen, and phosphorus analysis

Using the powdered plant material, we measured the total C and N concentration in different plant parts using the method of dry combustion (at 1000 °C) in an elemental analyzer (LECO Truspec CHNS) calibrated with the pattern LECO 502-278 of wheat (C=45.00% and N=2.68%). Meanwhile, the P concentration (of acid digests) was measured by spectrophotometer (model SP-1105) using the molybdenum antimony colorimetric method following the methods described by Bataglia et al. (1978). Using the C, N, and P concentrations in different plant organs, we calculated the C:P, C:N, and N:P ratios.

2.6. Statistical analysis

We used the two-way analysis of variance (ANOVA) model after checking for homogeneity of variances. Shapiro-Wilks W-test was used to test the normality of the data. In this study, we tested the main effects of two levels of soil water availability (W) and the effects of two canopy temperatures (T), as well as their interactions (T×W). We compared the average values using least significant difference (LSD) Fisher test with a significance level of $p<0.05$. All statistical analyses were conducted using the SAS program, version 8.02 (SAS, System 1999).

3. Results

Our data revealed interactive effects between water and temperature of [N] in leaves, stems, and inflorescences (Fig. 2-C). Leaf and stem [N] increased under higher temperatures and in well-watered conditions, while in rainfed conditions and ambient temperature, leaf [N] decreased in inflorescences. However, under wSeT conditions we observed that the higher temperature exacerbated the negative impact of drought on leaf and stem [N] (Fig. 2A, 2C).

Meanwhile, in wSeT plots, higher temperatures led to higher [N] in inflorescences than those in wSaT plots; no differences from sWeT plots were noted (Fig. 2B). Leaf and stem [P] reduced due to the isolated effects of W and T; however, the interactive effect of W and T increased [P] under wSeT conditions in both organs (Fig. 2D, 2F). However, the inflorescence [P] increased under sWaT conditions but decreased in the non-irrigated plots (Fig. 2E). Interestingly, leaf and inflorescence [C] increased under wS conditions regardless of the temperature (Fig. 2G, 2H).

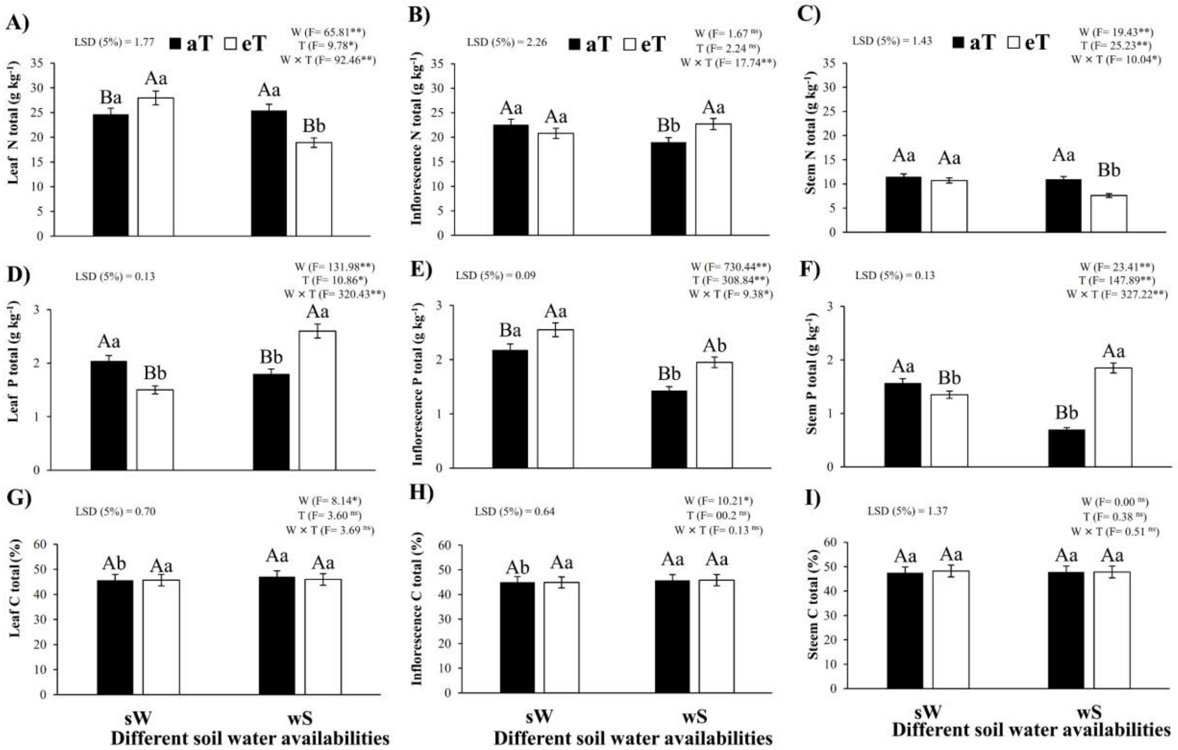


Fig. 2. Concentrations of elements in leaves, inflorescences, and stems of *Stylosanthes capitata* grown under different canopy temperatures and soil water availability treatments: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT). Element concentrations are marked as follows: nitrogen concentration of leaf (A), inflorescence (B), and stem (C); phosphorus concentration of leaf (D), inflorescence (E), and stem (F); carbon concentration of leaf (G), inflorescence (H), and stem (I). Different uppercase letters indicate significant differences between same soil water availabilities at different temperatures, while lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. Significant differences are marked as follows: * $p \leq 0.05$; ** $p \leq 0.01$; ns not significant. W×T marks the interactive effects of water and temperature; LSD marks the least significant difference. Error bars show standard errors based on the average values of three replicates.

Leaf C:N ratio reduced due to the effects of higher temperatures in irrigated plots; however, an interactive effect increased C:N ratio under wSeT conditions (Fig. 3A). Our data showed that higher temperatures caused the decrease of C:N ratio in inflorescences, especially under wSeT conditions (Fig. 3B). However, stem C:N ratio increased due to the isolated effects of W and T, and a synergistic effect was observed under wSeT conditions (Fig. 3C). Leaf C:P ratio decreased under wSeT conditions due to an interactive effect of higher temperature and lower soil water availability, reversing the isolated effect of drought (Fig. 3D). In inflorescences and stems, we observed the isolated effect of drought increased C:P ratio (p≤0.01) (Fig. 3E, 3F). Leaf N:P ratio decreased due to an interactive effect under wSeT conditions (Fig. 3G), while in inflorescences and stems we observed that the isolated effect of drought increased N:P ratio (Fig. 3H, 3I).

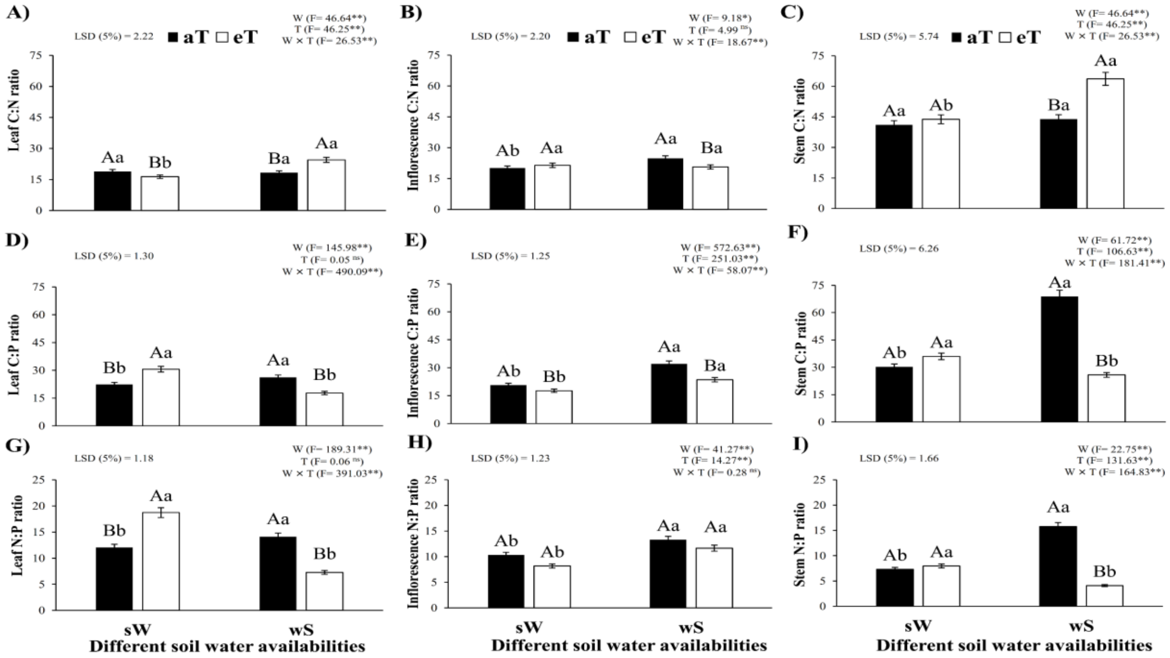


Fig. 3. Element ratios in leaves, inflorescences and stems of *Stylosanthes capitata* grown under different canopy temperatures and soil water availability treatments: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT). Element ratios are marked as follows: leaf C:N ratios (A), inflorescence C:N ratios (B), stem C:N ratios (C), leaf C:P ratios (D), inflorescence C:P ratios (E), stem C:P ratios (F), leaf N:P ratios (G), inflorescence N:P ratios (H), and stem N:P ratios (I). Different uppercase letters indicate significant differences between the same soil water availabilities at different temperatures, while lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. Significant differences are marked as follows: * p≤0.05; ** p≤0.01; ns not significant. W×T marks the interactive effects of water and temperature; LSD marks the least significant difference. Error bars show standard errors based on the average of three replicates.

For leaf biomass production, we observed an antagonistic effect between W and T, with higher temperature increasing the plant’s biomass (33% increase), and drought decreasing it (30% decrease). Under wSeT conditions, the effect of drought was more pronounced, and leaf biomass production decreased compared to sWeT conditions (Fig. 4A).

Inflorescence dry mass production also increased at higher temperatures, while in drought conditions it decreased. However, under the combined effects of higher temperatures and drought (wSeT), inflorescence dry mass increased compared to wSaT conditions (Fig. 4A).

Stem biomass accumulation decreased under wS conditions regardless of the temperature (Fig 4A). The changes in plant living conditions changed the allocation of biomass between plant organs (Fig. 4B, 4C). Leaf/inflorescence ratio decreased in warmer plots, and this effect was most pronounced under the combined wSeT conditions (Fig. 4B). Leaf/stem ratio increased due to the isolated effect of higher temperatures, while it decreased in drought conditions. However, when combined, higher temperatures and drought decreased the leaf/stem ratio compared to sWeT conditions (Fig. 4C).

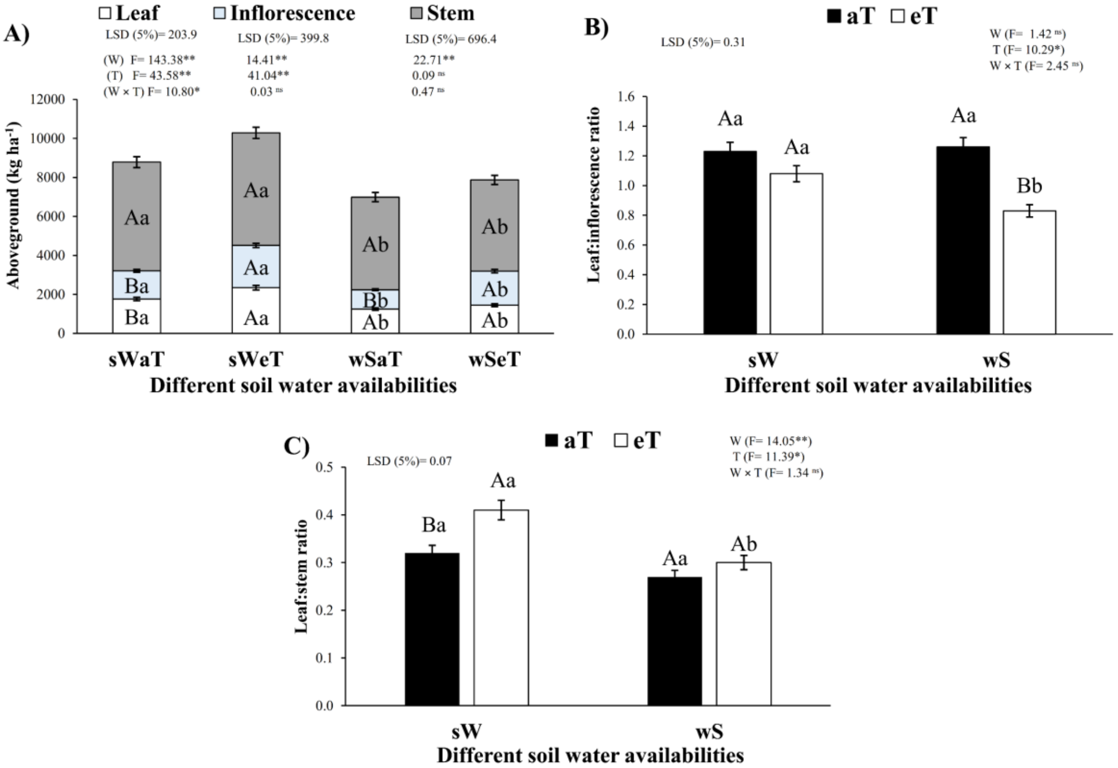


Fig. 4. Dry biomass of leaves, inflorescences and stems (A), leaf/inflorescence ratio (B), and leaf/stem ratio (C) of *Stylosanthes capitata* under different canopy temperature and soil water availability treatments: irrigation and ambient canopy temperature (sWaT), irrigation and elevated canopy temperature (sWeT), non-irrigation and ambient canopy temperature (wSaT), and non-irrigation and elevated canopy temperature (wSeT). Different uppercase letters indicate significant differences between the same soil water availabilities at different temperatures, while lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. Significant differences are marked as follows: * p≤0.05; ** p≤0.01; ns not significant. W×T marks the interactive effects of water and temperature; LSD marks the least significant difference. Error bars show standard errors based on the average of three replicates.

4. Discussion

Although important for the comprehending many acclimation mechanisms, studies that research the impact of isolated abiotic factors have limited extrapolation capacity to field conditions. Therefore, research encompassing combinations of abiotic factors may be more useful for understanding plant responses to climate change conditions. Plants are able to allocate certain organic and inorganic compounds between different tissues, modifying the mineral composition and carbon accumulation in different tissues. Therefore, we explored the interacting effects of two abiotic factors on stoichiometric ratios of C:N:P and biomass production of *S. capitata* in different plant organs under field conditions.

The ANOVA analysis showed that the water stress considerably affected [N] in leaves and stems of *S. capitata* (Fig. 2A, 2C). However, under wSeT conditions, this effect was more pronounced, suggesting that higher temperatures exacerbate the effects of drought, in agreement with earlier studies (Allison and Treseder 2008; Habermann et al. 2019b). The reduced plant [N] is an important indicator suggesting that the uptake, transport or redistribution of nitrogen is limited (Rouphael et al. 2012). Furthermore, low soil moisture may decrease the nutrient supply through reduced mineralization rates (Sanaullah et al. 2012), reduced soil microbial activity (Borken and Matzner 2009), and reduced mass flow and nutrient diffusion in the soil (Lambers et al. 2008). In contrast, under warmer and well-watered conditions (sWeT), leaf and stem [N] increased (Fig. 2A, 2C), which is associated with higher transpiration rates and better water-use efficiency. These results suggest greater homeostatic stability of N in vegetative structures (leaves and stems) compared to reproductive structures (inflorescences), since N regulates the development of plant organs and exerts stronger control over vegetative than over reproductive growth (Marschner and Marschner 2012; Peng et al. 2016).

On the other hand, under isolated effects of W and T, the [P] decreased in different plant tissues (Fig. 2D-F), likely due to reduced soil moisture, which decreases the contact surface area between the root and the soil, hampering nutrient absorption (Barber 1995; Rouphael et al. 2012). Consequently, nutrient transport to leaves was also reduced, decreasing the leaf [P] (Sardans et al. 2006, 2008c; Sardans and Peñuelas 2007). However, under wSeT conditions, [P] increased (Fig. 2D, 2F), suggesting an offset effect of warming on phosphorous metabolism, even though plant [P] responses to drought are still poorly understood (Sardans et al. 2008b; Sardans and Peñuelas 2012). This happens because [N] and [P] depend on the balance between the absorption of N and P, carbon assimilation rate, and the loss of N and P in the plant-soil system (Luo et al. 2016). Under arid conditions, P and N cycles tend to decouple, decreasing soil [N] while increasing soil [P] (Delgado-Baquerizo et al. 2013). However, in irrigated plots, P concentrations were higher in inflorescences compared to leaves and stems (Fig. 2D-F). P regulates plant flowering and seed formation (Marschner and Marschner 2012), and it is a crucial nutrient for the reproductive structures (Peng et al. 2016).

Regarding C allocation to different tissues, we did not observe any interactions between W and T (Fig. 2G-I). However, there was an increase in [C] in leaves and inflorescences of plants grown under wS conditions regardless of the temperature (Fig. 2G, 2H), suggesting that C was allocated to those organs because of drought (Fig. 1B). This effect could be explained by the fact that plants produce carbon-rich compounds, such as proline and glycine betaine, in addition to synthesizing more fibers and lignin in leaves, to protect themselves against the effects of drought (Fiasconaro et al., 2019; Selim et al., 2019; Olivera Viciedo et al., 2019; Sardans et al., 2006).

A wide range of changes in C:N ratio can occur in response to higher temperatures depending on the duration and intensity of heat, plant species composition, climate type, and different abiotic factors (Sardans et al. 2012). In this study, we found that C:N ratio increased under wSeT conditions in leaves and stems (Fig, 3A, 3C), and no differences were found in inflorescences (Fig. 3B). These results are in agreement with a study conducted on a C_4 grass *Panicum maximum* (Poaceae) under wSeT conditions in the field, in which the authors observed an increase in C:N and C:P ratios (Olivera Viciedo et al. 2019). C:N ratio is also related to the senescence process in leaves (Chen et al. 2015). However, it is still unclear how plant N:P ratios change in response to drought (Sardans et al. 2008b; Sardans and Peñuelas 2012).

C:P and N:P ratios increased under drought conditions in different organs compared to irrigated conditions (Fig. 3D-I), probably as result of decreased [P] (Fig. 2D-F) and plant protection mechanisms against drought, as this has been found in certain Mediterranean tree species (Inclán et al. 2005; Matías et al. 2011). These findings indicate that drought conditions have a tendency to increase C:P and N:P ratios regardless of the climate zone (tropical or temperate). Most of the drought-avoidance responses enable the plant to adjust to low availability of resources (water and minerals) (Poorter and Nagel 2000). In fact, in addition to the reduction in available water, soil dryness decreases nutrient availability, with strong interactive effects on plant growth and metabolism (Chaves et al. 2003). On the contrary, under sWeT conditions, there was a high level of stoichiometric homeostasis in all plant parts except for the inflorescences (Fig. 3D-F). The increase in N:P ratio under sWeT conditions could be associated with the increased mineralization and nitrification of N, increasing the N availability in the soil (Melillo et al. 2011). However, drought can increase N:P ratio solely as a consequence of decrease in [P] in different plant tissues, with no changes in [N] (Fig. 2A-F). Therefore, our results indicate that reduced soil moisture can change biomass production rate and N:P and C:P ratios in this tropical grass.

We observed that leaf biomass increased under well-watered and warmer conditions. This positive effect on biomass accumulation has previously been reported (Martinez et al. 2014; Gonzalez-Meler et al. 2017). The underlying mechanisms of this response are probably associated with an improved photosynthesis rate and PSII performance with a moderate level of canopy warming (Martinez et al., 2014). Habermann et al. (2019a) demonstrated that even with a moderate level of warming, rainfed conditions, and low soil moisture, the photosynthetic performance was improved and plant water status remained stable. Aside from this effect, warming has been shown to affect plants by modifying fiber synthesis and consequently changing the digestibility of leaves and stems (Wilson et al. 1991). In a recent study with *Panicum maximum* plants exposed to moderately warm conditions, the plants' biomass production and fiber content increased, while crude protein content decreased, leading to a reduction of forage digestibility (Habermann et al. 2019b).

These results indicated that even with improved foliage production, pastures might be negatively affected due to reduced nutritional quality and digestibility of the plants. The increased leaf/stem ratio under sWeT conditions indicated that a part of the plant’s biomass is being allocated to leaves, which suggests a lesser effect of higher temperatures on stem biomass. In warmer plots, regardless of SWC level, we have also observed some level of biomass allocation from leaves to inflorescences, which was expected since the plants have reached anthesis and were investing in the production of flowers. This positive effect of higher temperature on flower production has been reported in many studies and has a great potential to change pollination and reproductive potential of this species in pastures (Jagadish et al. 2016).

However, in this study we showed that under rainfed conditions, leaf biomass production is not consistent in warmer plots. We hypothesize that under rainfed conditions, carbon is being allocated to stems, which is indicated by the reduced leaf/stem ratio caused by wS conditions. Due to the antagonistic strategies of carbon allocation in the conditions of higher temperatures and dry soil (wSeT), leaf dry mass production decreased less than under wSaT conditions. Therefore, we found a lower level of stoichiometric homeostasis in non-irrigated plots (wSaT and wSeT) compared to the irrigated plots, which could be associated with decreased biomass production (Fig. 4A). Higher temperatures in dry environments can increase the rate of soil drying and limit nutrient and water intake (Link et al. 2003; Allison and Treseder 2008).

These results partially corroborate our second and third hypotheses, and are in agreement with other experiments (Olivera Viciedo et al. 2019). In contrast, *S. capitata* plants grown under sWeT conditions had higher levels of stoichiometric homeostasis and biomass production, corroborating our first hypothesis. According to Fahey et al. (1994), temperature is the main factor influencing the nutritive value of forage plants. Some recent studies have shown that in a tropical pasture with sufficient water and soil nutrient availability, a rise in temperature tends to increase plant growth and biomass production (Martinez et al. 2014; Gonzalez-Meler et al. 2017; Habermann et al. 2019b; Olivera Viciedo et al. 2019). This increases leaf/stem ratios due to higher leaf appearance and leaf elongation rates, even though this does not necessarily result in a higher quality of the forage (Prado et al. 2016). Therefore, the effect of warming on biomass production could be related to growth conditions, phenology, and the ability of plants to maintain a relatively stable composition of elements regardless of environmental changes and to remain in homeostasis.

5. Conclusions

Our results indicated that drought and high temperatures change the stoichiometric ratios of C:N:P of *S. capitata* under field conditions. Our findings showed that the change in soil water availability changes the biomass production of this species under moderate warming, as reported in other studies. Rainfed conditions decreased biomass production up to 30% in wSaT plots, and reduced [P], which suggests that the growth and production rates of *S. capitata* may be more responsive to changes in soil [P] than to the changes in soil [N]. However, higher temperatures combined with drought decreased [N], increasing the C:N ratio. Meanwhile, higher temperatures combined with well-watered conditions increased leaf biomass production up 33%, indicating a higher stoichiometric homeostasis of this tropical forage legume in well-watered conditions.

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Author contributions

D.O.V. wrote the manuscript, with input from all authors. R.M.P and M.C.P. contributed to the materials and analysis. D.O.V., E.H., A.C.H. and R.F.B. collected the data in the field and processed it. C.A.M. conceived and supervised the T-FACE experiment. C.A.M., M.C.P. and R.M.P. supervised the laboratory and field work of graduate students and postdocs. All authors contributed to the revision of the manuscript. DOV is responsible for the manuscript as a whole.

Competing Interests

The authors declare no competing interests.

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CHAPTER 4- Water stress and elevated canopy temperature impact the nutrient-use efficiency of *Megathyrsus maximus* (Jacq.) B.K.Simon & Jacobs under field condition

Abstract

Human-induced global warming is expected to increase the intensity and duration of drought, heat waves and other extreme weather events, leading to negative impacts on plant growth and yield. Plant responses to abiotic stresses are typically being studied under artificial growth conditions. However, field conditions is very different from the controlled conditions used in laboratory studies, and often involves the simultaneous exposure of plants to more than one abiotic and/or biotic stress factors. Predicted climate change might affect the nutrient-use efficiency (*NUE*) and growth of *Megathyrsus maximus* Jacq B.K.Simon & Jacobs *cv*. 'Mombaca'. Therefore, we aim to evaluate the isolated and combined effects of warming (eT) and water stress (wS) on the accumulation of nutrients, *NUE*, and biomass production of *M. maximus* under field conditions. Temperature control was performed by a temperature free-air-controlled enhancement (T-FACE) system. The wSaT treatment decreased *NUE* of K, Ca, and B and the leaves dry biomass, while root growth was stimulated. Under sWeT treatment increased the leaves dry biomass, root dry biomass, root length density, root surface area and the *NUE* of N, P, K, Ca, Mg, Cu, Mn, and Zn. Meanwhile, in the wSeT treatment, biomass production was not affected presumably because of the increased *NUE* of N, P, K, Ca B, Fe and Zn, enhanced root length and the absence of negative effects on other nutrients compared with isolated drought. We concluded that warming buffered the negative impacts of drought on mineral composition of leaves, NUE and biomass production of *M. maximus* under field conditions.

Keywords: Tropical pasture, gas exchange, biomass production, nutrients uptake, root growth

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1. Introduction

Human-induced climate change is considered one of the greatest concerns for future societies because of its effects on worldwide food production. The Intergovernmental Panel on Climatic Change (IPCC) has shown that anthropogenic global warming is already occurring (IPCC, 2018). The RCP 6.0 climate scenario of IPCC estimate that the global mean surface air temperature could reach values of 2.3±0.3°C by 2046-2065 and 2.8±0.4°C by 2081-2100 (Brown and Caldeira, 2017). Climate change is occurring because of the intense emissions of greenhouse gases, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), which increase the global surface temperature. These changes are increasing the intensity and severity of heat waves (Wu et al., 2011), droughts, and floods around the world (Rizhsky et al., 2004; Shah and Paulsen, 2003; Xu and Zhou, 2005).

Temperature and other abiotic factors, such as solar radiation, water availability, and mineral nutrition, are crucial to maintaining crop production (Taiz et al., 2013). However, studies regarding the effects of climate change on plant species have been restricted to only a few species and temperate ecosystems. For C4 tropical forage species, studies are even rarer (Habermann et al., 2019a) although these species are widely used in different parts of the world. Brazil has about 180 million hectares of pastures and is one of the largest commercial producers of world cattle, which depends on the pasture (Galindo et al., 2017; Silva et al., 2009). The grass species *M. maximus* (Guinea-grass) is one of the most cultivated pasture grasses for livestock farming because high productive potential around 33 t ha^{-1} of dry biomass (Freitas et al., 2009).

A previous experiment conducted in pots showed that drought reduced the accumulation and *NUE* of N, P, and K in *M. maximus* leaves, resulting in a decreased aboveground biomass production (Dias-Filho et al., 1992). Reduced mineral absorption under water stress occurs because of the reduced contact between roots and nutrients, since nutrient movement through soil, either by diffusion or mass flow, depends on the soil water content (Potters et al., 2007; Shao et al., 2008) and leaf transpiration rate (*E*). During periods of water shortage, *E* is reduced (Ng et al., 1975), impairing the mass flow between roots and soil.

For C4 species, such as *M. maximus*, the optimum growth temperature is 30-40°C (Yamori et al., 2014). Thus, it is possible that a moderate warming of 2°C will not harm the growth of this species, and depending on other environmental interactions as the high temperature under irrigation and nutrient, guinea-grass could even be benefited (Prado et al., 2016). Recent studies have been carried out on *M. maximus* under field conditions using the T-FACE system (Habermann et al., 2019a; Prado et al., 2016). The authors showed that an increase of 2°C in canopy temperature with adequate soil moisture and mineral supply, enhanced the biomass production by 22% presumably due to a new starch remobilization strategy. However, under rainfed conditions, it seems that only under a warmed and CO_2-enriched atmosphere aboveground biomass is improved (Habermann et al., 2019b).

Nonetheless, the combined effects of warming and water stress on the performance of tropical forage crops is not well documented in the literature. In a study performed with *Panicum coloratum* (Poaceae) cultivated in pots, it was demonstrated that an increase in temperature to 40°C was not harmful to the growth of this forage species even under water stress (Bade et al., 1985). Recent efforts have been made in order to understand *M. maximus* responses to future climate change variables.

Under combined conditions of warming and drought, soil water deficit is the only factor that reduces photosynthesis (Habermann et al., 2019a) and stoichiometric homeostasis of *M. maximus* (Olivera Viciedo et al., 2019). Evidences also indicated that both abiotic factors (drought and warming) decreases the forage quality and digestibility of guinea-grass due to increased content of fibers and lignin in leaves and reduced crude protein amount (Habermann et al., 2019a). However, the impacts of climate change variables on mineral composition of *M. maximus* and the strategies of nutrient uptake by roots are unknown.

Therefore, the aim of this study was to evaluate the isolated and combined effects of warming and soil water deficit on the accumulation of nutrients, *NUE*, and growth of

M. maximus under field conditions. Here, we tested three hypotheses: (1) soil water deficit will decrease the accumulation and *NUE* of most of the macro and micronutrients, whilst root growth will be stimulated as a strategy to cope with the soil water deficit; (2) an increase of 2°C in canopy temperature plus adequate soil moisture will enhance whole-plant biomass production, accumulation and *NUE* of most of the nutrients; (3) warming will exacerbate the negative impact of water stress.

2. Material and methods

2.1. Study site and system

The experiment was conducted at the Trop-T-FACE facility, located at the University of Sao Paulo, Ribeirão Preto campus, São Paulo state, Brazil (S 21° 10′ 8″ W 47° 51′48″) using the tropical forage specie *Megathyrsus maximus* (Jacq.) B.K.Simon & Jacobs cv. ‘Mombaca’. According to the classification of Köppen-Geiger, the climate of the experimental area is tropical with dry winters and rainy summers (Alvares et al., 2013). The experimental area is characterized by an annual average temperature of 22°C, an average yearly precipitation of 1508 mm (Climate-Data, 2016).

The soil is classified as Rhodic Ferralsol, with clay texture (IUSS - WRB, 2014). Plants were exposed to four treatments: canopy ambient temperature and irrigated soil (sWaT); canopy warming (2°C above the ambient canopy temperature) and irrigated soil (sWeT); soil moisture deficit and ambient canopy temperature (wSaT) and soil moisture deficit and canopy warming (wSeT). Experimental plots were arranged in four randomized blocks with four treatments in each block.

2.2. T-FACE description

Plants were warmed using the T-FACE (*Temperature free-air controlled enhancement*) system under field conditions with a set-point of 2ºC above ambient canopy temperature (Kimball et al., 2008). In each warmed plot, six Salamander TFE 750-240 infrared heaters (Mor Electric Heating, Comstock Park, MI, USA) mounted on Salamander ALEX-F reflectors (Mor Electric Heating, Comstock Park, MI, USA) were placed in a hexagonal pattern of 2 m diameter. The IR Heaters were mounted on horizontal aluminum bars and were positioned 0.8 m above the plant canopy.

The canopy temperature of each plot was monitored by an infrared thermometer model SI-1H1-L20, (Apogee Instruments, EUA) (Campbell Scientific, UT, USA). The T-FACE system used a proportional-integrative-derivative (PDI) feedback control system in a model CR1000 datalogger with AM25T multiplexors (Campbell Scientific, Logan, UT, USA) to maintain an approximately 2°C increase over the ambient canopy temperature (Kimball et al., 2008). Dummy heaters were installed in plots with no warming to produce the same shading between the plots. A computer with the Loggernet software was used to access and collect data from the T-FACE system during the experiment. The air temperature was measured in each plot at canopy height using the HOBO ProV2 U23-002 sensors (Onset, Hobo Dataloggers, USA).

2.3. Irrigation

Before initiating the treatments, all plots were irrigated equally to maintain soil water content at 80% field capacity. Water stress was applied by the suspension of watering in the wSaT and wSeT plots. The soil water content (SWC) were monitored hourly with Theta Probe ML2xe (Delta-T Devices Ltd), and ST2 sensors located at the center of each plot at 10 cm depth and connected to a datalogger model DL2e (Delta-T Devices Ltd) (Fig. 1).

The average of SWC of the sWaT and sWeT plots were 0.51 and 0.46 m^3 m^{-3}, respectively. Meanwhile the average soil water content of both wSaT and wSeT plots was 0.37 m^3 m^{-3}, and during the maximum period of water stress was reached 0.25 m^3 m^{-3}. The maximum air temperatures registered during the growing season were 37.7, 39.9, 40.5, and 40.4°C under the sWaT, wSaT, sWaT and wSeT treatments, respectively (Fig. 2a). The minimum air temperatures during the experiment were 13.1, 10.1, 11.8, and 12.67°C under the sWaT, wSaT, sWeT, and wSeT treatments, respectively (Fig. 2b).

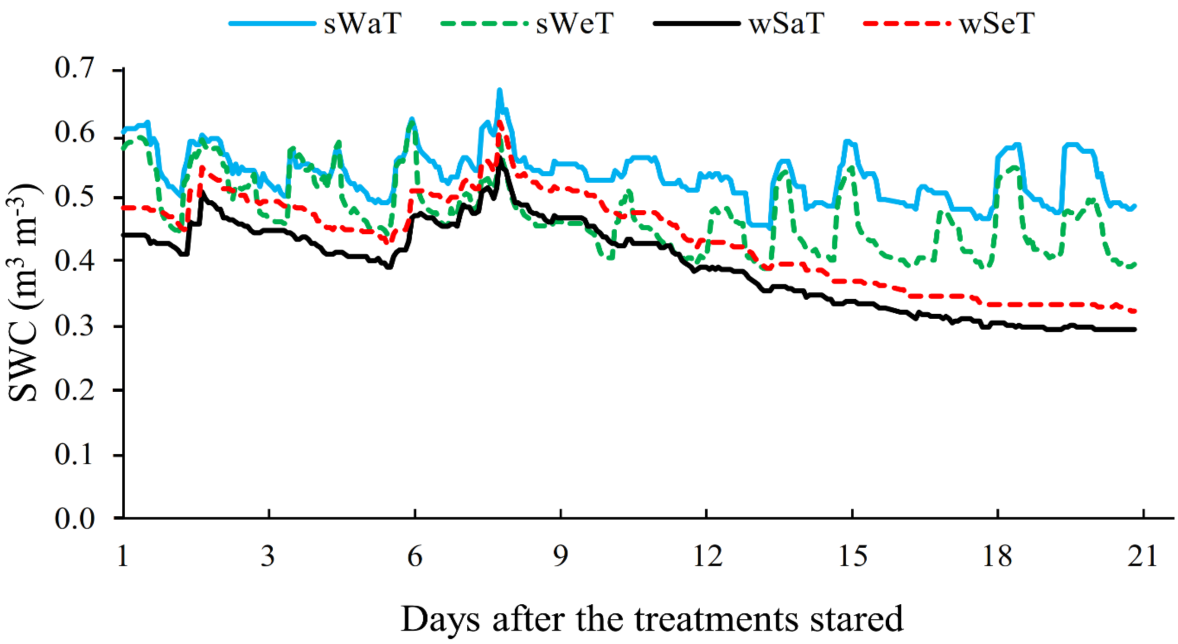

Fig 1. Soil water content registered during the experimental period with *Megathyrsus maximus.* Treatments: canopy ambient temperature and irrigated soil (sWaT); canopy warming (2°C above the ambient canopy temperature) and irrigated soil (sWeT); soil moisture deficit and ambient canopy temperature (wSaT) and soil moisture deficit and canopy warming (wSeT).

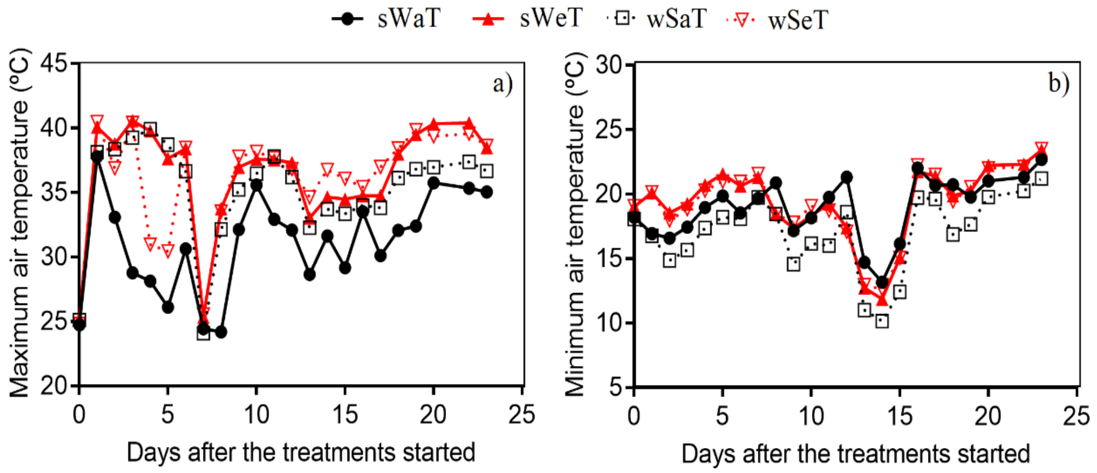

Fig 2. Air temperature in each treatment registered during the experimental period with *Megathyrsus maximus*. (a) Maximum air temperatures. (b) Minimum air temperatures. Treatments: canopy ambient temperature and irrigated soil (sWaT); canopy warming (2°C above the ambient canopy temperature) and irrigated soil (sWeT); soil moisture deficit and ambient canopy temperature (wSaT) and soil moisture deficit and canopy warming (wSeT).

2.4. Pasture management

Soil fertilization was carried out using 40, 100, 60 and 3 kg ha^{-1} of N, P_2O_5, K_2O and Zn respectively. Each plot was differentially fertilized according to the soil analysis and following the recommendations by (Raij et al., 1997). The seeds of *M. maximus* were sowed in 16 plots (4 m × 4 m), and supplemental irrigation was performed during seedling growth. Approximately three months after the establishment of the pasture, the plants were clipped 30 cm above ground (Santos and Corrêa, 2009). Subsequently, the treatments with elevated temperature and soil water deficit were initiated and applied for 21 days.

2.5. Aboveground biomass, root growth and nutrient analysis

Aboveground biomass production was estimated using a quadrat of 0.2 m × 0.2 m (an area of 0.04 m^2). We collected three samples per plot. Samples were dried in a stove at 60°C until constant weight and powdered in an electric mill (Willey type). The powdered samples were used to measure the content of macronutrients (N, P, K, Ca, and Mg) and micronutrients (B, Cu, Fe, Mn, and Zn) (Bataglia et al., 1978). According to the nutrient content and dry mass of plants, we calculated the aboveground accumulation of each nutrient. Using the accumulation of nutrients and biomass production, we calculated the *NUE* (Siddiqi and Glass, 1981).

To analyze root growth, we collected soil samples from two regions of each plot using a soil-sampling probe (Sondaterra, Brazil) with volume of 1908 cm^3. Samples were taken from the superficial soil layers (from 0 to 20 cm deep). We washed each sample with an alcohol solution 20% and roots were separate with a sieve (0.5 mm). Digital images of roots were taken with a commercial scanner (HP, Scanjet G 4050, USA). Using the software Safira (Stonway, Brazil), we determined the root length (mm cm^{-3}), root volume (mm cm^{-3}) and surface area (cm^2). Subsequently, they were oven dried at 70°C during 72 hours to measure root dry matter (g m^2).

2.6. Statistical analysis

Data were subjected to a two-way analysis of variance (ANOVA) after checking the homogeneity of variance. The Shapiro-Wilks W test was used to test the normality of the data. The mean values were compared using the least significant difference (LSD) Fisher test with a significance level of p-value less than 0.05. We used the Statistical Analysis System (SAS version 8.0) software to conduct the analysis (SAS, System, 1999).

3. Results

Our data showed interactive effects between W × T for the accumulation all of macronutrients, except P (Fig. 3a-e) and the micronutrients Cu and Mn (Fig. 4b, d). We observed that for most of the macronutrients and micronutrients, warming improved nutrients accumulation, while drought decreased it. Therefore, when combined under wSeT, warming partially mitigated the negative impact of drought on accumulation of N, P, K, Ca, and Mg (Fig. 3a-e) and micronutrients, except for Fe (Fig.4c). Meanwhile, under sWeT, showed a synergy effect of W × T on the accumulation of N, P, K, Ca (Fig. 3a, b, c, d) and B, Cu, Fe and Mn (Fig. 4a, b, c, d), and with no differences for Mg (Fig. 3b, e), and Zn (Fig. 4e).

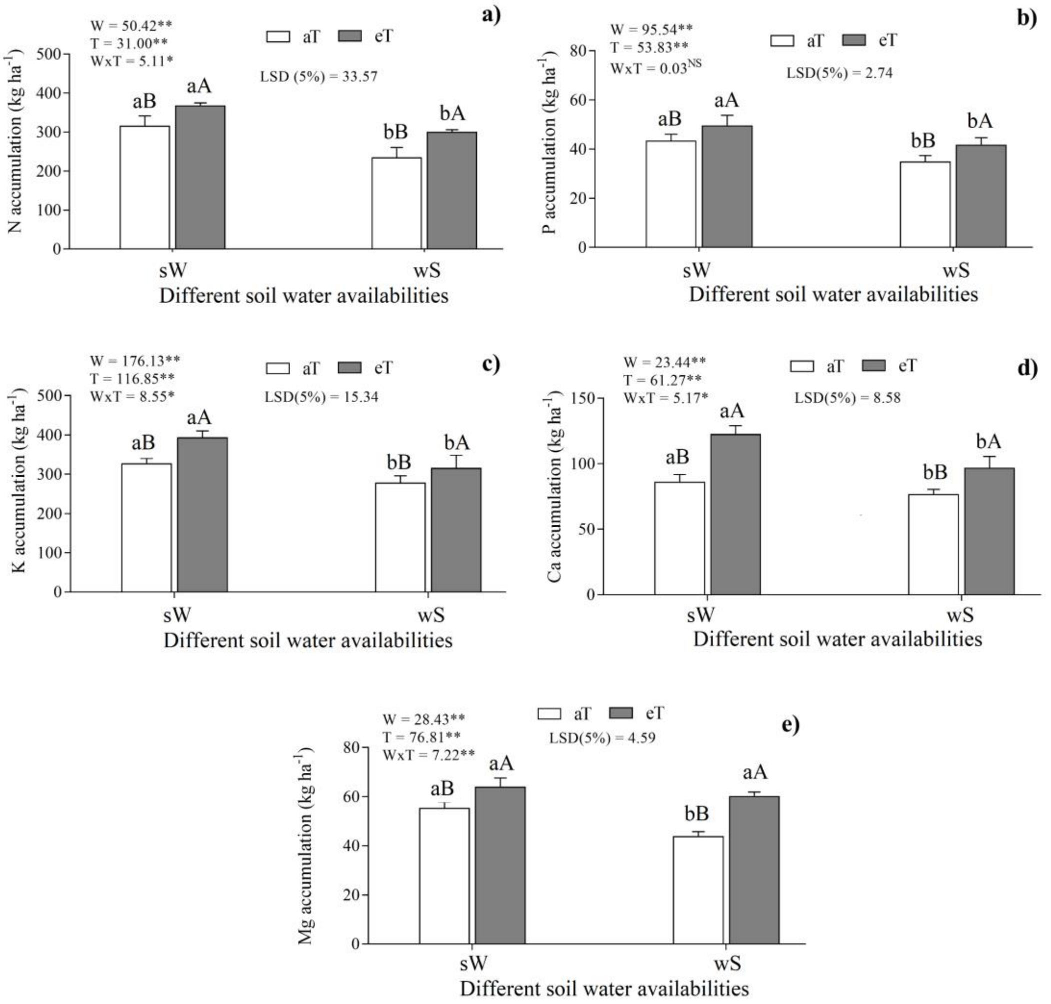

Fig 3. Accumulation of macronutrients in leaves of *Megathyrsus maximus* during the experimental period. Accumulation of: (a) N, (b) P, (c) K, (d) Ca and (e) Mg. Treatments: canopy ambient temperature and irrigated soil (sWaT); canopy warming (2°C above the ambient canopy temperature) and irrigated soil (sWeT); soil moisture deficit and ambient canopy temperature (sWaT) and soil moisture deficit and canopy warming (sWeT). Different uppercase letters indicate significant differences between the same soil water availabilities at the different temperatures, and lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. *p ≤ 0.05; ** p ≤ 0.01; NS, not significant; W × T, water–temperature interaction; LSD, least significant difference. Stack bars show the standard error based on the average of four replicates.

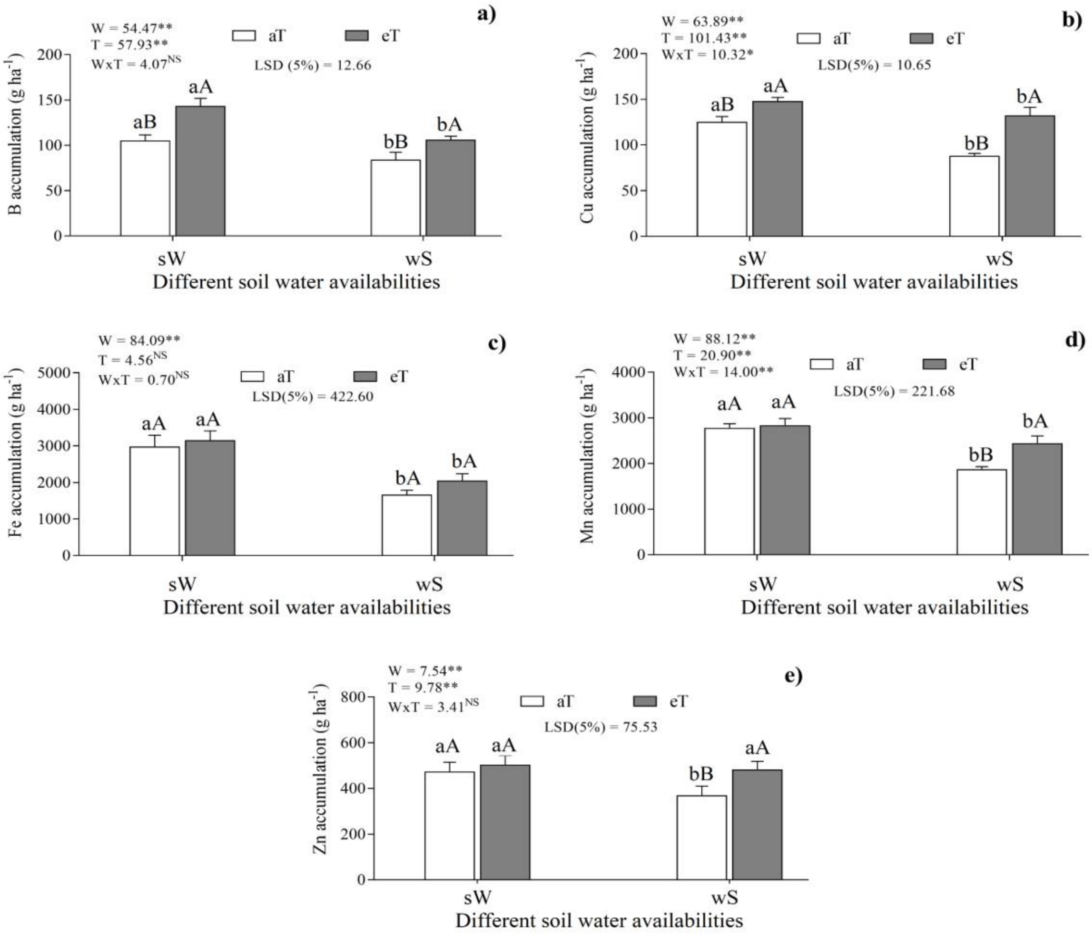

Fig 4. Accumulation of micronutrients in leaves of *Megathyrsus maximus* during the experimental period. Accumulation of (a) B, (b) Cu, (c) Fe, (d) Mn and (e) Zn. Treatments: Treatments: canopy ambient temperature and irrigated soil (sWaT); canopy warming (2°C above the ambient canopy temperature) and irrigated soil (sWeT); soil moisture deficit and ambient canopy temperature (sWaT) and soil moisture deficit and canopy warming (sWeT). Different uppercase letters indicate significant differences between the same soil water availabilities at the different temperatures, and lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. *$p \leq 0.05$; ** $p \leq 0.01$; NS, not significant; W × T, water–temperature interaction; LSD, least significant difference. Stack bars show the standard error based on the average of four replicates.

For nutrient use efficiency (NUE) we also observed contrary effects between T × W for most of the nutrients, except for Fe (Fig. 5, Fig. 6). While drought decreased the NUE of macronutrient and micronutrients, warming increased NUE. Therefore, under wSeT, warming mitigated the negative impact of isolated drought and increased the NUE of N, P, K, Ca (Fig. 5a, b, c, d) and B, Fe, Zn (Fig. 6a, c, e) of *M. maximum* leaves. Meanwhile, under irrigation conditions and elevated temperature (sWeT) the NUE of N, P, K, Ca, Mg (Fig. 5a, b, c, d, e), Cu, Mn, and Zn (Fig. 6b, d, e) increased in relation to isolated drought (wSeT) plots, with no differences for B and Fe (Fig. 6a, c).

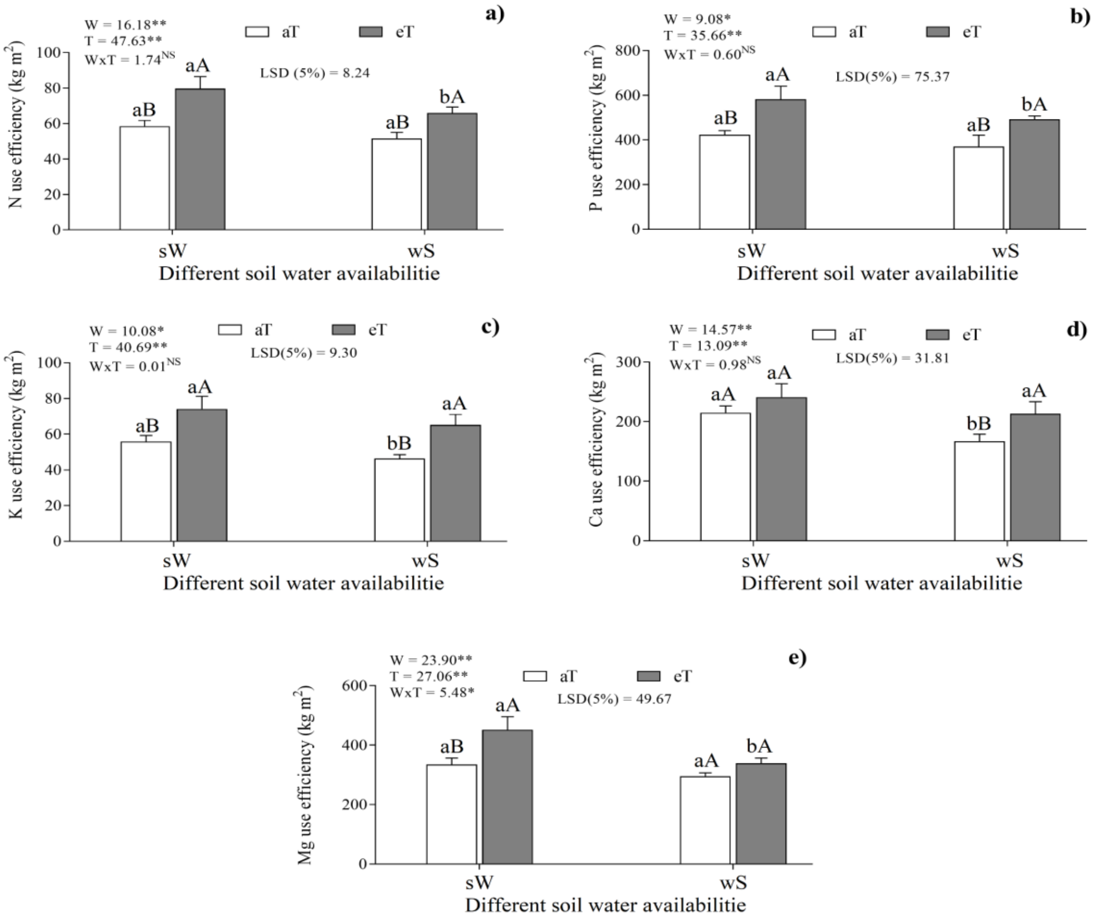

Fig 5. Use efficiency of macronutrients in leaves of *Megathyrsus maximus* during the experimental period. Use efficiency of (a) N, (b) P, (c) K, (d) Ca, and (e) Mg. Treatments: Treatments: canopy ambient temperature and irrigated soil (sWaT); canopy warming (2°C above the ambient canopy temperature) and irrigated soil (sWeT); soil moisture deficit and ambient canopy temperature (sWaT) and soil moisture deficit and canopy warming (sWeT). Different uppercase letters indicate significant differences between the same soil water availabilities at the different temperatures, and lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. *$p \leq 0.05$; ** $p \leq 0.01$; NS, not significant; W × T, water–temperature interaction; LSD, least significant difference. Stack bars show the standard error based on the average of four replicates.

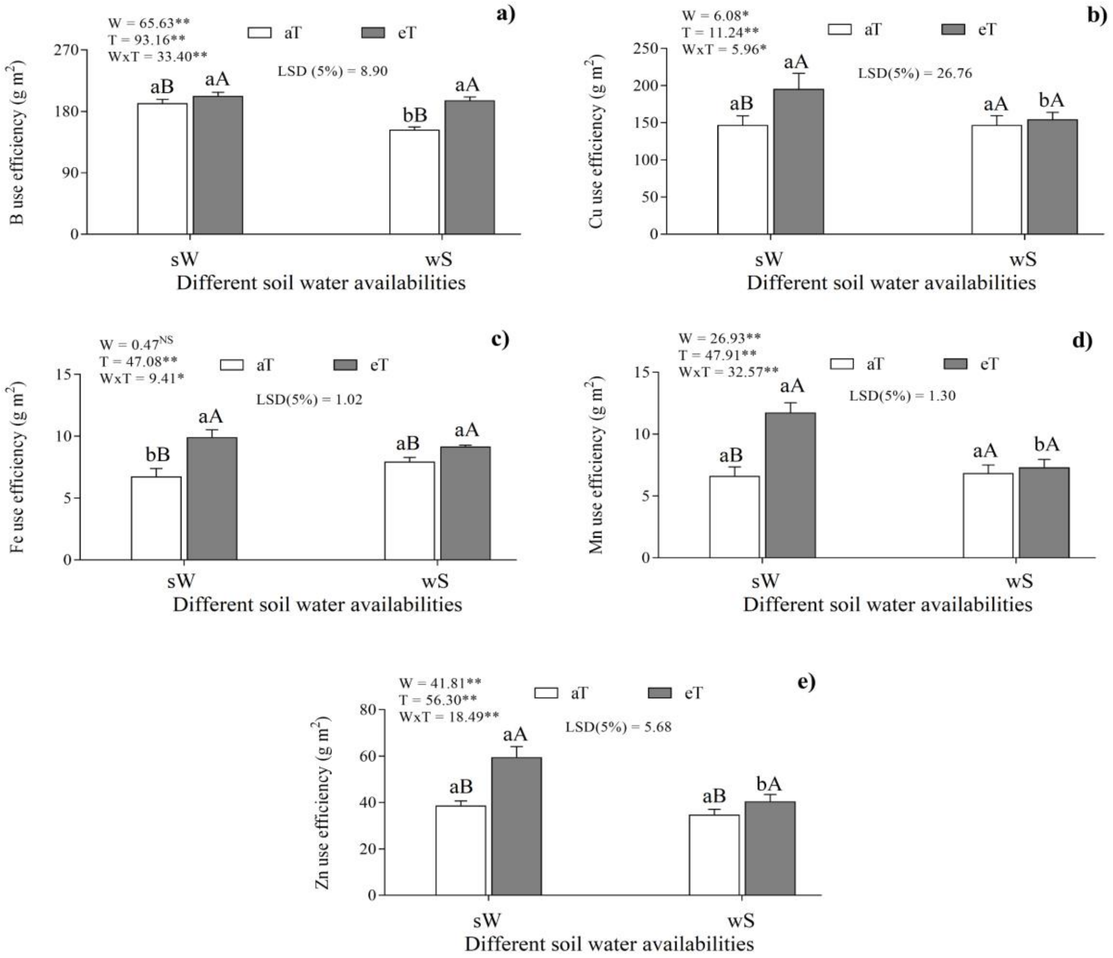

Fig 6. Use efficiency of nutrients in leaves of *Megathyrsus maximus* during the experimental period. Use efficiency of (a) B, (b) Cu, (c) Fe, (d) Mn, and (e) Zn. Treatments: Treatments: canopy ambient temperature and irrigated soil (sWaT); canopy warming (2°C above the ambient canopy temperature) and irrigated soil (sWeT); soil moisture deficit and ambient canopy temperature (sWaT) and soil moisture deficit and canopy warming (sWeT). Different uppercase letters indicate significant differences between the same soil water availabilities at the different temperatures, and lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. *$p \leq 0.05$; ** $p \leq 0.01$; NS, not significant; W × T, water–temperature interaction; LSD, least significant difference. Stack bars show the standard error based on the average of four replicates.

We also observed contrary effects between W and T for leaf biomass production (Fig 7a). While drought decreased leaf dry mass, warming improved biomass productivity. Therefore, under wSeT treatment, warming reversed the negative impact of isolated drought on biomass production of *M. maximus* (Fig. 7a). In contrast, root dry biomass (Fig. 8b) increased in approximately 28% under isolated drought (wSaT) compared with sWaT, probably due to a greater root volume (Fig. 8d) and root surface area (Fig. 8e). However, under rainfed conditions, warming exacerbated the drought effect and decreasing root dry biomass in approximately 38% (Fig. 8b). Although, under wSeT plots, root length increased when compared to isolated drought (Fig. 8c).

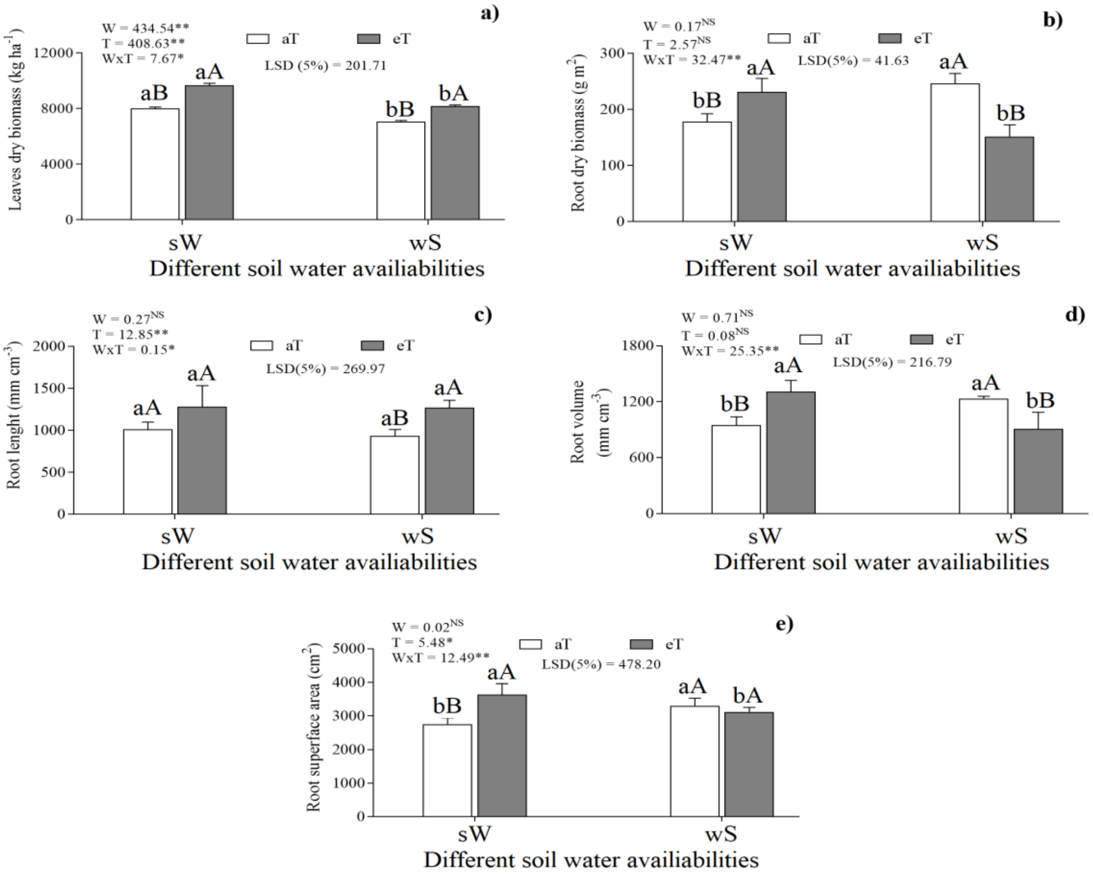

Fig 7. Whole-plant biomass production at the end of experimental period. (a) Leaves dry biomass. (b) Root dry biomass. (c) Root length. (d) Root volume. (e) Root surface area. Treatments: canopy ambient temperature and irrigated soil (sWaT); canopy warming (2°C above the ambient canopy temperature) and irrigated soil (sWeT); soil moisture deficit and ambient canopy temperature (sWaT) and soil moisture deficit and canopy warming (sWeT). Different uppercase letters indicate significant differences between the same soil water availabilities at the different temperatures, and lowercase letters indicate significant differences between different soil water availabilities at the same temperature, according to the F test. *$p \leq 0.05$; ** $p \leq 0.01$; NS, not significant; W × T, water–temperature interaction; LSD, least significant difference. Stack bars show the standard error based on the average of four replicates.

4. Discussion

We studied how mineral composition, nutrient use efficiency and biomass production of guinea-grass responses to the isolated and combined effects of warming and soil water deficit under tropical field conditions. Our main findings suggest that both factors have contrary effects on mineral composition and biomass production of *M. maximus* leaves. However, when both factors were combined, warming reversed the negative impacts of drought on plants, contradicting our third hypotheses.

Drought stress was the only factor that impaired plant development and nutritional quality of guinea-grass. Under soil water deficit, roots are unable to get optimal amounts of nutrients from the soil, resulting in metabolic disruption and negative effects on plant growth (Waraich et al., 2011). Soil moisture greatly impact the degradation of organic matter in the soil, soil microbiota activity and nutrient diffusion, which may decrease plant access to nutrients. E.g. Nitrate reductase is a key enzyme for N acquisition by plants and due to more-severe drought conditions might have a negative effect on nutrient availability, compromising the current structure of enzymes (Hueso et al., 2012). In addition, drought stress reduces the uptake of phosphorus, increasing P-limitation in plants (Olivera Viciedo et al., 2019; Sardans et al., 2008). The plant response mechanisms to the conditions of soil water deficit is complex and change according to plant type and growth duration, as well as with the period of water restriction (Farooq et al., 2009).

During the same experiment reported here, Habermann et al. (2019a) showed that net photosynthesis rate, maximum carboxylation rate of Rubisco, and stomatal conductance were impaired under drought regardless temperature level, while PSII functioning remained intact. Therefore, we hypothesized that reduced leaf N concentration could be contributing to photosynthesis limitation of plants under wS plots. In contrast, the beneficial effect of warming is often the result of an increased root development (Mackay and Barber, 1984), as we observed in this study (Fig. 8b, c, d, e). Our data showed that under sWeT treatment, root volume, surface area and length greatly enhanced, resulting in higher root dry and leaf dry biomass production (Fig. 7a, b). These results indicated that elevated temperature induced root soil exploration, presumably enhancing nutrients and water absorption (Sharp et al., 2004). Increased soil temperature could increase the N- and P-uptake capacities to a greater extent in plant species from warm and fluctuating soil habitats than in species from cold and stable soil environments (Bassirirad, 2000).

The increased root growth may be result of new regulation of starch broken and remobilization, since it was found that starch content decrease in *M. maximus* leaves during a moderate warming (Habermann et al., 2019a). Therefore, our hypothesis is that sugars are being translocated to roots to sustain belowground growth and to new leaves increasing leaves biomass. Plants respond to environmental changes partitioning biomass to different organs as a mechanism to enhance resources utilization and growth. Our data indicated that under wSaT treatment, carbon was remobilized to sustain root growth, a response often associated to a search for water resources at deeper soil layers (Hodge et al., 2009). This improvement in root growth may explain the buffering effect of warming when combined with drought at wSeT treatment, since transpiration rate and water use efficiency were both not increased due to warming effect (Habermann et al., 2019).

The dry biomass yield is the result of complex interactions between different physiological processes. Most of these processes are negatively affected by drought stress. Drought impairs cell mitosis and elongation, resulting in limited growth (Hussain et al., 2008), mainly due to the loss of turgor (Taiz et al., 2013). The drought-induced reduction in yield might also occur due to other factors, such as decreased rate of photosynthesis (Saud et al., 2017), disturbed assimilate partitioning (Gulías et al., 2003), or poor flag leaf development (Rucker et al., 1995). The negative effect of drought on leaf biomass production was associated with a reduction in the absorption of macronutrients, such as N, P, K, Ca and Mg (Fig. 3a, b, c, d, e), and micronutrients (Fig. 4), as well as by an impairment of the *NUE* of K, Ca (Fig. 5c, d), and B (Fig. 6a). Similar effects were observed in *Kentucky bluegrass*, in which growth was adversely influenced by drought conditions (Saud et al., 2017).

Meanwhile, under warming and irrigated plots (sWeT), we observed an increase in leaves dry biomass production. This beneficial effect was presumably associated with a high leaf homeostatic capacity of plant (Olivera Viciedo et al., 2019), a higher content of amino acids related to drought tolerance (Wedow et al., 2019) and a higher accumulation of N, P, K, Ca, Mg and B, Cu, Fe, Mn (Fig. 3a, c, d and Fig. 4a, b, c, d) and an increase in the *NUE* of macronutrients (Fig. 5a-e), and micronutrients as Cu, Mn, and Zn (Fig. 6b, d, e). Suggest that the increased N in C4 grasses leading to increased biomass production under warming (Sardans et al., 2012).The enhanced shoot development was coupled with a greatest root growth. This synchronization between shoot-root grown is necessary since a greater aerial growth increase the demand for more water and nutrients uptake from soil (Hodge et al., 2009). In the warmed plots, the air temperature in the growing season reached up to 40°C, which was higher than that observed under the control conditions (Fig. 2a). This increase in temperature was beneficial for *M. maximus* since the optimum growth temperature for this species ranges between 30 and 40°C (Yamori et al., 2014). Previous experiments showed that elevated temperatures (35°C in daytime and 30°C during the night) increased photosynthetic nitrogen use in *Panicum. coloratum* (Dwyer et al., 2007). Our results help to explain the 22% increase in the aboveground biomass of *M. maximus* observed under the conditions similar to those used in the present study; however, leaf nutrients were not measured in their study (Prado et al., 2016).

The mineral composition of leaves are important aspects of forage quality for livestock. Some important insights regarding future animal feeding can be discussed from our data, because forage quality depends on nutrient concentration, which determines digestibility, partitioning of metabolized products in the digestive tract and forage intake; it thus strongly affects animal performance (Dumont et al., 2014).

5. Conclusions

Our study indicated that under future conditions of high temperature and soil water deficit similar to those found here, an increase of nutrients accumulation and NUE could contribute to a greater mineral quality of guinea grass. We observed that warming and drought have contrary effects on mineral composition, NUE and biomass production of M. maximus under field tropical conditions. The positive effect of warming completely buffered the negative impacts of drought, suggest leading to a greater biomass under future conditions.

Author contributions

DOV wrote the manuscript, with input from all authors and RMP contributed to the materials and analysis. DOV, EH, ACH and RBFB collected the data in the field and processed it. CAM conceived and supervised the T-FACE experiment. CAM and RMP supervised the laboratory and field work of graduate students and postdocs. All authors contributed to the revision of the manuscript. DOV is responsible for the manuscript as a whole.

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