NAYARA MESQUITA MOTA

RELAÇÕES PLANTA-SOLO E PROCESSOS ECOSSISTÊMICOS SOB REDUÇÃO DE CHUVA EM TRÊS FITOFISIONOMIAS DO CERRADO

Tese apresentada a Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ecologia, para obtenção do título de *Doctor Scientiae*

Orientador: João Augusto Alves Meira-Neto

Coorientadores: Alexandra Rodríguez Pereiras
Jorge Durán Humia

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Assentimento:

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Ao Cerrado,
a esperança de um futuro bom,
a minha família e amigos.
A Deus e a N. Sra. Aparecida.

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In environments in which there is a recurrence of natural hazards, populations may spend most of their time recovering from the hazards…

(Harper, 1967)

Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito.

(Martin Luther King Jr.)

RESUMO

MOTA, Nayara Mesquita, D.Sc., Universidade Federal de Viçosa, março de 2020. **Relações planta-solo e processos ecossistêmicos sob redução de chuva em três fitofisionomias do Cerrado.** Orientador: João Augusto Alves Meira Neto. Coorientadores: Alexandra Rodríguez Pereiras e Jorge Durán Humia

Devido à sua grande biodiversidade e superfície, o Cerrado é responsável por serviços ecossistêmicos relacionados à ciclagem de energia, água e nutrientes em escala regional e global. Entretanto, cenários futuros indicam uma redução entre 20-70% dos regimes de chuvas no Cerrado o que alterará a umidade e dinâmica do solo, e consequentemente processos ecossistêmicos. Portanto, buscamos entender as relações entre plantas, microrganismos e nutrientes do solo, e avaliar o efeito da redução de chuva em processos biogeoquímicos. Para isso, alocamos cinco parcelas de 7x7m emparelhadas (redução de chuva e controle) em três fisionomias de Cerrado na Floresta Nacional de Paraopeba (Cerradão Distrófico, Cerrado *stricto sensu* denso e Cerrado *stricto sensu*). No Capítulo 1, avaliamos a vegetação (jovens e adultos), as comunidades microbianas e o estado nutricional do solo, bem como as relações entre eles. No Capítulo 2, estimamos a disponibilidade *in situ* de nitrogênio (N) e fósforo (P) no solo ao longo de dois anos, em condições ambientais e sob redução de chuva. No Capítulo 3, usamos duas abordagens (redução das chuvas in situ e incubações em laboratório a 80% e 40% da capacidade de retenção de água no solo [CRA]) para investigar os efeitos das mudanças na umidade do solo na diversidade funcional microbiana e no funcionamento do solo (fluxos potenciais solo-atmosfera de gases de efeito estufa [GEE; N_2O, CO_2 e CH_4] e taxas de mineralização de N). Nossos resultados revelaram que a disponibilidade de NH_4 no solo foi um determinante da estrutura das plantas jovens e da diversidade funcional microbiana do solo. A diversidade vegetal não foi relacionada às variáveis medidas, enquanto a biomassa foi negativamente relacionada à diversidade microbiana e aos nutrientes do solo. Nossos resultados sugerem fortes ligações positivas entre o estado nutricional do solo e a diversidade funcional microbiana. A disponibilidade de N e P variou entre fisionomias, estações e anos, com interações notáveis entre alguns desses fatores. O Cerrado *stricto sensu* apresentou valores mais baixos de disponibilidade de nutrientes, emissões de CO_2 e diversidade funcional microbiana. O Cerradão Distrófico exibiu um melhor estado nutricional geral, as maiores taxas de mineralização e emissão de N_2O. Tanto a sazonalidade quanto o tratamento de redução das chuvas reduziram a disponibilidade de N, mas não a de P, sugerindo uma maior sensibilidade da disponibilidade de N do que a de P às mudanças na umidade do solo. Entretanto, diferentes fisionomias mostraram diferentes sensibilidades à redução das chuvas. Por outro lado, verificamos que o teor de N no solo foi maior e a mineralização de amônio foi menor sob exclusão de chuva. Mudanças na umidade do solo (80% vs. 40% CRA) não influenciaram a diversidade funcional microbiana, entretanto, modularam as relações da biodiversidade microbiana com a respiração do solo e as taxas de transformação de N. Os fluxos de GEE foram menores em solos com 40% de CRA do que aqueles com 80% de CRA. Portanto, mudanças relacionadas à umidade do solo e no domínio relativo das diferentes fisionomias do Cerrado provavelmente alterariam a capacidade desse bioma de mineralizar e estocar N, e de trocar GEE com a atmosfera.

Palavras-chave: Biodiversidade. Atividade microbiana. Processos biogeoquímicos. Umidade do solo. Mudanças climáticas.

ABSTRACT

MOTA, Nayara Mesquita, D.Sc., Universidade Federal de Viçosa, March, 2020. **Plant-soil relationships and ecosystem processes under rainfall reduction in three Cerrado physiognomies.** Advisor: João Augusto Alves Meira Neto. Co-Advisors: Alexandra Rodríguez Pereiras and Jorge Durán Humia.

Cerrado savanna is among the most important ecosystems worldwide due to its great biodiversity and extension. The Cerrado is responsible for key ecosystem services related to energy, water, and nutrients cycling at the regional and global scale. However, these ecosystem services may be altered due to climate change. Future climate scenarios indicate a rainfall reduction between 20-70% in the Cerrado that will consequently alter the content and dynamics of soil moisture. We sought to understand the effects of forecasted rainfall reduction in important ecosystem processes, as well as on the relationships among nutrients, plant and microbial communities. To do so, we placed five 7x7m paired (one rainfall reduction and one control) plots in each of the dominant Cerrado physiognomies present in the Paraopeba Reserve (woodland, intermediate and open savannah). In Chapter 1, we evaluated the soil nutritional status and the saplings communities, tree and soil microorganisms, as well as the relationships among all of them. In Chapter 2, we estimated in-situ soil nitrogen (N) and phosphorus (P) availability along two years under ambient and rainfall reduction conditions. In Chapter 3, we used two different approaches (i.e. the *in situ* rainfall reduction and lab incubations at 80% and 40% of the soil water holding capacity [WHC]) to investigate the effects of changes in soil moisture on the soil microbial functional diversity and functioning (i.e. potential soil-atmosphere greenhouse gases fluxes [GHG; N_2O, CO_2 and CH_4] and N mineralization rates). Our results revealed that soil NH_4 availability was a key determinant of the structure of plant saplings and of soil microbial functional diversity. Plant richness was not significantly related to any of our measured variables, but saplings biomass was negatively related to microbial communities and soil nutrients. Our results suggest strong positive links between the soil nutritional status and microbial functional diversity. Nutrient availability varied between physiognomies, seasons and years, with noteworthy interactions among some of these factors. The open savannah showed overall lower nutrient availability values, CO_2 emissions and microbial functional diversity and evenness. The woodland savannah exhibited overall better nutritional status, the highest N mineralization and N_2O emission rates. Both the dry season and the rainfall reduction treatment decreased N availability but not that of P, suggesting a higher sensitivity of N availability than that of P to changes in soil moisture. However, different physiognomies showed different sensitivities to the rainfall reduction. On the other hand, we found that soil N content was higher, and ammonium mineralization was lower, under the rainfall exclusion than in control plots. Changes in soil moisture (80% vs 40% WHC) did not influence microbial functional diversity and evenness, but they did modulate the relationships of microbial functional biodiversity with soil respiration and N transformation rates. Soil-atmosphere GHG fluxes were lower in soils at 40% WHC than in those at 80%WHC. Altogether, our results indicate that climate change-related changes in soil moisture and in the relative dominance of the different Cerrado physiognomies would likely change the capacity of this biome to mineralize and stock N, and to exchange GHG with the atmosphere.

Keywords: Biodiversity. Microbial activity. Biogeochemical processes. Soil moisture. Climate change.

SUMÁRIO

INTRODUÇÃO GERAL

O Cerrado é o segundo maior bioma do Brasil, ocupando mais de dois milhões de km^2, e um dos *hotspots* mundiais de biodiversidade, devido a sua vegetação ser extremamente rica em espécies endêmicas e também devido a ameaça que sofre pelos impactos das ações antrópicas (KLINK; MACHADO, 2005; MITTERMEIER et al., 2005; RATTER; RIBEIRO; BRIDGEWATER, 1997; STRASSBURG et al., 2017). Além disso, como resultado de sua grande distribuição e biodiversidade, o Cerrado fornece importantes serviços ecossistêmicos relacionados à energia, água e nutrientes, tanto em escala regional quanto global (BUSTAMANTE et al., 2006, 2012; MIRANDA et al., 1997; RESENDE et al., 2011). Apesar da sua magnitude e importância, estudos sobre as características e funcionamento dos solos do Cerrado, e suas relações com a biota, ainda são escassos (BUTLER et al., 2012; GRACE et al., 2006). Em um cenário de mudanças climáticas, estudos desse tipo tornam-se urgentes, visto que a ameaça a biodiversidade é evidente (HANNAH, 2012). Portanto, avaliar as alterações nos processos ecossistêmicos nos irá fornecer ideias e hipóteses sobre o futuro desses ecossistemas.

Na maioria das regiões do Cerrado, os modelos climáticos preveem uma redução anual do período de chuvas, com prolongamento da estação seca (BUSTAMANTE et al., 2012). De forma geral, estima-se uma diminuição de 20 a 70% dos valores atuais de precipitação (BUSTAMANTE et al., 2012; MARENGO et al., 2010). As projeções menos severas do Quinto Relatório de Avaliação do Painel Intergovernamental sobre Mudanças Climáticas (IPCC) preveem uma redução de 30% das chuvas em 2100 nas regiões central e sul do Cerrado (BUSTAMANTE et al., 2012). A redução na precipitação poderia alterar a umidade do solo, bem como a ciclagem e a disponibilidade de nutrientes (GUO et al., 2002; RODRÍGUEZ et al., 2011, 2019). Apesar disso, são escassos os estudos com esta abordagem em savanas tropicais (AUGUSTINE; MCNAUGHTON, 2006; OKACH et al., 2019; SANKARAN, 2019). Falta também uma compreensão dos impactos das mudanças climáticas na microbiota dos solos do Cerrado, que são fundamentais para a ciclagem de nutrientes, e que influenciam diretamente a vegetação (BUSTAMANTE et al., 2012).

Considerando esse cenário, avaliar o impacto da redução de chuvas no funcionamento dos processos ecossistêmicos relacionados ao carbono (C), nitrogênio (N) e fósforo (P) nas diferentes fisionomias do Cerrado é crucial para a conservação e o manejo do Cerrado. Alguns estudos procuraram entender como as mudanças na precipitação podem afetar os ecossistemas (PEIXOTO et al., 2018; RODRÍGUEZ et al., 2017, 2019; SMITH, 2011). Tanto experimentos de redução de chuvas em campo (HOMYAK et al., 2017; LI et al., 2018; SAYER et al., 2017) quanto incubações do solo em laboratório em diferentes condições de umidade (GARTEN; CLASSEN; NORBY, 2009; SUN; LEI; CHANG, 2019) demonstraram contribuir efetivamente para a compreensão do impacto das mudanças climáticas no funcionamento do solo (SRIVASTAVA et al., 2017). Assim, experimentos manipulativos tornam-se necessários para entender os efeitos da redução das chuvas em diferentes características do ecossistema (ASBJORNSEN et al., 2018; SMITH, 2011). Para o Cerrado, até onde sabemos, não existem tais trabalhos.

O Cerrado compreende uma grande variedade de fisionomias, que diferem em estrutura e composição, e que podem ser classificadas de acordo com a proporção de árvores e gramíneas, variando de campos a formações florestais (COUTINHO, 1978; FURLEY, 1999; OLIVEIRA-FILHO; RATTER, 2002; RIBEIRO; WALTER, 1998). Os fatores ambientais que explicam a distribuição dessas fisionomias incluem precipitação, drenagem e fertilidade do solo, concentração de alumínio no solo, regime de fogo e flutuações climáticas do Quaternário, como indicado pelos estudos realizados nesse bioma (NERI et al., 2012; OLIVEIRA-FILHO; RATTER, 1995; RIBEIRO; WALTER, 1998). As fisionomias do Cerrado variam desde coberturas florestadas, como o Cerradão, savânicas, como o Cerrado *stricto sensu,* até campestres (RIBEIRO; WALTER, 1998). O Cerradão é uma formação florestal com árvores escleróﬁlas de 12 a 15 m com 50 a 90% de cobertura, apresenta sub-bosque esparso e a fertilidade dos solos define os seus subtipos, distrófico e mesotrófico (NERI et al., 2013; RIBEIRO; WALTER, 1998). Já o Cerrado *stricto sensu* é um tipo de cerrado com cobertura arbórea entre 5 e 70%, sem formação de dossel, e cobertura herbácea proeminente (NERI et al., 2013; RIBEIRO; WALTER, 1998). Em geral, a vegetação do Cerrado se torna mais fina e mais curta à medida que a capacidade de troca catiônica diminui na camada superficial do solo (NERI et al., 2013). Assim, os solos do Cerradão são muito argilosos e com reservas de matéria orgânica e P relativamente altos (GIÁCOMO et al., 2015; NERI et al., 2013), enquanto os solos de formações de savana mais abertas (como o Cerrado *stricto sensu*) são arenosos e contêm menor teor de fósforo (NERI et al., 2013). Assim como em muitos ecossistemas terrestres, a disponibilidade de N e P do solo está entre os principais fatores limitantes da produtividade primária do Cerrado (ELSER et al., 2007; HARIDASAN, 2001). Em geral, os solos do Cerrado tendem a ser mais limitados por P do que por N, pois são altamente intemperizados (ABRAHÃO et al., 2019; BUSTAMANTE et al., 2012, 2006;

NARDOTO et al., 2013; RESENDE et al. , 2011) e o P do solo é dominado por formas oclusas (RESENDE et al., 2011).

Assim, como cada fisionomia do Cerrado apresenta características bióticas e abióticas distintas, elas provavelmente apresentarão diferentes respostas à redução das chuvas. Por exemplo, os solos argilosos com maior matéria orgânica do Cerradão Distrófico poderiam reter mais umidade do que os solos mais arenosos da vegetação mais aberta do Cerrado (ABDULLAH; MADZHI; ISMAIL, 2015; ASSIS et al., 2011). Da mesma forma, é provável que as comunidades microbianas de cada fisionomia sejam afetadas de maneira diferente pelas mudanças no regime de chuvas e consequentemente na umidade do solo, o que por sua vez poderá alterar a disponibilidade de nutrientes no solo (HORZ et al., 2005).

Nesse sentido, o objetivo geral deste trabalho é entender o funcionamento e as respostas de três diferentes fitofisionomias do Cerrado aos cenários de mudanças climáticas previstas. Para isso, avaliamos a diversidade funcional de microrganismos do solo; a diversidade, composição e estrutura das comunidades vegetais; e algumas variáveis relacionadas a processos ecossistêmicos e ao funcionamento do solo, tais como a produção de serapilheira, o teor de matéria orgânica, a disponibilidade de N e P, a mineralização do N e fluxos potenciais de gases do efeito estufa (GEE; CO_2, CH_4 e N_2O) em três fisionomias dominantes do Cerrado (Cerradão Distrófico, Cerrado *stricto sensu* denso e Cerrado *stricto sensu*) na Floresta Nacional de Paraopeba. A partir disso, essa tese foi dividida em três capítulos. No primeiro capítulo investigamos as relações entre as comunidades vegetais (jovem e adulta), a diversidade funcional microbiana e o status nutricional do solo (NH_4^+, NO^-_3, matéria orgânica, produção de serapilheira). No segundo e no terceiro capítulo avaliamos processos ecossistêmicos em um cenário de mudanças climáticas futuras, ou seja, sob redução de chuva em campo ou através da diminuição da umidade do solo em condições controladas no laboratório. Especificamente, no segundo capítulo, avaliamos a disponibilidade de N e P nas três fisionomias do Cerrado sob condições ambientais (parcelas controle) e de redução de chuva (parcelas tratadas). Enquanto que no terceiro capítulo, exploramos as relações entre o funcionamento do solo (mineralização do N, atividade microbiana e fluxos potenciais de GEE) sob o experimento de redução de chuva em campo e no laboratório. O experimento de redução de chuva em campo consistiu na instalação de seis faixas plásticas transparentes suspensas nas parcelas tratamento, e o do laboratório consistiu na incubação dos solos coletados em campo (parcela controle) em 40% e 80% da capacidade de campo.

As principais hipóteses dessa tese são: (i) diferentes fisionomias do Cerrado apresentam diferentes comunidades vegetais e microbianas, bem como diferentes atributos do solo; (ii) a maior disponibilidade de nutrientes no solo poderia suportar comunidades microbianas e vegetais do solo mais diversas; (iii) as fisionomias mais abertas (ou seja, com menor cobertura vegetal) teriam menor disponibilidade de N e P do que as fisionomias mais fechadas; (iv) a disponibilidade de N aumentaria durante a estação chuvosa em relação à estação seca (devido a um aumento na mineralização de N), enquanto a disponibilidade de P diminuiria (devido a um aumento na absorção de plantas). Pelas mesmas razões, esperamos que o tratamento de redução de chuvas (v) diminua a disponibilidade de N, mas aumente a de P, e que (vi) esse efeito seja mais forte durante a estação chuvosa. Finalmente, também esperamos que (vii) a disponibilidade de água influencie a mineralização do N do solo, os fluxos de GEE e a diversidade funcional microbiana no Cerrado; mas que cada fisionomia responda de forma diferente à redução da água disponível.

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CHAPTER 1*: Plant-soil-microorganism interactions in three phytophysiognomies of Cerrado (Brazilian Savannah)

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Abstract

We investigated interactions among plants (i.e. richness and abundance of adult trees and saplings), soil microorganisms (i.e. functional diversity), and soil nutritional status (i.e. litter production, soil organic matter, microbial biomass, and nitrogen and phosphorus availability) in three Cerrado phytophysiognomies (woodland savannah, intermediate savannah, and open savannah). The intermediate savannah showed the highest microbial and plant diversities. Diversity of niches of this phytophysiognomy can behind of these results. Adults and saplings richness were positively related, whereas adults and saplings biomasses were negatively related likely because competition by light. Adults communities were associated with litter production; suggesting strong relationships between plant community's composition and litter production. Soil ammonium was positively related to soil microbial functional diversity and the saplings structure what may indicate intense competition between plants and microorganisms for ammonium in the Cerrado. Further, the negative associations between microbial and saplings attributes indicate likely competitive interactions among those groups of organisms, or alternatively, the negative effect of soil pathogens on plant development. Also, our results suggest strong positive links between the soil nutritional status and microbial functional diversity, but not with saplings diversity.

Key words: diversity; biomass; saplings; microorganisms; nitrogen.

Introduction

The Cerrado is considered the largest and most diverse tropical savannah region in the world, covering the central plateau of Brazil in approximately 23% of the national territory (Ratter et al. 1997; Ribeiro and Walter 1998), and possessing more than 12000 vascular plant species registered (Mendonça et al. 2008). This diversity is associated with a large variety of phytophysiognomies (hereafter physiognomies), including grasslands, savannahs, woodlands and forests (Ribeiro and Walter 1998; Oliveira-Filho and Ratter 2002). The vegetation cover of each physiognomy could distinctly impact their soil microbial communities via changes in soil microclimate (e.g. shading-driven reductions in soil temperature; Valentini et al. 2009; de Carvalho Mendes et al. 2012; Castro et al. 2016). Similarly, increasing litter production may reduce thermal amplitude and enhance the availability of some soil nutrients (Loydi et al. 2014). Indeed, past studies have shown that different Cerrado physiognomies differ in terms of soil nutrient and water availability, soil organic matter and aluminum content, and soil pH

(Neri et al. 2013; De Castro et al. 2016). However, we are still far from achieving a full understanding of plant-soil-microorganism relationships in the Cerrado biome. Cerrado soils can store up to 90% of the total C of the ecosystem (Morais et al. 2013). Hence, a deeper understanding of the intricate plant-soil-microorganism feedbacks is key to comprehend the Cerrado carbon and nutrient cycles in a future carbon-enriched atmosphere scenario.

Species diversity depends on the existence of mechanisms that prevent a competitive dominant species from excluding all other ones (Reynolds et al. 2003). Plant-soil-microorganism interactions are an important organizing force that can limit plant competition and promote coexistence mechanisms through niche partitioning activated by soil microbes (Reynolds et al. 2003). On the other hand, diverse plant communities are expected to have high root trait diversity, resulting in the exudation of a more diverse range of organic compounds into the soil, thereby sustaining high microbial biomass and diversity (Reynolds et al. 2003; Thakur et al. 2015; Ren et al. 2018). Thus, previous studies have shown positive associations between plant diversity and microbial biomass (Thakur et al. 2015), diversity (Ren et al. 2018), and abundance (Leloup et al. 2018). Other known mechanisms by which plants influence the soil microbial community operate through changes in biomass production, litter quality, belowground C allocation, and nutrient movements (Wardle et al. 2004; Dennis et al. 2010; Leloup et al. 2018). In these cases, not only diversity but also plant biomass could influence microbial communities.

Microbial diversity and abundance are key to the maintenance of important ecosystem functions and services, such as organic matter decomposition and nutrient cycling (Delgado-Baquerizo et al. 2016). The availability of N and P is particularly relevant for ecosystem functioning since both are critical components of plant and microbial nutrition and growth (Ratnam et al. 2008; Khan et al. 2011). Nutrient cycling rates are tightly controlled by interactions among plants and soil microbes (Frank and Groffman 2015; Delgado-Baquerizo et al. 2016). For instance, the plant litter characteristics influence microbial abundance and decomposer communities (Wardle et al. 2006; Thoms et al. 2010), and that soil microbial abundance, microsite conditions and nutrient content are highly dependent on the decomposition of plant litter. Thus, litter accumulation has strong effects on soil communities, microsite conditions and nutrient contents (Loydi et al. 2014; Leloup et al. 2018). Likewise, soil properties have been consistently shown to be a primary factor determining bacterial community structure and function (Girvan et al. 2003; Wakelin et al. 2008). For instance, N availability largely affects bacterial diversity, abundance, and community composition (Ren et al. 2018). Similarly, organic C content drives soil activity and functional diversity in the way that soils with higher organic C content often shows a higher catabolic activity (Lagomarsino et al. 2007). Therefore, soil, vegetation, and microorganisms are largely interconnected.

Two main plant layers characterize the Cerrado vegetation (Oliveira-Filho and Ratter 2002): the woody layer, which includes trees and larger shrubs; and the understory layer, which comprises seedlings and saplings of trees together with specialist understory species. Each Cerrado physiognomy has its own specific understory vegetation. For instance, herbaceous plants are absent in the understory of woodland savannah, whilst in the open savannah is characterized by well-defined herb and shrubs layers (Neri et al. 2012, 2013). Despite varying greatly between physiognomies, understory vegetation, mainly saplings, is rarely studied in the Cerrado, and, to our knowledge, there are no studies relating saplings, adult trees, microbial communities and soil nutritional status in this biome.

To shed light on the plant-soil feedbacks in the Cerrado, we explored the links among adults and saplings communities, soil nutritional status and microbial functional diversity in three plant community-soil contrasted physiognomies. We hypothesized that different Cerrado physiognomies would show different plant and soil microbial communities, as well as different soil attributes. Also, as a higher soil nutrient availability might support more diverse soil microbial and plant communities, as well as more saplings individuals (and larger), we expected to find positive relationships among soil nutritional status, soil functional diversity, and plant diversity, biomass and abundance.

Methods

Study area

The study was carried out in the Paraopeba National Reserve (PNR), located in southeastern Brazil (19º20 'S; 44º20' W), and covering a continuous area of 200 ha. The PNR is a federal conservation unit for sustainable uses that aims to guarantee the multiple use of vegetation resources and scientific research, focusing on strategies for sustainable exploitation of native species (Neri et al. 2012). There are no records of clear-cutting or wildfires since 1952 and 1963, respectively (Meira-Neto et al. 2017). The climate of the region is tropical humid, Aw by the Köppen climate classification, with a rainy summer from October to March and a dry winter from April to September (Neri et al. 2012). Annual average temperature and precipitation are 20.9 °C and 1328 mm, respectively, and the altitude varies between 740 and 760 m (Neri et al. 2012; Meira-Neto et al. 2017).

Different physiognomies can be found at the PNR, varying in an edaphic gradient (Neri et al. 2012, 2013). For this study, we considered the following physiognomies woodland savannah (locally known as *Cerradão*), intermediate savannah (locally known as Cerrado *stricto sensu* dense), and open savannah (locally known as Cerrado *stricto sensu*). The woodland savannah has higher plants with canopies forming a dense cover on “Red” Latosol (oxisols in United States classification; Figure 1). The intermediate savannah shows greater densification of shrub-tree species over herbaceous and sub-shrub species on “Red-Yellow” Latosol. The open savannah is characterized by the presence of well-defined herbaceous-shrub and shrub-tree strata on “Yellow” Latosol. In August 2015, we placed five representative 7 m x 7 m permanent plots in each of the three Cerrado physiognomies (Figure 1).

Plant biomass and diversity

We evaluated the richness and biomass of the adult tree community (all woody individuals with circumference at ground level > 10 cm) in all plots. We considered as richness the number of species of each plot (7 m x 7 m). The biomass of this component (hereafter tree biomass) was calculated for the total volume of woody individuals sampled within each plot, using the equation proposed by CETEC (1995):

$$\mathrm{VFCC} = 0.00539009 + 0.0000341998 \times \mathrm{DGL}^2 \times \mathrm{H}$$

Where VFCC stands for the stem volume with bark of woody individuals, DGL for diameter at ground level, and H for total height.

To estimate the diversity, biomass and abundance of the sapling trees of the understory vegetation, we carried out a survey in 2m x 2m plots in the middle of the 7 m x 7 m plots. We considered as saplings all woody individuals with a height fewer than 2 meters and circumference at ground level < 10 cm (Table S1). All saplings were measured in height (saplings height) and stem diameter (saplings diameter) at ground level. Here, we also considered as richness the number of species of each plot and calculated the Shannon Index (*H’*). We estimated the biomass of all the individuals of this component using the equation above (stem diameter measured at ground level), hereafter saplings biomass.

Soil sampling

On July 2017, we collected three soil samples (5 cm diameter x 15 cm of depth) in each plot to create a single composite soil sample per plot to assess microbial functional diversity and nutritional status. The composite soil samples were homogenized by hand and passed through a 2-mm sieve to remove rocks, roots and other large organic particles.

Soil microbial functional diversity (MicroResp-CLPP)

To assess the soil microbial functional diversity in the different physiognomies, we determined the catabolic response profile of soil microbial communities using the MicroResp system (MicroResp™, Macaulay Scientific Consulting Ltd., Aberdeen, UK) as described in Campbell et al. (2003). MicroResp™ offers a convenient, rapid and sensitive method for the determination of Community-Level Physiological Profiles (Chapman et al. 2007). We used these functional activities by microorganisms as a surrogate of soil microbial functional diversity. This method provides this information because different C sources correspond to the catabolic attributes of diverse soil microbial functional groups (García-Palacios et al. 2011; Delgado-Baquerizo et al. 2013; Morillas et al. 2015). We specifically tested the respiratory response to seven C sources: one amino acid (gamma-aminobutyric acid), one amino sugar (N-acetyl-glucosamine), two neutral sugars (d-galactose, and D (+)-glucose), and three carboxylic acids (L-malic acid, alpha ketogluterate, and citric acid). These analyses were carried out in soil samples previously incubated at 80% of their WHC and at 25 °C for 15 days. MicroResp plates with 96-wells were set up following Morillas et al. (2015). The soil sample was distributed to a microtitre plate where each well contain a small amount of water with a dissolved C substrate. The value of absorbance from each well was corrected by subtracting the blank well (inoculated but without a substrate). The gel plates sealed were incubated for 6 h and read at 570 nm. To estimate the evolved CO_2, a colorimetric method relying on the change in the pH of a gel-based solution of bicarbonate was used (Delgado-Baquerizo et al. 2013; Morillas et al. 2015). We calculated the microbial functional diversity through the Shannon Index (*H'*) and the microbial functional evenness (*E*) such as in Rodríguez et al. (2017).

Soil nutritional status

We estimated the litter production and the soil organic matter, microbial biomass nitrogen (MBN), dissolved organic nitrogen (DON) and N and P availability in all plots. All these variables were selected because they are good proxies of the nutritional status of the system, and because they are known to be of paramount importance for the maintenance of key ecosystem functions and services.

In each plot of 7 x 7 m we installed two suspended litter collectors made with nylon screen of 2 mm mesh opening and with one m² of area to assess the litter production during two years (from August 2015 to July 2017). The litter was collected every 40 days, dried in a kiln for 72 hours at 65°C and then weighed in an analytical balance (0.01 g). We used the two-years average values to represent the overall, plot-based litter production.

Soil organic matter was determined weighting the soil samples before and after passing by muffle furnace at 500°C for eight hours (complete combustion). Soil microbial biomass-N was analyzed using the fumigation-extraction method (Brookes et al. 1985). First, we activated the soil microbial community by wetting the dried soil samples at 60% of their WHC and incubating them at ambient temperature for four days. After this activation period, a 5 g soil subsample was immediately extracted with 100 ml of 0.5 M K_2SO_4 and filtered through a 0.45 μm Millipore filters, while another 5 g soil subsample was exposed to chloroform for five days before being extracted and filtered. Total N in the extracts was converted to nitrate using the persulphate oxidation technique, and the nitrate in the digest was reduced to ammonium and the concentration was estimated by the colorimetric method as described by Rodríguez et al. (2007). Total N from non-fumigated samples was subtracted from fumigated ones in order to estimate microbial biomass-N. DON was analyzed by persulfate oxidation of non-fumigated K_2SO_4 extracts and subtraction of mineral N from those extracts (Rodríguez et al. 2007).

To estimate soil available N and P in-situ production, ion exchange membranes (IEMs) were incubated in the soil as described in Rodríguez et al. (2009). IEMs are reliable in estimating nutrient availability for plant and soil microbes (Qian and Schoenau, 2002) and simulate the flux of nutrients into plant roots (Huang and Schoenau, 1997). Incubations of IEMs into the soil were carried out for two years, between August 2015 and July 2017. Specifically, the membranes were incubated in the soil for 30-40 days four times year (see chapter 2 for more details). We used the average values for these two years to represent the overall, plot-based nutrient availability. Upon retrieval, the membranes were individually transported to the lab, air-dried, and cleaned of soil particles. We extracted NH^+_4, NO^-_3, and PO^{3-}_{4-} from the membranes with 2 M KCl as explained in Rodríguez et al. (2009), and analyzed the amount of NH^+_4, NO^-_3, and PO^{3-}_{4-} in the extract by colorimetry using a microplate reader. Concentrations of NH^+_4-N and NO^-_3-N were determined by the indophenol blue method, and PO_4-P concentrations by the molybdene blue method. Data were expressed as μg N or P dm^{-2} resin surface day^{-1}.

Data analysis

We explored the relationships between saplings and adult trees (richness, individual's number, and biomass) using Pearson's correlation (r) analysis. As both were tightly correlated (Figure S1), we used only saplings to test the effect of the physiognomy on plant communities and to analyze relationships with microbial communities and soil nutritional status.

Differences in the plant and soil microbial diversity between physiognomies were analyzed by using one-way analysis of variance (ANOVA; for normally distributed data) followed by a post hoc Tukey's test ($p < 0.05$). Data that did not present normal distribution were square root transformed.

We carried out multivariate analyses (redundancy analysis - RDA) to determine the relative importance of soil nutritional status variables (i.e. litter production, organic matter, MBN, DON, and NH_4^+-N, NO_3^--N and PO_4^{-3}-P availability) influencing species composition and abundance. The primary matrix was composed of species abundance data for each site, while the secondary matrix included the soil nutritional status variables. Subsequently, we made a PERMANOVA (Permutational multivariate analysis of variance) to verify the significance of the variables in explaining the ordering. The relationship between plant and microbial diversity/biomass variables and soil nutritional status variables was also analyzed using Pearson's correlation (r) analysis. All analyses were carried out in R 3.3.3 (R Development Core Team 2013), with a significance level of $P<0.05$.

Results

Saplings and adult trees relationships

Sapling and adult riches were significantly and positively correlated, whereas saplings diameter and adult biomass were negatively related. Moreover, the greater the number of adults, the lower the sapling biomass (Figure S1).

Differences among Cerrado physiognomies

We found a significant effect of the physiognomy on saplings richness and *H'* index, and on microbial functional *H'* and *E* indexes ($p<0.05$). Saplings *H'* and richness was higher in the intermediate savannah than in the other two physiognomies (Figure 2a). Microbial functional *H'* was significantly higher in the woodland and intermediate savannah than in the open savannah (Figure 2b). Microbial functional *E* was significantly higher in the intermediate savannah than in the open savannah, with the woodland savannah showing intermediate values (Figure 2c). The microbial community of each physiognomy responded in a similar way to the seven substrates (C sources; figure S2), with significant lower substrate utilization values in the open savannah than in the other two physiognomies.

The RDA of microbial C sources utilization showed a marginally significant separation between the open savannah and the other two physiognomies (F=3.37, P=0.059; Figure 3a). Similarly, the RDA for plant communities showed a marginally significant separation among physiognomies (F=1.23, P=0.063 for saplings, F=2.98, P=0.088 for adults; Figure 3b and 3c, respectively). Soil NH_4-N availability was identified as a significant driver of the separation among physiognomies in the RDA of microbial C sources utilization (F=8.68, P<0.05) and saplings structure (F=2.02, p=0.008), whereas litter production was the most important contributor to the RDA physiognomy ordination for adults structure (F=5.96, P<0.05). Soil NO_3-N availability was also a factor correlated with microbial C sources utilization (F=6.19, P<0.05).

Plant-soil-microorganism interactions

Microbial functional *H'* was significantly and negatively related to saplings diameter (r=0.75; P<0.005; Figure 4a), but significantly and positively related to soil NO_3^--N (r=0.56; P<0.05; Fig 5b), soil dissolved organic nitrogen (r=0.79; P<0.005; Fig 5c), PO_4^{-3}-P availability (r=0.87; P<0.001; Fig 5d), and litter production (r=0.79; P<0.001; Fig 5f). Likewise, microbial functional *E* was significantly and negatively related to saplings diameter (r=0.69; P<0.05; Fig. 4b), and positively related to PO_4^{-3}-P (r=0.67; P<0.01; Fig 5e). Sapling biomass was negatively related to soil NH_4-N (r=0.62; P<0.05) and NO_3-N (r=0.53; P<0.005) availability (Figure 6a, b and c). Saplings diameter was also significantly and negatively related to litter production (r=0.69; P<0.001), DON (r=0.55; P<0.05), soil NH_4-N (r=0.53; P<0.05) and PO_4-P availability (r=0.61; P<0.005) availability (Figure 6d, e and f).

Discussion

Different Cerrado physiognomies had different saplings and soil microbial diversities, but they were not directly related. On the other hand, we did find negative relationships between saplings biomass and variables such as adult biomass, and dissolved organic nitrogen content and microbial functional diversity. These negative relationships may indicate both a specialization of the scrubby layer to high light and low nutrient availability (Barbosa et al.

1999; Ronquim et al. 2003, 2009, 2013), and resources competition between plant and microbial communities (Xi et al. 2018). Indeed, soil ammonium was the main driver of saplings and microbial communities, whereas litter production had strong influence on adult structure. Altogether, these results indicate a central importance of nutrient availability and turnover (mainly N) for plants and microorganisms in this ecosystem. Agreeing with this, we found that microbial functional diversity was significantly lower in the open than in the woodland and intermediate savannahs. As total N in the litter and soil layers also increases with forest development in Cerrado (Pellegrini et al. 2014), this low diversity in open savannah corroborates our hypothesis that high nutrient availability might support more diverse microbial communities. Further, the higher microbial activity (e.g. Table 1 and Figure S2) in the forest-like Cerrado physiognomies (i.e. woodland Cerrado; likely due to higher litter inputs), point towards positive links between microbial functional diversity and functioning (de Carvalho Mendes et al. 2012).

Interestingly, the woodland savannah, which showed consistently greater levels of soil nutritional status than the other physiognomies (Table S1), and had lower saplings diversity and richness than intermediate savannah. This unexpected result may be related with a particularly low specialization of Cerrado plant species to shady conditions (Barbosa et al. 1999; Ronquim et al. 2003, 2009, 2013). The dense tree cover of the woodland cerrado is likely to lead to relatively better (as compared to other physiognomies) nutrient conditions (see above), but also particularly low light availability, constraining the development of understory species.

On the other hand, the intermediate savannah, a physiognomy in between the open and the woodland savannah, presented both the most diverse microbial and saplings communities, and the highest microbial functional evenness values. These results support the idea that intermediate levels along resource gradients may present greater niche diversification, and consequently more diverse communities (Ranjeameni 2003). In the intermediate savannah, soils are not as poor as the soils of the open savannah, but also not rich enough to support a forest community like that of the woodland savannah (Neri et al. 2012, 2013). Thus, these soils allow the densification of the vegetation, but not the development of a closed canopy, which allows the arrival and establishment of plant species adapted to a wide range in light conditions, from full sun up to shaded conditions (see Neri et al. 2012, 2013).

Although open savannah presented the lowest saplings diversity among the Cerrado physiognomies, we found there the highest saplings biomass. Again, light and nutrient availability may be behind these results. Open savannah, with lower nutritional levels than the woodland and intermediate savannahs, has a particularly high light availability because of its open canopy (lower tree cover). These conditions might lead to a strong development of understory species adapted to the low nutrient availability and the high radiance of this physiognomy (Barbosa et al. 1999; Ronquim et al. 2003, 2009, 2013; Neri et al. 2013; de Souza et al. 2017). These species possess strong nutrient-conserving traits and slow-growth strategy (Abrahão et al. 2019). One example is *Miconia albicans* (Sw.) that possess various individuals in open savanna (Table S1) and high ability to branch, contributing to high biomass in this physiognomy. This species is able to establish itself successfully in environments with high levels of aluminum, and it has competitive advantages over other species in these environments (Haridasan et al. 1986). Our results add further evidence that different ecosystem attributes, such as biomass or diversity, might be affected in different ways by different drivers (Beguin et al. 2011; Harpole et al. 2017; van der Sande et al. 2018), and that the relative importance of these drivers might vary among the different Cerrado physiognomies.

In terrestrial ecosystems, plants and microorganisms are related by strong and intricate interactions that can be positive or negative, among other things because soil microorganisms can act both as a sink and a source of nutrients (Singh et al. 1989). Several studies have shown that plants compete with soil microorganisms for N, even on relatively fertile soils (Kaye and Hart 1997; Xu et al. 2011; Dijkstra et al. 2015; Xi et al. 2018). We found that, in the Cerrado, sapling biomass was negatively related to microbial functional diversity and dissolved organic N content. These results suggest the dominance of competitive interactions among soil microorganisms and plants for soil resources, or alternatively, the negative effect of soil pathogens on plant development (Xi et al. 2018). In any case, the consistent relationships among microbial diversity, soil nutrient content, and plant attributes anticipate that any disturbance affecting one component of the Cerrado or a change in the relative dominance of one of the physiognomies could cause cascade effects in many components of this biome (McNaughton 1992; Klumpp et al. 2009; Pugnaire et al. 2019).

According to RDA analysis, soil ammonium availability was one important driver shaping microorganisms and plant saplings communities. Soil ammonium is the predominant form of inorganic N in the Cerrado (Frazão et al. 2010), and its availability is responsible for variations in net N mineralization (Nardoto and Bustamante 2003). Cerrado is known to have a conservative N cycle and, as soon as the organic N is mineralized to ammonium, plants consume this inorganic N form (Nardoto and Bustamante 2003; Bustamante et al. 2012). Our results corroborate that competition between plants and microorganisms for ammonium in the Cerrado is intense (Nardoto and Bustamante 2003), which explains why this nutrient is particularly important in shaping these communities (Kaye and Hart 1997; Moreau et al. 2019). Indeed, soil organic matter content and microbial biomass N were not directly related to diversity and biomass of the communities evaluated, despite strong variations among physiognomies (Table 1). These results suggest that the availability and turnover of a particular form of a nutrient (i.e. ammonium) may be more important than the total amount of soil organic matter in this highly conservative system.

From the above, we conclude that plant and soil biodiversity differ among the different Cerrado physiognomies, with the highest levels of biodiversity consistently found in the intermediate savannah. This study also shows intense but intricate relationships among microbial diversity, soil nutrient content, and plant attributes. These relationships, likely driven by competition for light and soil resources, anticipate that any disturbance affecting one component of the Cerrado or a change in the relative dominance of one of the physiognomies could cause cascade effects in many components of this biome.

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Figures and tables

Figure 1: Sampling of saplings (2 x 2 meters plots). A: Plot in woodland savannah; B: Plot in intermediate savannah; C: Plot in open savannah, detail for litter collector.

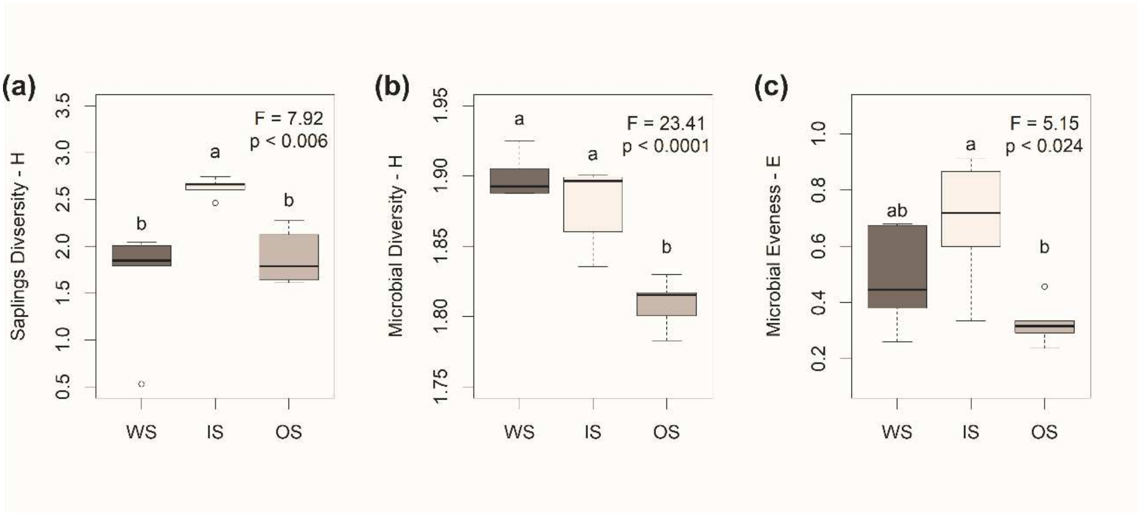


Figure 2: Boxplots of (a) saplings diversity (Shannon – *H'*) and soil microbial functional (b) diversity (Shannon – *H'*) and (c) evenness (*E*) indexes in the three Cerrado physiognomies. Boxes, the horizontal line inside the boxes and the whiskers represent the second and third quartiles, the median value, and the lower and the upper quartile, respectively. WS: woodland savannah; IS: intermediate savannah; OS: open savannah. Different letters indicate statistical differences among physiognomies ($p < 0.05$).

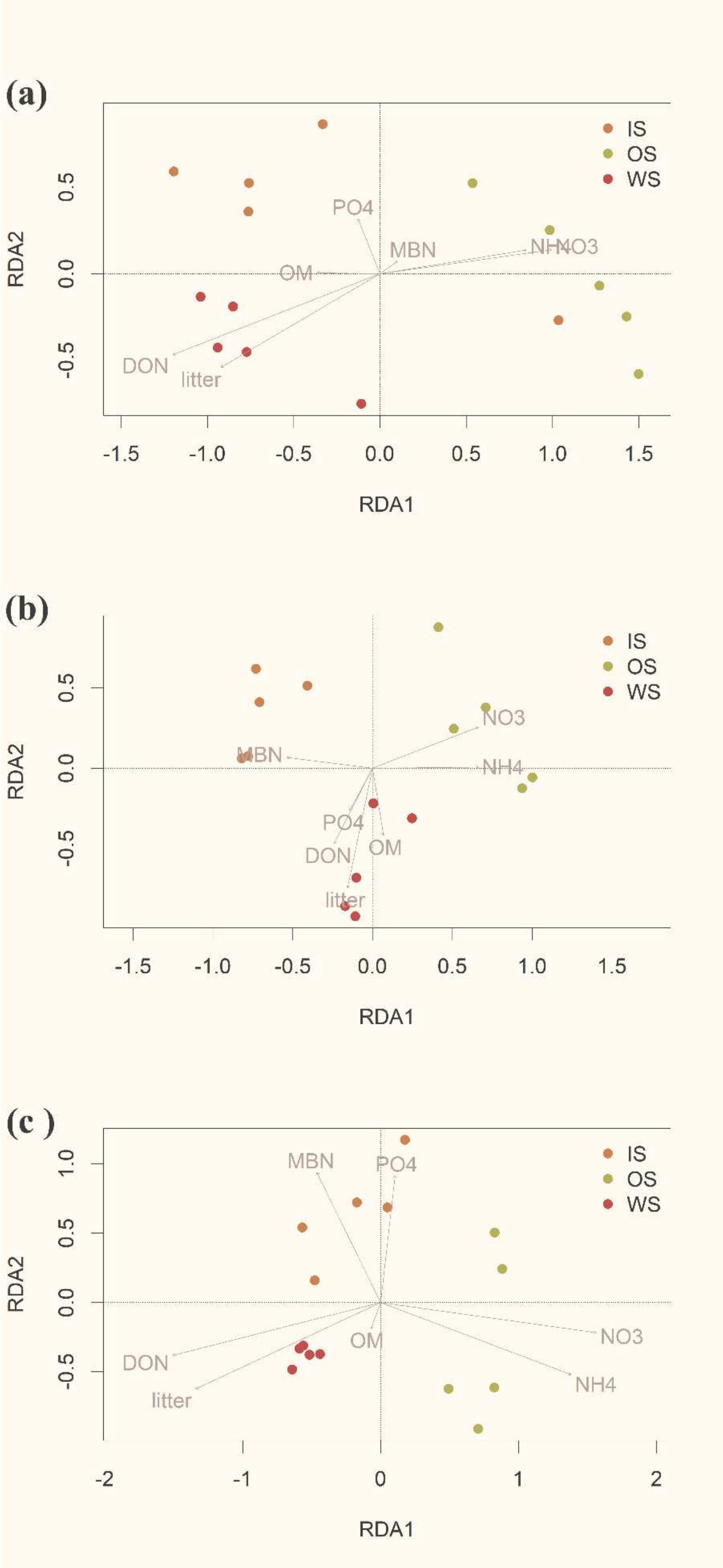


Figure 3: Ordination plots from the redundancy analysis for (a) microbial C sources utilization with the MicroRespTM method and for (b) saplings and (c) adult trees composition

in the three Cerrado physiognomies, and the contribution of soil nutritional status variables on the ordinations (arrows). WS: woodland savannah; IS: intermediate savannah; OS: open savannah.

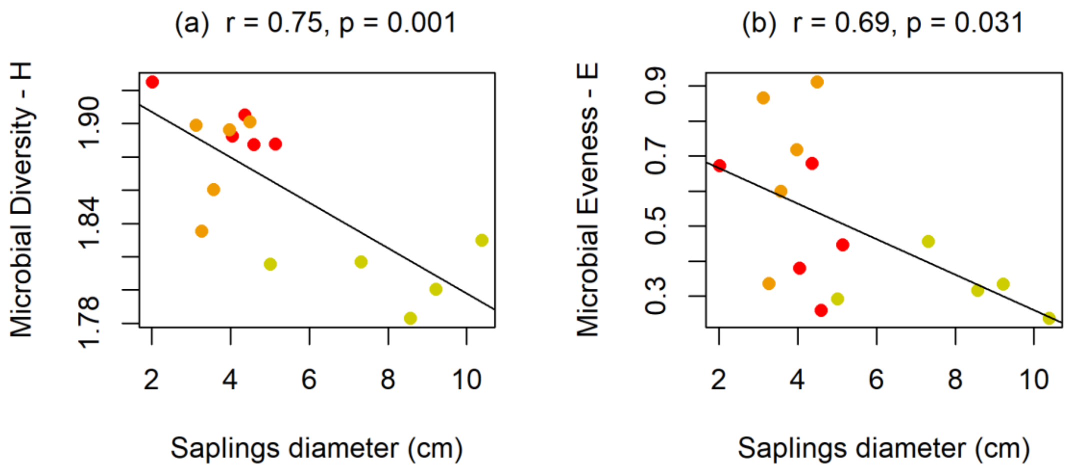


Figure 4: Correlations of microbial functional (a) diversity (Shannon – *H'*) and (b) Evenness (*E*) with saplings diameter. Red dots: woodland savannah; Orange dots: intermediate savannah; Yellow dots: open savannah.

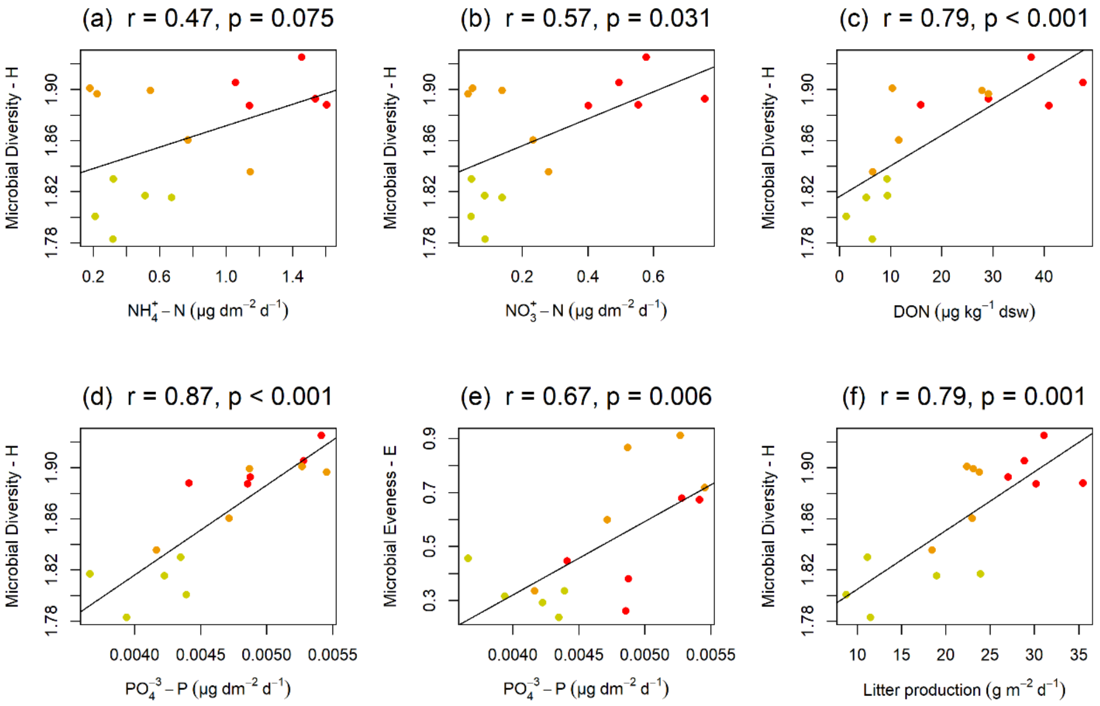


Figure 5: Correlations of microbial functional diversity (Shannon – *H'*) and Evenness (*E*) with different soil nutrient status variables (NH_4^+-N, NO_3^--N and PO_4^{-3}-P availability,

dissolved organic nitrogen [DON], and litter production). Red dots: woodland savannah; orange dots: intermediate savannah; yellow dots: open savannah.

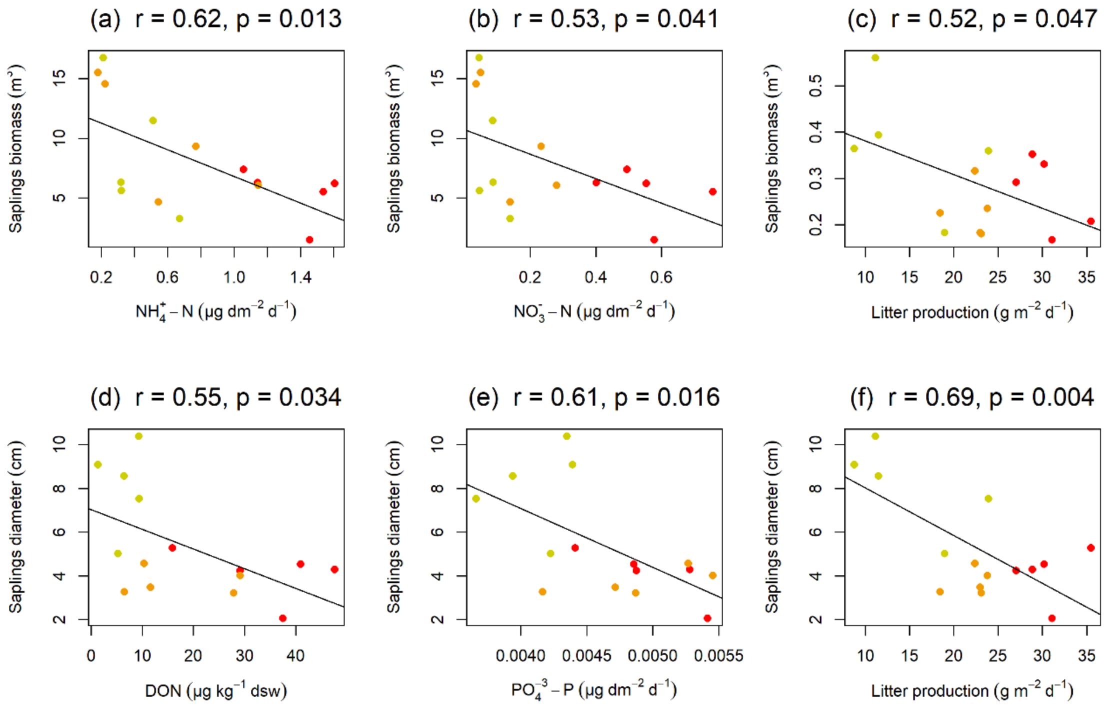


Figure 6: Correlations of saplings biomass and diameter with different soil nutrient status variables (NH_4^+-N, NO_3^--N and PO_4^{-3}-P availability, dissolved organic nitrogen [DON], and litter production). Red dots: woodland savannah; orange dots: intermediate savannah; yellow dots: open savannah.

Table 1: Soil nutritional status in the three physiognomies of Cerrado.* Values represent the mean (±SE). Different letters in each variable represent significant differences among physiognomies ($P < 0.05$).

Variables	woodland savannah	intermediate savannah	open savannah
NH_4^+-N (µg dm^{-2} d^{-1})	1.36±0.06 a	0.57±0.10 b	0.41±0.05 b
NO_3^--N (µg dm^{-2} d^{-1})	0.56±0.03 a	0.15±0.02 b	0.08±0.01 b
PO_4^{-3}-P (ng dm^{-2} d^{-1})	4.95±0.10 a	4.89±0.07 b	4.12±0.10 a
DON (mg kg^{-1} dsw)	34.20±5.46 a	17.10±4.74 b	6.34±1.49 b
MBN (mg kg^{-1} dsw)	288.99±24.52 b	484.86±49.24 a	278.14±40.15 b
Litter production (g m^{-2})	2.19±0.09 a	1.33.9±0.06 b	0.90±0.18 b
Organic matter (%)	5.86 (0.21) a	4.78 (0.11) b	3.16 (0.22) c

*Data from chapter two and from Lacerda (2019)

Supplementary material

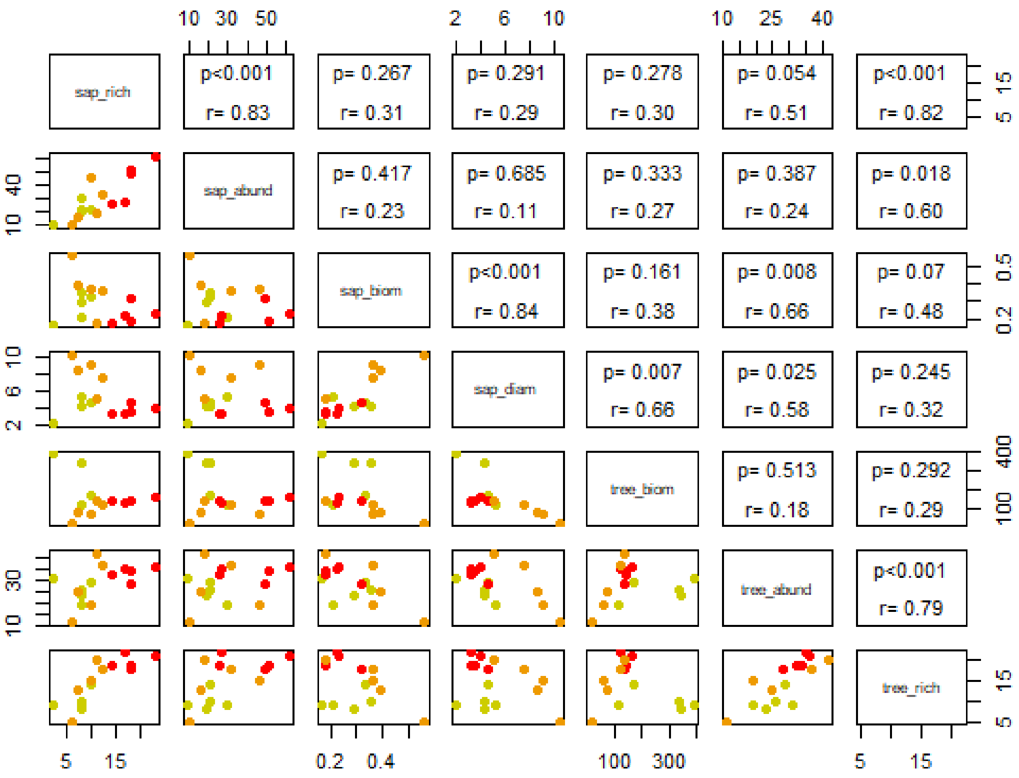
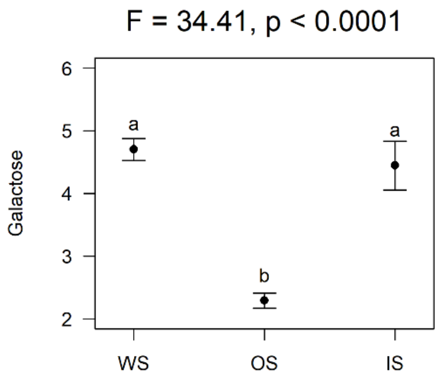
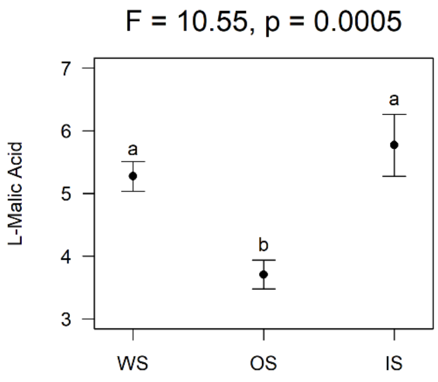
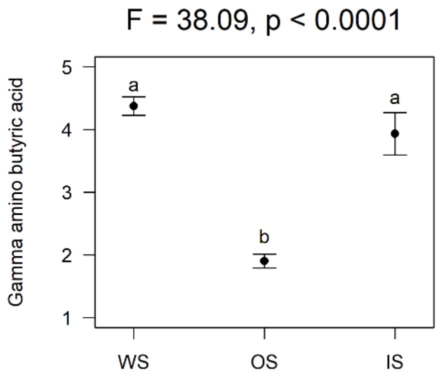
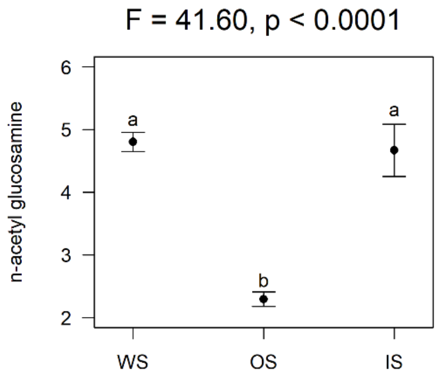


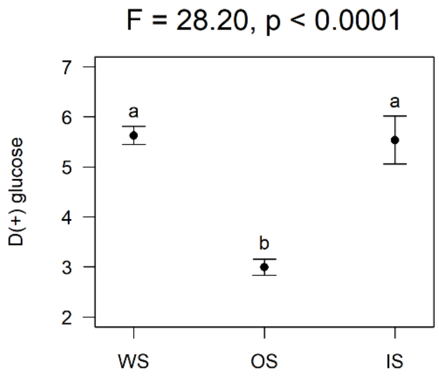
Figure S1: Correlations between saplings and adult trees parameters. Sap_rich: number of sapling species. Sap_abund: number of sapling individuals. Sap_biom: sapling biomass (stem volume with bark). Sap_diam: sapling stem diameter. Tree_VFCC: tree biomass (stem volume with bark). Tree_abund: number of tree individuals. Tree_rich: number of tree species. Red dots: woodland savannah; orange dots: intermediate savannah; yellow dots: open savannah.

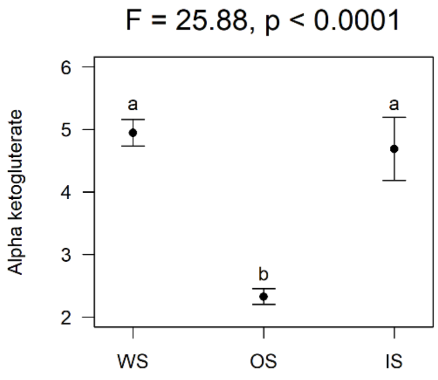

F = 34.41, p < 0.0001
Galactose
6
5
4
3
2
a
b
a
WS
OS
IS


F = 10.55, p = 0.0005
L-Malic Acid
7
6
5
4
3
a
b
a
WS
OS
IS


F = 38.09, p < 0.0001
Gamma amino butyric acid
5
4
3
2
1
a
b
a
WS
OS
IS


F = 41.60, p < 0.0001
n-acetyl glucosamine
6
5
4
3
2
a
b
a
WS
OS
IS


F = 28.20, p < 0.0001
D(+) glucose
7
6
5
4
3
2
a
b
a
WS
OS
IS


F = 25.88, p < 0.0001
Alpha ketogluterate
6
5
4
3
2
a
b
a
WS
OS
IS

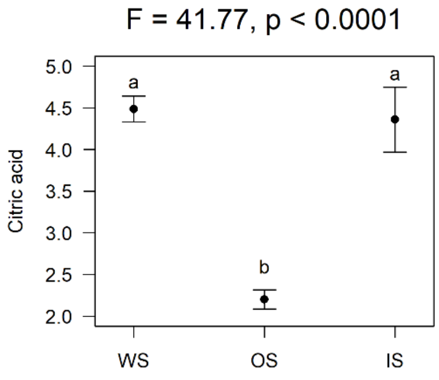

F = 41.77, p < 0.0001
Citric acid
5.0
4.5
4.0
3.5
3.0
2.5
2.0
a
b
a
WS
OS
IS

Figure S2: Carbon substrate utilization (mean ± SE) by soil microorganisms in the three physiognomies of Cerrado and statistic results testing the physiognomy effect (ANOVA). WS: woodland savannah; IS: intermediate savannah; OS: open savannah. Different letters indicate significant differences among physiognomies ($p < 0.05$).

Table S1: Species list of the saplings in the three physiognomies of Cerrado in Paraopeba Reserve (Minas Gerais state, Brazil southeast). The absence of the value indicates the absence of the species in the area. WS: woodland savannah; IS: intermediate savannah; OS: open savannah.

Family/Species	**WS**	**IS**	**OS**	**Total**
Anacardiaceae		**10**		**10**
Tapirira guianensis Aubl.		10		10
Annonaceae	**1**	**2**	**1**	**4**
Xylopia aromatica (Lam.) Mart.	1	2	1	4
Asteraceae	**1**		**7**	**8**
Asteraceae 1	1			**1**
Baccharis platypoda DC.			3	3
Lessingianthus elegans (Gardner) H.Rob.			1	1
Sabicea brasiliensis Wernham.			3	3
Combretaceae		**2**		**2**
Terminalia argentea Mart. & Zucc.		2		2
Dilleniaceae		**1**	**2**	**3**
Curatella americana L.			1	1
Dilleniaceae 1			1	1
Dilleniaceae 2		1		1
Erythroxylaceae	**5**	**18**	**18**	**41**
Erythroxylum campestre A. St.-Hil.	4	14	7	25
Erythroxylum daphnites Mart.		1		1
Erythroxylum sp.	1		11	12
Erythroxylum suberosum A.St.-Hil.		3		3
Fabaceae	**38**	**25**	**7**	**70**
Bauhinia dumosa Benth.		2		2
Bauhinia sp.		12		12
Chamaecrista sp.			2	2
Copaifera langsdorffii Desf.	27	7		34
Dalbergia miscolobium Benth.	2	3		5
Dimorphandra mollis Benth.			1	1
Fabaceae 1			1	1
Plathymenia reticulata Benth.			2	2
Platypodium elegans vog.	8	1	1	10
Swartzia sp.	1			1
Malpighiaceae			**5**	**5**

Byrsonima crassifolia (L.) Kunth.			1	1
Byrsonima sp.			1	1
Malpighiaceae 1			3	3
Malvaceae		**2**	**2**	**4**
Guazuma ulmifolia Lam.		2		2
Pavonia sp.			2	2
Melastomataceae	**1**	**9**	**40**	**50**
Miconia albicans (SW.) Triana		1	37	38
Miconia rubiginosa (Bonpl.) DC.	1	3		4
Miconia sp.		3		3
Miconia sp.2		2		2
Miconia sp.3			1	1
Pleroma granulosum (Desr.) D. Don			2	2
Myrtaceae	**12**	**65**	**4**	**81**
Campomanesia velutina (Cambess.) O.Berg		4		4
Eugenia dysenterica DC.		1		1
Eugenia sp.		3		3
Myrcia fenzliana O.Berg		5		5
Myrcia guianensis (Aubl.) DC.		16	4	20
Myrcia sp.	2	8		10
Myrcia sp.2		1		1
Myrcia sp.3		1		1
Myrcia splendens (Sw.) DC.	10	21		31
Myrtaceae 1		4		4
Myrtaceae 2		1		1
Nyctaginaceae	**1**	**3**		**4**
Guapira noxia (Netto) Lundell	1	3		4
Ochnaceae	**2**	**1**		**3**
Ouratea castaneifolia (DC.) Engl.	2	1		3
Peraceae		**1**	**5**	**6**
Pera glabrata (Schott) Poepp. ex Baill.		1	5	6
Primulaceae	**10**	**2**		**12**
Cybianthus detergens Mart.	8			8
Myrsine sp.		2		2
Primulaceae 1	2			2
Proteaceae	**5**	**3**		**8**
Roupala montana Aubl.	5	3		8
Rubiaceae	**8**	**50**	**11**	**69**
Alibertia edulis (Rich.) A.Rich. ex DC.	4	30	5	39
Chiococca alba (L.) Hitchc.	1	4		5
Coussarea cornifolia (Benth.) Müll. Arg.	3	2		5
Palicourea rigida Kunth		4	6	10
Rudgea viburnoides (Cham.) Benth.		10		10
Salicaceae		**1**		**1**
Maytenus salicifolius Reissek		1		1

Siparunaceae	**7**	**1**	**2**	**10**
Siparuna guianensis Aubl.	7	1	2	10
Styracaceae		**8**		**8**
Styrax camporum Pohl		8		8
Symplocaceae		**2**		**2**
Symplocos nitens (Pohl) Benth.		2		2
Vochysiaceae		**2**	**3**	**5**
Qualea cordata Spreng.		2		2
Qualea grandiflora Mart.			2	2
Qualea multiflora Mart.			1	1
Undetermined	**9**	**7**	**15**	**31**
Undetermined 1		4		4
Undetermined 2	6			6
Undetermined 3			1	1
Undetermined 4			1	1
Undetermined 5			5	5
Undetermined 6			8	8
Undetermined 7		1		1
Undetermined 8		1		1
Undetermined 9		1		1
Undetermined 10	3			3
Total	**100**	**215**	**122**	**437**

CHAPTER 2*: Nutrient availability in three Cerrado physiognomies under ambient and rainfall reduction conditions

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*This chapter follows the standards of bibliographic formatting of the journal *Geoderma.*

Abstract

Climate models forecast rainfall decreases for the Cerrado, a highly diverse and threatened savanna located in Central South America. Studies on the fate of the key soil nutrients availability for the Cerrado under these climate change scenarios are still scarce. We sought to assess nitrogen (N) and phosphorus (P) availability in the three dominant Cerrado physiognomies (woodland savanna, intermediate savanna, and open savanna) under ambient (control plots) and rainfall reduction (treated plots) conditions. Ion exchange membranes were incubated in the soil of each plot for 30-40 days in four different times (mid and late rainy and dry seasons) during two years. Nutrient availability varied between physiognomies, seasons and years, with noteworthy interactions among some of these factors. The open savanna showed overall lower nutrient availability values than the other two physiognomies, but it strongly depended on the season and the year. Both seasonality and the rainfall reduction treatment decreased N availability but not that of P, suggesting a higher sensitivity of N availability than that of P to changes in soil moisture, and pointing towards a likely climate change-driven decoupling of the P and N cycles. However, different physiognomies showed different sensitivities to the rainfall reduction, with the woodland savanna consistently showing the strongest responses. Our results highlight the need for more and longer experimental studies to better understand the responses of this important and understudied ecosystem to future climate change scenarios, and reinforce the need of considering its different physiognomies in future studies.

Key words: nitrogen, phosphorus, seasonality, soil moisture, climate change, Brazilian savanna.

Introduction

The Cerrado, as the Neotropical Savanna is commonly known, is the second largest biome of South America, covering about 2 million km^2 of its territory, approximately 23% of the Brazilian's land surface (Ratter et al., 1997). This biome is one of the world's biodiversity hotspots (Mittermeier et al., 2005). Its vegetation is extremely rich with many endemic species, but approximately 50% of its territory has already been deforested or transformed in the last decades by human action (Klink and Machado, 2005; Mittermeier et al., 2005; Strassburg et al., 2017). As a result of its large distribution and biodiversity, the Cerrado provides important ecosystem services related to energy, water, carbon (C) storage, and nutrients cycling both at regional and global scale (Batlle-bayer et al., 2010; Bustamante et al., 2012, 2006; Miranda et al., 1997; Resende et al., 2011).

The Cerrado landscape encompasses a number of physiognomic formations that differ in structure and composition, and that can be classified according to the proportion of trees and grasses, ranging from grassland to forest formations (Coutinho, 1978; Furley, 1999; Ribeiro and Walter, 1998). The distribution of plant species in the Cerrado is highly correlated with soil fertility (Neri et al., 2013, 2012). Consequently, a phytophysiognomic gradient with varying fertility can be found within this biome (Goodland and Pollard, 1973; Neri et al., 2013). In general, trees become thinner and shorter as the cation-exchange capacity decreases in the superficial soil layer (Neri et al., 2013). Thus, soils from woodland savanna formations (i.e *Cerradão*) are very clayey and with relatively high organic matter and P reserves (Giácomo et al., 2015; Neri et al., 2013). Whilst soils from more open savanna formations (i.e. Cerrado *stricto sensu*) are sandy and contain lower P content (Neri et al., 2013). The availability of soil N and P are among the main limiting factors of primary productivity in many terrestrial ecosystems, including savannas (Elser et al., 2007; Haridasan, 2001). In general, the Cerrado soils tend to be more limited by P than by N, as they are highly weathered (Abrahão et al., 2019; Bustamante et al., 2012, 2006; Nardoto et al., 2013; Resende et al., 2011), and soil P is dominated by occluded forms (Resende et al., 2011).

The Cerrado is among the world's most humid savannahs, yet climate in the Cerrado is seasonal with defined dry and rainy seasons (Neri et al., 2012). Approximately 90% of rainfall is concentrated from October to April, which induces large seasonal fluctuations in nutrients, water and C fluxes (Grace, 2000). Thus, nutrient cycling in the Cerrado mainly occurs during the rainy season (Bustamante et al., 2012), whereas the low water availability during the dry season is a growth- and nutrient cycling-limiting factor (Haridasan, 2001). Despite this strong seasonality, few studies have aimed to analyze the temporal variability of nutrient cycling in the Cerrado (Bustamante et al., 2012; Coletta et al., 2009). Understanding how P and N cycles changes with seasonality (water availability) in different Cerrado physiognomies could help to reveal the behavior of biogeochemical cycles in climate change scenarios.

In most regions dominated by Cerrado formations, climate models forecast a decrease in the number of rainy days per year, with up to a 20-70% decrease of the current values of rainfall (Bustamante et al., 2012; Marengo et al., 2010). The less severe projections of the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) anticipates a 30% rainfall reduction for 2100 in the central and southern parts of the Cerrado biome (Bustamante et al., 2012). This rainfall reduction is likely to decrease soil moisture, and consequently to modify nutrient cycling and availability (Guo et al., 2002; Rodríguez et al., 2019, 2011). However, little has been studied about the impact of rainfall reductions on nutrient cycling and availability in tropical savannas (Augustine and Mcnaughton, 2006; Okach et al., 2019; Sankaran, 2019), and, to our knowledge, there are no such studies in the Cerrado.

Herein, we aimed to study how N and P availability changes throughout the year in the Brazilian Cerrado, and how it will be influenced after reductions in rainfall. To do so, we set up a field-based rainfall reduction experiment in the three more common physiognomies of Cerrado, and estimated soil N and P availability using ion exchange membranes (IEMs) along two years. We hypothesized that: i) more open (i.e. with lower plant cover) physiognomies would have less N and P availability than more closed physiognomies, and ii) under reduced soil moisture (i.e. dry season and rainfall reduction treatment) there would be less N (because of a decrease in N mineralization) and more P (because of an decrease in plant uptake) available. We also hypothesized that the effect of rainfall reduction would be stronger during the rainy season because of higher differences in soil moisture between control and treated plots. Finally, we also expected that the three physiognomies would have different seasonal and rainfall reduction treatment sensitivities due to different biotic (e.g. plant and microbial communities) and abiotic (e.g. soil texture) characteristics.

Methods

Study area

The study was carried out in the Paraopeba National Reserve (southeast of Brazil; 19°16'S 44°23'W), with a total area of 200 ha (Neri et al., 2013, 2012). The climate is categorized as Aw according to the Koppen classification, with rainy summers and dry winters, total annual rainfall of 1328 mm and average annual temperature of 20.9 ° C (Neri et al., 2013, 2012).

In the Paraopeba Reserve, different vegetation types (i.e. phytophysiognomies) of Cerrado are organized in an edaphic gradient. However, the entire area has the same parent rock material, a very small altitudinal range, and the same climatic conditions, which is optimal to test our hypotheses. This study was carried in the three dominant Cerrado physiognomies, all of them present in the Paraopeba Reserve: woodland savanna (i.e *Cerradão*) on red latosol (oxisols in United States classification), intermediate savanna (i.e. *Cerrado strictu sensu denso*) on yellow red latosol, and open savanna (i.e. Cerrado *strictu* *sensu*) on yellow latosol (Neri et al., 2013). The woodland savanna presents high plants and sparse herbaceous/scrubby layer; the intermediate savanna has a great densification of shrub-tree species over herbaceous and sub-shrub species; and the open savanna is characterized by the presence of well-defined herbaceous-shrub and shrub-tree strata (Neri et al., 2013). Soils of woodland savanna are more clayey, acid, and with higher organic matter values in comparison to other physiognomies (Table 1). For more details on the characterization of soil and physiognomies, see Neri et al. (2013).

Experimental design

In August 2015, we placed five 7 x 7 m paired (one rainfall reduction and one control) plots in each of the three dominant Cerrado physiognomies present in the Paraopeba Reserve, totaling 15 paired plots. The manipulated rainfall reduction experiment consisted in the installation of six suspended transparent plastic bands (dimensions: 50 cm wide and 10 m long) in the treatment plots (Figure 1). The bands were separated equidistantly from each other and covered ~42% of the total soil surface. The last two meters of each plastic band were outside the plot. The bands were installed with ~8° of inclination to facilitate the drainage of rainwater. The orientation of the bands slope followed the topography of each plot, and the minimum height of the bands (at the bottom of the plot) was ~80 cm. Moreover, at the lowest end of each plastic band, we attached a 15 cm perimeter and 2 m length PVC (Polyvinylchloride) pipe that brought the collected rainwater out of the plot. We monitored (every hour) soil moisture in two paired plots per physiognomy (Figure S1) by using soil moisture sensors (CR10X-ECH2O; Decagon Devices, Inc., Pullman WA, USA) connected to data loggers (HOBO® H08-006-04; Onset, Pocasset, MA, USA). Soil moisture of treated plots was estimated from sensors installed both under and out the plastic bands. Rainfall reduction (RR) in each treated plot was estimated as:

$$\boxed{RR(\%) = \frac{(SM_c - SM_t)}{SM_c} \times 100}$$

Where SM_c is the soil moisture of the control plot, and SM_t is the soil moisture of the treated plot.

Soil sampling and laboratory analysis

To estimate soil N and P availability, ion exchange membranes (IEMs) were incubated in the soil following Rodríguez et al. (2009). Nutrient accumulation on the IEMs during the incubation period simulates the flux of nutrients into roots (Huang and Schoenau, 1997). Further, IEMs are a reliable method to estimate inorganic N production in the soil, due to their strong relationships with the N mineralization process (Durán et al., 2013). This methodology causes minimal damage to the soil, is easy to apply, and allows intensive sampling at various time periods at the same sites (Cain et al., 1999).

Incubations of IEMs into the soil were carried out seasonally (mid [July] and late [September] dry season and mid [January] and late [April] rainy season) for two years. The first incubation was carried out in the late dry season of 2015 and the last in the mid dry season of 2017, totaling eight incubations. At each plot, five IEMs were incubated for 30-40 days depending on the season (Figure 1). Upon retrieval, the IEMs were individually transported to the lab, air-dried, and cleaned of soil particles. We extracted and analyzed NH_4^+, NO_3^- and PO_4^{-3} from the membranes according to Rodríguez et al. (2009) by shaking them in 50mL of 2M KCl for 1h at 200rpm in an orbital shaker. From these extracts we calculated the amount of NH_4-N, NO_3-N and PO_4-P by colorimetry, and absorbance was measured with a microplate reader (Durán et al., 2009). Total inorganic nitrogen (TIN) was calculated as the sum of NH_4-N and NO_3-N. Data were expressed as $\mu g\ dm^{-2}$ resin surface day^{-1}. We extract all the IEMs from each plot separately, and we then estimated the mean from the five IEMs to have just one value per plot.

Data analysis

We first tested the effect of physiognomy on the two-years average values of soil N and P availability of control plots by using one-way ANOVA. We then used linear mixed-effect models to test the effects of the physiognomy, the season, and the year, as well as the interactions among all these factors, on the N and P availability of control plots. Here, plot was considered as a random factor to account for likely temporal dependencies. We also used linear mixed-effects models to evaluate the effects of the RR treatment and the physiognomy, as well as the interaction between both factors, on the two-years N and P availability average values. Moreover, we also performed linear mixed-effects models to test whether season and/or year modulated the effect of RR treatment on the N and P availability in each physiognomy separately. The control-treated paired plot was considered as a random factor in these two latest types of models to account for likely spatial and temporal dependencies. Finally, we tested pairwise statistical differences among different levels of our fixed factors using the Tukey’s test.

All statistical analyses were performed in R 3.3.3 (R Development Core Team 2013). Linear mixed-effects models and simultaneous tests for general linear hypotheses were carried out using the statistical packages lme4 (Bates et al., 2015) and multcomp (Bretz et al., 2011), respectively.

Results

Effects of physiognomy, season and year on soil N and P availability

We found significant effects of physiognomy, season and year on most of the studied variables, as well as noteworthy interactions between some of these factors (Table 2). Soil N availability was significantly higher in the woodland savanna than in the other two physiognomies (Figure 2a, b), but these differences among physiognomies disappeared in the mid dry season as a consequence of a sharp decrease in the soil N availability of the woodland savanna (Figure 3a-c). Thus, N availability was significantly lower in the dry than in the other seasons (Figure 3a-c). Interestingly, the N availability also changed between years, with lower values of NH_4-N and TIN in the first than in the second year, but only in the woodland savanna (Figure 3).

Soil P availability was significantly higher in the woodland and intermediate savanna than in the open savanna (Fig. 2c). However, these differences among physiognomies in P availability were only observed in the rainy and dry seasons of the first year, and in the late dry season of the second year (Figure 3). In the woodland and open savanna, P availability tended to show the highest values in the dry and late dry seasons. However, the intermediate savanna showed different seasonal P dynamics, with the greatest values from the rainy season of the first year to the late dry season of the second year (Figure 3d). Interestingly, P availability was consistently higher in the first than in the second year, regardless of the physiognomy (Figure 3d).

Effects of rainfall reduction on soil N and P availability

The effect of the rainfall reduction treatment on soil moisture varied among physiognomies. On average, the rainfall reduction structures reduced soil moisture between 10-32% in the open savanna, and between 4-8% in the woodland savanna (Table 3). Surprisingly, our results suggest that the structures resulted in a consistent increase (between 12 and 117%) in the soil moisture of the intermediate savanna plots (Table 3).

The rainfall reduction treatment significantly reduced the two-years average values of soil NO_3-N and TIN availability, considering all physiognomies together (Figure 4). The effect was similar but not significant for soil NH_4-N ($p=0.064$; Figure 4a). The direction of the effect of the rainfall reduction treatment was the same for all physiognomies, and we found the same significant differences among physiognomies in control and treated plots (Figure 4). Thus, we did not find a significant interaction between the physiognomy and the rainfall reduction treatment ($p > 0.05$). However, considering physiognomies separately, the effect of the rainfall reduction on N availability was only significant in the woodland savanna, and for all the N forms (Table 4). Further, considering also the year and the season in the full model, we only found significant differences between control and treated plots in soil NO_3-N and NH_4-N availability of the woodland savanna in the late rainy season of the second year (Figure 5).

The rainfall reduction treatment did not significantly affect P availability considering the physiognomies neither together ($p=0.248$) nor separately (Table 4). However, we found a significant interaction between physiognomy and rainfall reduction (Chi=9.63; $p=0.008$), as the differences observed among physiognomies in the control plots (see above) disappeared in the treated plots ($p=0.057$; Figure 4d).

Discussion

Our results confirmed the hypothesis of open physiognomies (i.e. open savanna) having less soil N and P available than closed physiognomies (i.e. woodland savanna). Except in the dry season, the woodland savanna consistently showed the highest values of soil N availability along the two years of our study. In the Cerrado biome, the production of litter is highest in areas with more tree cover, increasing the soil organic matter and nutrients content (Pellegrini et al., 2014). These high soil organic matter and total N content of the woodland savanna (Table 1) are likely to favor N mineralization and availability (Hossain et al., 1995; Li et al., 2003). On the other hand, specific characteristics of woodland savanna soils such as high depths and clayey textures favor soil water and nutrient retention and storage, increasing nutrient availability and favoring the tree species establishment and growth (Neufeldt et al., 2002). Thus, our results align with the idea that plant-soil interactions determine and maintain the physiognomic specificities within the Cerrado biome.

Of particular interest are the low values of nitrate in relation to ammonium that we found in all physiognomies. The low availability of NO_3-N in soils of the Cerrado, despite the observed high NH_4-N availability, could be related to low nitrification rates linked to the particularly low populations of nitrifying bacteria in these soils (Bustamante et al., 2006). Alternatively, this could be also attributed to a great demand of nitrate from soil microbiota and plants (Parron et al., 2003). In any case, NH_4-N as the dominant form of inorganic N can be interpreted as a sign of a particularly conservative N cycle in the Cerrado (Pinto et al., 2002).

The open savanna showed the lowest levels of soil P availability among all physiognomies. Several mechanisms could be behind this result. First, latosols with lower clay contents, as those of the more open physiognomies (Table 1), have lower total soil P contents (Neri et al., 2013; Resende et al., 2011). Second, the lower amount of soil organic matter and C in the open savanna compared to those of the other physiognomies could also be behind its lower P availability (Bezerra et al., 2015; Eberhardt et al., 2008; Lardy et al., 2002). Third, litter quality (i.e. nutrient content) may also help to explain these differences. Different physiognomies have different species composition with different litter qualities (Neri et al., 2012; Ribeiro and Walter, 1998). For instance, the amount of P in the litter of trees from the denser physiognomies tend to be higher than that of more open physiognomies, as a result of a higher resorption efficiency linked to lower soil P availability (Ratnam et al., 2008; Vourlitis et al., 2014). Finally, higher Al^{3+} contents in the open savanna soils (Neri et al., 2013) might occlude soil organic P hindering its mineralization and therefore decreasing its availability (Eberhardt et al., 2008; Neri et al., 2013).

As we hypothesized, soil N (TIN, NH_4-N and NO_3-N) availability was significantly lower in the dry season than in the other seasons, particularly in the woodland savanna. Soil moisture has been recognized as one of the main drivers of N mineralization in savannas (Bustamante et al., 2006). Even in gallery forests inside the Cerrado biome (under the same seasonal climate of the studied areas), the N mineralization presents seasonal dynamics, with reductions in the driest period (Bustamante et al., 2006; Parron et al., 2011, 2003). Our results reflect the soil moisture-dependence of N mineralization and availability. The fact that this seasonal pattern was not significant in the intermediate and open savanna supports the hypotheses that different physiognomies have different sensitivities to the typical seasonality of this biome. More importantly, they also suggest that different physiognomies might respond differently to forecasted changes in soil moisture due to climate change (see below). This different response may be at least partially related to differences in soil N content among these three physiognomies (Table 1). The lower soil N contents in the intermediate and open savanna could limit plant and microbial growth along the year, driving to a faster N uptake and cycling when it becomes available (Nardoto & Bustamante, 2003). Thus, although N mineralization and nitrification rates could be also higher during the rainy season in these two physiognomies, a higher N demand may decrease this new available N, counteracting the positive effect of the rainy season.

The seasonal pattern of soil P availability was not as clear as that of soil N availability. During the first year of the study, soil P availability showed, as we expected, the lowest values in the rainy season. Phosphorus is the most limiting nutrient in savannas worldwide (Abrahão et al., 2019; Grace et al., 2006; Pellegrini, 2016; Resende et al., 2011). Consequently, plants and microorganisms are likely to strongly compete for it during the most active (rainy) season, therefore decreasing its availability (Abrahão et al., 2019; Bustamante et al., 2012, 2006; Nardoto et al., 2013; Resende et al., 2011). However, when we considered the physiognomies separately, the intermediate savanna did not follow this seasonal pattern, confirming again the different sensitivities among physiognomies to seasonality. In addition, available P values dropped dramatically from the rainy season of the second year of the study. We cannot explain all these different temporal patterns of soil P availability, as they were related neither to soil water content nor to any of the physiognomy-related variables we studied. Different forms of soil P interact with several biotic (e.g. plants, microorganisms) and abiotic (e.g. minerals, organic matter, climate) factors, which makes particularly challenging to assess both its fate and main drivers (Ruttenberg, 2003). Also, the Cerrado biome is known for a dependence on the organic P turnover (Resende et al., 2011). As a result, it is likely that the availability of P could be more controlled by biotic processes (such as plant uptake and microbial mineralization, immobilization, and solubilization) than by seasonal changes on soil water content (Bezerra et al., 2015; Cross and Schlesinger, 1995; Pavinato and Rosolen, 2008). In any case, this study adds evidence to the inherent complexity of the P cycle, highlighting the need for more studies assessing its main mechanistic drivers and its potential responses to the ongoing environmental changes.

Similar to what we found with the effect of seasonality on soil N and P availability, our rainfall reduction structures influenced N and P availability in opposite ways, tending to decrease N availability, but to increase that of P, with exception of the intermediate savanna, where P availability also tended to decrease. These antagonistic effects on the soil N and P availability under a soil moisture reduction can have direct effects on organisms and on ecosystem structure and function by themselves, but also indirect effects via alterations in the

N:P ratio (Peñuelas et al., 2013, 2012; Yuan and Chen, 2015). In a study carried out in a wide aridity gradient, Delgado-baquerizo et al. (2018) found that aridity is negatively correlated with available N but positively with available P in soil, which could lead to a decoupling of these two elements. Here we add experimental evidence that changes in water availability could affect in opposite directions to soil N and P availability, which could influence ecosystem functioning in the medium term and promote evolutionary long-term changes in the stoichiometry of the whole ecosystem (Delgado-baquerizo et al., 2018; Peñuelas et al., 2012).

As we observed with seasonality, the woodland savanna was the most responsive physiognomy to the rainfall reduction in terms of soil N availability, despite having lower rainfall reduction values (4-8%) than the open savanna (10-32 %). The reasons are likely linked to the differences in the nutritional status of both physiognomies (see above). More importantly, these results add novel experimental evidence to the idea that different physiognomies might respond differently to forecasted changes in soil moisture due to climate change. Regarding the response of P availability to the rainfall reduction treatment, the intermediate savanna did not follow once again the general pattern observed in the other two physiognomies. This response of the intermediate savanna could be due to the particularly low efficiency of the rainfall reduction structures in this physiognomy (Table 3). In the denser physiognomies (i.e. woodland and intermediate savanna) a significant fraction of the rain may be intercepted by the canopy and flow through the branches and trunk towards the soil (Klaassen et al., 1996), which would reduce the efficiency of our structures in excluding part of the rainwater. On the other hand, contrary to what we would have expected in terms of soil characteristics (e.g. soil organic matter content, clay content), the soil moisture registered in the open savanna was much higher than that in the intermediate and woodland savanna (Table 3, Figure S1). The high levels of silt in this physiognomy may be behind this result as silt (fine) particles account for greater water retention (Ferrari et al., 2016). In addition, in grasslands, there may be more moisture in the soil surface layer due to entry of moisture from nocturnal dew (Ferrari et al., 2016), which is common in the study area. All these facts suggest that using soil moisture sensors in the most superficial soil layers may not be the best way of evaluating soil water availability and the efficiency of our rainfall exclusion structures. These insights should be considered in future studies that seek to simulate climate change in the different Cerrado physiognomies. However, the consistently different responses of P availability in the intermediate savanna and the highest responses of N in the woodland savanna to both seasonality and the rainfall reduction treatment, provide strong evidences that soil nutrient availability of the different Cerrado physiognomies may be affected in different ways by forecasted changes in climate, thus reinforcing the need of considering this particular characteristic of the Cerrado in future studies.

Interestingly, the second year showed higher soil moisture values than the first year in the woodland savanna (0.129 and 0.110 m^3 m^{-3}, respectively), and slightly higher in the intermediate savanna (0.111 and 0.106 m^3 m^{-3}, respectively), but not in the open savanna (0.185 and 0.203 m^3 m^{-3}, respectively). This could explain why we observed a higher N availability and a higher response of this nutrient to the rainfall reduction treatment in the second than in the first year only in the woodland savanna. All our physiognomies are located on the same site with the same climatic conditions, so observing or not differences in soil moisture among years is likely to be dependent on physiognomy-specific plant-soil interactions. Thus, our results indicate that particular plant-soil features of the woodland savanna could make this physiognomy more sensible to short- (season and annual) and long-term (climate change) changes in soil water availability than the other two physiognomies. For instance, this sensitivity could lead to a restriction of N-dependent soil species and an increase in leguminous species, changing the species composition in this physiognomy.

In summary, our study shows that the availability of N and P in the Cerrado varies with physiognomy, season and year. Further, our results suggest that rainfall reductions could decrease N availability while increasing P availability in this important biome, although the intensity and the direction of the response are likely to vary among physiognomies. Longer and more experimental studies will be however necessary to better understand the responses of this important ecosystem to future climate change scenarios. Particularly, future work should address the implications of these changes on the microbial and plant functioning of Cerrado.

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Figures

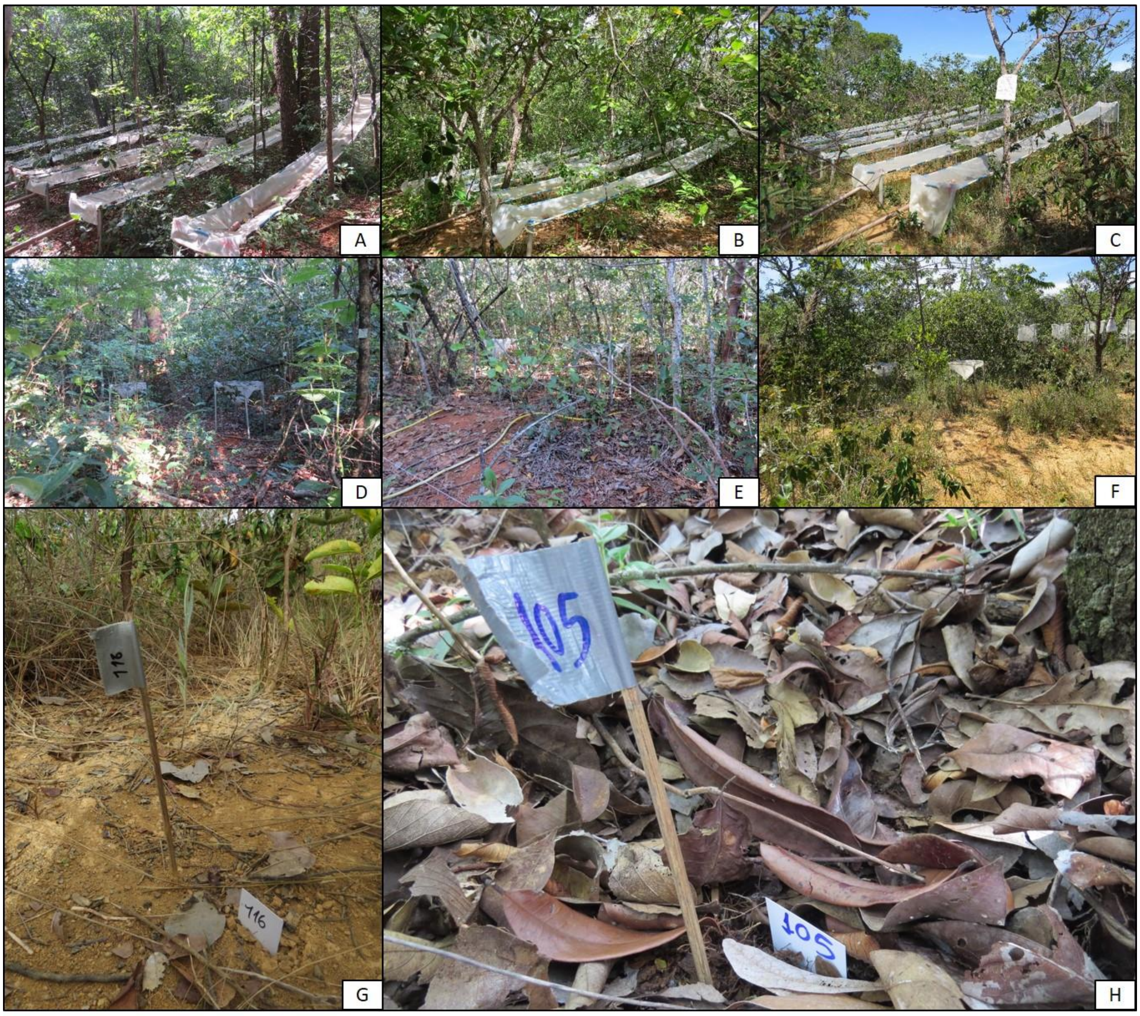

Figure 1: Rainfall reduction experiment in three physiognomies in the Paraopeba Reserve. A: treated plot in the woodland savanna. B: treated plot in the intermediate savanna. C: treated plot in the open savanna. D: control plot in the woodland savanna. E: control plot in the intermediate savanna. F: control plot in the open savanna. G: Ion exchange membrane incubated in the open savanna (treated plot). H: Ion exchange membrane incubated in the Intermediate savanna (control plot). Pictures: Mota, NM.

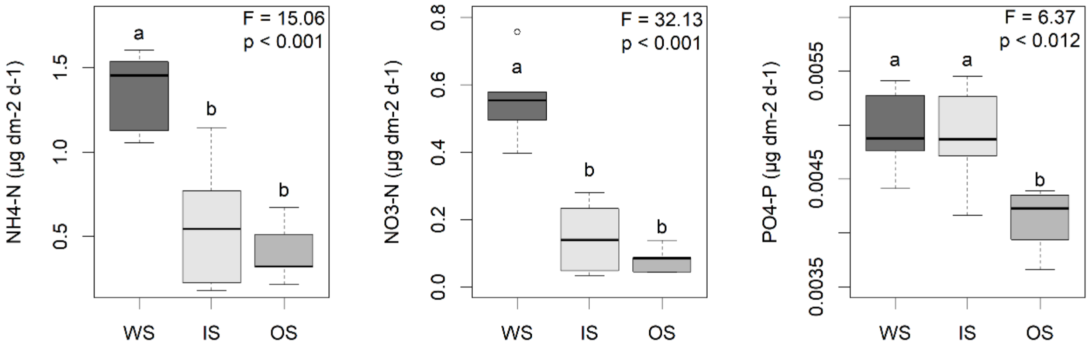

Figure 2: Boxplots of the two-years average values of soil N and P availability in the three Cerrado physiognomies. Boxes, the horizontal line inside the boxes and the whiskers represent the second and third quartiles, the median value, and the lower and the upper quartile, respectively. WS: woodland savanna; OS: open savanna; IS: intermediate savanna. Different letters indicate significant differences among physiognomies ($p < 0.05$).

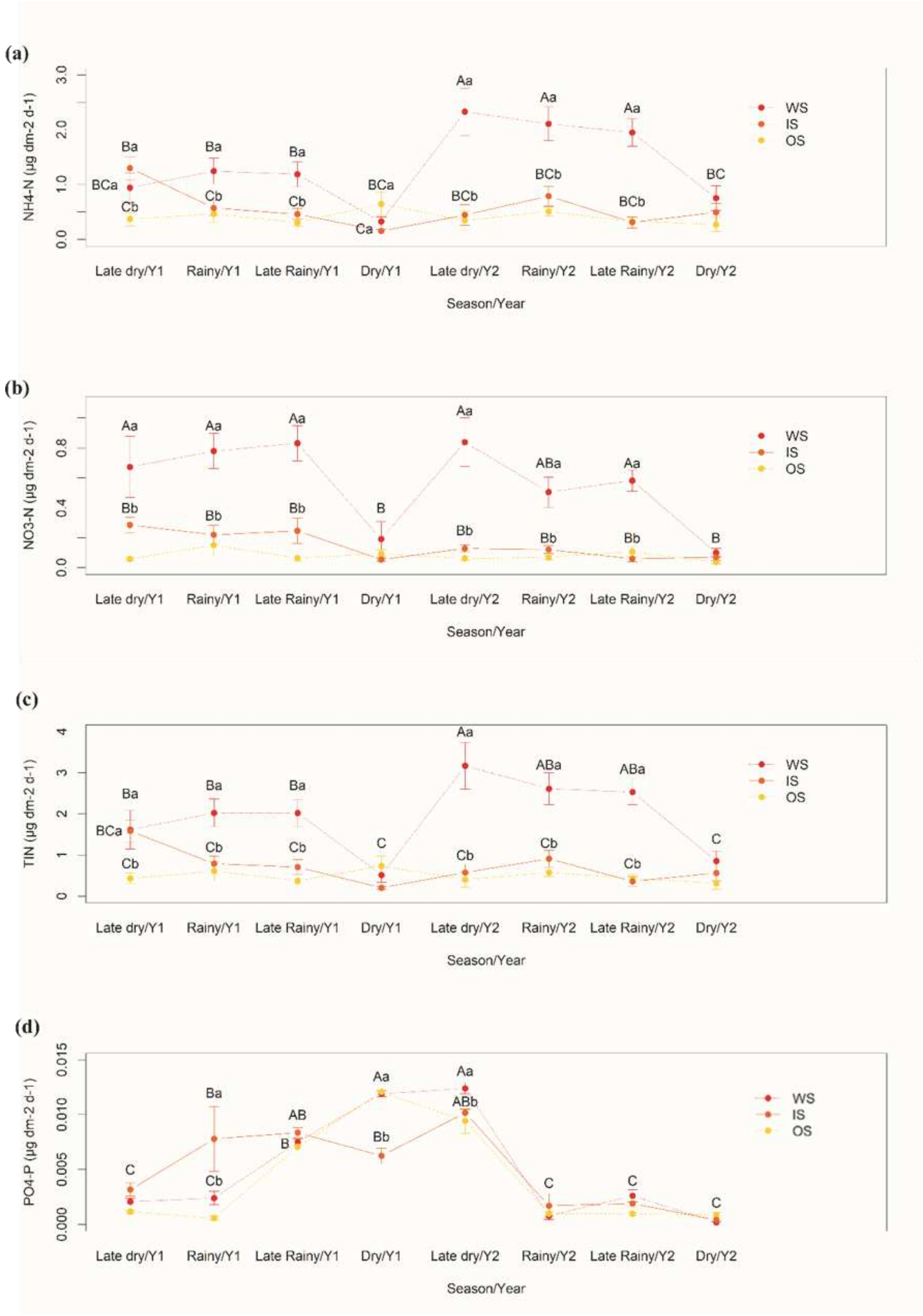

Figure 3: Soil N and P availability throughout the two years of study in the three Cerrado physiognomies. TIN: total inorganic nitrogen. WS: woodland savanna; OS: open savanna; IS: intermediate savanna. Dots and horizontal bars are means and standard errors, respectively.

Uppercase letters indicate differences among seasons in the same physiognomy; lowercase letters indicate difference among physiognomies in the same season and year.

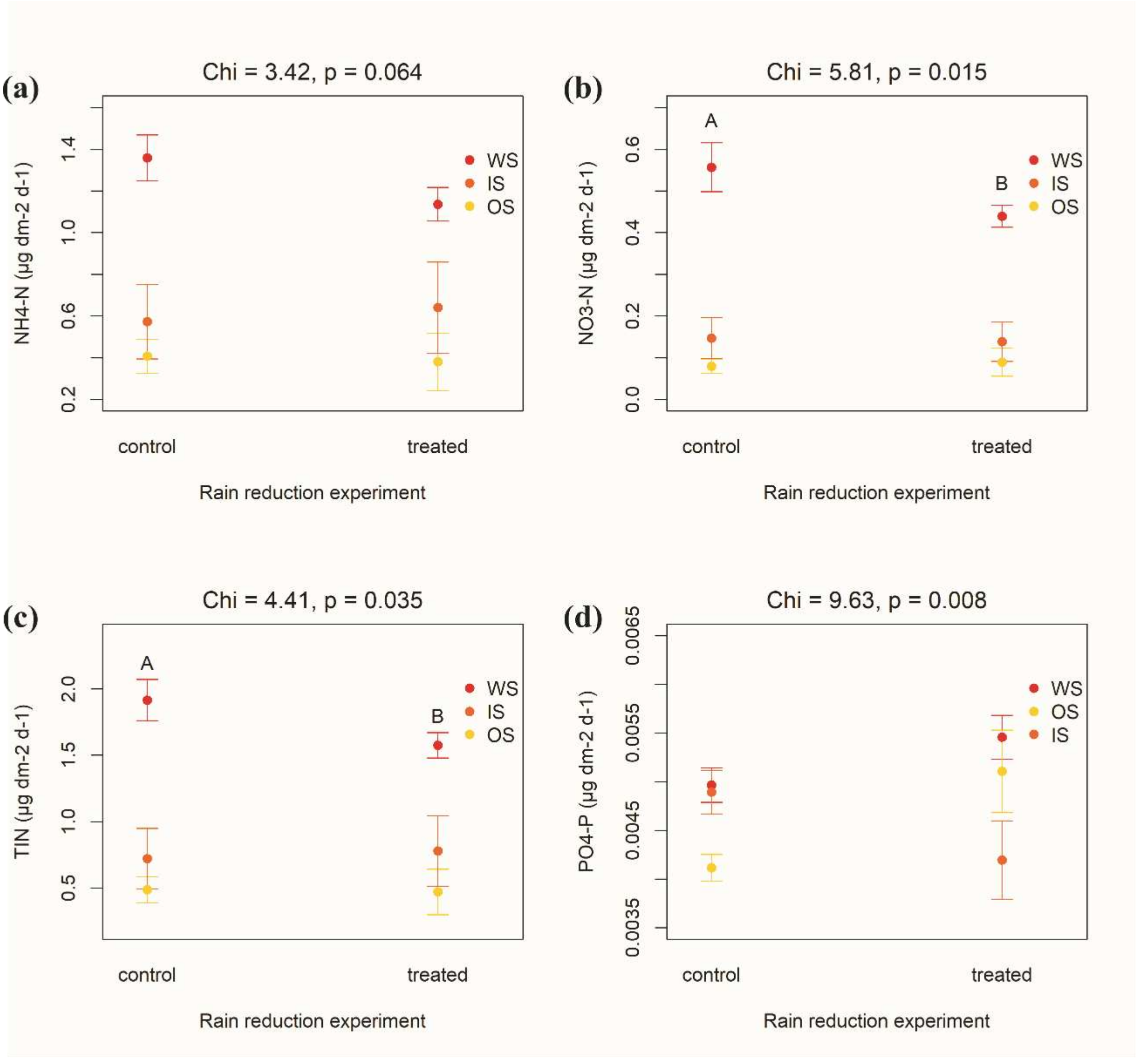

Figure 4: Effect of the rainfall reduction treatment in the two-years average values of soil N and P availability for the three Cerrado physiognomies. Dots and horizontal bars are means and standard errors, respectively. Different letters indicate significant differences ($p < 0.05$) between control and treated plots for each physiognomy separately. TIN: total inorganic nitrogen.

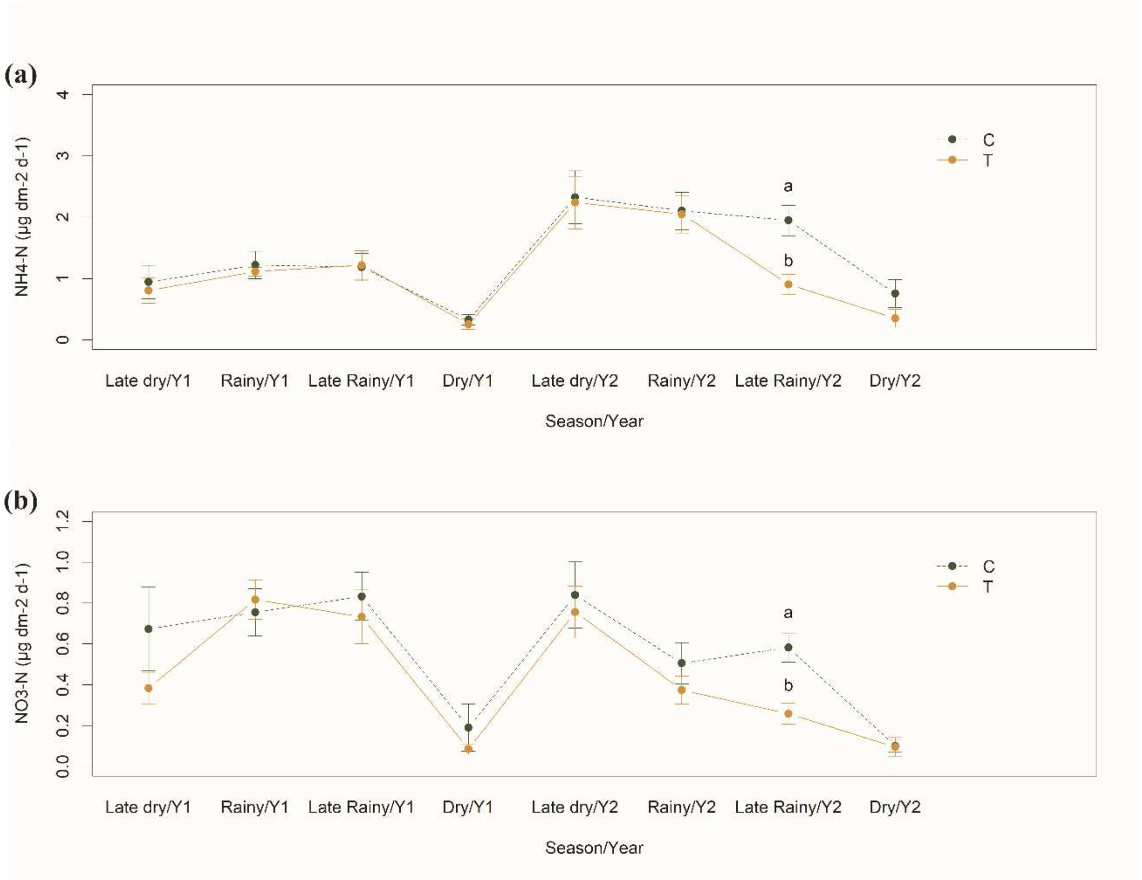

Figure 5: Nitrate (NO_3^-) and ammonium (NH_4^+) availability in woodland savanna throughout the two years under the rainfall reduction experiment. C: control plots; T: treated plots. Dots and horizontal bars are means and standard errors, respectively. Different letters indicate differences between treatments in the same season and year.

Tables

Table 1: Physicochemical variables for each Cerrado physiognomy*. Values represent the mean (±1SE). Statistically significant effects of physiognomy are represented by bold P values (PERMANOVA). Different letters in each variable represent significant differences among physiognomies ($P < 0.05$). SWC= soil water content; OM= organic matter; TC= total C, TN= total N; TIN= total inorganic N; CEC= cationic exchange capacity; WHC= water holding capacity; WS: woodland savanna; IS: intermediate savanna; OS: open savanna.

					Permutations	
	n	WS	IS	OS	*P*	Pseudo-F
SWC (%)	30	16.4 (1.17)	16.09 (0.38)	13.51 (0.47)		
Sand (%)	5	3.06 (0.59)	2.56 (0.21)	2.82 (0.27)	0.847	0.197
Silt (%)	5	22.8 (2.40) a	39.3 (4.02) b	42.6 (0.99) b	**0.001**	15.03
Clay (%)	5	74.1 (2.94) a	58.2 (3.89) b	54.6 (1.11) b	**0.002**	11.84
OM (%)	30	5.86 (0.21) a	4.78 (0.11) b	3.16 (0.22) c	**0.001**	43.37
TC (%)	5	3.55 (0.34) a	2.57 (0.11) b	1.70 (0.07) c	**0.001**	31.29
TN (%)	5	0.30 (0.05) a	0.25 (0.01) a	0.14 (0.03) b	**0.018**	5.701
C:N	5	12.6 (1.28)	10.4 (0.66)	16.2 (4.42)	0.399	0.971
pH	30	4.58 (0.13) a	4.99 (0.08) b	5.03 (0.07) b	**0.012**	6.998
NH_4^+-N (mg kg^{-1} soil)	30	31.9 (4.33) a	11.6 (2.64) b	9.25 (2.11) b	**0.003**	10.51
NO_3^--N (mg kg^{-1} soil)	30	43.3 (10.2) a	10.4 (6.43) b	15.1 (5.68) b	**0.019**	5.403
TIN (mg kg^{-1} soil)	30	75.2 (11.8) a	22.0 (7.45) b	24.4 (5.65) b	**0.004**	8.275
PO_4^{-3}-P (mg dm^{-3})	30	1.59 (0.17) a	1.29 (0.06) a	0.65 (0.12) b	**0.003**	12.86
CEC (cmol dm^{-3})	30	11.0 (0.44) a	10.1 (0.34) a	7.35 (0.33) b	**0.000**	28.62
WHC (%)	5	46.3 (1.10) b	48.4 (0.31) b	50.2 (0.29) a	**0.007**	7.879

*Data of Durán et al. (unpublished)

Table 2: Effects of physiognomy (Phy), season (S), and year (Y), as well as their interactions, on soil N and P availability. Statistically significant effects are represented by bold P values.

Mixed models	NH_4-N		NO_3-N		TIN		PO_4-P	
	X^2	P	X^2	P	X^2	P	X^2	P
Phy	27.52	**<0.0001**	65.01	**<0.0001**	34.97	**<0.0001**	6.05	**0.0485**
S	54.61	**<0.0001**	41.02	**<0.0001**	65.26	**<0.0001**	85.52	**<0.0001**
Y	3.89	**0.0486**	6.38	**0.0115**	0.71	0.3992	53.06	**<0.0001**
Phy x S	38.89	**<0.0001**	43.94	**<0.0001**	50.01	**<0.0001**	52.57	**<0.0001**
Phy x Y	29.54	**<0.0001**	1.62	0.4436	17.58	**0.0001**	1.35	0.5080
S x Y	5.44	0.1424	4.07	0.2530	2.67	0.4450	465.41	**<0.0001**
Phy x S x Y	27.60	**0.0001**	9.16	0.1646	24.37	**0.0089**	41.37	**<0.0001**

Table 3: Means of soil moisture (SM) and rainfall reduction (RR) in the three Cerrado physiognomies for each season and year. Soil moisture of treated plots (SM_t) was estimated from sensors installed both under and out the plastic band. Bold RR values denote soil moisture reductions whereas negative RR values denote increases in soil moisture. Late-dry: September, October and November; Rainy: December, January and February; Late-rainy: March, April and May; Dry: June, July and August. WS: woodland savanna; IS: intermediate savanna; OS: open savanna.

			Soil moisture (m^3/m^3 VWC)		
Year/Season		**Physiognomy**	**SM_c (m^3/m^3 VWC)**	**SM_t (m^3/m^3 VWC)**	**RR (%)**
First year	Late-dry	WS	0.1011	0.1031	- 2
		IS	0. 0972	0.109	- 12
		OS	0.1947	0.1581	**19**
	Rainy	WS	0.1501	0.1942	- 29
		IS	0. 1571	0.1855	- 18
		OS	0.2874	0.2186	**24**
	Late-rainy	WS	0.1056	0.1330	- 26
		IS	0.1238	0.1556	- 26
		OS	0.2225	0.1514	**32**
	Dry	WS	0.0822	0.0787	**4**
		IS	0. 0457	0.0994	- 117
		OS	0.1079	0.0811	**25**
Second year	Late-dry	WS	0.1426	0.1197	**8**
		IS	0. 1061	0.1347	- 27
		OS	0.1623	0.1153	**29**
	Rainy	WS	0.1568	0.1711	**9**
		IS	0. 1542	0.1656	- 7
		OS	0.2218	0.1988	**10**
	Late-rainy	WS	0.1257	0.1154	- 8
		IS	0. 1204	0.1501	- 25
		OS	0.2034	0.1580	**22**
	Dry	WS	0.0902	0.0840	**7**
		IS	0. 0620	0.1186	- 91
		OS	0.1531	0.1275	**17**

Table 4: Effects of the year (Y), season (S), and the rainfall reduction (RR) treatment, as well as their interactions, on soil N and P availability in the three Cerrado physiognomies. Statistically significant effects are represented by bold P values. WS: woodland savanna; IS: intermediate savanna; OS: open savanna.

Mixed models	WS		IS		OS	
NH_4-N	X^2	P	X^2	P	X^2	P
Y	18.46	**<0.0001**	0.79	0.3739	1.17	0.2788
S	112.44	**<0.0001**	6.85	0.0767	6.94	0.0739
RR	4.70	**0.0302**	0.67	0.4141	0.45	0.5029
S x RR	1.26	0.7392	2.16	0.5391	0.97	0.8068
S x Y	14.83	**0.0020**	7.55	0.0561	2.61	0.4561
Y x RR	3.38	0.0661	1.78	0.1818	1.98	0.1593
S x Y x RR	3.37	0.3374	6.54	0.0882	2.49	0.4770
NO_3-N	X^2	P	X^2	P	X^2	P
Y	4.45	**0.0350**	0.22	0.6368	0.02	0.8903
S	122.31	**<0.0001**	18.00	**0.0004**	7.91	**0.0479**
RR	9.20	**0.0024**	0.11	0.7507	0.03	0.8526
S x RR	5.97	0.1130	1.14	0.7666	2.94	0.4017
S x Y	26.67	**<0.0001**	1.71	0.6338	4.76	0.1901
Y x RR	0.00	0.9840	0.16	0.6933	1.01	0.3159
S x Y x RR	3.42	0.3311	6.05	0.1090	1.33	0.7210
TIN	X^2	P	X^2	P	X^2	P
Y	6.20	**0.0128**	1.65	0.1988	0.79	0.5395
S	158.94	**<0.0001**	4.21	0.2391	6.85	0.0767
RR	5.75	**0.0302**	0.37	0.5395	0.67	0.4141
S x RR	2.29	0.5133	1.36	0.4231	2.16	0.5391
S x Y	14.85	**0.0019**	1.76	0.1149	7.55	0.0561
Y x RR	2.19	0.1388	1.65	0.1724	1.78	0.1818
S x Y x RR	3.06	0.3827	2.36	0.0561	6.54	0.0882
PO_4-P	X^2	P	X^2	P	X^2	P
Y	3634.68	**<0.0001**	26.65	**<0.0001**	2007.38	**<0.0001**
S	371.59	**<0.0001**	1378.95	**<0.0001**	2392.56	**<0.0001**
RR	0.00	0.94	0.21	0.6463	0.54	0.4624
S x RR	1.26	0.74	2.81	0.4223	3.57	0.3117
S x Y	1742.25	**<0.0001**	489.54	**<0.0001**	1367.92	**<0.0001**
Y x RR	0.27	0.6065	0.03	0.8649	2.49	0.1146
S x Y x RR	3.82	0.2818	5.96	0.1134	5.15	0.1614

Supplementary material

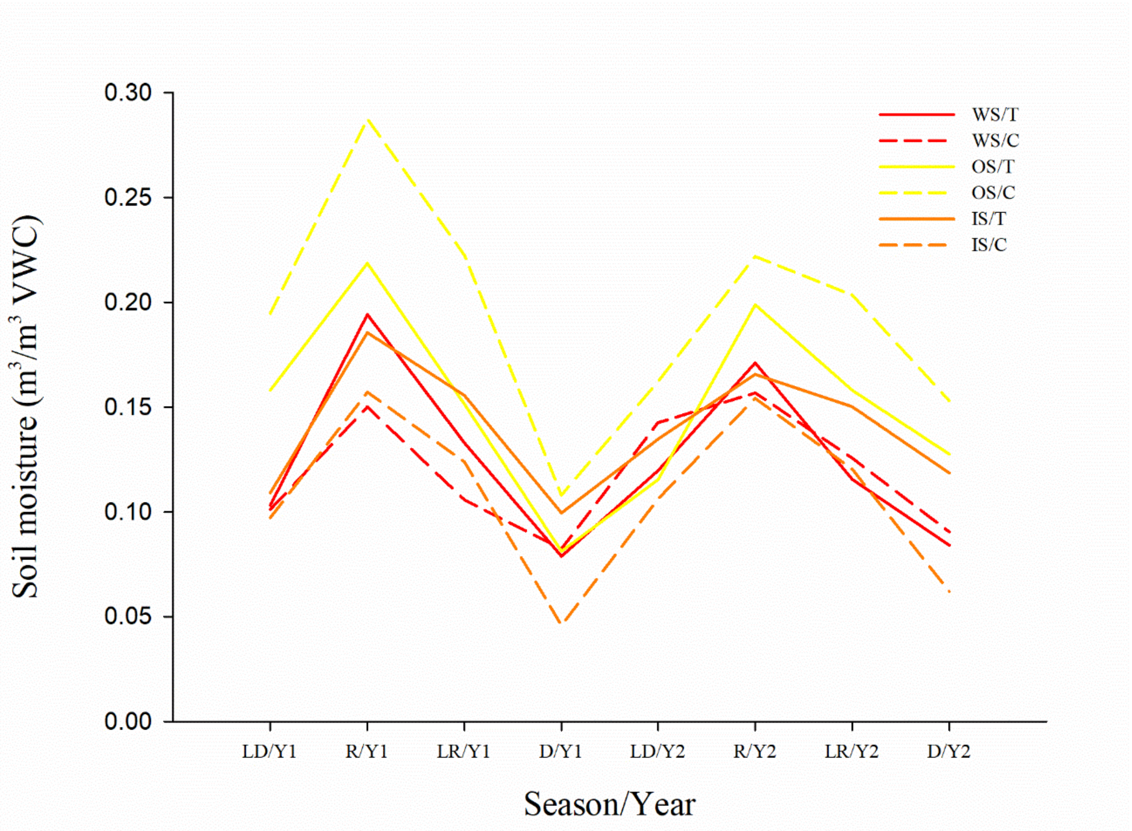

Figure S1: Means of soil moisture for each season and year in control (C) and treated (T) plots of the three Cerrado physiognomies (WS: woodland savanna; IS: intermediate savanna; OS: open savanna). LD (Late-dry): September, October and November; R (Rainy): December, January and February; LR (Late-rainy): March, April and May; D (Dry): June, July and August. Y1: first year of experiment; Y2: second year of experiment.

CHAPTER 3*: Soil functioning under experimental water reduction in three physiognomies of Cerrado (Brazilian savannah)

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Abstract

Carbon (C) and nitrogen (N) dynamics are likely to change with forecasted climate change. In most Cerrado (Brazilian savannah) regions, climate models project decreases in current precipitation rates from 20 to 70%. We used two different approaches (i.e. an *in situ* rainfall reduction and laboratory incubations) to investigate the effects of changes in soil moisture on soil functioning in the three dominant physiognomies of Cerrado (i.e. woodland, intermediate and open savannah). The woodland savannah exhibited the highest N mineralization and N_2O emission rates, whereas the open savannah showed the lowest CO_2 emissions and microbial functional diversity and evenness. *In situ* rainfall reduction resulted in a significant increase in soil N contents, and lower soil ammonification in comparison to control plots, regardless of the physiognomy type. Decreases in soil moisture through laboratory incubations resulted in increased nitrification rates, but only in soils from the woodland savannah, and decreased soil respiration and N_2O fluxes in all physiognomies. Changes in soil moisture from 80% to 40% WHC affected microbial functional evenness in the intermediate savannah soil (higher at 40% than 80% WHC), and they modulated the relationships of surrogates of microbial biodiversity with soil respiration and N transformation rates. Potential soil CH_4 fluxes did not differ among physiognomies or with *in situ* rainfall reduction, but CH_4 uptake during laboratory incubations was lower at 80%WHC than at 40%WHC, regardless of physiognomy type. Altogether, our results indicate that climate change-related changes in soil moisture and in the relative dominance of the different Cerrado physiognomies would likely change the capacity of this biome to mineralize and stock N, and to exchange GHG with the atmosphere.

Key words: soil moisture, GHG emissions, biogeochemical processes, microbial functional diversity, soil respiration.

Introduction

Savannahs cover 20% of the world's land surface and account for almost 30% of global net primary production (Hutley and Setterfield, 2008). Moreover, savannahs constitute the largest surface C pool accounting for about 10 to 30% of the world soil C stock (Bernoux et al., 2006; Grace et al., 2006). The soils of the Brazilian savannah (i.e. Cerrado), which is the largest neotropical savannah, can store up to 90% of the total C of the ecosystem (Morais et al., 2013). Changes in soil characteristics and functioning in these ecosystems can lead to changes in N and C stocks and alterations in net greenhouse gases emissions (i.e. CO_2, CH_4, and N_2O) to the atmosphere (Bernoux et al., 2001; Lal, 1997). However, studies on the functioning of Cerrado soils are still scarce (Butler et al., 2012; Grace et al., 2006).

The Cerrado is characterized by high spatial heterogeneity, which is a result of different edaphic attributes (e.g. soil texture, nutrient availability, heavy metals content) and intricate plant-soil interactions that determine the size and composition of vegetation (Goodland and Pollard, 1973; Walter et al., 2008). Therefore, the distribution of plant species is strongly associated with soil fertility and there are strong links among the different Cerrado vegetation formations (hereafter physiognomies) and soil functioning (de Carvalho Mendes et al., 2012; Meira-Neto et al., 2017; Neri et al., 2012). The Cerrado physiognomies vary from forests (woodland savanna) to open physiognomies with a prominent herbaceous cover (Cerrado *sensu stricto*; Ribeiro & Walter, 1998). In other savannah ecosystems, grasses and trees have contrasting influences on soil functioning (e.g. dominant grasses might inhibit nitrification processes, whereas trees dominance might stimulate them; Srikanthasamy et al., 2018). Thus, it is expected that different Cerrado physiognomies show different values of key ecosystem processes and aspects such as greenhouse gases (GHGs) fluxes, C and N cycling, and microbial functional diversity.

The Cerrado is among the world's wettest savannahs, yet it has a very seasonal climate characterized by dry winters with little or no rain that last for five months (Neri et al., 2012). In most Cerrado regions, climate models forecast a decrease in the average precipitation from 20 to 70% of the current values, and a decrease in the number of rainy days per year, prolonging the dry season (Bustamante et al., 2012; Marengo et al., 2010). Soil water availability drives a myriad of biogeochemical processes, including C and N cycling, as well as the exchange of GHGs between the soil and the atmosphere (Cardenas et al., 1993). Thus, forecasted changes in precipitation will likely have significant impacts on many biogeochemical processes (Austin et al., 2004). However, each Cerrado physiognomy may respond differently to changes in soil moisture, as they are characterized by particular soil features and unique plant and soil microbial communities (de Carvalho Mendes et al., 2012; Neri et al., 2013). Thus, to effectively manage and conserve the Cerrado ecosystem, it is crucial to specifically evaluate the impact of climate change on the different Cerrado physiognomies functioning (Bustamante et al., 2012). Many studies aimed to understand how changes in precipitation could affect different ecosystems, but most of these studies are observational, and in some cases, carried out due to the occurrence of extreme climatic events, being therefore rarely replicable (Smith, 2011). More manipulative experiments are still necessary to understand the effects of rainfall decrease on different ecosystem features (Asbjornsen et al., 2018; Smith, 2011). This is particularly true for the Cerrado ecosystem, where, to our knowledge, no such studies had yet been conducted.

Both field-based rainfall reduction experiments (e.g. Homyak et al., 2017; Li et al., 2018; Sayer et al., 2017) and laboratory soil incubations at different moisture conditions (e.g Garten et al., 2009; Sun et al., 2019) have been shown to effectively contribute to the understanding of the impact of forecasted climate changes to soil functioning (Srivastava et al., 2017). In this study we combined both experimental approaches to investigate the potential effects of decreases in water availability on different soil C- and N-related processes in different Cerrado physiognomies. We hypothesized that water availability would influence soil N mineralization, GHG fluxes, and microbial functional diversity in the Cerrado; but that the effect would be different among the three dominant Cerrado physiognomies. We also investigated whether the relationships among soil N mineralization, GHG fluxes, and microbial functional diversity varied among physiognomies, and whether soil water availability modulates these relationships.

1. Materials and methods

1.1. Study site

The study was carried out in the Paraopeba Reserve (*Floresta Nacional de Paraopeba*; southeast of Brazil; 19º20'S, 44º20'W), which has a total area of 200 ha (Neri et al., 2012). Among the physiognomies present in the Paraopeba Reserve (Figure 1; Neri et al., 2013, 2012), we chose the three dominant ones: woodland savannah, intermediate savannah, and open savannah. According to Neri et al. (2013), the woodland savannah (or dystrophic Cerradão) is a woodland formation developed on the Red Latosol (Oxisols) soil type, with high Al^{3+} and organic matter contents, as well as very clayey soil texture. The intermediate savannah (or closed savannic Cerrado) is a type of savannic Cerrado developed on the Yellow Red Latosol soil type, with greater density of woody plants than that of the graminoid layer. The open savannah (or Cerrado *sensu stricto*) is characterized by well-defined graminoid, shrub, and tree layers developed on the typical Yellow Latosol soil type. Altogether, these three physiognomic types occupy ~70% of the entire Cerrado biome (Eiten, 1972).

1.2. Experimental design and soil sampling

In August 2015, we established 15 rainfall reduction plots (7 m x 7 m) equally distributed among the three dominant physiognomies in the Paraopeba Reserve (i.e. five per physiognomy; Figure 2). For each treatment plot, a control plot of the same size was established at approximate distance of 10 meters, totalling 30 plots.

The manipulated rainfall reduction experiment consisted in six transparent plastic bands (50 cm wide and 10 m long) per plot, which were connected to PVC tubes (15 cm of perimeter and 2 m of length) which drained a share of the precipitation out of the plot (Figure 2). Soil moisture and temperature (Table 1 and S1, respectively) were monitored with soil moisture sensors (CR10X-ECH2O; Decagon Devices, Inc., Pullman WA, USA) connected to data loggers (HOBO® H08-006-04; Onset, Pocasset, MA, USA), and temperature sensors (copper-constantan thermocouple). Rainfall reduction (RR) in each treated plot was estimated as:

$$RR(\%) = \frac{(SM_c - SM_t)}{SM_c} \times 100$$

Where SM_c is the soil moisture of the control plot, and SM_t is the soil moisture of the treated plot (under the plastic bands – rainfall exclusion).

In July 2017, two years after the beginning of the experiment, we collected three soil samples (5 cm diameter x 15 cm depth) in each control and treated (under plastic bands) plot. Soil samples were then homogenized by hand and sieved through a 2 mm sieve in order to remove rocks, roots, and other large organic particles. Then, we made one composite sample from the three samples of each control and treated plot totalling 30 soil samples. We used these composite soil samples for downstream analyses.

1.3. Potential GHG fluxes, N transformation rates and microbial functional diversity

We first calculated soil water holding capacity (WHC) for each soil sample following Morillas et al. (2015). To assess the effect of two years of rainfall exclusion on potential soil functioning rates and microbial functional diversity in the different physiognomies, we placed 60 g of each of the 30 samples in glass jars (750 ml) and incubated them for 15 days in the dark at 25°C and 80% WHC. In order to avoid soil drying but allowing gas exchange, the jars were closed with plastic wraps (with 10 holes approximately 2 mm in diameter) secured with rubber bands. Samples were weighed daily so any moisture loss was compensated by adding an equivalent amount of deionized water. Additionally, to estimate the direct effects of changes in soil moisture on potential soil functioning rates and microbial functional diversity in the different physiognomies, we also made incubations of soil samples from the control plots at 40% WHC. To avoid methodological biases, both the location of the samples within the incubation chambers and the measurement order were fully randomized.

Soil inorganic N was analysed before and after soil incubations to estimate potential N mineralization rates. First, soil inorganic N (NH_4-N and NO_3-N) was extracted with 2M KCl, shaking for 1 h at 200 rpm in an orbital shaker. Then, we calculated the amount of NH_4-N and NO_3-N in the extracts by colorimetry using a microplate reader (MB-580; see Durán et al., 2009 for futher details on this methodology).

To determine soil–atmosphere CO_2, N_2O, and CH_4 fluxes, we carried out six gas collections during the 15 days of incubation. To do so, in the collection day, we covered the glass jars with aluminum caps and a rubber septum (which allowed gas sampling), and incubated the soil samples for 60 min at 25°C. To estimate the net gas exchange between the soil and the headspace, we collected 22 ml gas samples of the jar headspace at the start and at the end of each incubation period using a fine needle polypropylene syringe. The collected gases were transferred to evacuated 20-ml glass vials (i.e., overpressured to prevent dilution by the ambient atmosphere) and stored at room temperature. The amount of N_2O, CO_2, and CH_4 in the vials were analyzed with a mass selective detector (model 5973; Agilent, USA) connected to an Agilent 6890N gas chromatograph. The column used was a CP Pora Plot Q capillary column (Variant INC., USA). Helium was used as the carrier gas. We calculated fluxes from the change in gas concentration in the jar, the jar headspace volume, and the soil weight using the following formula:

$$GHG\ Rate = \frac{\mathrm{ppm} * \frac{\mathrm{P} * \mathrm{V}}{\mathrm{R} * \mathrm{T}} * \mathrm{M}}{\mathrm{time}\ (min) * \mathrm{soil\ sample\ weight}\ (g)}$$

Where *ppm* is the gas concentration in the glass vial, *P* is the atmospheric pressure (atm), *V* is the headspace volume in the jar (L), *T* is the temperature (K), *R* is the universal constant of ideal gases (0.08206 atm L/mol K^{-1}), and *M* is the molar mass of the gas (g/mol). For statistical analysis, we used the average values from the six measurements collected as estimations of potential GHG fluxes throughout the incubation period.

We determined the catabolic response profile of soil microbial communities using the MicroResp soil respiration system (MicroResp™, Macaulay Scientific Consulting Ltd., Aberdeen, UK) as described in Campbell et al. (2003). Eight substrates (D-(+)-galactose, L-malic acid, gamma amino butyric acid, n-acetyl glucosamine, D-(+)-glucose, alpha ketogluterate, citric acid and water - basal respiration) were used for respiration measurements. We used these microbial activities as a surrogate of soil microbial functional diversity. MicroResp plates with 96-wells were set up following Morillas et al. (2015). The soil sample was distributed to a microtitre plate where each well contain a small amount of water with a dissolved C substrate. Briefly, the sealed gel plates were incubated for 6 h and read at 570 nm. The absorbance of each well was corrected by subtracting the absorbance of the blank well (inoculated, but without a substrate) from the absorbance of each well. To estimate the evolved CO_2, a colorimetric method relying on the change in the pH of a gel-based solution of bicarbonate was used (Delgado-Baquerizo et al., 2013; Morillas et al., 2015). For logistical reasons, these analyses were carried out in the soil samples collected from the control plots and subjected to the laboratory experiment (i.e. incubated at 40% and 80% of their WHC), but not with the soil samples collected from the treated plots of the *in situ* rainfall experiment. Microbial functional diversity (Shannon *H'* Index) and evenness (*E*) were calculated as described in Rodríguez et al. (2017).

1.4. Data analysis

We used linear mixed-effects models to test the effects of physiognomy, soil moisture treatment, as well as the interactions among these factors, on potential N transformation rates and GHG fluxes in both the *in situ* rainfall reduction and the lab incubation approaches. Similarly, we used linear mixed-effects models to test the effects of physiognomy, soil moisture treatment (lab incubation approach), as well as the interactions among these factors, on the soil microbial functional diversity and evenness. We considered the pair control-treated plot as a random factor due to likely spatial dependencies between the plots of each pair.

We carried residual analyses and evaluations of the adequacy of error distribution of the response variable (Crawley 2013). In this case, full models were constructed and simplified by removing non-significant explanatory variables (physiognomy, treatment, physiognomy x treatment) using the P value until the minimal adequate model was obtained. As a criterion for stopping the removal of variables, we used the change in F-statistics. We used the Chi-square test to evaluate significance of the data. Finally, we tested pairwise statistical differences among different levels of our fixed factors using the Tukey’s test.

We also explored the relationships among microbial functional diversity/evenness and soil functioning (N mineralization rates and GHG fluxes) in samples collected from control plots and subjected to the two soil moistures (40% and 80% WHC) by using Pearson's correlation (r) analysis.

All analyses were carried out in R 3.3.3 (R Development Core Team 2013), with a significance level of $p<0.05$. Linear mixed-effects models and simultaneous tests for general linear hypotheses were carried out using the statistical packages lme4 (Bates et al., 2015) and multcomp (Bretz et al., 2011), respectively.

2. Results

In the second year of the study, on average, our rainfall exclusion structures reduced soil moisture between 16-28% in the open savanna, and between 8-31% in the woodland savanna (Table 1). Unexpectedly, our results indicate that, on average, in the intermediate savannah, soil moisture was higher in the treated (between 1 and 13%) than in the control plots, only detecting a decrease (10%) in soil moisture during the rainy season (Table 1).

2.1. Soil inorganic N content and N transformation rates

Soil NH_4-N and NO_3-N contents differed among the different Cerrado physiognomies. Woodland and intermediate savannah soils had significantly higher NH_4-N contents than those from the open savannah (Figure 3a). Soil NO_3-N contents were higher in the woodland savannah than in the other two physiognomies (Figure 3b). Considering all physiognomies together, the *in situ* rainfall reduction treatment significantly increased soil NH_4-N and NO_3-N (Fig. 3c and d, respectively), with no significant interaction between physiognomy and the rainfall reduction treatment ($p>0.05$).

Potential N mineralization rates also varied among physiognomies and between control and treated plots ($p<0.05$; Figure 4). Potential ammonification rates were higher in the woodland and intermediate savannah physiognomies compared to the open savannah (Chi=7.65, $p=0.021$; Figure 4a), whilst potential nitrification rates were higher in the woodland savannah than in the other two physiognomies (Chi=45.78, $p<0.01$; Figure 4b). Potential ammonification rates were higher in the control than in the treated plots (Chi=5.64, $p=0.017$; Figure 4c). We did not find a significant effect of the rainfall exclusion on potential nitrification rates ($p=0.18$). Again, there was no significant interaction between physiognomy and treatment for N transformation rates ($p>0.05$).

We did not find a significant effect of soil moisture conditions (i.e. 40% vs 80% WHC) on potential ammonification rates, nor did we find a significant interaction between soil moisture and physiognomy ($p>0.05$), meaning that the differences observed among physiognomies in samples incubated at 80% WHC were similar to those observed in samples incubated at 40% WHC. However, we found a significant interaction for potential nitrification rates, with significantly higher levels in soils incubated at 40% WHC compared to those incubated at 80% WHC, but only in the woodland savannah (Figure 4d).

2.2. *Greenhouse gases fluxes*

Soil N_2O emissions were higher in the woodland savannah than in the other physiognomies (Figure 5), but we did not find a significant effect of the rainfall exclusion ($p>0.05$; Figure S1). Likewise, CO_2 emissions varied among physiognomies, with the intermediate savannah showing the highest CO_2 fluxes (Figure 5), but we did not observe significant differences between control and treated plots ($p>0.05$; Figure S1). We did not find a significant effect of the physiognomy or the rainfall treatment on CH_4 fluxes ($p>0.05$), nor significant interactions between these factors ($p>0.05$). However, we found a significant effect of soil moisture (i.e. 40% vs. 80% WHC) on potential CH_4, N_2O, and CO_2 fluxes (Figure 6). In general, GHG fluxes were consistently higher in soils under the 80% WHC treatment than in the soils incubated at 40% WHC (Figure 6 and S2) with no significant interactions with physiognomy ($p>0.05$). At 40% WHC differences of N_2O among physiognomies were not significant, but CO_2 remained lower in open savannah in comparison to the other physiognomies (Figure 6d).

2.3. *Microbial functional diversity and evenness under water reduction treatment*

Microbial functional diversity was higher in the woodland and intermediate savannahs than in the open savannah (Chi 20.98; $p<0.001$), whereas microbial functional evenness was higher in the intermediate savannah than in the other two physiognomies (Chi=16.55; $p<0.001$). We did not find a significant effect of soil moisture (40% vs. 80% WHC) on microbial functional diversity (Chi=2.90; $p=0.088$), nor significant interactions between physiognomy and soil moisture conditions (Chi=0.52; $p=0.769$). Similarly, soil moisture did not influence microbial functional evenness (Chi=0.74; $p=0.388$), but we did find a significant soil moisture x physiognomy interaction (Chi=8.38; $p=0.015$), with higher evenness values at 40% than at 80% WHC in soils of the intermediate savannah but not in soils of the other two physiognomies.

2.4. *Relationships among soil functioning and microbial functional diversity and evenness under water reduction treatment*

At 80% WHC, soil microbial diversity (Shannon index) was positively correlated with N mineralization (Figure 7a and c), whereas soil microbial functional evenness was positively correlated with soil CO_2 fluxes and ammonification (Figure 7e and g). However, at 40%WHC these relationships changed, losing significance (Figure 7b, 7d, 7f and 7h).

3. Discussion

The forecasted decreases in water availability in coming decades can have strong impacts on C and N dynamics (Guo et al., 2002; Rodríguez et al., 2019, 2011; Srivastava et al., 2017), but little is known about how the Cerrado ecosystem would respond to climate change. In the present study, we found that experimental decreases in water availability are likely to cause increases in soil inorganic N content in the Cerrado, and decreases in GHG fluxes. Interestingly, and somehow unexpectedly, most of the effects of changes in soil moisture were consistent across the three types of physiognomies explicitly considered, which provides experimental evidence of the strong capacity of this climate change driver to influence the entire Cerrado biome.

We have also observed clear differences in the soil attributes and potential N cycling and GHGs exchange rates of the different physiognomies, suggesting that any climate change-related variation in the relative dominance of any of these physiognomies will also likely result in changes in the overall Cerrado ecosystem functioning. For instance, soil N contents were consistently higher in the woodland savannah (and in the intermediate for soil ammonium) than in the open savannah. Total N in the litter and in soil layers has been shown to increase from the open savannah to the woodland savannah, the latter being more similar to forest environments (Pellegrini et al., 2014). In addition, litter production is higher in the woodland savannah than that in the more open Cerrado physiognomies, which is known to be positively related to soil C and N stocks (Lacerda, 2019). Thus, the higher levels of plant cover of the woodland savannah, as compared to the intermediate and the open savannahs, would be responsible for higher overall soil fertility levels and N cycling capacity via substrate inputs (e.g. Gallardo *et al.* 2000; Tang & Baldocchi 2005). Indeed, our results show consistently lower N transformation rates in the open savannah. The positive relationships among our indices of microbial diversity and our potential N transformation rates suggest that more diverse (functionally) microbial communities in this biome might sustain higher N cycling rates (Delgado-Baquerizo et al., 2016). An alternative explanation has to do with litter quality, as different savannah species have different mechanisms of nutrient conservation

(Vourlitis et al., 2014). Thus, woodland and intermediate savannah comprises some species of dry forests (Meira-Neto et al., 2017), which may contribute to soil fertility, N cycling and microbial diversity through higher litter quality as compared with the open savannah, whose litter tends to be nutritionally poorer. Furthermore, in the woodland savannah, symbiotic bacteria associated with nitrogen-fixing leguminous species may be related to higher N pools and transformation rates, because these species are capable of promoting soil ammonization (Meira-Neto et al., 2017).

In any case, it is also likely that the high N availability and N transformation rates in the woodland savannah are responsible for its high rates of N_2O emissions, as denitrification. Although highly controlled by the presence of oxygen in the soil matrix (Morse et al., 2015), it is also strongly dependent on the amount of available soil N (Stark and Firestone, 1995). On the other hand, potential soil CO_2 emissions picked in the intermediate savannah. Thus, assuming that climate change might favor open physiognomies at the expense of woodland formations in the Cerrado (Siqueira and Peterson, 2003), our results anticipate that the second largest biome of South America will likely face decreases in soil fertility but also decreases in the potential N_2O emissions from these soils. However, our results also suggest that any change in the vegetation structure towards intermediate physiognomies could lead to increases in microbial respiration (i.e. CO_2 emissions).

Our findings showed that the *in situ* rainfall exclusion altered the soil N contents and transformation rates. The soils in the treated plots had higher N contents and lower ammonification rates than those of the control plots. The highest values of N in soils from treated than from control plots may be a result of drier conditions, which could lead to a decrease in the absorption of nitrogen by plants, mainly because gross primary production is limited by soil water content (Homyak et al., 2017; Zanella De Arruda et al., 2016). On the other hand, soil samples were collected in the dry season when plant activity is low and microbial communities promote the accumulation of N in biologically active forms (Bustamante et al., 2006; Nardoto and Bustamante, 2003; Parron et al., 2011). In this period, increased N immobilization by microorganisms contributes to the reduction of soil inorganic N pools (Neff et al., 2002; Parron et al., 2003). Thereby, an alternative explanation for our results would be related to the effect of drier conditions on the dynamics and activity of soil microbial communities, which in turn would impair the ability of microbial communities to immobilize N and it would justify the highest N content in the treated plots. In any case, the reduced soil moisture seems to cause a decrease in the cycling and immobilization of N. Thus, the projected climate change-driven reductions of the rainy season and increases in frequency and intensity of extreme events (e.g. storms) could decrease the biota biomass stock and cause generalized losses of nitrogen from the soil by leaching.

However, we did not find significant differences in potential nitrification rates between soils of the control plots and those of the treated plots. This could indicate that either (i) the differences in nitrate concentration due to the rainfall reduction are more related to changes in plant activity (that are reduced in the dry season) than in microbial functioning, or (ii) the rewetting of the soil reactivated the nitrifiers community (Bustamante et al., 2006; Nardoto and Bustamante, 2003; Parron et al., 2003), reducing the differences in inorganic N stocks between the control and treated samples in our incubations. Microbial communities have the capacity to quickly recover after a drought period (Bustamante et al., 2006; Nardoto and Bustamante, 2003; Parron et al., 2003). Thus, after rewetting the soil samples in the laboratory, it is likely that microbial communities started to transform ammonium to nitrate.

On the other hand, in the woodland savannah soil samples, we found lower potential nitrification rates at 80% WHC than at 40% WHC. This result may mean that there was a higher nitrate uptake in the soil at 80%WHC than in soils at 40%WHC, due to a great demand of nitrate from soil microbiota (Parron et al., 2003). Alternatively, this result may be due to greater denitrification under higher soil moisture conditions, due to an increase in the anaerobic conditions (Homyak et al., 2017), as suggested by the higher N_2O emissions found at 80% WHC. The fact that the net CH_4 uptake (which is a ratio between the aerobic CH_4 oxidation and its anaerobic production) also decreased in the 80% WHC incubation (indicating an increase in the relative dominance of anaerobic processes) supports this idea (Lafuente et al., 2019).

Similarly, the laboratory soil moisture decreases also resulted in lower CO_2 emissions. In general, CO_2 emissions are directly affected by soil moisture (Flores-Rentería et al., 2018). Lower water availability as a consequence of climate change can potentially impact soil C dynamics (Srivastava et al., 2017), but previous studies have indicated that the effect of soil moisture on soil respiration is highly variable (Liu et al., 2018; Wood et al., 2013; Zhang et al., 2015; Zhou et al., 2014). Our results support the hypothesis that, in savannahs, CO_2 emissions might decrease with decreasing moisture availability (Liu et al., 2018; Siqueira Neto et al., 2011; Werner et al., 2014). Thus, altogether, our study suggests that forecasted decreases in soil water availability due to climate change might result in significant decreases in the potential capacity of the Cerrado to emit N_2O and CO_2 to the atmosphere, but also in increases in their capacity to uptake atmospheric CH_4. Probably because N_2O emissions are more affected by the negative effects of decreased precipitation, as an unfavourable aerobic environment for denitrifiers, than by the positive effects, such as increase in C and N in the substrate (Li et al., 2019). Further, drought has legacy effects on soil microbial communities including the decomposers (Pugnaire et al., 2019). These results, strengthened by the absence of significant interactions with physiognomy, would represent a negative biotic feedback to climate change (Li et al., 2019; Pugnaire et al., 2019). However, it is necessary to consider that if the Cerrado vegetation becomes more open, there would be, at least initially, a strong positive feedback to climate change due to the loss of biomass. Furthermore, considering other climate change drivers (such as increases in temperature, the intensification of drying-rewetting cycles, or extreme drought events), as well as in-situ measurements (Hoover et al., 2016; Hoover and Rogers, 2016; Morillas et al., 2015) is critical to fully understand the fate of the GHGs emissions under future climate change scenarios.

It is worth mentioning that the microbial functional diversity and evenness were positively correlated with the potential N mineralization and CO_2 fluxes rates at 80%WHC, but not at 40%WHC. Thus, our results suggest that functionally diverse microbial communities might not be enough to overcome the stress associated to decreases in water availability (Amundson and Davidson, 1990; Pinto et al., 2002). These results support that the responses of the microbial community to drought might be limited, and that the physiological costs imposed on the soil microbes may cause large shifts in the allocation and fate of C and N (Schimel et al., 2007). However, as microbial communities are responsible for the control and responses to these adverse conditions, a more thorough characterizing of the soil microbial communities under rainfall reduction treatments would be necessary to understand the relationships among soil microbial communities and key soil functioning rates in the Cerrado under different climate change scenarios.

4. Conclusions

- Different Cerrado physiognomies show different N contents and potential cycling rates.
- Closed formations showed an overall better nutritional status and higher potential N mineralization, as well as higher N_2O and CO_2 emissions rates than more open physiognomies.

- Forecasted vegetation shifts towards more open Cerrado physiognomies are likely to result in decreased N contents and cycling rates in this biome.
- Similarly, any climate change-driven increase in the dominance of more open Cerrado physiognomies is likely to decrease the capacity of this biome to emit greenhouse gases to the atmosphere, which would represent a negative biotic feedback to climate change.
- *In situ* rainfall reduction increased soil inorganic N content and decreased potential ammonification rates.
- Laboratory decreases in water availability increased potential nitrification rates in woodland savannah and microbial functional evenness in intermediate savannah.
- Laboratory decreases in water availability consistently decreased soil CO_2 and N_2O emissions, and increased CH_4 uptake, which represents another paradigmatic negative biotic feedback to climate change.
- Most of the effects of changes in soil moisture were consistent across the three types of physiognomy, providing experimental evidence of the strong capacity of this climate change driver to influence the entire Cerrado biome.

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Figures and tables

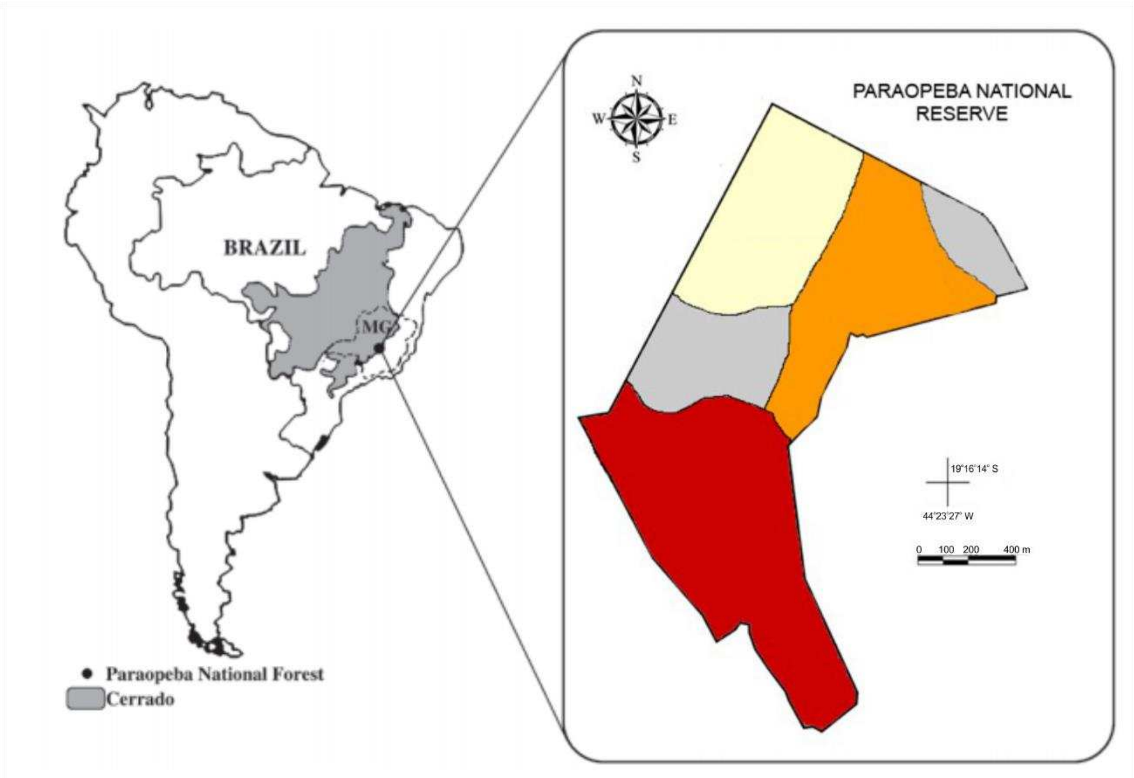


Figure 1: Geographic location of the study area in the Paraopeba Reserve (*Floresta Nacional de Paraopeba*), Minas Gerais (MG), southeast of Brazil. Red colour: woodland savannah on Red Latosol; orange colour: intermediate savannah on Yellow red Latosol; and yellow colour: open savannah on Yellow Latosol. Figure modified from Neri et al. (2013).

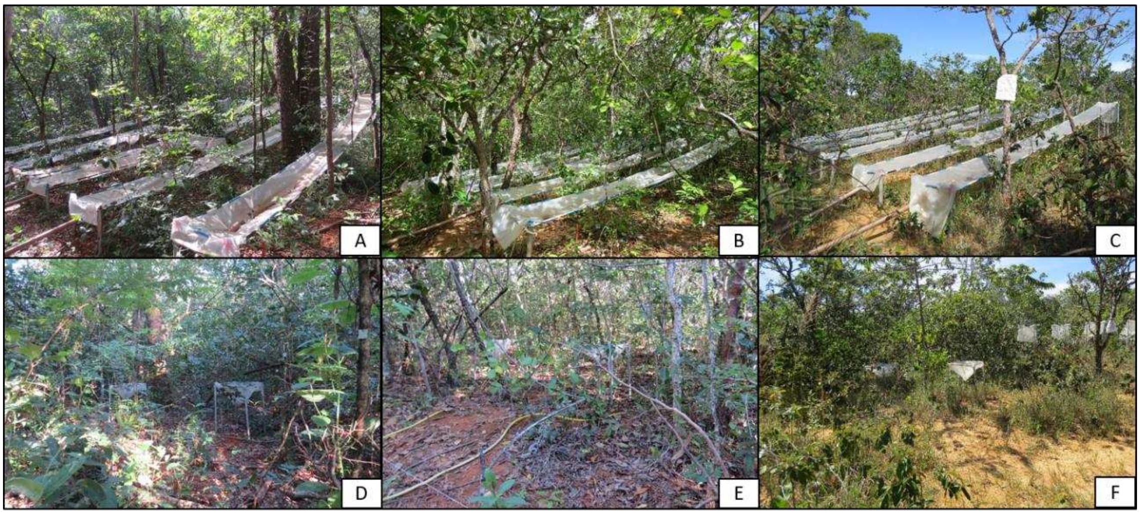


Figure 2: Rain reduction experiment in three physiognomies in the Paraopeba Reserve. A: treatment in the woodland savannah; B: treatment in the intermediate savannah; C: treatment

in the open savannah; D: control in the woodland savannah; E: control in the intermediate savannah; F: control in the open savannah. Pictures by Nayara Mesquita Mota.

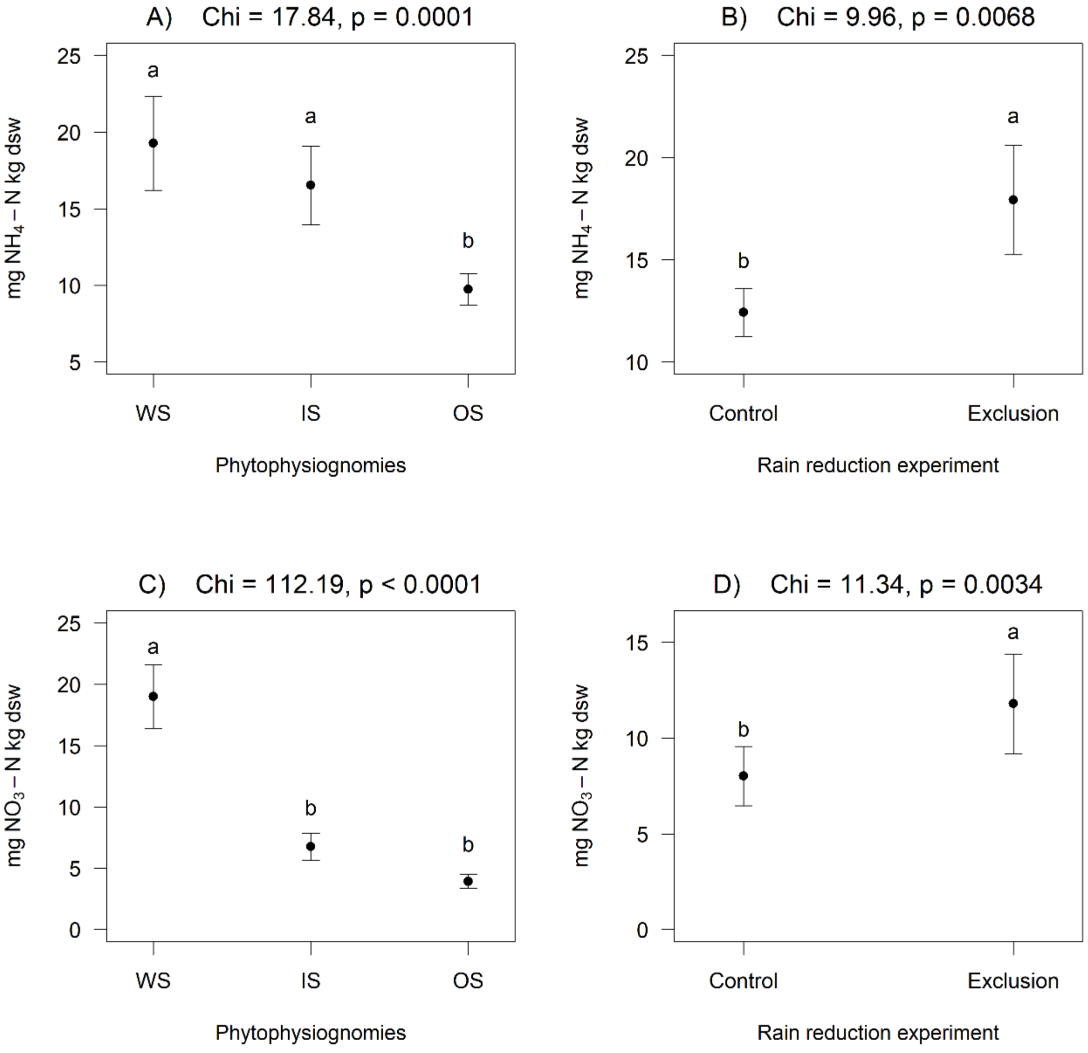


Figure 3: Soil NH_4-N and NO_3-N contents (mean±SE) for control and treated plots together in each of the three Cerrado physiognomies (WS: woodland savannah; IS: intermediate savannah; OS: open savannah) (A and C, respectively), and in the control and treated (Exclusion) plots for all physiognomies together (B and D, respectively). Lowercase letters indicate significant differences ($p < 0.05$) between physiognomies (A and C) and treatments (B and D).

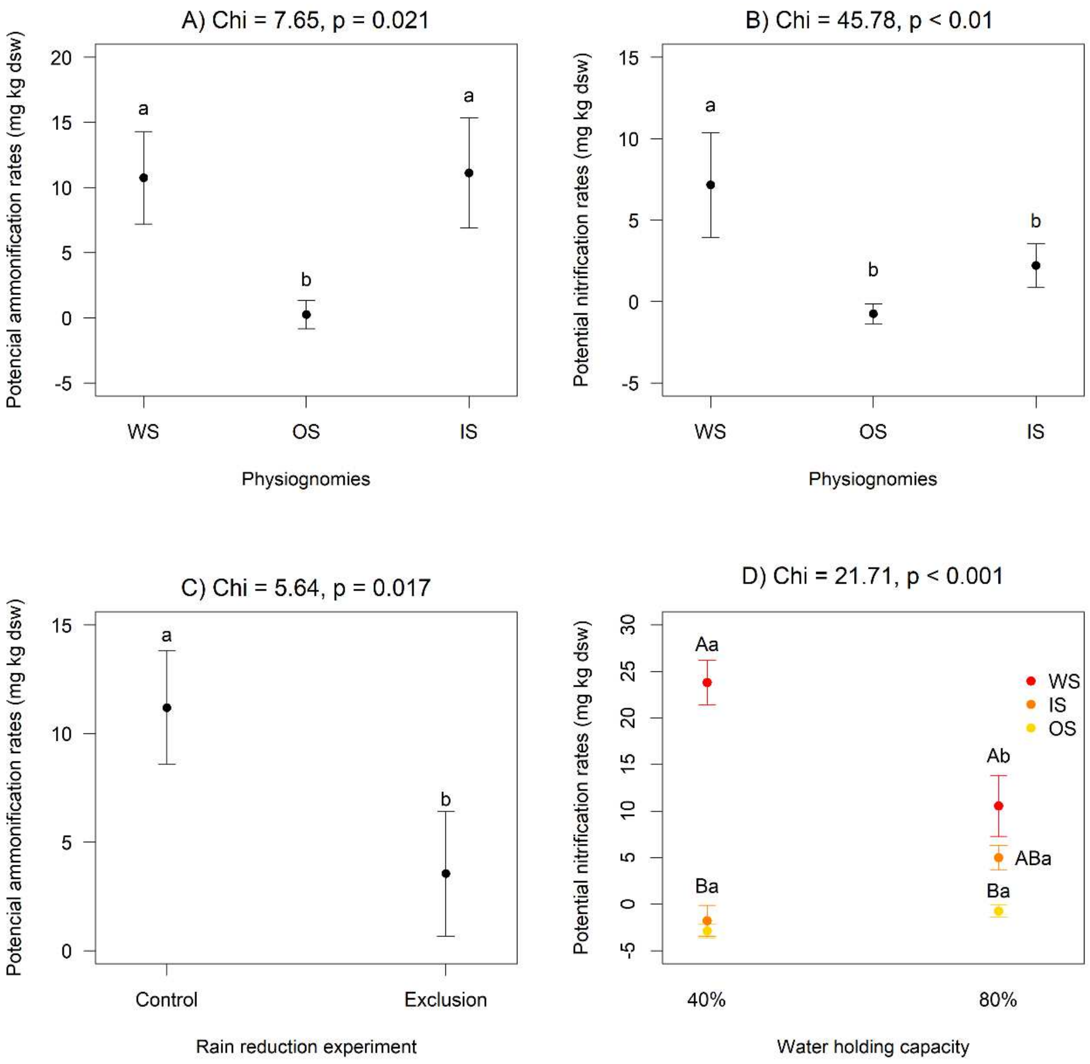


Figure 4: Soil net N mineralization rates (mean±SE) for: control and treated plots together in each different Cerrado physiognomy (WS: woodland savannah; IS: intermediate savannah; OS: open savannah) (A and B, respectively), for the control and treated (Exclusion) plots for all physiognomies together (C); and for the soils collected from the control plots in each physiognomy and subjected to the two soil moisture treatments (40% and 80% WHC) during the laboratory incubations (D). Lowercase letters indicate significant differences ($p < 0.05$) between physiognomies for control and treated plots together (A and B), between treatments for all physiognomies together (C), and for each incubation treatment in the same physiognomy (D). Uppercase letters indicate significant differences between physiognomies in the same incubation treatment (D).

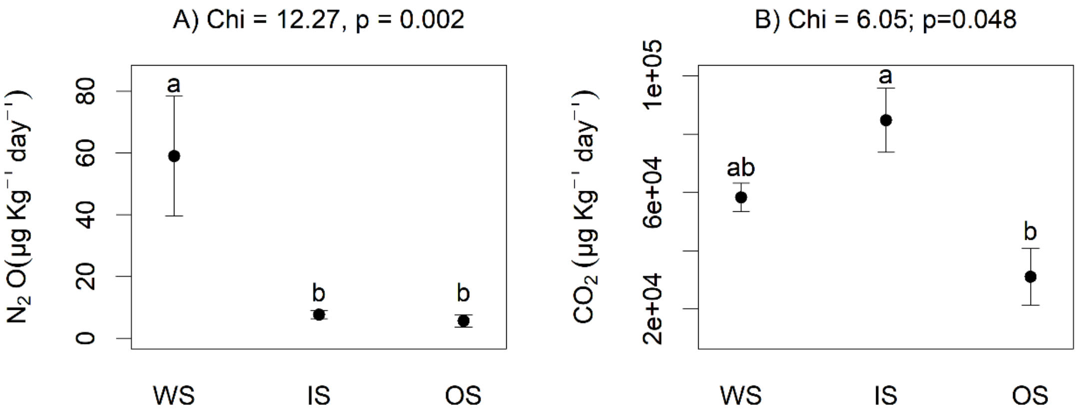
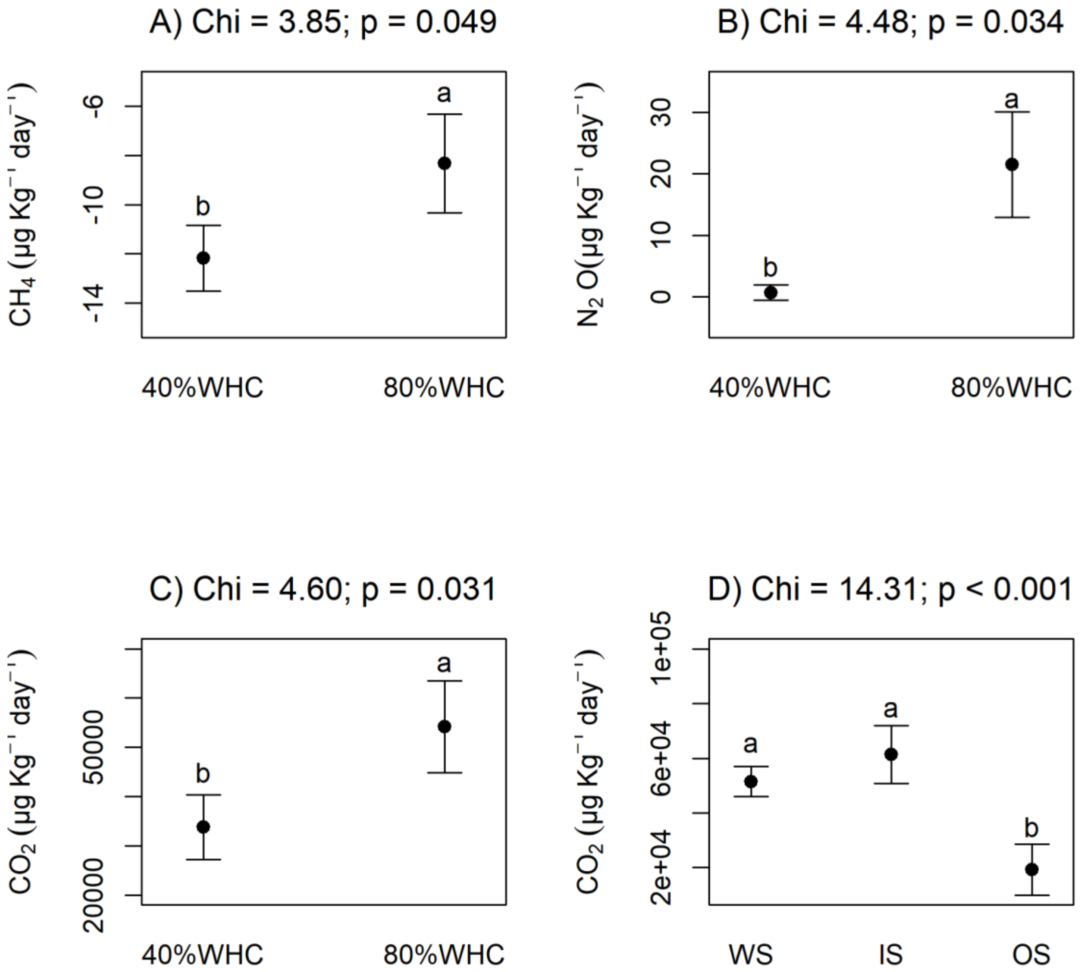


Figure 5: Average N_2O and CO_2 fluxes rates during the 15-days incubations at 80% WHC of soils from control plots of the three different Cerrado physiognomies (WS: woodland savannah; IS: intermediate savannah; OS: open savannah) (error bars: standard error; n=5). Different letters indicate significant differences at $p < 0.05$.



A) Chi = 3.85; p = 0.049

B) Chi = 4.48; p = 0.034

C) Chi = 4.60; p = 0.031

D) Chi = 14.31; p < 0.001

Figure 6: Average CH_4, N_2O and CO_2 fluxes rates during the 15-days soil incubations at 40% and 80% WHC for all physiognomies together (error bars: standard error; n=5) (A, B, C) and average CO_2 fluxes rates at 40% WHC in three different Cerrado physiognomies (D). WS: woodland savannah; IS: intermediate savannah; OS: open savannah. Different letters indicate significant differences ($p < 0.05$).

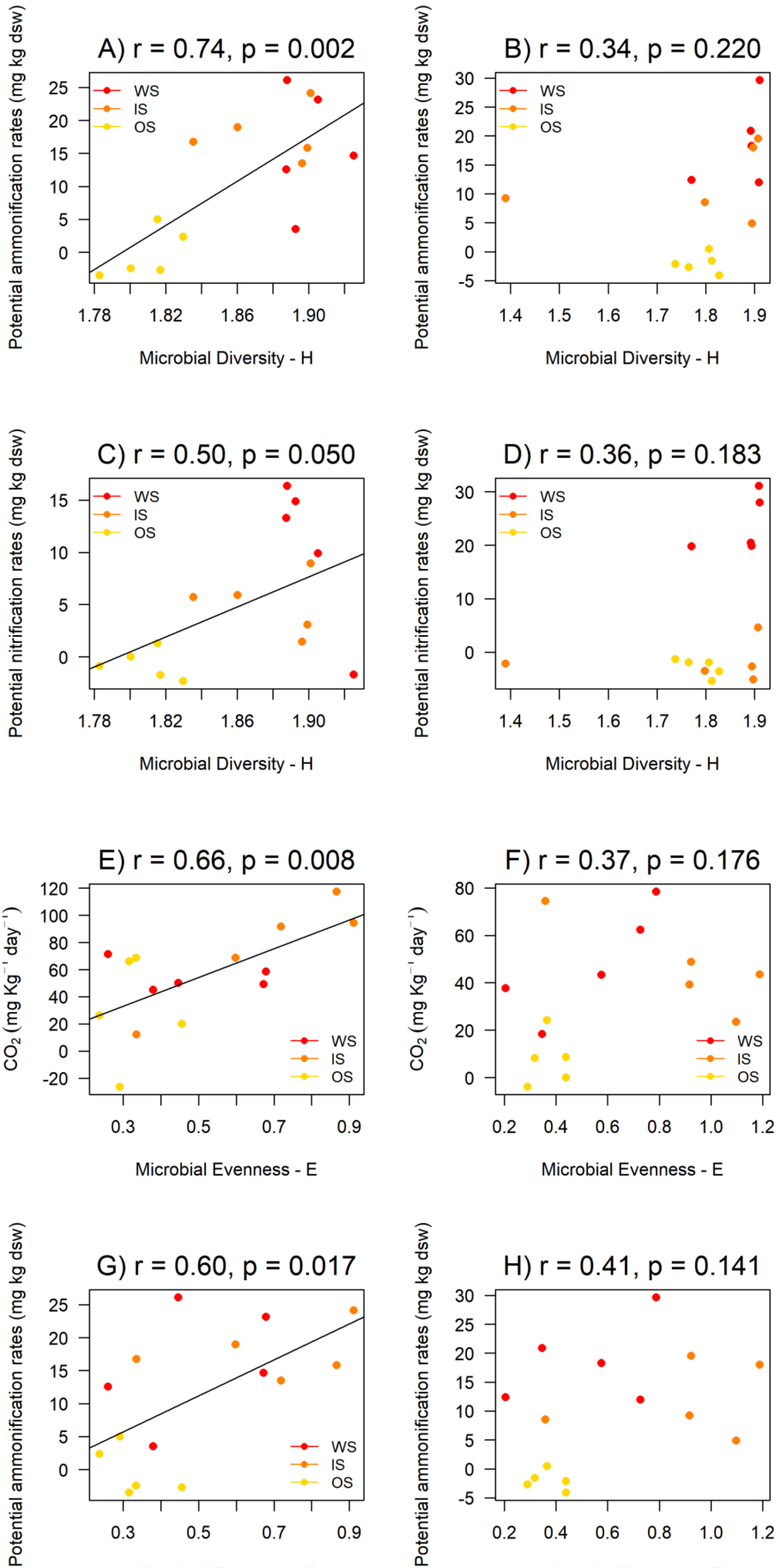

A) r = 0.74, p = 0.002
B) r = 0.34, p = 0.220
Potential ammonification rates (mg kg dsw)
Microbial Diversity - H
C) r = 0.50, p = 0.050
D) r = 0.36, p = 0.183
Potential nitrification rates (mg kg dsw)
Microbial Diversity - H
E) r = 0.66, p = 0.008
F) r = 0.37, p = 0.176
CO_2 (mg Kg^{-1} day^{-1})
Microbial Evenness - E
G) r = 0.60, p = 0.017
H) r = 0.41, p = 0.141
Potential ammonification rates (mg kg dsw)
Microbial Evenness - E
WS
IS
OS
1.78
1.82
1.86
1.90
1.4
1.5
1.6
1.7
1.8
1.9
0.3
0.5
0.7
0.9
0.2
0.4
0.6
0.8
1.0
1.2

Figure 7: Correlations of microbial functional diversity (Shannon – *H*') and Evenness (*E*) with different soil functioning variables (potential CO_2 fluxes and ammonification and nitrification rates) in soils collected from the control plots of the three different Cerrado physiognomies (IS: intermediate savannah; OS: open savannah; WS: woodland savannah) and incubated at 80%WHC (A, C, E and G) and at 40 % WHC (B, D, F and H).

Table 1: Means of soil moisture (SM) and rainfall reduction (RR) in the three Cerrado physiognomies for each season in the second year of field experiment. The soil moisture values of the treated plots correspond to the data collected under the plastic bands. Late-dry: September, October and November; Rainy: December, January and February; Late-rainy: March, April and May; Dry: June, July and August. WS: woodland savanna; IS: intermediate savanna; OS: open savanna.

		Soil moisture (m^3/m^3 VWC)		
Season	**Physiognomy**	**$SM_{control}$ (m^3/m^3 VWC)**	**$SM_{treated}$ (m^3/m^3 VWC)**	**RR (%)**
Late-dry	WS	0.1426	0.0688	**8**
	IS	0.1347	0.1174	- 13
	OS	0.1623	0.1170	**28**
Rainy	WS	0.1568	0.1087	**22**
	IS	0.1656	0.1391	**10**
	OS	0.2218	0.1865	**16**
Late-rainy	WS	0.1257	0.1155	**31**
	IS	0.1501	0.1322	- 12
	OS	0.2034	0.1656	**19**
Dry	WS	0.0902	0.0705	**30**
	IS	0.1186	0.1173	- 1,1
	OS	0.1531	0.1219	**20**

Supplementary material

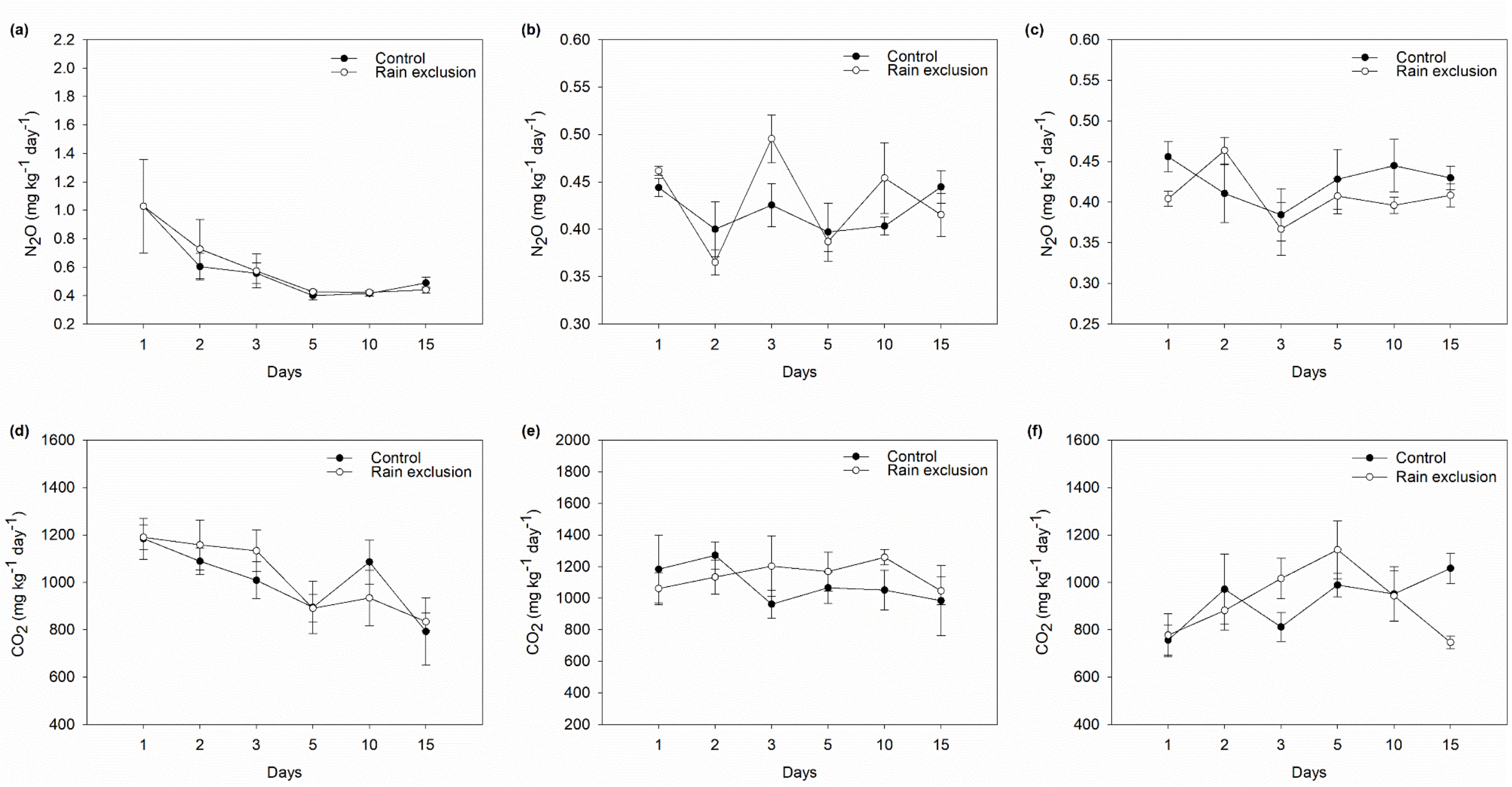


Figure S1: Average N_2O and CO_2 fluxes per physiognomy (a and d) woodland savannah; (b and e) intermediate savannah; (c and f) open savannah) during 15 days of soil incubation (error bars: standard error; n=5). Control: soil samples collected in control plots. Rain exclusion: samples collected from under the plastic bands in treated plots.

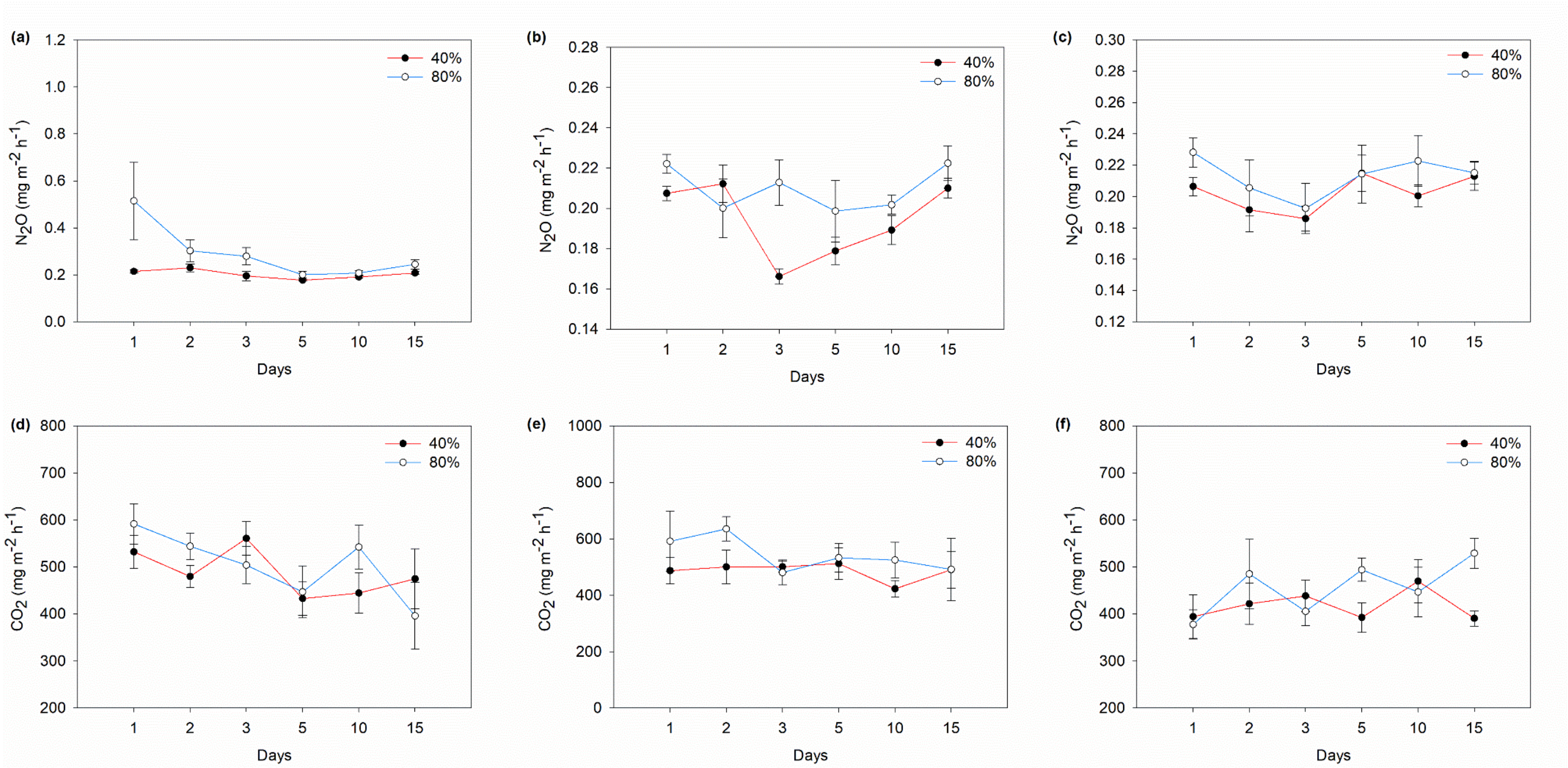


Figure S2: Average N_2O and CO_2 fluxes from soils collected in (a and d) the woodland; (b and e) the intermediate; and (c and f) the open savannah during the 15-days soil incubations (error bars: standard error; n=5) at 40% and 80%WHC.

Table S1: Mean values of soil temperature in the three Cerrado physiognomies in July 2017, when soil samples were collected. "Control": samples in control plots; "Out": samples outside of the plastic bands in the treated plots; "Under": samples under the plastic bands in the treated plots.

	Temperature (°C)	
Physiognomy	**Control**	**Under**
Woodland savannah	17.09	17.38
Intermediate savannah	16.21	17.19
Open savannah	17.77	18.49

CONCLUSÕES GERAIS

Este trabalho evidencia que a diversidade de plantas e do solo difere entre as fisionomias do Cerrado, e que a fisionomia Cerrado *stricto sensu* denso possui uma maior biodiversidade. Esse estudo também expõe relações intensas, mas complexas, entre diversidade microbiana, conteúdo de nutrientes do solo e atributos das plantas. Essas relações, provavelmente motivadas pela competição por recursos de luz e solo, antecipam que qualquer perturbação que afete um componente do Cerrado (ou uma mudança na dominância relativa de uma das fisionomias) podem causar efeitos em outros componentes desse bioma.

Nossos resultados também mostraram que a disponibilidade de N e P no Cerrado varia com a fisionomia, estação e ano. Além disso, as reduções de chuva previstas podem diminuir a disponibilidade de N e aumentar a disponibilidade de P nesse bioma, embora a intensidade e a direção da resposta provavelmente variem entre as fisionomias.

As fisionomias do Cerrado avaliadas também diferem em conteúdo de N e possíveis taxas de ciclagem. As formações fechadas apresentaram um melhor estado nutricional geral e maior mineralização potencial de N, bem como taxas mais altas de emissões de N_2O e CO_2 do que fisionomias mais abertas. Assim, as mudanças da vegetação previstas para fisionomias mais abertas do Cerrado provavelmente resultarão em menor conteúdo de N e taxas de ciclagem neste bioma em longo prazo. Em médio prazo, as perdas de carbono devido às transformações de fisionomias fechadas em fisionomias mais abertas provavelmente representarão um *feedback* biótico positivo à mudança climática. Entretanto, em longo prazo, qualquer aumento impulsionado pela mudança climática no domínio relativo de fisionomias mais abertas do Cerrado provavelmente diminuirá a capacidade desse bioma de emitir gases de efeito estufa na atmosfera, o que representaria um *feedback* biótico negativo à mudança climática.

A partir do exposto, nós concluímos que a redução das chuvas in situ aumentou o teor de N inorgânico no solo e diminuiu as taxas potenciais de amonificação, enquanto que reduções experimentais na disponibilidade de água diminuíram consistentemente as emissões de CO_2 e N_2O do solo e aumentaram a captação de CH_4, o que também representa um feedback biótico negativo paradigmático às mudanças climáticas. Para entender melhor o destino das emissões de GEE em cenários futuros de mudança climática, torna-se necessário considerar outros fatores de mudança climática (como aumentos de temperatura, a intensificação dos ciclos de secagem e umedecimento ou eventos extremos de seca), bem como medições in situ.

Sendo assim, estudos mais longos e experimentais são cada vez mais necessários para entender por completo as possíveis respostas deste importante bioma aos futuros cenários de mudanças climáticas. Particularmente, trabalhos futuros devem abordar as implicações dessas mudanças no funcionamento das comunidades vegetais e microbianas do Cerrado. Além disso, como as comunidades microbianas são responsáveis pelo controle e respostas a essas condições adversas, estudos futuros devem buscar caracterizar as comunidades microbianas do solo sob tratamentos de redução de chuvas para entender profundamente as relações entre as comunidades microbianas do solo e as principais taxas de funcionamento do solo no Cerrado.