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SOIL SCIENCE

Ecosystem functions in different physiognomies of Cerrado through the Rapid Ecosystem Function Assessment (REFA)

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Abstract: The assessment of ecosystem functions in Cerrado is important to implement practices of conservation. Recently, a 'rapid ecosystem function assessment' (REFA) for measuring ecosystem functions has been proposed and tested as a suitable method. Thus, this study aimed to assess the proxies of ecosystem functions of three physiognomies of Cerrado through REFA. This method was applied in three different preserved physiognomies of Cerrado from Northeastern, Brazil, namely: *Campo Graminoide* (CG), *Cerrado Stricto Sensu* (CSS), and *Cerradão* (CD). All proxies for the selected ecosystem functions differed between sites and seasons. The above- and belowground primary productivity and microbial biomass C were higher in CD than in CSS and CG. The above- and belowground secondary productivity and decomposition were higher and similar in CD and CSS as compared to CG. The principal component analysis explained 89.8% of the data variation and clustered the majority of ecosystem functions with CD, in both seasons and CSS in the wet season. The proxies of ecosystem functions measured through REFA showed differences between the physiognomies of Cerrado. Since each physiognomy of Cerrado presents different plant richness and diversity, and soil conditions, these characteristics contribute to influencing multiple ecosystem functions.

Key words: Brazilian savannah, ecosystem services, soil properties, microbial biomass.

INTRODUCTION

As the second-largest Brazilian biome, the Cerrado presents a high biological diversity (Amaral et al. 2006). One distinct characteristic of this biome is its different physiognomies distributed from grassland to arboreal formations. However, the main physiognomies found in Cerrado are '*Campo Graminoide*', '*Cerrado Stricto Sensu*', and '*Cerradão*' (Coutinho 1978) that present different types of vegetation and soil conditions (Lucena et al. 2014). Briefly, *Campo Graminoide* is dominated by grasses, while *Cerrado Stricto Sensu* presents the dominance of grasses, shrubs, and woody stratum, and *Cerradão* is dominated by shrubs and woody stratum (Coutinho 1978).

The differences found in each different Cerrado physiognomies have contributed to the different status of vegetation (Oliveira et al. 2007), macro and microfauna (Nunes et al. 2019), and also soil microorganisms (Araujo et al. 2017a, b, 2018). Regarding plant vegetation, some studies have shown different plant species and diversity according to the physiognomies of Cerrado (Oliveira et al. 2007, Lenza et al. 2015). These differences influence the distribution and composition of soil organisms, such as microbes. Indeed, recent studies have reported differences in fungi, bacterial, and archaeal diversities in

different physiognomies of Cerrado (Araujo et al. 2017a, b, 2018). Also, some studies have estimated important ecosystem processes in Cerrado, such as primary production (Batmanian & Haridasan 1985), decomposition (Valenti et al. 2008), and nutrient cycling (Alves et al. 2018).

Although studies have been done to estimate important ecosystem processes, the estimation of multiple ecosystem functions in preserved physiognomies of Brazilian Cerrado remains scarce. Ecosystem functions are important to provide fundamental environmental services that are driven by the biotic components, such as clean water provisioning and soil erosion control (Millennium Ecosystem Assessment 2005). Therefore, the knowledge of the ecosystem functions is important to provide environmental policies for conservation practices (Kollmann et al. 2016). However, it is necessary to use methods that investigate multiple ecosystem functions (Hillebrand & Matthiessen 2009). Recently, a 'rapid ecosystem function assessment' (REFA) for measuring ecosystem functions has been proposed and tested as a suitable method (Meyer et al. 2017, Leidinger et al. 2017). REFA measures potential ecosystem functions through a set of easy and standardized proxies (Meyer et al. 2015). This method provides information about multiple ecosystem functions and has been applied in marine and terrestrial ecosystems (Lefcheck et al. 2016, Leidinger et al. 2017). Therefore, this study applied REFA to assess the approximate ecosystem functions in different physiognomies of Cerrado.

MATERIALS AND METHODS

The assessment of ecosystem functions was applied in a preserved gradient of Cerrado located at "Parque Nacional de Sete Cidades", Piauí State, Brazil. (04°02'-08'S and 41°40'-45'W). The climate is sub-humid with two distinct seasons (wet and dry) and presents an annual average temperature of 25°C and rainfall of 1,558 mm. Three preserved sites of Cerrado were selected, namely: a) *Campo Graminoide* (CG); b) *Cerrado Stricto Sensu* (CSS); and c) *Cerradão* (CD) (Table I).

Each site was divided into four transects (considered here as replicates) and they were evaluated in April (wet season) and October (dry season), 2019. In each transect, the measurements of ecosystem functions were done in three points distanced by 50m. We applied a sampling strategy according to REFA (Meyer et al. 2017). In each site, we measured proxies of the ecosystem functions described below (REFA; Meyer et al. 2017, Leidinger et al. 2017):

a) Aboveground primary productivity (AGPP): it was approximated by peak standing biomass in 20 cm x 50 cm plots. Biomass was selected at ground level, including dead biomass and woody components of vegetation. A total of 12 samples per site were dried for 48 hours (70 °C) before weighing.

b) Belowground primary productivity (BGPP): volumetric soil samples of 10 cm depth and a diameter of 20 cm were taken with an auger. A total of 12 soil samples per site were collected and the roots were separated by washing the soil sample through a sieve and then dried for 48 hours (70 °C) before weighing.

c) Aboveground secondary productivity (AGSP). Invertebrates were sampled using pitfall traps. The traps were made from polyethylene terephthalate (PET) bottles containing two units of different sizes. The first unit, 15 cm in height and 10 cm in diameter (capture area), was buried leaving the border level with the ground, and the second unit, with a height of 10 cm and a diameter of 8 cm, (placed inside the larger container) was used as a collector, two-thirds

Table I. Vegetation indexes (Oliveira et al. 2007), chemical variables and granulometry of the soil (Rocha et al. 2019) of the evaluated sites.

	Campo Graminoide	Cerrado Stricto Sensu	Cerradão
Plant richness*	4.7	11	17
Plant diversity**	0.2	0.85	1.10
Plant density***	4.7	27.1	35.0
Plant species****	a	b	c
Soil N (%)	1.2	2.0	2.9
Soil P (mg kg^{-1})	3.5	4.1	3.9
Soil K (mg kg^{-1})	13.2	18.1	18.9
Sand (%)	72	70	69
Silt (%)	22	23	22
Clay (%)	6	7	9

*** species/100 m^2; ** H/100 m^2; *** individual/100 m^2; H – Shannon-Weaver index.**

****** a -** ***Andropogon fastigiatus; Aristida longifolia; Eragrostis maypurensis.*** **b -** ***Andropogon fastigiatus; Aristida longifolia; Terminalia fagifolia; Magonia pubescens; Hymenaea courbaril; Plathymenia reticulata; Qualea grandiflora; Combretum mellifluum; Lippia origanoides; Anacardium occidentale; Simarouba versicolor; Vatairea macrocarpa.*** **c -** ***Aspidosperma discolor; Parkia platycephala; Terminalia fagifolia; Piptadenia moniliformis; Plathymenia reticulata; Qualea parviflora; Anacardium occidentale; Copaifera coriacea; Thiloa glaucocarpa; Casearia grandiflora.***

full of solution (water, detergent, and NaCl) for the capture and death of the invertebrates. The top part of the larger bottles was used as a funnel and fitted into the collector. A total of 12 pitfall traps per site were used and stayed in the field for one week, and then the samples were transferred to 70% ethanol. The invertebrates were separated from plant material in the laboratory before being counted.

d) Belowground secondary productivity. A total of 12 soil cores with a diameter of 20 cm and a depth of 10 cm were taken in each site. The soil fauna was extracted from the soil cores for 10 days using a Tullgren-Funnel with a 30 W light bulb and collected in ethylene glycol. After sample cleaning, the number of individuals was counted as described above.

e) Decomposition. A total of 12 standardized and previously dried and weighed wooden sticks (115 mm x 10 mm x 2 mm) were placed per site. These sticks were buried horizontally at a depth of approximately 10 cm. After a mean exposure time of 45 days, the sticks were retrieved, washed, dried at 70 °C for 48 hours, and then weighed.

f) Microbial biomass carbon (MBC). In each site, 12 soil samples from 10 cm deep soil cores of 1.6 cm diameter (approximately 30 g) were taken per site. MBC was determined by the chloroform fumigation-extraction method according to Vance et al. (1987). Briefly, 25 g soil was fumigated with 500 mL chloroform. The remaining 25 g soil was non-fumigated and treated as control. C from fumigated and non-fumigated soils were extracted with 100 mL 0.5 mol L^{-1} K_2SO_4 (soil:extractant = 1:4) and shaken for 30 minutes on a shaker. The extraction efficiency coefficient of 0.38 was used to convert

the difference in C between fumigated and non-fumigated soil in MBC.

In addition, soil pH, phosphorus (P), and potassium (K) were determined and measured using standard laboratory procedures (Embrapa 1999). Soil pH was estimated in water (1:2.5 v:v) and measured by a pH-meter. Available P and exchangeable K were extracted using the Mehlich-1 extraction method and determined by colorimetry and photometry, respectively. Total organic carbon (TOC) was determined by the wet combustion method using a mixture of potassium dichromate and sulfuric acid under heating (Yeomans & Bremner 1988). At each soil sampling, the soil temperature was measured for 5 minutes at 10 cm depth using a probe thermometer.

The data obtained were statistically analyzed with R (Version 3.6.1). Firstly, we evaluated the variables by a two-way MANOVA using Cerrado physiognomies, season, and their interaction. The variables that presented statistical differences were evaluated using a simple linear model (two-way ANOVA). Linear mixed-effects models were used with site, season, and interactions as random factors, and the ecosystem functions as fixed ones. For further multivariate analysis, the functional data matrix was transformed via Box-Cox transformation to obtain a normal distribution of the data (Box & Cox 1964). Then, we used the principal component analysis (PCA) to compare the ecosystem functional profile between the sites. To test the significance of the sample clustering in the PCA plot we used the permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001). Later, to assess the contribution (%) of each functional factor and season to the dissimilarity between the sites we run the SIMPER analysis. The PCA plot was generated using the software Canoco 4.5 (Biometrics, Wageningen, The Netherlands), Box-cox transformation and PERMANOVA were calculated using PAST3 software (Hammer et al. 2001), and SIMPER analysis was conducted using PRIMER6 software (PrimerE, Ivybridge, United Kingdom).

RESULTS

All proxies for the selected ecosystem functions differed between sites and seasons (Table II). Considering the interactions, AGPP, BGPP, and microbial biomass C showed significance. Regarding the evaluated soil properties, TOC, moisture, and temperature presented differences between the sites and seasons. The exception was soil pH, which did not show differences between sites and seasons.

Soil pH did not vary between sites (Figure 1a), while TOC was higher in CD than CSS and CG (Figure 1b). Soil moisture was higher in CD than in CSS and CG (Figure 1c), while soil temperature was higher in CG than in CSS and CD, in the dry season. In the wet season, soil temperature was similar between CG and CSS, and lower in CD (Figure 1d).

In both seasons, AGPP were higher in CD (971 $\pm$ 32 and 1408 $\pm$ 61 g m^{-2} in the dry and wet season, respectively) than in CSS (501 $\pm$ 34 and 726 $\pm$ 41 g m^{-2} in the dry and wet season, respectively) and CG (302 $\pm$ 29 and 439 $\pm$ 34 g m^{-2} in the dry and wet season, respectively) (Figure 2a). Similarly, BGPP were higher in CD (378 $\pm$ 22 and 511 $\pm$ 31 g m^{-2} in the dry and wet season, respectively) than in CSS (104 $\pm$ 12 and 141 $\pm$ 14 g m^{-2} in the dry and wet season, respectively) and CG (79 $\pm$ 10 and 107 $\pm$ 12 g m^{-2} in the dry and wet season, respectively) (Figure 2b). AGSP were higher and similar in CD (18 $\pm$ 4.8 and 31 $\pm$ 6.5 individuals m^{-2} in the dry and wet season, respectively) and CSS (15 $\pm$ 3.4 and 26 $\pm$ 4.2 individuals m^{-2} in the dry and wet season, respectively) as compared to CG (9 $\pm$ 1.6 and 16 $\pm$ 2.3 individuals m^{-2} in the dry and

Table II. Linear mixed models on the effects of sites (CG, CSS, and CD), seasons (dry and wet), and interactions on ecosystem functions.

Variable	Site	Season	Interaction
Aboveground primary productivity	$F_{2,18}$=1268***	$F_{1,18}$=381.1***	$F_{2,18}$=42.75***
Belowground primary productivity	$F_{2,18}$=880.2***	$F_{1,18}$=74.43***	$F_{2,18}$=19.50***
Aboveground secondary productivity	$F_{2,18}$=28.94***	$F_{1,18}$=55.54***	$F_{2,18}$=1.985^{ns}
Belowground secondary productivity	$F_{2,18}$=22.80***	$F_{1,18}$=88.24***	$F_{2,18}$=2.093^{ns}
Decomposition	$F_{2,18}$=34.52***	$F_{1,18}$=177.3***	$F_{2,18}$=2.324^{ns}
Microbial biomass C	$F_{2,18}$=190.8***	$F_{1,18}$=290.8***	$F_{2,18}$=15.58***
Total organic C	$F_{2,18}$=164.3***	$F_{1,18}$=24.44***	$F_{2,18}$=1.393^{ns}
Soil moisture	$F_{2,18}$=67.00***	$F_{1,18}$=683.9***	$F_{2,18}$=4.273*
Soil pH	$F_{2,18}$=0.386^{ns}	$F_{1,18}$=0.534^{ns}	$F_{2,18}$=2.319^{ns}
Temperature	$F_{2,18}$=30.32***	$F_{1,18}$=154.7***	$F_{2,18}$=13.61***

ns – non significant, * p < 0.05; *** p < 0.001.

wet season, respectively) (Figure 2c). The values of BGSP followed the same trend, being higher in CD (24 ± 3.9 and 46 ± 4.7 individuals m^{-2} in the dry and wet season, respectively) and CSS (22 ± 3.1 and 41 ± 4.2 individuals m^{-2} in the dry and wet season, respectively) as compared to CG (14 ± 1.5 and 27 ± 1.9 individuals m^{-2} in the dry and wet season, respectively) (Figure 2d). Soil microbial biomass was significantly higher in CD (207 ± 32 and 374 ± 41 mg kg^{-1} C in the dry and wet season, respectively) than in CSS (171 ± 21 and 308 ± 29 mg kg^{-1} C in the dry and wet season, respectively) and CG (79 ± 12 and 107 ± 11 mg kg^{-1} C in the dry and wet season, respectively) (Figure 2e). The decomposition (total weight loss) was similar and higher in CD (150 ± 18 and 255 ± 21 mg in the dry and wet season, respectively) and CSS (131 ± 12 and 224 ± 17 mg in the dry and wet season, respectively) as compared to CG (100 ± 11 and 170 ± 16 mg in the dry and wet season, respectively) (Figure 2f).

The PCA revealed that the ecosystem functional profiles were distinct among sites, clustering according to their responses to sites and seasons (Figure 3). The analysis explained 89.8% of the data variation, being distributed as 81.2% and 8.6% in axes 1 and 2, respectively. The PCA analysis grouped the data according to the physiognomies and season, as supported by PERMANOVA (Site - F = 156.65, *P* = 0.0001; Season – F = 164.07, *P* = 0.0001). The majority of ecosystem functions clustered with CD, in both seasons and CSS in the wet season. These sites were characterized by high above- and belowground primary and secondary productivities, soil microbial biomass and decomposition. In contrast, CG clustered in a separate group with the influence of soil pH and temperature. The SIMPER analysis indicated that the factors AGPP, MBC, and BGPP were the main contributors to the differences among the physiognomies. Regarding the season, the factors MBC, moisture, and decomposition contributed to almost 50% of the data variation.

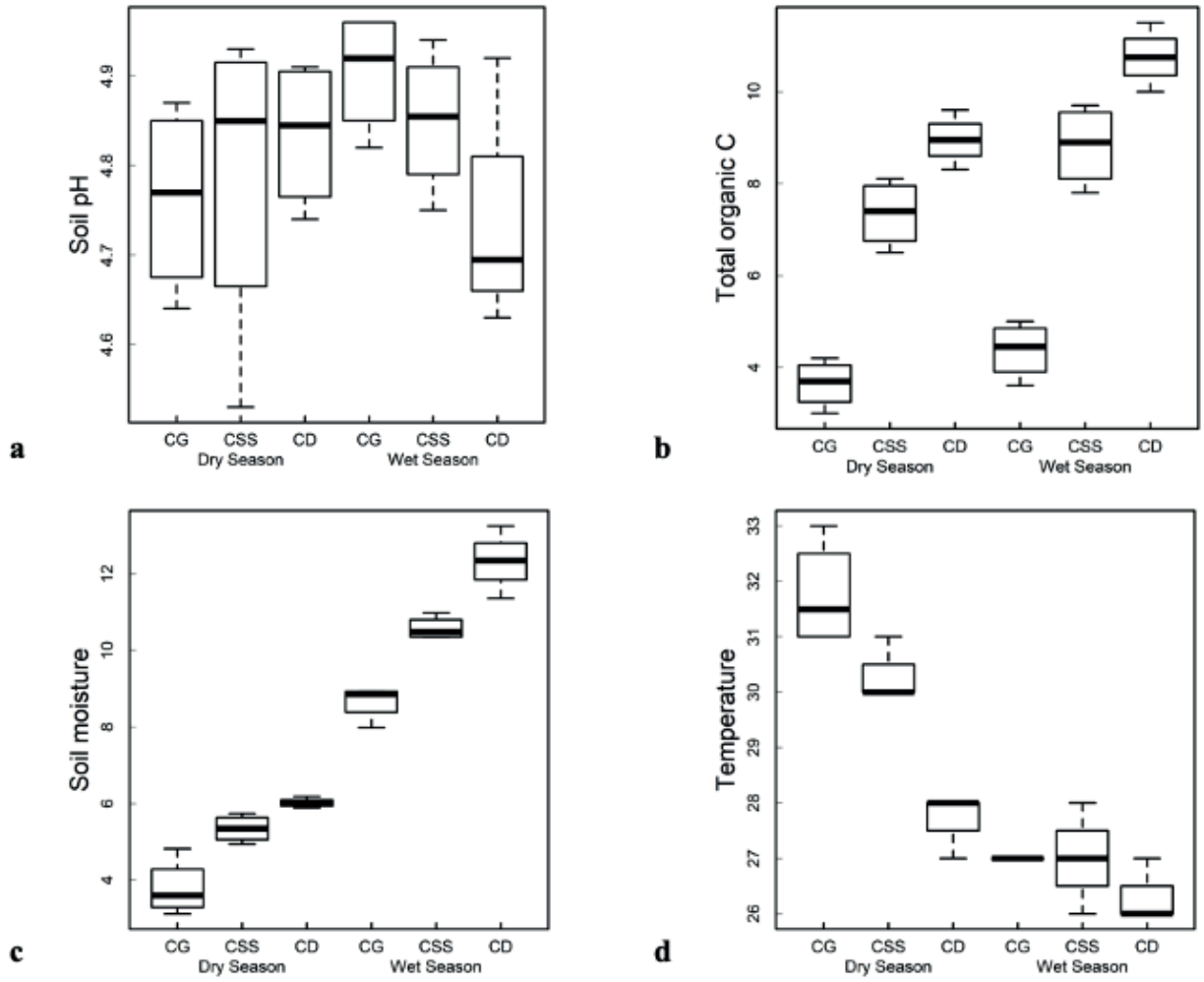

Figure 1. Drivers for ecosystem function between Cerrado physiognomies: Soil pH (a), total organic C (b), moisture (c), and temperature (d). Error bars represent the standard deviation of four independent replicates.

DISCUSSION

In this study, REFA was applied in different physiognomies of Cerrado and showed that some important proxies of ecosystem function differed between sites. The main advantage of using REFA is that the evaluated proxies present high efficiency in detecting changes in the ecosystem status (Leidinger et al. 2017). In addition, this method uses standardized ecosystem functions proxies instead of the functions themselves (Meyer et al. 2015). Here, we presented the first study that applied REFA under Cerrado and the results showed how different are the ecosystem functions in the different physiognomies of this biome. Thus, the results found in this study corroborate and add novel information to previous studies that observed differences in the composition, structure, and diversity of plants, animal, and microorganisms (Oliveira et al. 2007, Araujo et al. 2017a, b, 2018, Nunes et al. 2019).

For all evaluated ecosystem functions, Cerrado physiognomies and seasons showed significant effects. The effects were more pronounced when we compared *Cerradão* and *Campo Graminoide*, while *Cerrado Stricto Sensu* showed intermediate effects, as illustrated in

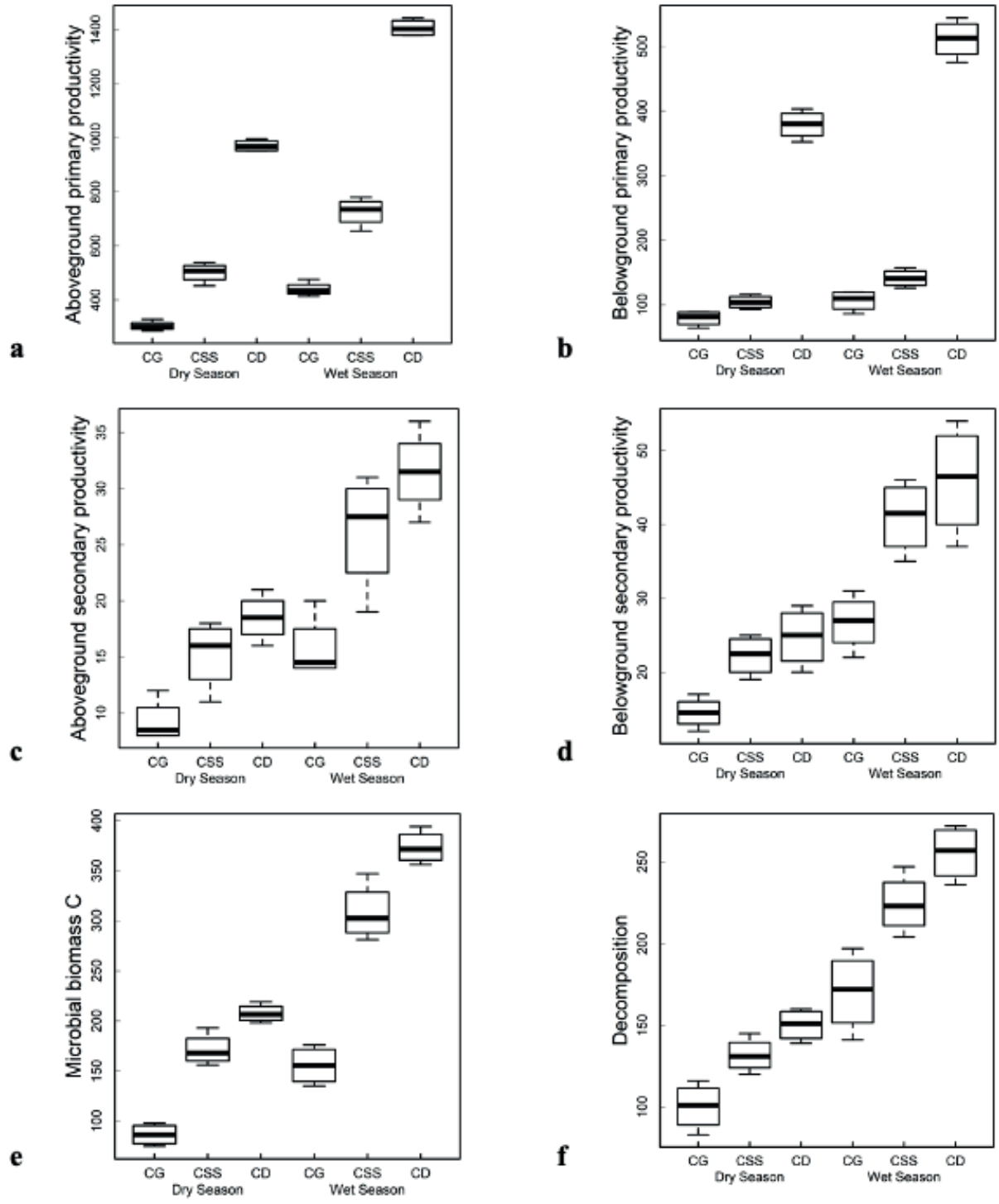

Figure 2. Ecosystem function between Cerrado physiognomies: Aboveground primary productivity (a), belowground primary productivity (b), aboveground secondary productivity (c), belowground secondary productivity (d), microbial biomass C (e) and decomposition (f). Error bars represent the standard deviation of four independent replicates.

the PCA analysis. These significant differences between *Cerradão* and *Campo Graminoide* are a reflection of the different composition and diversity of plant species (Table I) and soil and environmental conditions (Table II, Figure 2). A previous study across a gradient of physiognomies of Cerrado has found *Cerradão* with higher richness and diversity of plants (Oliveira et al. 2007), which contributes for higher input of plant litter (Nardoto & Bustamante 2003), and better environmental conditions (Rocha et al. 2019) when compared to *Campo Graminoide*. In addition, Koch et al. (2016) reported that the composition of plants is an important variable influencing ecosystem function. Indeed, Leidinger et al. (2017) found differences in ecosystem functions between permanent and secondary grasslands. Soil conditions, i.e. soil properties and environmental conditions, are drivers of ecosystem functions (Leidinger et al. 2017). For example, total organic C is important to soil microbial biomass and activity, i.e. decomposition (Rocha et al. 2019), and secondary productivity (Lavelle et al. 2006). Generally, the high abundance of invertebrates (secondary productivity) is dependent on the quality, quantity, and availability of plant biomass (primary productivity) that stimulated the activity of invertebrates (Araujo et al. 2015, Leidinger et al. 2017). Soil moisture is another important driver of ecosystem function (Jing et al. 2015) as it influences soil microbial biomass (Rocha et al. 2019) and primary and secondary productivity (Meza et al. 2018). Thus, it could explain the differences in the proxies of ecosystem functions found between *Cerradão* and *Campo Graminoide*. Besides, *Campo Graminoide*, which is dominated by grassland, presented lower organic C and moisture and it corroborates Baer et al. (2002) who found grasslands having reduced total organic C and belowground primary productivity.

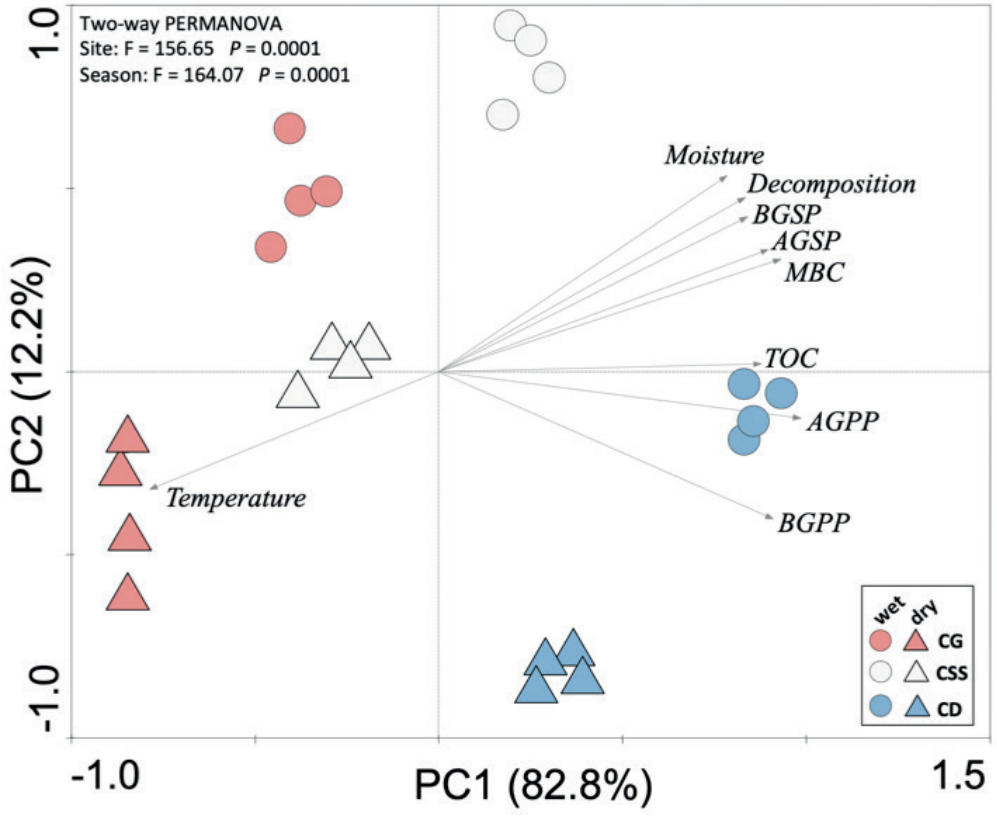

Figure 3. Principal component analysis (PCA) biplot based on the measured proxies of the ecosystem functions determined by REFA in a gradient of Cerrado physiognomies.

In this study, we assessed the ecosystem functions in different physiognomies of the Cerrado. The results add important information about the Cerrado that, together with previous studies on plant diversity (Oliveira et al. 2007), macro and microfauna (Nunes et al. 2019), and soil microorganisms (Araujo et al. 2017a, b, 2018) are important to the development of policies and practices of conservation of native ecosystems. Also, we showed that the assessment of ecosystem functions by using REFA can be a useful method for measuring potential ecosystem service that can be evaluated, such as nutrient cycling (decomposition, AGSP, BGSP, and microbial biomass C), biomass production (AGPP and BGPP); control of erosion (AGPP, BGPP, and TOC) and climatic regulation (AGPP, and TOC), that are linked to native and preserved ecosystems (Constanza et al. 1997).

CONCLUSION

The proxies of ecosystem functions measured through REFA showed differences between physiognomies of Cerrado. Since each physiognomy of Cerrado presents different plant richness and diversity, and soil conditions, these characteristics contribute to influencing multiple ecosystem functions. In this study, total organic C, moisture, and temperature of soil varied and can have influenced the differences in all measured proxies of ecosystem functions in Cerrado from Northeastern, Brazil. Finally, the results found in this study could contribute with information about potential ecosystem functions in Cerrado and, consequently, with conservation policies and ecosystem services.

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Author contributions

ASFA coordinated the study, wrote and discussed the paper. SMBR conducted the study and collected data. JELA conducted the study, data collection and analysis. FFA helped in data analysis and writing. LWM helped in data analysis, wrote and discussed the paper.