Fiber fractions, multielemental and isotopic composition of a tropical C₄ grass grown under elevated atmospheric carbon dioxide

Adibe L. Abdalla Filho¹, Geovani T. Costa Junior², Paulo M.T. Lima³, Amin Soltangheisi⁴, Adibe L. Abdalla³, Raquel Ghini⁵ and Marisa C. Piccolo¹

¹ Universidade de São Paulo, Centro de Energia Nuclear na Agricultura—Laboratório de Ciclagem de Nutrientes, Piracicaba, São Paulo, Brazil
² Universidade de São Paulo, Centro de Energia Nuclear na Agricultura—Laboratório de Instrumentação Nuclear, Piracicaba, São Paulo, Brazil
³ Universidade de São Paulo, Centro de Energia Nuclear na Agricultura—Laboratório de Nutrição Animal, Piracicaba, São Paulo, Brazil
⁴ Universidade de São Paulo, Centro de Energia Nuclear na Agricultura—Laboratório de Ecologia Isotópica, Piracicaba, São Paulo, Brazil
⁵ Embrapa Meio Ambiente, Jaguariúna, Sao Paulo, Brazil

ABSTRACT

Background: Brazil has the largest commercial herd of ruminants with approximately 211 million head, representing 15% of world’s beef production, in an area of 170 million hectares of grasslands, mostly cultivated with Brachiaria spp. Although nutrient reduction due to increased atmospheric carbon dioxide (CO₂) concentration has already been verified in important crops, studies evaluating its effects on fiber fractions and elemental composition of this grass genus are still scarce. Therefore, a better understanding of the effects of elevated CO₂ on forage quality can elucidate the interaction between forage and livestock production and possible adaptations for a climate change scenario. The objective of this study was to evaluate the effects of contrasting atmospheric CO₂ concentrations on biomass production, morphological characteristics, fiber fractions, and elemental composition of Brachiaria decumbens (cv. Basilisk).

Methods: A total of 12 octagonal rings with 10 m diameter were distributed in a seven-ha coffee plantation and inside each of them, two plots of 0.25 m² were seeded with B. decumbens (cv. Basilisk) in a free air carbon dioxide enrichment facility. Six rings were kept under natural conditions (≅390 μmol mol⁻¹ CO₂; Control) and other six under pure CO₂ flux to achieve a higher concentration (≅550 μmol mol⁻¹ CO₂; Elevated CO₂). After 30 months under contrasting atmospheric CO₂ concentration, grass samples were collected, and then splitted into two portions: in the first, whole forage was kept intact and in the second portion, the leaf, true stem, inflorescence and senescence fractions were manually separated to determine their proportions (%). All samples were then analyzed to determine the fiber fractions (NDF, hemicellulose, ADF, cellulose, and Lignin), carbon (C), nitrogen (N), potassium (K), calcium (Ca), sulfur (S), phosphorus (P), iron (Fe), and manganese (Mn) contents and N isotopic composition.

How to cite this article: Abdalla Filho AL, Costa Junior GT, Lima PMT, Soltangheisi A, Abdalla AL, Ghini R, Piccolo MC. 2019. Fiber fractions, multielemental and isotopic composition of a tropical C₄ grass grown under elevated atmospheric carbon dioxide. PeerJ 7:e5932 DOI 10.7717/peerj.5932

Copyright 2019 Abdalla Filho et al. Distributed under Creative Commons CC-BY 4.0
**Results:** Elevated atmospheric CO$_2$ concentration did not influence biomass productivity, average height, leaf, stem, senescence and inflorescence proportions, and fiber fractions ($p > 0.05$). Calcium content of the leaf and senescence portion of $B. \text{decumbens}$ were reduced under elevated atmospheric CO$_2$ ($p < 0.05$). Despite no effect on total C and N ($p > 0.05$), lower C:N ratio was observed in the whole forage grown under elevated CO$_2$ ($p < 0.05$). The isotopic composition was also affected by elevated CO$_2$, with higher values of $\delta^{15}N$ in the leaf and stem portions of $B. \text{decumbens}$ ($p < 0.05$).

**Discussion:** Productivity and fiber fractions of $B. \text{decumbens}$ were not influenced by CO$_2$ enrichment. However, elevated CO$_2$ resulted in decreased forage Ca content which could affect livestock production under a climate change scenario.

**Subjects** Agricultural Science, Climate Change Biology

**Keywords** Brachiaria decumbens, Climate change, FACE, Livestock, Calcium

**INTRODUCTION**

Fossil fuel combustion, land use changes, and the expansion of population and industry have significantly contributed to the global carbon dioxide (CO$_2$) rise, from the preindustrial level of 280 ppm to the current level of 400 ppm (International Panel on Climate Change (IPCC)—Climate Change, 2014; Broberg, Högy & Pleijel, 2017), and this increase is expected to continue. According to the representative concentration pathways (RCPs) of International Panel on Climate Change (2014), the atmospheric CO$_2$ concentration is estimated to reach the range of 420 ppm (RCP2.6) to 1,300 ppm (RCP8.5) in the next decades. Such increases in CO$_2$ concentration is expected to have cascading effects on numerous aspects of plant biochemistry, since plant productivity is strongly tied to atmospheric CO$_2$ through photosynthesis (Dietterich et al., 2015).

Experimental studies simulating future scenarios predict that C$_4$ species are less responsive to elevated CO$_2$ conditions in comparison with C$_3$ species due to the differences in their photosynthetic mechanism (Ehleringer & Björkman, 1977; Sage & Kubien, 2007; Reich et al., 2018). C$_4$ species have a C-accumulation strategy, which minimizes photorespiration through biochemical and anatomical specializations. Using this strategy, C$_4$ species can concentrate CO$_2$ at the active site of the ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) (Sage, 2004) and be virtually CO$_2$ saturated even at the current atmospheric CO$_2$ concentration (Tom-Dery et al., 2018). In addition, photosynthetic capacity of many plant species reduced, when they were exposed to elevated CO$_2$ due to an inhibition mechanism called photosynthetic acclimation, generally attributed to an alteration in the balance of the supply and sink of assimilates leading to increased nonstructural carbohydrates content in the leaves (Drake, González-Meler & Long, 1997; Porras et al., 2017). However, indirect effects of elevated CO$_2$ can increase leaf CO$_2$ assimilation rate and growth of C$_4$ species via increases in intracellular partial pressure, changes in fixation patterns, improvements of shoot water relations, and increases in leaf temperature (Ghannoum et al., 2000). In addition, recent results from a 20-
year free air carbon dioxide enrichment (FACE) experiment showed a much more positive response of C\textsubscript{4} species to elevated atmospheric CO\textsubscript{2} concentration compared to C\textsubscript{3} grasses after 12 years of exposure (Reich et al., 2018).

The response of grasslands to climate change is complex due to the interactions between water availability and management practices (Chang et al., 2017). Higher concentrations of atmospheric CO\textsubscript{2} have the potential to alter food and fodder nutritional quality (Porteous et al., 2009; Abdelgawad et al., 2014; Myers et al., 2014; Dumont et al., 2015). Some authors have already noticed that elevated CO\textsubscript{2} can improve pasture productivity at the expense of a decreased nutritional quality of forage (Milchunas et al., 2005; Mueller et al., 2016; Augustine et al., 2018) and this could result in low production and reproduction rates of grazing livestock since these animals usually depend exclusively on forages to meet their nutritional requirements (Ezzat, Fadlalla & Ahmed, 2018).

Changes in production and chemical composition of plants have significant impact on ecological processes (Damatta et al., 2010; Sanz-Sáez et al., 2012; Abdelgawad et al., 2014). Effects of CO\textsubscript{2} enrichment on plants have been intensively investigated in C\textsubscript{3} species (Lara & Andreo, 2011), but some recent studies have investigated these effects on tropical C\textsubscript{4} grasses (McGranahan & Yurkonis, 2018; Santos et al., 2014; Tom-Dery et al., 2018). These C\textsubscript{4} grasses are the main foders in the Brazilian livestock production system, having the largest commercial herd of ruminants with nearly 211 million head (15% of world’s beef production) in an area of approximately 170 million hectares of grasslands (Instituto Brasileiro de Geografia e Estatística, 2012; Barneze et al., 2014; Dick, Da Silva & Dewes, 2015; Cerri et al., 2016). Most of this area is under native African C\textsubscript{4} grass species, mostly belonging to the genus Brachiaria (Gracindo et al., 2014). Although the livestock sector in Brazil is one of the largest in the world, little information is available regarding the changes in pasture productivity and forage nutritional quality due to enhanced atmospheric CO\textsubscript{2}.

Therefore, the main objective of this study was to investigate changes in pasture productivity and forage nutritional quality of Brachiaria decumbens (cv. Basilisk) in response to 30-month exposure to elevated atmospheric CO\textsubscript{2}. We hypothesized that elevated concentration of atmospheric CO\textsubscript{2} may result in increased pasture productivity, changes in morphological characteristics and changes in nutritional quality of the forages.

**MATERIALS AND METHODS**

**Study site and FACE facility**

This experiment was carried out in an experimental area belonging to the Brazilian Agricultural Research Corporation (Embrapa—Meio Ambiente) located in the municipality of Jaguariúna (22°43’S, 47°01’W, 570 m a.s.l), State of São Paulo, Brazil. According to the Köppen classification, the climate is humid subtropical (Cfa), with hot rainy summers and cold dry winters (Fig. 1). The soil is classified as dystrophic Red Latosol with clayey texture, according to the Brazilian soil classification system. Soil physico-chemical properties of the site are described in Maluf et al. (2015).

A total of 12 octagonal rings with 10 m diameter were established in a seven-ha coffee plantation (Coffee arabica cv. Catuai vermelho IAC 144) and forage growth was
investigated in a FACE experiment. Six rings were considered as control under normal atmospheric CO$_2$ (390 μmol mol$^{-1}$ CO$_2$; $\delta^{13}$C = −8‰) and the other six rings were under elevated atmospheric CO$_2$ (550 μmol mol$^{-1}$ CO$_2$; $\delta^{13}$C = −10.6‰). The level of atmospheric CO$_2$ concentration in elevated atmospheric CO$_2$ treatments was increased with an arrangement of tubes and wireless network controlled by environmental sensors (WXT520 climate sensor and GMM343 CO$_2$ sensor from Vaisala Co., Helsinki, Finland) through the injection of pure CO$_2$ ($\delta^{13}$C = −30.7‰) as described by Ghini et al. (2015). CO$_2$ enrichment was began in August 2011. The level used in this study (550 μmol mol$^{-1}$ CO$_2$) is based on the intermediate scenario (RCP6.0) by 2070 (International Panel on Climate Change, 2013) and have already affected some crops (McGranahan & Poling, 2018).

Forage sampling and chemical analysis

Within each ring, two experimental square plots (0.25 m$^2$) were cultivated with *B. decumbens* (cv. Basilisk) sown in the last week of October 2011, and after a cut for standardization in January 2012, forage availability was evaluated every 21 days (giving priority to forage nutritional quality) from February 2012 to January 2014 (Abdalla et al., 2016). In October 2012, and October 2013, the experimental plots were fertilized with 40 kg ha$^{-1}$ of N, 82 kg ha$^{-1}$ of P$_2$O$_5$ and 41 kg ha$^{-1}$ of K$_2$O.

A total of 30 months after cultivation under normal and elevated CO$_2$ concentrations, average height of grasses was measured with a graduated ruler. All the plants inside the squared plots (0.25 m$^2$) were cut at 20 cm above soil surface with scissor.

Biomass production at the field scale was estimated by weighing the collected samples and then they were immediately moved to Animal Nutrition Laboratory (LANA/CENA)
for analysis. Samples were then split into two portions; the first portion, whole forage was kept intact and the second, the leaf, true stem, inflorescence, and senescence fractions were manually separated. All samples were dried at 55 °C for 72 h. Forage dry matter biomass was weighed, and proportions (%) of leaf, stem, inflorescence, and senescence material were calculated.

For chemical composition analysis, the whole forage and the different fractions were ground in a Wiley mill through a one mm screen. Organic matter (OM) concentrations were determined according to AOAC (2011). Neutral detergent fiber (aNDFom) was analyzed according to Mertens (2002), and acid detergent fiber (ADFom) and lignin (Lignin (sa)) were determined sequentially following the methodology of Van Soest, Robertson & Lewis (1991) using a fiber analyzer (Tecnal - Equipamentos para Laboratórios, Tecnal TE-149, Piracicaba, Brazil) and Ankom filter bags (ANKOM Technology, Ankom F-57, Macedon, NY, USA). Hemicellulose and cellulose were calculated by the differences between aNDFom, ADFom, and Lignin-sa.

To determine the total content of C and N, and N isotopic composition in the forage, samples were ground to pass through a 0.15 mm sieve, sealed in tin capsules and loaded into an elemental analyzer (CH-1110; Carlo Erba, Milan, Italy) for combustion under continuous flow of He. The gases generated from the combustion (CO₂-C and N₂-N) were passed directly through the inlet of a mass spectrometer (Thermo Scientific, Delta Plus; Bremen, Germany) and the stable isotopic ratio was expressed using the following equation:

\[ \delta^{15}N(\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3 \]

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are \(^{15}\text{N}:/^{14}\text{N} \) ratios of the sample and the standard. Atmospheric N was used as standard for \( \delta^{15}\text{N} \).

Energy dispersive X-ray fluorescence (Shimadzu EDX 720 spectrometer, furnished with a 50 W Rh Anode X-ray tube) technique was used for elemental analysis of potassium (K), calcium (Ca), sulfur (S), phosphorus (P), iron (Fe), and manganese (Mn). The ground samples \((n = 24)\) were analyzed under vacuum using a Rh X-ray tube at 50 kV and auto-tunable current adjusted for a detector deadtime below 30% and a collimator with three mm beam size. The X-ray spectrum of the sample was acquired utilizing a Si (Li) detector for 300 s and the quantification was carried out using the fundamental parameters approach.

**Statistical analysis**

The experiment was a completely randomized block design (spatial distribution of the rings within the experimental area) with two treatments (Control and Elevated CO₂) and six replications, and the statistical analysis was performed using SAS software, version 9.4 (SAS Institute Inc., Cary NC, USA). The data were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure considering block and treatment as fixed effects and the least square means were compared with LSD \((p < 0.05)\).
RESULTS

Elevated CO$_2$ had no effect on biomass productivity, average height, and proportions of leaf, stem, senescence, and inflorescence (Table 1). The OM and fiber fractions of the whole forage, leaf, stem, and senescence portions were also not influenced by elevated CO$_2$ concentration (Table 2). Elevated CO$_2$ showed a nonsignificant ($p > 0.05$) 18% decrease in biomass productivity, 10% decrease in average height, 4% decrease in FDAom and 5% decrease in Lignin (sa) related to control.

Despite no significant effect on C and N concentrations, a decrease ($p < 0.05$) in the C:N ratio of the whole plant was observed under elevated CO$_2$ (Table 3). In addition, elevated CO$_2$ led to higher values of $\delta^{15}$N in the leaf and stem portions of $B.$ decumbens ($p < 0.05$). However, such increase (0.4‰ and 0.7‰ in leaf and stem portions, respectively) was generally lower or very close to 0.5‰, which is the analytical error of this analysis.

Elevated CO$_2$ did not influence the concentrations of K, S, P, Fe, and Mn in the whole plant, leaf, stem, and senescent portions (Figs. 2A–2E). However, lower concentration of Ca was observed in the leaf (12%) and senescence portion (18%) of $B.$ decumbens grown under elevated atmospheric CO$_2$ ($p < 0.05$) (Fig. 2F).

DISCUSSION

Even after two and half years of exposure to elevated atmospheric CO$_2$ concentration, the productivity, and morphological characteristics of $B.$ decumbens (cv. Basilisk) were not influenced, following the same pattern observed in our trial with $B.$ brizantha (cv. Marandu) in a short term (9 months) experiment (A. L. Abdalla Filho, P. M. T. Lima, G. Z. Sakita, T. P. D. Silva, W. S. Costa, R. Ghi, A. L. Abdalla, M. C. Piccolo, 2019, unpublished data). These results reject our initial hypothesis and are in line with Dumont et al. (2015) stating that only N content and nonstructural carbohydrates concentrations increased under elevated CO$_2$ in a meta-analysis. No effects of elevated

### Table 1 Biomass productivity, average height, leaf, stem, senescence, and inflorescence proportions of *Brachiaria decumbens* cv. Basilisk grown under contrasting atmospheric CO$_2$ concentrations.

| Parameters                     | Treatments               | 95% CI       | $p$-value |
|--------------------------------|--------------------------|--------------|-----------|
|                                | Control ($n=24$)         | Elevated CO$_2$ ($n=24$) |
| Biomass productivity (kg FM ha$^{-1}$) | 28,657.6 ± 4,008.26      | 26,765.4 ± 3,554.44 | [-14856–11072] | 0.7619 |
| Biomass productivity (kg DM ha$^{-1}$) | 5,654.5 ± 592.63         | 4,609.5 ± 610.67 | [-2811.18–721.05] | 0.2288 |
| Average height (cm)            | 60.2 ± 2.41              | 53.7 ± 3.84  | [-16.53–3.65] | 0.1960 |
| Leaf proportion (%)            | 38.5 ± 2.31              | 39.6 ± 2.21  | [-5.75–7.87] | 0.7471 |
| Stem proportion (%)            | 48.5 ± 3.36              | 44.7 ± 1.65  | [-11.94–4.19] | 0.3249 |
| Senescence proportion (%)      | 20.2 ± 1.89              | 21.1 ± 2.19  | [-5.17–6.93] | 0.7620 |
| Inflorescence proportion (%)   | 3.0 ± 0.58               | 3.8 ± 1.06   | [-1.58–3.16] | 0.4915 |

**Note:** Means ± standard error of the means; FM, fresh matter; DM, dry matter; Treatments: control, ambient conditions ($\approx$390 μmol mol$^{-1}$ CO$_2$), elevated CO$_2$, CO$_2$ fertilization ($\approx$550 μmol mol$^{-1}$ CO$_2$); CI, confidence interval.
CO$_2$ (950 μmol mol$^{-1}$ CO$_2$) on total biomass of the C$_4$ grass Cenchrus pedicellatus were also observed by Tom-Dery et al. (2018). However, a recent study conducted over 20 years in a FACE facility in Minnesota, USA, showed that after 12 years of exposure to elevated CO$_2$, the total biomass of several C$_4$ grasses enhanced (Reich et al., 2018). Effects of elevated CO$_2$ could also vary according to the evaluated cultivar. Using growth chambers and the same concentration level of our study (550 μmol mol$^{-1}$ CO$_2$), Santos et al. (2014) evaluated three cultivars of buffel grass (Cenchrus ciliaris) and found that elevated CO$_2$ did not affect the productive characteristics of Biloela, decreased Aridus forage mass and increased forage mass in West Australia. The reason for discrepancies between different

### Table 2 Organic matter and fiber fractions of Brachiaria decumbens cv. Basilisk grown under contrasting atmospheric CO$_2$ concentrations.

| Parameters (g kg$^{-1}$ DM) | Treatments | 95% CI | p-value |
|----------------------------|------------|--------|---------|
|                            | Control ($n = 24$) | Elevated CO$_2$ ($n = 24$) |        |
| Whole plant                |            |        |        |
| OM                         | 945.1 ± 1.70 | 944.4 ± 1.74 | [-5.49–4.20] | 0.7835 |
| aNDFom                     | 682.6 ± 8.02 | 671.9 ± 8.57 | [-35.88–14.41] | 0.3836 |
| ADFom                      | 398.5 ± 10.32 | 382.9 ± 8.80 | [-42.17–11.02] | 0.2983 |
| Lignin (sa)                | 81.5 ± 3.20 | 77.4 ± 3.08 | [-12.64–6.62] | 0.3413 |
| HEMI                       | 284.1 ± 6.07 | 289.0 ± 3.36 | [-10.38–20.16] | 0.5083 |
| CEL                        | 317.0 ± 8.78 | 305.5 ± 6.77 | [-35.00–11.92] | 0.3139 |
| Leaf portion               |            |        |        |
| OM                         | 939.5 ± 1.41 | 942.3 ± 1.07 | [-0.69–6.33] | 0.1090 |
| aNDFom                     | 555.1 ± 7.00 | 564.1 ± 6.55 | [-7.36–25.38] | 0.2619 |
| ADFom                      | 274.9 ± 3.92 | 279.8 ± 5.13 | [-8.10–17.85] | 0.4389 |
| Lignin (sa)                | 64.0 ± 4.99 | 67.6 ± 5.18 | [-8.36–15.40] | 0.5407 |
| HEMI                       | 280.2 ± 4.28 | 284.3 ± 5.43 | [-9.25–17.53] | 0.5230 |
| CEL                        | 210.9 ± 5.05 | 212.2 ± 6.26 | [-15.74–18.46] | 0.8689 |
| Stem portion               |            |        |        |
| OM                         | 957.4 ± 1.71 | 957.9 ± 1.50 | [-3.46–4.44] | 0.7964 |
| aNDFom                     | 778.0 ± 5.70 | 773.6 ± 7.40 | [-22.22–13.39] | 0.6075 |
| ADFom                      | 495.7 ± 6.16 | 491.7 ± 8.68 | [-27.35–19.27] | 0.7190 |
| Lignin (sa)                | 107.4 ± 5.64 | 118.7 ± 17.41 | [-28.50–51.13] | 0.5567 |
| HEMI                       | 282.3 ± 4.07 | 281.9 ± 3.13 | [-12.32–11.52] | 0.9444 |
| CEL                        | 388.3 ± 5.91 | 373.0 ± 15.56 | [-48.97–18.26] | 0.3486 |
| Senescence portion         |            |        |        |
| OM                         | 931.1 ± 2.05 | 926.6 ± 4.07 | [-12.52–9.67] | 0.7896 |
| aNDFom                     | 739.8 ± 4.31 | 730.1 ± 5.26 | [-23.43–11.12] | 0.4612 |
| ADFom                      | 458.9 ± 9.16 | 445.4 ± 6.47 | [-30.95–16.00] | 0.5094 |
| Lignin (sa)                | 91.0 ± 4.35 | 94.9 ± 3.54 | [-7.44–13.45] | 0.5502 |
| HEMI                       | 280.9 ± 5.71 | 284.6 ± 4.93 | [-13.34–16.03] | 0.8487 |
| CEL                        | 367.8 ± 5.89 | 350.5 ± 4.98 | [-29.60–8.65] | 0.2629 |

**Note:**
Means ± standard error of the means; DM, dry matter; OM, organic matter; aNDFom, neutral detergent fiber; ADFom, acid detergent fiber; Lignin (sa), Lignin; HEMI, hemicellulose; CEL, cellulose; Treatments: control, ambient conditions (≈390 μmol mol$^{-1}$ CO$_2$), elevated CO$_2$, CO$_2$ fertilization (≈550 μmol mol$^{-1}$ CO$_2$); CI, confidence interval.
studies is unknown but it is important to consider the differences in methodologies as a possible explanation (McGranahan & Yurkonis, 2018) since growth chambers may overestimate the effects of elevated CO2 on photosynthetic and plant growth parameters (Leakey et al., 2006).

Plants respond directly to higher atmospheric CO2 concentration through photosynthesis and stomatal conductance, and these are the basis for the higher biomass production (Long et al., 2006). However, in C4 plants, RuBisCO is localized in bundle sheath cells, in which CO2 is concentrated in levels of three to six times higher than those of the atmospheric CO2 concentration (Caemmerer & Furbank, 2003). Such CO2 enrichment is sufficient to saturate RuBisCO and prevent any increase in CO2 uptake with CO2 fertilization. In addition, under elevated CO2 condition, C4 plants can close their stomata to reduce water loss during photosynthesis (McGranahan & Poling, 2004).

| Parameters          | Treatments                  | 95% CI       | p-value |
|---------------------|-----------------------------|--------------|---------|
|                     | Control (n = 24)            | Elevated CO2 (n = 24) |
| Whole plant         |                             |              |         |
| C (%)               | 42.95 ± 0.228               | 42.22 ± 0.447 | [−1.78–0.32] | 0.1624 |
| N (%)               | 1.9 ± 0.072                 | 2.12 ± 0.094  | [−0.03–0.48] | 0.0885 |
| C:N                 | 22.97 ± 0.8                 | 20.36 ± 0.896 | [−5.17–0.04] | 0.0462 |
| δ15N (%)            | 4.4 ± 0.107                 | 4.4 ± 0.152   | [−0.42–0.43] | 0.9680 |
| K (%)               | 1.43 ± 0.11                 | 1.57 ± 0.066  | [−0.14–0.42] | 0.3308 |
| Leaf portion        |                             |              |         |
| C (%)               | 43.49 ± 0.221               | 43.76 ± 0.149 | [−0.31–0.85] | 0.3397 |
| N (%)               | 2.78 ± 0.074                | 2.84 ± 0.059  | [−0.14–0.27] | 0.5278 |
| C:N                 | 15.82 ± 0.49                | 15.46 ± 0.318 | [−1.62–0.96] | 0.5776 |
| δ15N (%)            | 4.2 ± 0.104                 | 4.6 ± 0.118   | [0.08–0.81]  | 0.0188 |
| K (%)               | 1.88 ± 0.071                | 1.83 ± 0.054  | [−0.24–0.13] | 0.5363 |
| Stem portion        |                             |              |         |
| C (%)               | 41.35 ± 0.181               | 41.57 ± 0.142 | [−0.18–0.61] | 0.2782 |
| N (%)               | 1.43 ± 0.051                | 1.43 ± 0.063  | [−0.17–0.16] | 0.9898 |
| C:N                 | 29.32 ± 1.097               | 29.76 ± 1.359 | [−3.19–4.08] | 0.7998 |
| δ15N (%)            | 4.2 ± 0.16                  | 4.9 ± 0.138   | [0.13–1.11]  | 0.0162 |
| K (%)               | 1.66 ± 0.086                | 1.72 ± 0.080  | [−0.20–0.32] | 0.6161 |
| Senescence portion  |                             |              |         |
| C (%)               | 40.8 ± 0.175                | 40.5 ± 0.36   | [−1.23–0.63] | 0.5006 |
| N (%)               | 1.24 ± 0.048                | 1.32 ± 0.048  | [−0.05–0.22] | 0.2386 |
| C:N                 | 33.15 ± 1.279               | 31.07 ± 1.23  | [−5.69–1.13] | 0.1755 |
| δ15N (%)            | 4.6 ± 0.151                 | 5.1 ± 0.163   | [−0.10–0.97] | 0.1053 |
| K (%)               | 1.2 ± 0.056                 | 1.29 ± 0.069  | [−0.11–0.30] | 0.3626 |

Note: Means ± standard error of the means; a,b Different letters in the same row indicate statistical difference; Treatments: control, ambient conditions (~390 μmol mol−1 CO2); Elevated CO2, CO2 fertilization (~550 μmol mol−1 CO2); CI, confidence interval.
Still, sufficient rainfall during the experimental period (Fig. 1) could limit gains from reduced transpiration (Fay et al., 2012). For these reasons and the fact that we used a FACE facility, we observed the lack of response to elevated CO$_2$ in our study.

Structural carbohydrates in *Brachiaria decumbens* were also not affected by the increased atmospheric CO$_2$ concentration, refuting our hypothesis. These results are in line with Dumont et al. (2015) but contradicts the findings of Abdalla et al. (2016) evaluating this cultivar under the same treatments (control and elevated CO$_2$) during the rainy season. Our results also contradict Tom-Dery et al. (2018) stating that elevated CO$_2$ reduced Lignin and increased ADF content of *Cenchrus pedicellatus*. It is noteworthy that in our study, in order to evaluate the effect of 30 month exposure to elevated atmospheric CO$_2$, the grass was kept under no grazing management (e.g., considering the concept of critical leaf area index to determine the time of sampling) for almost 6 months, which resulted in older plants with higher proportion of stem, as well as fiber fractions compared to the other studies evaluating the same cultivar (Pedreira, Braga & Portela, 2017; Lima et al., 2018).

By altering plant and microbial processes involved in the N cycle, elevated atmospheric CO$_2$ may change the isotopic signature of plant N (Polley et al., 2015). In our study, a slightly lower C:N ratio was found when the whole plant was analyzed (Table 3). Similarly, rather higher δ$^{15}$N values were found under elevated CO$_2$ in some plant parts. In another study, higher δ$^{15}$N in leaves of ponderosa pine with increasing...
atmospheric CO₂ was recorded (Johnson, Cheng & Ball, 2000); meanwhile, in our study the difference was significant but it was too small to be attributed to elevated CO₂.

The major difference was a reduced Ca content of the leaves and the senescence portion of *B. decumbens* under elevated atmospheric CO₂. Calcium is an essential macronutrient for plant growth, plays an important structural role in the cell wall and membranes, and acts as an intracellular messenger in the cytosol (White & Broadley, 2003). Lower levels of Ca in forage may have implications for animal nutrition since reduced availability of Ca to the rumen microbes decreases fiber digestion (Fielding & Miller, 1986). The Ca content of forages is the net result of absorption and translocation processes operating within the roots and shoots and such processes are being modulated by various environmental factors affecting plant growth and metabolism (Grunes & Welch, 1989).

Other studies found lower Ca concentration in sorghum and soybean under elevated atmospheric CO₂ due to the dilution effect caused by an increased biomass (Rogers, Runion & Krupa, 1994; Rogers et al., 1999), often referred as the “dilution hypothesis” (Loladze, 2014). As the yield was not changed due to the elevated CO₂ in our study, other mechanisms may be involved in reduced Ca concentration. Related to the flow of nutrients, the processes involved in the use of available water may be affected by elevated CO₂ concentration since under this condition, transpiration rates of plants may be reduced and water use efficiency in photosynthetic processes may be improved (Fay et al., 2012). In this study, the plots were kept under similar soil fertility, daily air temperature and rainfall conditions, and the only different parameter was the concentration of atmospheric CO₂, hence the reduced Ca content under elevated CO₂ is more related to an enhanced water use efficiency (parameter not evaluated here) rather than the dilution hypothesis. Despite the reduced Ca content of *B. decumbens* under elevated CO₂, it is important to emphasize that a possible Ca deficiency in ruminants can be easily ameliorated by feeding calcium-containing mineral supplements (e.g., limestone, steamed bone flour, and dicalcium phosphate) (McDonald et al., 2011).

The predicted world population of 9.6 billion in the next decades will result in 70% increase in the demand of animal derived foods and considering the current scenario of climate change, sustainable production of them to achieve food security will be a big challenge faced by humanity (Gerber et al., 2013; Cerri et al., 2016). Our results showed that the productivity and fiber fractions of *B. decumbens* were not impaired by elevated CO₂, suggesting that the tropical pasture-based beef production has the potential to overcome the above-mentioned challenges. A remarkable sustainable potential of grazing systems is also shown in recent studies (De Oliveira Silva et al., 2016; Dass et al., 2018).

**CONCLUSIONS**

We concluded that productivity, morphological characteristics, and fiber fractions of *B. decumbens* (cv. Basilisk) were not affected by elevated atmospheric CO₂ in 30 months. These results are of great importance since *B. decumbens* is one of the main fodders in the Brazilian livestock production system, where extensive grazing is predominant and the herds depend almost exclusively on these grasses to meet their nutritional
requirements. However, elevated CO$_2$ decreased forage Ca content, which can affect livestock production under a climate change scenario and needs further investigations.

**ACKNOWLEDGEMENTS**

The authors would like to thank the researchers and technicians of Embrapa Meio Ambiente, Laboratory of Animal Nutrition (LANA), Isotopic Ecology (LEI), Nuclear Instrumentation (LIN, Asst. Prof. H.W.P. Carvalho and Dr. E. Almeida for XRF analysis) and Nutrient Cycling (LCN) from the Center of Nuclear Energy in Agriculture (CENA/USP).

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Funding**
The National Council of Technological and Scientific Development (CNPq #141762/2015-2) and São Paulo Research Foundation (FAPESP #2015/14699-1) financially supported this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**
The following grant information was disclosed by the authors:
National Council of Technological and Scientific Development: CNPq #141762/2015-2.
São Paulo Research Foundation: FAPESP #2015/14699-1.

**Competing Interests**
The authors declare that they have no competing interests.

**Author Contributions**
- Adibe L. Abdalla Filho conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Geovani T. Costa Junior performed the experiments, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Paulo M.T. Lima performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Amin Soltangheisi prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Raquel Ghini conceived and designed the experiments, contributed reagents/materials/analysis tools, approved the final draft.
- Marisa C. Piccolo conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
Data Availability
The following information was supplied regarding data availability:

The raw data are provided in a Supplemental File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5932#supplemental-information.

REFERENCES

Abdalla AL, Ghini R, Natel AS, Abdalla Filho AL, Louvandini H, Piccolo MC, Torre Neto A, Nechet KL. 2016. Forage quality and methane production of the grazing portion of grass produced under elevated [CO₂]. Melbourne: Greenhouse Gas and Animal Agriculture Conference, 6.

Abdelgawad H, Peshev D, Zinta G, Van Den Ende W, Janssens IA. 2014. Climate extreme effects on the chemical composition of temperate grassland species under ambient and elevated CO₂: a comparison of fructan and non-fructan accumulators. PLOS ONE 9(3):e92044 DOI 10.1371/journal.pone.0092044.

AOAC. 2011. Official methods of analysis of AOAC international. Eighteenth Edition. Gaithersburg: AOAC International.

Augustine DJ, Blumenthal DM, Springer TL, Lecain DR, Gunter SA, Derner JD. 2018. Elevated CO₂ induces substantial and persistent declines in forage quality irrespective of warming in mixedgrass prairie. Ecological Applications 28(3):721–735 DOI 10.1002/eap.1680.

Barneze AS, Mazzetto AM, Zani CF, Misselbrook T, Cerri CC. 2014. Nitrous oxide emissions from soil due to urine deposition by grazing cattle in Brazil. Atmospheric Environment 92:394–397 DOI 10.1016/j.atmosenv.2014.04.046.

Broberg CM, Högy P, Pleijel H. 2017. CO₂-induced changes in wheat grain composition: meta-analysis and response functions. Agronomy 7(2):32 DOI 10.3390/agronomy7020032.

Caemmerer SV, Furbank RT. 2003. The C₄ pathway: an efficient CO₂ pump. Photosynthesis Research 77(2–3):191–207 DOI 10.1023/A:1023830019591.

Cerri CC, Moreira CS, Alves PA, Raucci GS, Castigioni BA, Mello FFC, Cerri DGP, Cerri CEP. 2016. Assessing the carbon footprint of beef cattle in Brazil: a case study with 22 farms in the State of Mato Grosso. Journal of Cleaner Production 112:2593–2600 DOI 10.1016/j.jclepro.2015.10.072.

Chang J, Ciais P, Vievy N, Soussana JF, Klumpp K, Sultan B. 2017. Future productivity and phenology changes in European grasslands for different warming levels: implications for grassland management and carbon balance. Carbon Balance and Management 12(1):11 DOI 10.1186/s13021-017-0079-8.

Damatta FM, Grandis A, Arenque BC, Buckeridge MS. 2010. Impacts of climate changes on crop physiology and food quality. Food Research International 43(7):1814–1823 DOI 10.1016/j.foodres.2009.11.001.

Dass P, Houlton BZ, Wang Y, Warlind D. 2018. Grasslands may be more reliable carbon sinks than forests in California. Environmental Research Letters 13(7):074027 DOI 10.1088/1748-9326/aacb39.

De Oliveira Silva R, Barioni LG, Hall JAJ, Matsuura FM, Albertini TZ, Fernandes FA, Moran D. 2016. Increasing beef production could lower greenhouse gas emissions in Brazil if decoupled from deforestation. Nature Climate Change 6(5):493–497 DOI 10.1038/NCLIMATE2916.
Dick M, Da Silva MA, Dewes H. 2015. Mitigation of environmental impacts of beef cattle production in southern Brazil—Evaluation using farm-based life cycle assessment. Journal of Cleaner Production 87:58–67 DOI 10.1016/j.jclepro.2014.10.087.

Dietterich LH, Zanobetti A, Kloog I, Huybers P, Leakey ADB, Bloom AJ, Carlisle R, Fernando N, Fitzgerald G, Hasegawa TN, Holbrook M, Nelson RL, Norton R, Ottman MJ, Raboy V, Sakai H, Sartor KA, Schwartz J, Seneveera S, Usui Y, Yoshinaga S, Myers SS. 2015. Impacts of elevated atmospheric CO$_2$ on nutrient content of important food crops. Scientific Data 2:150036 DOI 10.1038/sdata.2015.36.

Drake BG, González-Meler MA, Long SP. 1997. More efficient plants: a consequence of rising atmospheric CO$_2$? Annual Review of Plant Physiology and Plant Molecular Biology 48(1):609–639 DOI 10.1146/annurev.arplant.48.1.609.

Dumont B, Andueza D, Niderkorn V, Lüscher A, Porqueddu C, Picon-Cochard C. 2015. A meta-analysis of climate change effects on forage quality in grasslands: specificities of mountain and Mediterranean areas. Grass and Forage Science 70(2):239–254 DOI 10.1111/gfs.12169.

Ehleringer J, Björkman O. 1977. Quantum yields for CO$_2$ uptake in C$_3$ and C$_4$ plants. Plant Physiology 59(1):86–90 DOI 10.1104/pp.59.1.86.

Ezzat S, Fadlalla B, Ahmed H. 2018. Effect of growth stage on the macro mineral concentrations of forbs and grasses in a semi-arid region of Sudan. Journal of Rangeland Science 8:23–29.

Fay PA, Jin VL, Way DA, Potter KN, Gill RA, Jackson RB, Polley HW. 2012. Soil-mediated effects of subambient to increased carbon dioxide on grassland productivity. Nature Climate Change 2(10):742–746 DOI 10.1038/nclimate1573.

Fielding AS, Miller WJ. 1986. Calcium and fat interactions in ruminant nutrition. Professional Animal Scientist 2(2):28–32 DOI 10.15232/S1080-7446(15)32431-1.

Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A, Tempio G. 2013. Tackling climate change through livestock—A global assessment of emissions and mitigation opportunities. Rome: Food and Agriculture Organization of the United Nations (FAO).

Ghannoum O, Von Caemmerer S, Ziska LH, Conroy JP. 2000. The growth response of C$_4$ plants to rising atmospheric CO$_2$ partial pressure: a reassessment. Plant, Cell and Environment 23(9):931–942 DOI 10.1046/j.1365-3040.2000.00609.x.

Gracindo CV, Louvandini H, Riet-Correa F, Barbosa-Ferreira M, Castro MB. 2014. Performance of sheep grazing in pastures of Brachiaria decumbens, Brachiaria brizantha, Panicum maximum, and Andropogon gayanus with different protodioscin concentrations. Tropical Animal Health and Production 46(5):733–737 DOI 10.1007/s11250-014-0556-y.

Grunes DL, Welch RM. 1989. Plant contents of magnesium, calcium and potassium in relation to ruminant nutrition. Journal of Animal Science 67(12):3485–3494 DOI 10.2527/jas1989.67123485x.

International Panel on Climate Change (IPCC)—Climate Change. 2014. In: Synthesis report, contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change, Geneva, 151.

International Panel on Climate Change (IPCC)—Climate System Scenario Tables, Annex II: Climate Change. 2013. The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge, United Kingdom and New York: Cambridge University Press.
Instituto Brasileiro de Geografia e Estatística (IBGE). 2012. Produção da pecuária municipal - PPM. Rio de Janeiro, 2012. Available at http://www.ibge.gov.br/home/estatistica/economia/ppm/2012.

Johnson DW, Cheng W, Ball JT. 2000. Effects of CO$_2$ and N fertilization on decomposition and N immobilization in ponderosa pine litter. Plant and Soil 224(1):115–122 DOI 10.1023/A:1004606901550.

Lara MV, Andreo CS. 2011. C$_4$ plants adaptation to high levels of CO$_2$ and to drought environments. In: Shanker A, Venkateswarlu B, eds. Abiotic Stress in Plants—Mechanisms and Adaptations. Rijeka: InTech, 415–428.

Leakey ADB, Uribelarrea M, Ainsworth EA, Naidu SL, Rogers A, Ort DR, Long SP. 2006. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO$_2$ concentration in the absence of drought. Plant Physiology 140(2):779–790 DOI 10.1104/pp.105.073957.

Lima DM, Abdalla Filho AL, Lima PMT, Sakita GZ, Silva TPD, Macmanus C, Abdalla AL, Louvandini H. 2018. Morphological characteristics, nutritive quality, and methane production of tropical grasses in Brazil. Pesquisa Agropecuária Brasileira 53(3):323–331 DOI 10.1590/s0100-204x2018000300007.

Loladze I. 2014. Hidden shift of the ionome of plants exposed to elevated CO$_2$ depletes minerals at the base of human nutrition. Elife 3:e02245 DOI 10.7554/eLife.02245.001.

Long SP, Ainsworth EA, Leakey ADB, Nosberger J, Ort DR. 2006. Food for thought: lower than expected crop yield stimulation with rising CO$_2$ concentrations. Science 312(5782):1918–1921 DOI 10.1126/science.1114722.

Maluf HJGM, Ghini R, Melo LBB, Silva CA. 2015. Fertilidade do solo e estado nutricional do cafeeiro cultivado em atmosfera enriquecida com CO$_2$. Pesquisa Agropecuária Brasileira 50(11):1087–1096 DOI 10.1590/S0100-204X2015001100012.

McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA, Wilkinson RG. 2011. Animal Nutrition. Harlow: Pearson Education Limited.

McGranahan DA, Poling BN. 2018. Trait-based responses of seven annual crops to elevated CO$_2$ and water limitation. Renewable Agriculture and Food Systems 33(3):259–266 DOI 10.1017/S1742170517000692.

McGranahan DA, Yurkonis KA. 2018. Variability in grass forage quality and quantity in response to elevated CO$_2$ and water limitation. Grass and Forage Science 73(25):517–521 DOI 10.1111/gfs.12338.

Mertens DR. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International 85:1217–1240.

Milchunas DG, Mosier AR, Morgan JA, Lecain DR, King JY, Nelson JA. 2005. Elevated CO$_2$ and defoliation effects on a shortgrass steppe: forage quality versus quantity for ruminants. Agriculture, Ecosystems and Environment 111(1–4):166–184 DOI 10.1016/j.agee.2005.06.014.

Mueller KE, Blumenthal DM, Pendall E, Carrillo Y, Dijkstra FA, Williams DG, Follett RF, Morgan JA. 2016. Impacts of warming and elevated CO$_2$ on a semi-arid grassland are non-additive, shift with precipitation, and reverse over time. Ecology Letters 19(8):956–966 DOI 10.1111/ele.12634.

Myers SS, Zanobetti A, Klooq J, Huybers P, Leakey AD, Bloom AJ, Carlisle E, Dietterich LH, Fitzgerald G, Hasegawa T, Holbrook NM, Nelson RL, Ottman MJ, Raboy V, Sakai H, Sartor KA, Schwartz J, Seneweera S, Tausz M, Usui Y. 2014. Increasing CO$_2$ threatens human nutrition. Nature 510(7503):139–142 DOI 10.1038/nature13179.
Pedreira CGS, Braga GJ, Portela JN. 2017. Herbage accumulation, plant-part composition and nutritive value on grazed signal grass (Brachiaria decumbens) pastures in response to stubble height and rest period based on canopy light interception. *Crop and Pasture Science* 68(1):62–73 DOI 10.1071/CP16333.

Polley HW, Derner JD, Jackson R, Gill R, Procter AC, Fay PA. 2015. Plant community change mediates the response of foliar δ¹⁵N to CO₂ enrichment in mesic grasslands. *Oecologia* 178(2):591–601 DOI 10.1007/s00442-015-3221-x.

Porteaux F, Hill J, Ball AS, Pinter PJ, Kimball BA, Wall GW, Adamsen FJ, Hunsaker DJ, Lamorte RL, Leavitt SW, Thompson TL, Matthias AD, Brooks TJ, Morris CF. 2009. Effect of free air carbon dioxide enrichment (FACE) on the chemical composition and nutritive value of wheat grain and straw. *Animal Feed Science and Technology* 149(3–4):322–332 DOI 10.1016/j.anifeedsci.2008.07.003.

Porras ME, Lorenzo P, Medrano E, Sánchez-González MJ, Otálora-Alcón G, Piñero MC, Del Amor FM, Sánchez-Guerrero MC. 2017. Photosynthetic acclimation to elevated CO₂ concentration in sweet pepper (Capsicum annuum) crop under Mediterranean greenhouse conditions: influence of the nitrogen source and salinity. *Functional Plant Biology* 44(6):573–586 DOI 10.1071/FP16362.

Reich PB, Hobbie SE, Lee TD, Pastore MA. 2018. Unexpected reversal of C₃ versus C₄ grass response to elevated CO₂ during a 20-year field experiment. *Science* 360(6389):317–320 DOI 10.1126/science.aas9313.

Rogers HH, Runion GB, Krupa SV. 1994. Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* 83(1–2):155–189 DOI 10.1016/0269-7491(94)90034-5.

Sage RF. 2004. The evolution of C₄ photosynthesis. *New Phytologist* 161:341–370 DOI 10.1111/j.1469-8137.2004.00974.x.

Sanz-Sáez A, Erice G, Aguirreolea J, Muñoz F, Sánchez-Díaz M, Irigoyen JJ. 2012. Alfalfa forage digestibility, quality and yield under future climate change scenarios vary with Sinorhizobium meliloti strain. *Journal of Plant Physiology* 169(8):782–788 DOI 10.1016/j.jplph.2012.01.010.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74(10):3583–3597 DOI 10.3168/jds.S0022-0302(91)78551-2.

White PJ, Broadley MR. 2003. Calcium in Plants. *Annals of Botany* 92(4):487–511 DOI 10.1093/aob/mcg164.