

Different Cerrado Ecotypes Show Contrasting Soil Microbial Properties, Functioning Rates, and Sensitivity to Changing Water Regimes

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ABSTRACT

Soil moisture is among the most important factors regulating soil biodiversity and functioning. Models forecast changes in the precipitation regime in many areas of the planet, but how these changes will influence soil functioning, and how biotic drivers modulate such effects, is far from being understood. We evaluated the responses of C and N fluxes, and soil microbial properties to different soil water regimes in soils from the main three ecotypes of the world's largest and most diverse tropical savanna.

Further, we explored the direct and indirect effects of changes in the ecotype and soil water regimes on these key soil processes. Soils from the woodland savanna showed a better nutritional status than the other ecotypes, as well as higher potential N cycling rates, N₂O emissions, and soil bacterial abundance but lower bacterial richness, whereas potential CO₂ emissions and CH₄ uptake peaked in the intermediate savanna. The ecotype also modulated the effects of changes in the soil water regime on nutrient cycling, greenhouse gas fluxes, and soil bacterial properties, with more intense responses in the intermediate savanna. Further, we highlight the existence of multiple contrasting direct and indirect (via soil microbes and abiotic properties) effects of an intensification of the precipitation regime on soil C- and N-related processes. Our results confirm that ecotype is a fundamental driver of soil properties and functioning in the Cerrado and that it can determine the responses of key soil processes to changes in the soil water regime.

Received 27 September 2022; accepted 15 March 2023;
published online 24 April 2023

Supplementary Information: The online version contains supplementary material available at <https://doi.org/10.1007/s10021-023-00838-0>.

Author Contribution: JD designed the study, performed research, analyzed data, and wrote the paper. JM designed the study, and wrote the paper. MDB analyzed data, and wrote the paper. KH performed research, analyzed data, and wrote the paper. AEP performed research, analyzed data, and wrote the paper. AR designed the study, performed research, analyzed data, and wrote the paper.

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Key words: cerrado; ecotype; precipitation regime; C cycle; N cycle; greenhouse gases.

HIGHLIGHTS

- Cerrado ecotypes differed in most studied soil properties
- Ecotype modulated the effects of changes in the soil water regime
- Intermediate savanna soils responded more intensely to changes in soil water regime

INTRODUCTION

The Brazilian savanna (that is, Cerrado) is the largest and most diverse tropical savanna of the world (Mendonça and others 2008), covering about 24% of Brazil (approximately 2 million km²), and representing the second-largest biome of South America (Bonanomi and others 2019). Because of its extent and biodiversity, the Cerrado is important for maintaining multiple ecosystem processes related to water regulation, carbon (C) stocks, and nutrient cycling from regional to global scales (Miranda and others 1997; Santos and others 2004; Grace 2006; Gomes and others 2019). Paradoxically, the Cerrado is also one of the most important hotspots of land-use change worldwide with most of the area converted to soybean and beef production. As a result, many global change studies on the Cerrado have focused on the effects of land-use change (Varela and others 2004; Neto and others 2011; Silveira Sartori Silva and others 2019; Siqueira-Neto and others 2021). Still, close to 20% of the biome remains undisturbed (Siqueira-Neto and others 2021), and despite few previous efforts focused on the effects of different climate change components (Souza and others 2016; Peixoto and others 2018; de Oliveira and others 2019), we are far from understanding how this ecosystem will respond to forecasted changes in climate.

Soil moisture is one of the most important factors regulating soil functioning and microbial structure (for example, community composition, diversity or biomass) in many ecosystems (Moyano and others 2013; Blazewicz and others 2014; Rodríguez and others 2019b). This is also true for the Cerrado (Neto and others 2011; Bustamante and others 2012; Peixoto and others 2018), which besides being considered one of the world's most humid savannas, has a very seasonal climate that makes the C and nitrogen (N) cycles largely regulated by

the rainfall regime (Varela and others 2004; Bustamante and others 2012). As for many terrestrial ecosystems, regional- to global-scale climate models project drastic climate change-driven alterations of the hydrological cycle for the Cerrado (de Oliveira and others 2019; Masson-Delmotte and others 2021). These changes include increases in evapotranspiration rates, decreases of 20–70% in precipitation (depending on the region), reductions in the number of rainy days, and increases in the volume of the rainfall coming from storms during the wet season, as well as an intensification of the precipitation regime resulting in more intense rain events followed by longer dry periods (Huntington 2006). However, how changes in precipitation regimes will influence soil biodiversity and function remains little studied (Dijkstra and others 2012; Song and others 2017). To our knowledge, there is no such study in the Cerrado.

The Cerrado landscape includes a heterogeneous mosaic of contrasting phytophysionomies that differ in both plant structure and composition, but also in soil conditions (hereinafter ecotypes), and that can be classified according to the proportion of trees and grasses, ranging from open to woodland savannas (Coutinho 2006). Although potential drivers for the occurrence of these ecotypes are not yet well elucidated (Eiten 1972; Marimon Junior and Haridasan 2005; Ribeiro and Walter 2008; Assis and others 2011; Neri and others 2012b, 2013), the distribution of these ecotypes is usually correlated with soil properties such as soil texture and fertility (Neri and others 2012a, 2013). Thus, soils from woodland formations are usually clayey and with relatively high organic matter and nutrient reserves, whilst those from more open formations are usually sandier and with lower nutrient contents (Neri and others 2013; Giacomo and others 2015). Moreover, it is known that a denser and more continuous canopy cover is related to greater litter production which, in turn, is associated to greater soil C stocks and nutrient contents as compared with more open ecotypes (Campos and others 2006; Souza and others 2010; de Castro and others 2016; Lacerda 2019). The relative coverage of the different Cerrado ecotypes is expected to change in response to climate change, likely increasing the most open formations at the expense of the closest ones (Maksic and others 2022). Further, the simulations performed by Maksic and others (2022) show an expansion of the Cerrado into the current rainforest area. All these changes in the distribution of Brazilian biomes would lead to changes in soil physicochemical properties (Neri and others 2012a, 2013). Different Cerrado ecotypes are likely to have

different soil microbial communities with different historical exposure to soil water regime changes and therefore different sensitivity to drying-rewetting events (de Castro and others 2016; Liu and others 2017; Rodríguez and others 2019a).

Here, we aimed to evaluate the responses of soil functioning (that is, C and N fluxes) and microbial properties (that is, bacterial abundance, richness and community composition) to changes in the soil water regime (that is, drying-rewetting frequency and intensity) in soils from three different Cerrado ecotypes (woodland, intermediate and open savanna) occurring in similar soil type and with the same climate. We conducted a laboratory experiment based on soil incubations to estimate different functioning rates of soils from different ecotypes under different soil water regimes (that is, constant moisture, two mild drying-rewetting cycles, and one single and intense drying-rewetting cycle). As soil functioning variables, we focused on C and N mineralization rates and soil:atmosphere greenhouse gases (GHG) exchange because they are intimately related to soil fertility and nutrient cycling, and are key to the maintenance of important ecosystem services (Maestre and others 2012; Soliveres and others 2014). Moreover, the exchange of strong GHG such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) between the soil and the atmosphere can strongly influence ongoing climate change (Martins and others 2015). Further, we mechanistically explored whether changes in the ecotype and the intensification of the soil water regime influence these key soil processes directly and indirectly through changes in soil biotic (bacterial abundance, diversity and community structure) and abiotic (for example, soil texture, pH, total C and N concentrations) variables. We hypothesized that (i) potential C and N transformation rates, as well as the potential soil:atmosphere GHGs exchange would be significantly higher in closer ecotypes (woodland and intermediate savannas) than in the open savanna; (ii) the open savanna would respond less than the other ecotypes to changes in the soil water regime; and (iii) the differences in responses to the intensification of the soil water regime among different ecotypes would be at least partially mediated by changes in soil biotic and abiotic properties.

METHODS

Study Site

The study was conducted in the Paraopeba National Reserve (PNR; Minas Gerais, Brazil; 19°20' S;

44°20' W). The PNR has been actively protected from cutting and fire at least since 1963 (Neri and others 2012a), covers an area of 200 ha., and has a tropical climate characterized by rainy summers from September to April and dry winters from May to August (climate classified as type Aw—tropical humid—by the Koeppen system). Mean annual temperature and precipitation are 20.9 °C and 1328 mm, respectively. The elevation varies between 740 and 760 m.

The PNR landscape is composed of a vegetation mosaic, and the dominant ecotypes are the three predominant phytophysiognomies of the Cerrado domain (that is, open savanna, woodland savanna, and an intermediate physiognomy here denominated intermediate savanna). According to Neri and others (2012a), the open savanna is characterized by the presence of well-defined herbaceous-shrub and shrub-tree strata; the intermediate savanna presents greater densification of shrub-tree species over the herbaceous layer; and the woodland savanna has higher trees, with canopies that form a denser cover, and a sparse herbaceous layer. Together, these three ecotypes occupy more than 70% of the entire Cerrado biome (Eiten 1972). In the PNR, the woodland and intermediate savannas have significantly higher values of canopy cover (89% and 86%, respectively) than the open savanna (64%), whereas the intermediate savanna has the highest plant abundance and richness (Lacerda 2019). Moreover, the woodland savanna is dominated by N-fixing leguminous trees (Meira-Neto and colleagues, 2016). Soils are acid oxisols (pH values ranging from 4.5 to 5) and range from clayey in the woodland to silty clayey in the intermediate and open savannas (see Table 1 for a complete description of the three ecotypes).

Experimental Design and Soil Analyses

In September 2014 (end of the dry season) we selected 15 independent (separated by more than 50 m) plots (20 × 20 m), five plots in each ecotype. Then, we established 6 soil sampling points 5 m from the center of each plot following N, NNE, SSE, S, SSW and NNW directions. Three soil samples were collected in each sampling point to create one composite sample per plot (five composite soil samples per ecotype, 15 in total). Soil samples were collected after removing the litter layer by using a 5 cm (∅) metal corer that allowed us to collect the first 12 cm of the soil profile. All soil samples were immediately sieved (2 mm mesh size), thoroughly homogenized in field-moist conditions and analyzed for soil water content (SWC) by oven-drying

a subsample of 5 g at 60 °C to constant mass. A 20 g soil subsample of each composite sample was immediately kept at -20 °C for future analyses of microbial diversity and abundance, whereas the rest of the sample was kept at 4 °C until analysis (less than one week). We analyzed all 15 soil composite samples for soil organic matter content (SOM) by loss on ignition at 450 °C for 4 h (Nelson and Summers 1996), pH (soil-to-deionized water ratio of 1:2.5 m/v), water holding capacity (WHC) following Rey and Jarvis (2006), and soil texture following Robinson's pipette method (Dane and others 2002). The amounts of soil total C and N (TC and TN, respectively) were analyzed by dry combustion with an elemental analyzer (LECO TruSpec CN). We extracted soil total inorganic N [TIN = ammonium (NH_4^+ -N) + nitrate (NO_3^- -N)] by mixing 7 g of soil with 35 mL of 1 M KCl. The concentration of TIN in the extracts was determined by colourimetric methods following Durán and others (2009). Soil extractable phosphorus (PO_4^{3-} -P) was determined by using the Mehlich-1 extractor, recommended for acid and clayey soils with low cation exchange capacity and analyzed by colourimetry (Medeiros and others 2021). Cation exchange capacity (CEC) was determined in an external laboratory by summing the amount of K, Ca, Mg and Al present in the 1 M KCl extracts following Rengasamy and Churchman (1999).

Then, we carried out 21-day laboratory incubations to estimate potential soil C and N mineralization and soil:atmosphere greenhouse gases (GHGs) exchange rates. Fresh subsamples (~ 100 g) of each of the five composite samples per ecotype were incubated in 0.6 L glass jars at 25 °C temperature in darkness and one of three different soil water regime treatments (that is, constant moisture, two mild drying-rewetting cycles, and one single and intense drying-rewetting cycle; 5 composite samples \times 3 ecotypes \times 3 treatments = 45 jars). To avoid soil drying while allowing gas exchange, tins were closed with plastic wrap secured with a rubber band and were weighed daily to make sure that the soil moisture was at the appropriate level. Our three water regime treatments consisted of the same amount of water but differently distributed throughout the incubation by allowing the soils subjected to the drying-rewetting cycles regimes to dry up to a certain level and then re-wetting them, whereas soils of the constant treatment remained with steady soil moisture during the 21-day incubation (Radu and Duval 2018; Barel and others 2021). As the Cerrado is a highly seasonal biome with 90% of the rainfall (and therefore soil activity), concen-

trated during the rainy season (Grace and others 2006; Bustamante and others 2012), we focused on changes in the soil water regime during this season. Thus, in one of the regimes, we kept steady soil moisture corresponding to the 70% WHC of each soil sample (hereinafter constant regime). This constant moisture regime was selected based on the optimal soil moisture conditions commonly used in laboratory soil incubations (60%—80% WHC) (Zheng and others 2019). Then, the timing and amount of soil water added in the drying-rewetting cycle regimes were adjusted to have two mild drying-rewetting cycles (hereafter 2c regime) and one single more intense drying-rewetting cycle (hereafter 1c regime) throughout 21 days by leaving the soils to dry at an optimal temperature (25 °C). Thus, soil moisture oscillated between 77% WHC and 62% WHC in the 2c regime and between 85% WHC and 55% WHC in the 1c regime (Figure S1). The exchange of GHGs between the soil and the atmosphere, that is, CO_2 (heterotrophic respiration), N_2O , and CH_4 , were determined by collecting gas samples in all soil water regimes on days 0, 1, 7, 8, 12, 13, 14, 15, 20 and 21 following (Morillas and others 2015) (see below). Moreover, in the case of the 1c and 2c regimes, we rewetted soils on day 11 (after they reached 55% WHC) and on days 7 and 14 (after they reached 62% WHC), respectively, and, besides the measurements done on the days above mentioned, GHGs fluxes were also measured one hour and one day after each rewetting (Figure S1). For each gas collection, we closed the glass jars for 60 min and sampled twice (at the beginning and the end of the 60 min) through a rubber septum placed on the jar lid with fine needle polypropylene syringes equipped with a valve system that allowed both the collection of 20 mL of air and the storage of the air sample in the syringe. One of the samples was transferred to evacuated 12 mL glass vials (that is, over-pressured to prevent dilution by the ambient atmosphere) and stored at room temperature until analyses (less than 15 days) of N_2O concentration by cavity ringdown spectroscopy (LGR $\text{N}_2\text{O}/\text{CO}$ Analyzer, Los Gatos Research, Mountain View, CA, US). The other sample was immediately analyzed for CO_2 and CH_4 concentrations using near-infrared cavity ring-down spectroscopy (Picarro, Santa Clara, CA, US). For each measurement, we used the linear rate of change in gas (CO_2 , N_2O or CH_4) concentration in the jar headspace throughout the 60-min incubation and the ideal gas law equation to convert the net increase in gas concentration (ppm) to mass of gas (m) as follows:

$$m = \frac{\text{ppm} \times P \times V \times M}{R \times T}$$

where P and V are, respectively, the air pressure (ATM) and the known headspace volume in the jar (L), M is the atomic mass of the respective gas (g mol^{-1}), R is the universal constant of gases ($0.08206 \text{ ATM L mol}^{-1} \text{ K}^{-1}$) and T is the temperature (K) at the measurement time. Then we estimated the rate fluxes of CO_2 , N_2O and CH_4 for each soil water regime by interpolation between measurement dates. The CO_2 emission rate (that is, heterotrophic respiration) was used as a surrogate of potential C mineralization. All GHGs fluxes were normalized by dry soil mass. Potential N transformation rates for each soil water regime were estimated by assessing the increase in $\text{NH}_4^+\text{-N}$ (that is, ammonification), $\text{NO}_3^-\text{-N}$ (that is, nitrification) and total inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$; that is, N mineralization) over the incubation period (Jenkinson and Powlson 1980; Durán and others 2013a). To do so, we measured soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ before and after the incubation of the soil samples as above explained. Potential N transformation rates were also normalized by dry soil mass.

Total genomic DNA was extracted from 0.5 g soil subsamples from the five composite samples per ecotype, as well as from the samples incubated under the different treatments (at the end of the incubation), using a commercial DNA extraction kit (PowerSoil DNA Isolation Kit®, Mobio). Soil DNA was extracted from 0.5 g of defrosted soil samples using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the instructions provided by the manufacturer. The abundance of bacteria was quantified using quantitative PCR and the 341F/805R primer set (Herlemann and others 2011). The diversity of bacteria was determined using amplicon sequencing and the bacterial 16S rRNA gene (341F-805R) at the Western Sydney University NGS facility (Sydney, Australia) using Illumina MiSeq 2×300 bp paired-end sequencing. Bioinformatic processing was performed using a combination of MOTHUR (Schloss and others 2009), UCHIME (Edgar and others 2011) and UPARSE (Edgar 2013). Operational taxonomic units (OTU) were identified at the 97% identity level. The richness of bacteria was determined from a rarefied OTU table. The bacterial community structure was determined through the relative abundance of each OTU. We focused on bacterial communities because they are the most abundant and diverse organisms thriving in the soil systems.

Statistical Analyses

Differences in bacterial community structure among ecotypes were explored with non-metric multidimensional scaling (nMDS) using the Euclidean distance measure after root square transformation of the data. We explored the effect of ecotype on the rest of the different variables using the permutational ANOVA (PERMANOVA) and a posteriori permutational pairwise comparisons (Anderson and others 2008). PERMANOVA and permutational pairwise comparisons were performed using 9999 permutations and Bray–Curtis similarity resemblances, using the Monte Carlo resampling approach to obtain permutational P -values. We used the same procedure to explore the effect of the soil water regime for each ecotype separately, as well as the interactions between ecotype and soil water regime, on potential soil C and N mineralization and soil:atmosphere GHG exchange rates, and on the % of change (between pre-incubation and post-incubation values) of soil bacterial properties (that is, abundance, richness, diversity and community structure). The permutational approach was chosen to overcome the fact that our data did not always meet the requirements of more classical parametric analyses. All these analyses were carried out with software Primer 6 and PERMANOVA + (PRIMER-E Ltd, Plymouth, UK).

Then, we used structural equation modelling (SEM) to evaluate the direct and indirect effects of ecotype (from woodland to more open formations), intensification of the soil water regime (that is, from lower to higher intensity: control, 2 mild drying-rewetting cycles and 1 more intense drying-rewetting cycle), and soil properties (pH, silt and C concentrations, and bacterial diversity and abundance) on potential C and N mineralization and soil:atmosphere GHGs exchange rates (Grace 2006). The a priori structural equation model used in this study can be found in the supplementary material (Figure S2). There is no single universally accepted test of the overall goodness of fit for SEM. Here we used the chi-squared test (χ^2 ; the model has a good fit when χ^2/df is low, that is, no greater than about 2, and P is high, traditionally > 0.05), the root-mean-square error of approximation (RMSEA; the model has a good fit when RMSEA is indistinguishable from zero, and P is high, traditionally > 0.05), as well as the Bollen-Stine bootstrap tests (Schermerh-Engel and others 2003; Hooper and others 2008). After verifying the adequate fit of our model, we were free to interpret the path coefficients of the model and their associ-

Table 1. Physicochemical Properties for Each Cerrado Ecotype ($n = 5$).

	Woodland savanna	Intermediate savanna	Open savanna	Permutations	
				<i>P</i>	Pseudo-F
Sand (%)	3.06 (0.59)	2.56 (0.21)	2.82 (0.27)	0.847	0.197
Silt (%)	22.8 (2.40) b	39.3 (4.02) a	42.6 (0.99) a	0.001	15.03
Clay (%)	74.1 (2.94) a	58.2 (3.89) b	54.6 (1.11) b	0.002	11.84
SOM (%)	5.86 (0.21) a	4.78 (0.11) b	3.16 (0.22) c	0.001	43.37
TC (%)	3.55 (0.34) a	2.57 (0.11) b	1.70 (0.07) c	0.001	31.29
TN (%)	0.30 (0.05) a	0.25 (0.01) a	0.14 (0.03) b	0.018	5.701
C:N	12.6 (1.28)	10.4 (0.66)	16.2 (4.42)	0.399	0.971
pH	4.58 (0.13) b	4.99 (0.08) a	5.03 (0.07) a	0.012	6.998
NH ₄ ⁺ -N (mg kg ⁻¹ soil)	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)	43.3 (10.2) a	10.4 (6.43) b	15.1 (5.68) b	0.019	5.403
TIN (mg kg ⁻¹ soil)	75.2 (11.8) a	22.0 (7.45) b	24.4 (5.65) b	0.004	8.275
PO ₄ ⁻³ -P (mg kg ⁻¹ soil)	1.59 (0.17) a	1.29 (0.06) a	0.65 (0.12) b	0.003	12.86
CEC (cmol dm ⁻³)	11.0 (0.44) a	10.1 (0.34) a	7.35 (0.33) b	0.000	28.62
WHC (%)	46.3 (1.10) b	48.4 (0.31) b	50.2 (0.29) a	0.007	7.879

Values represent the mean (\pm 1SE). Statistically significant effects of ecotype are represented by bold *P* values ($P < 0.05$; PERMANOVA). Different letters in each property represent significant differences among ecotypes ($P < 0.05$). SOM soil organic matter, TC total C, TN total N, TIN total inorganic N, CEC cationic exchange capacity, WHC water holding capacity.

ated *P*-values. A path coefficient is analogous to the partial correlation coefficient or regression weight and describes the strength and sign of the relationships between two variables (Grace 2006). The probability that a path coefficient differs from zero was tested using bootstrap tests, as our data were not always normally distributed (Schermelleh-Engel and others 2003; Kline 2011). We calculated the standardized total effects of all drivers on the selected response variables (Grace 2006). The net influence that one variable had upon another was calculated by summing all direct and indirect pathways (effects) between two variables. All SEM analyses were conducted using AMOS 24.0 (IBM SPSS, Chicago, IL, USA).

RESULTS

Soils from the different ecotypes showed significant chemical, physical, biotic and functional differences (Table 1). Soils from the woodland savanna had a higher concentration of clay, organic matter, TC and TN, TIN, and PO₄⁻³-P, as well as a higher CEC, but lower silt content and pH than soils from the intermediate and the open savanna (Table 1). We also found significantly higher soil bacterial abundance in the woodland savanna than in the intermediate and open savannas (Figure 1a). Soil bacterial richness was significantly higher in the open savanna than in the woodland savanna (Figure 1b), and the nMDS revealed a significant separation of the soil bacterial communities among

Cerrado ecotypes (Figure 1d). Specifically, although the three ecotypes shared the most dominant groups, both at the phylum and family level, the relative abundances of each varied among ecotypes (Figure S3). At the phylum level, Proteobacteria, Acidobacteria, Verrucomicrobia, Actinobacteria, and Chloroflexi were the dominant groups (Figure S2a). Proteobacteria, Acidobacteria and Actinobacteria decreased their dominances from the woodland to the open savanna, whereas Verrucomicrobia and Chloroflexi increased them. At the family level, Chthoniobacteraceae and Amoebophilaceae were clearly the dominant groups and decreased and increased their dominances, respectively, as the ecotype became more open (Figure S3b). Similarly, soil incubations revealed significant differences among ecotypes for all N transformation rates and GHG fluxes, as well as for the % of change of bacterial richness, diversity and community structure (Table 2; Figure S4). Soils from the woodland savanna showed consistently higher potential (that is, at constant moisture) N transformation rates (Figure 2) and N₂O emissions (Figure 3a) than soils from the other ecotypes. However, potential soil CO₂ emissions and CH₄ consumption peaked in the intermediate savanna (Figure 3b, c; Figure S5).

The soil water regime did not significantly affect potential N mineralization rates considering all physiognomies together (Table 2), but we did find a significant effect of the soil water regime on potential N transformation rates of the intermedi-

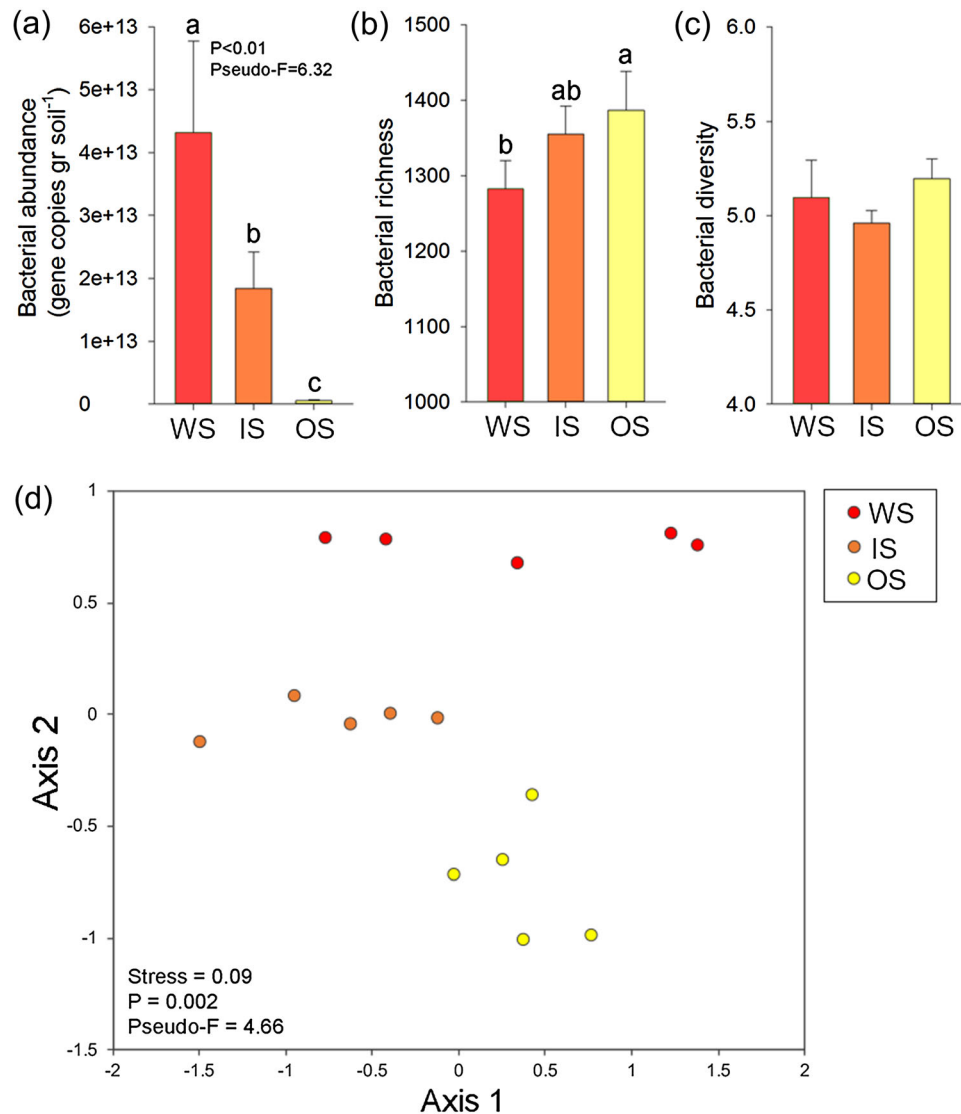


Figure 1. Bacterial abundance (a), richness (b), diversity (c), and community structure (d; non-metric multidimensional scaling; nMDS) in the different Cerrado ecotypes ($n = 5$). Bars and error bars in plots a, b and c represent means and standard errors (1SE), respectively. Different letters represent significant differences among ecotypes ($P < 0.05$). *WS* woodland savanna (in red), *IS* intermediate savanna (in orange), *OS* open savanna (in yellow).

ate savanna separately (Figure 2). Thus, the high intensity (1c) drying-rewetting regime increased the ammonification rates as compared to the constant regime (Figure 2a), whereas the low intensity (2c) drying-rewetting regime increased the NO_3^- -N and TIN immobilization compared to the constant regime (Figure 2b, c, respectively). Moreover, we found an overall significant effect of the soil water regime on all GHGs fluxes and the % of change of the soil bacterial community structure (Table 2), but the magnitude of these effects varied among ecotypes (Figure 3 and S3). Thus, when considering the different ecotypes separately, N_2O emissions were significantly higher in the samples

subjected to the high-intensity 1c regime than in those incubated at constant moisture, but only in the woodland and the open savanna (Figure 3a). Contrastingly, soil CO_2 emissions were significantly higher in the constant than in the rest of the regimes, independently of the ecotype, although the differences were less marked in the woodland than in the intermediate and the open savanna (Figure 3b). On the other hand, soil CH_4 uptake was significantly lower in the high-intensity 1c regime than in the other two regimes but only in the intermediate and the open savanna (Figure 3c). Soil bacterial richness and diversity from the intermediate and the open savannas significantly

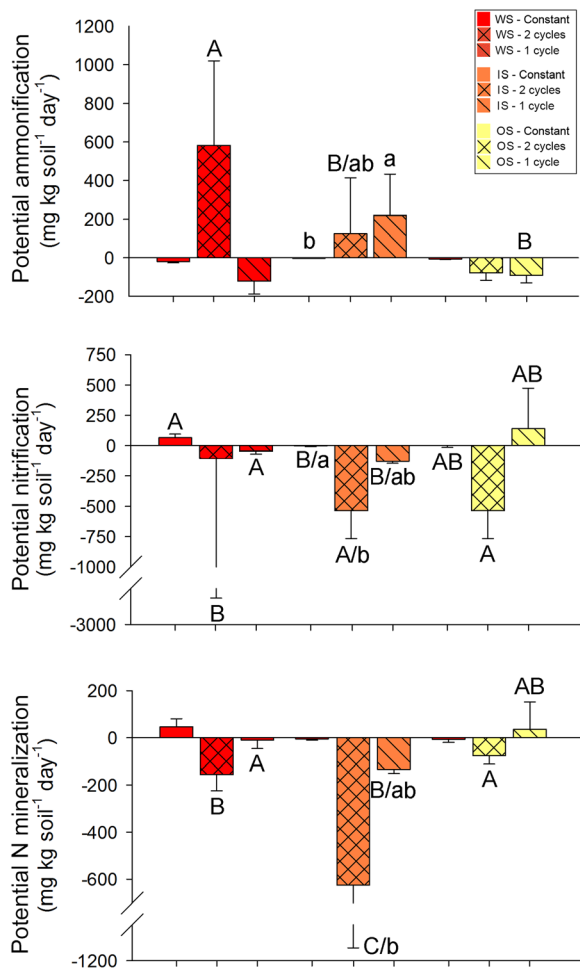


Figure 2. Potential soil N transformation rates of the three ecotypes during the three soil water regime treatments. Bars and error bars represent means ($n = 5$) and standard errors (1SE), respectively. Different capital letters represent significant differences among ecotypes within each treatment, and different lowercase letters represent differences among treatments within each ecotype ($P < 0.05$). *WS* woodland savanna (in red), *IS* intermediate savanna (in orange), *OS* open savanna (in yellow).

changed throughout the incubations under the cycle regimes, particularly under the 2c intermediate regime (Figure S4). However, neither a significant effect of the soil water regime treatment nor a significant treatment \times ecotype interaction was found for the % change of soil bacterial abundance, richness, or diversity (Table 2). Interestingly, the soil water regime did significantly affect the % change of soil bacterial community structure (that is, the % change of the relative abundance of each OTU before and after the incubation), with significant interaction with ecotype (Table 2, Figure S4). The % change of bacterial

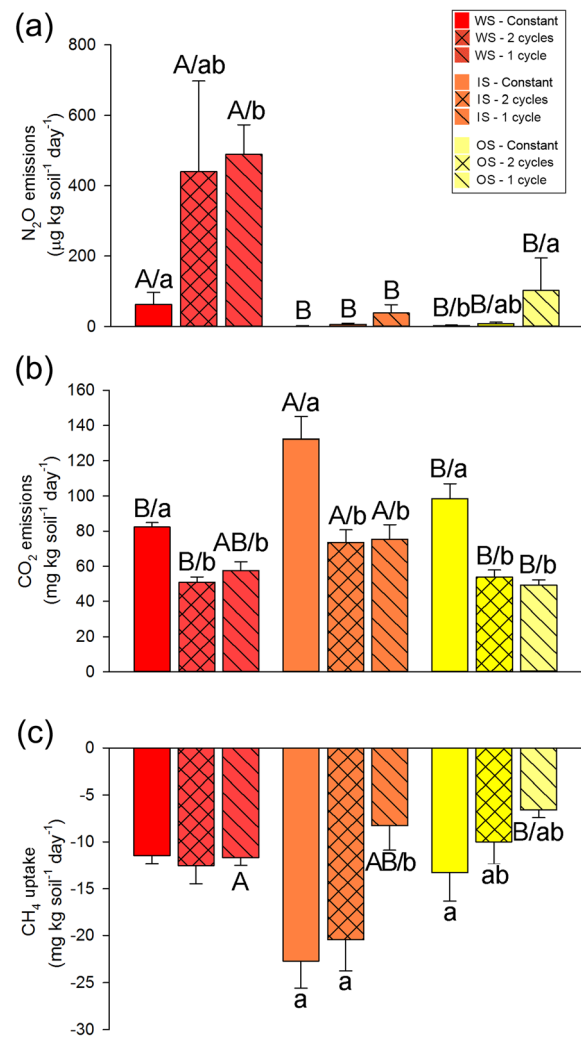


Figure 3. Greenhouse gas fluxes of soils collected from the three ecotypes during the three soil water regime treatments. Bars and error bars represent means ($n = 5$) and standard errors (1SE), respectively. Different capital letters represent significant differences among physiognomies within each treatment, and different lowercase letters represent differences among treatments within each physiognomy ($P < 0.05$). *WS* woodland savanna (in red), *IS* intermediate savanna (in orange), *OS* open savanna (in yellow).

community structure was more affected by the constant than by the cycle regimes, but only in the intermediate (different from both the 2c and 1c regimes) and open savanna (different from the 2c regime; Figure S4).

Our SEMs were able to explain 68, 67, 46 and 73% of the variability of N_2O , CO_2 , CH_4 , and nitrification fluxes, respectively (Figure 4) and provided evidence of opposite associations, both direct and indirect (via soil bacterial diversity and soil abiotic properties) of more open ecotypes and

Table 2. Statistical Results of PERMANOVA Testing the Effects of the Cerrado Ecotype (E), the Soil Water Regime (SWR), and the Interaction of Both (E x SWR) on Soil Functioning (Rates During Incubations) and the % of Change (% ch.) of Bacterial (B) Properties (Between Pre- and Post-incubation Values).

	Ecotype		SWR		E x SWR	
	Pseudo-F	<i>P</i>	Pseudo-F	<i>P</i>	Pseudo-F	<i>P</i>
Ammonification	11.6	< 0.001	0.66	0.532	0.24	0.930
Nitrification	11.3	< 0.001	1.10	0.346	0.99	0.419
N mineralization	4.87	< 0.01	2.06	0.121	0.82	0.537
N ₂ O flux	13.7	< 0.001	5.45	< 0.01	0.99	0.428
CO ₂ flux	30.6	< 0.001	76.6	< 0.01	2.31	0.074
CH ₄ flux	5.40	< 0.01	3.31	< 0.05	1.93	0.095
B. abundance (% ch.)	1.59	0.173	0.82	0.521	1.867	0.067
B. richness (% ch.)	7.83	< 0.005	2.92	0.055	0.24	0.945
B. diversity (% ch.)	12.12	< 0.001	1.07	0.358	1.14	0.347
B. comm. structure (% ch.)	1.645	< 0.05	3.344	< 0.001	1.5617	< 0.005

Statistically significant effects are represented by bold *P* values ($P < 0.05$).

the soil water regime intensification (SWRI; that is, less but more intense drying-rewetting events) on GHG fluxes. Thus, although nitrous oxide fluxes were affected directly and positively by the SWRI, these fluxes were consistently lower in more open ecotypes. These negative associations between N₂O and open ecotypes were both direct and indirect through increases in bacterial diversity and decreases in soil silt and C concentrations (which were negatively related to soil pH; Figure 4a and S6a). We also found a strong and negative direct effect of the SWRI on CO₂ emissions, but that was partially compensated by the positive effects of change from the woodland savanna to the open savanna, and particularly to the intermediate savanna (Figure 4b and S6b). These positive effects of the change in ecotype on CO₂ emissions were both direct and indirect via increases in soil pH and decreases in soil C and silt concentrations, which were negatively related to soil pH (Figure 4c, d). The intensification of the soil water regime was positively related to CH₄ fluxes (that is, less uptake) both directly and indirectly through decreases in bacterial abundance (Figure 4c and S6c). However, we also found negative indirect associations between more open ecotypes and CH₄ fluxes (that is, more uptake) through increases in soil pH (Figure 4c and S6c). Finally, the SWRI did not significantly relate with the potential nitrification rate (either directly or indirectly). However, this soil process tended to show lower rates in the more open ecotypes (Figure 4d and S6d). This negative effect was both direct and indirect through 1) negative effects on soil silt and C concentrations, which were positively related to soil bacterial

diversity; and 2) positive effects on soil pH, which negatively correlated with soil silt and C concentrations.

DISCUSSION

The results of this study show that soils from different ecotypes, despite coming from areas that shared similar bedrock and climate conditions, differed in most of the chemical, physical and biological variables analyzed. Further, they support our hypothesis of an overall higher soil functioning potential in closer ecotypes (that is, woodland and intermediate savannas) than in open ecotypes. Specifically, soils from the woodland savanna showed higher amounts of clay and organic matter than the other two ecotypes, and particularly than the open savanna, as well as an overall better nutritional status, with higher total C and N concentrations, higher potential N cycling rates and soil bacterial abundance, but lower bacterial richness. Although understanding the causes of these differences in soil attributes is beyond the objectives of this study, we can speculate that differences in soil texture among ecotypes may be caused by differences in the sedimentary parent rock even at the small scale of our study, with differences in texture observed depending on whether the parent rock was slate or limestone, the two parent rocks of the study area (Anderson 1988; da Silva and others 2022). Additionally, higher rates of wind and water erosion of fine particles in areas with lower vegetation density may also be behind the textural differences between ecotypes (Zuazo and Pleguezuelo 2008; Wu and others 2020). On the other hand, the

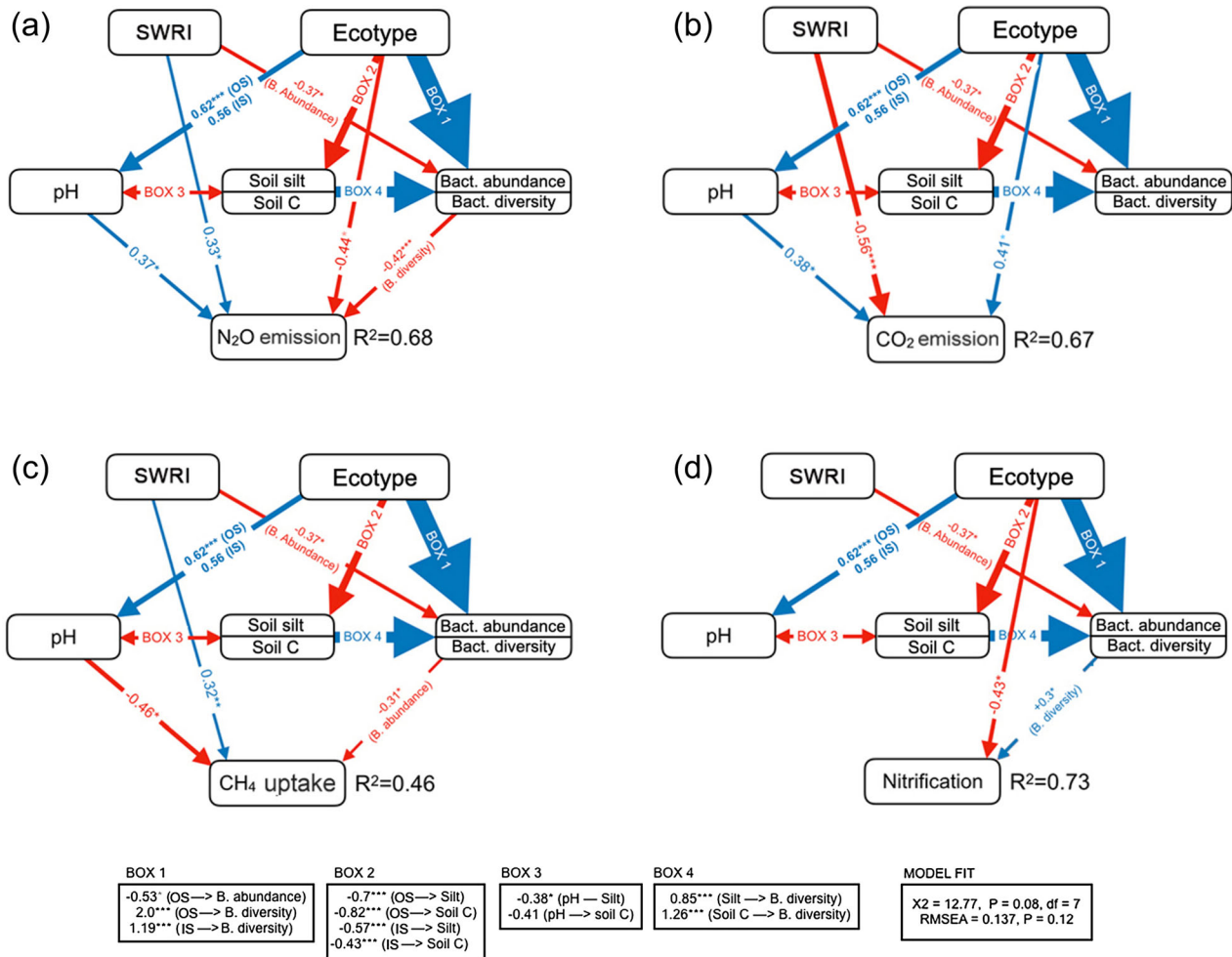


Figure 4. Effects of change in the ecotype from woodland to intermediate (IS) and open savanna (OS), the intensification of the soil water regime (SWRI), and key soil properties (pH, C and silt concentrations, and bacterial abundance and diversity) on soil functioning. The soil functioning surrogates represented are potential soil:atmosphere N₂O (a), CO₂ (b), and CH₄ (c) fluxes, and potential nitrification rate (d). Numbers on arrows and width of lines are indicative of the effect size of the relationship. Continuous and dashed arrows indicate significant and not significant relationships, respectively. Positive and negative relationships are represented in blue and red, respectively. Significance levels are as follows: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

vegetation in our site and plots, which have been protected from cutting and fire at least since 1963 (Neri and others 2012a), is old enough to have significantly influenced some biotic and chemical soil attributes (You and others 2014). Thus, the tropical vegetation growing in the dystrophic soils of the woodland savanna, with higher levels of plant cover than the intermediate and open savannas, can produce higher amounts of litter (Peixoto and others 2018; Lacerda 2019), and thus maintain higher overall soil fertility via substrate inputs (Gallardo and others 2000; Tang and Baldocchi 2005). Higher soil organic matter content can in turn maintain larger but less diverse microbial populations, as well as higher potential N

transformation rates (Xue and others 2018). Additionally, the dominance of N-fixing leguminous species in the woodland savanna, known to provide the soil with higher litter quality and N content, but also higher microbial biomass content (Neto and others 2011) could also help to explain the higher potential N transformation rates (Rodríguez and others 2007; Meira-Neto and others 2017). Further, both nitrification and denitrification, the two processes that determine net soil N₂O emissions, are known to strongly depend on the amount of available soil N (Stark and Firestone 1995). Thus, it is also likely that these higher N transformation rates in the woodland savanna are responsible for its higher rates of N₂O emissions.

Although our experimental design does not allow us to unveil the mechanistic processes behind these results, our study confirms that, even if they are in very close proximity, soils from open physiognomies of the Cerrado can show lower soil fertility, microbial abundance, capacity to mineralize N, and potential N₂O emissions than soils from more closed physiognomies. Interestingly, our results also suggest that intermediate ecotypes showed the highest levels of microbial respiration (that is, CO₂ emissions), but also of the capacity of these soils to uptake atmospheric CH₄. Considering the intermediate values of SOM and bacterial abundance and diversity for the intermediate savanna, our results also suggest the existence of a more labile SOM (Gregorich and others 2006) as well as a greater and/or more active (due to appropriate soil conditions) methanotrophic community (Malghani and others 2016; Ni and Groffman 2018). Results from our SEMs support these two assumptions (see below).

Moisture was the soil variable that interfered most with GHG fluxes in a study carried out on different land uses (including native vegetation) in the Cerrado biome (Neto and others 2011). Our study goes further and provides evidence that the Cerrado ecotype determines the responses of soil biodiversity and functioning (including GHG fluxes) to changing soil water regimes, suggesting a dissimilar sensitivity of soils from different ecotypes to the forecasted intensification of the hydrological regime (Marengo and others 2010; Seddon and others 2016). Moreover, we found that the modulator role of the ecotype in the response of soil functions and microbes to changes in soil water regime depends on the studied function and microbial properties. For instance, N₂O emissions responded more to the intensification of the soil water regime in soils from the woodland savanna than from the other ecotypes, perhaps due to the higher content in mineralizable organic matter and therefore potentially stronger pulses of available N (Stark and Firestone 1995; Durán and others 2013a). However, the rest of the studied variables (for example, N mineralization rates, CH₄ and CO₂ fluxes, bacterial properties) showed the highest responses to the intensification of the soil water regime in soils from the other ecotypes, particularly from the intermediate savanna. More specifically, the intensification of the soil water regime in soils from the intermediate savanna significantly increased the microbial immobilization of N, decreased the CO₂ emissions and CH₄ uptake, and changed the microbial community structure. These results partially support our hypothesis of open

ecotypes having lower sensitivity to changes in the soil water regime than closer ecotypes (open savanna as compared to the intermediate savanna). This lower sensitivity could likely be due to soil microbial communities better adapted (that is, higher resilience) to drying-wetting stresses, as well as to the observed highest bacterial richness and the poorest nutritional status of soils from the open savanna (Rodríguez and others 2019). However, our results also point out an unexpected overall potential resistance of the soil functioning in the woodland savanna to changes in the soil water regime, likely linked to a strong and more resistant soil microbial community (Figure 4). In any case, our results suggest that any change in dominance of any of the Cerrado ecotypes might result in a different overall response of this biome to the forecasted intensification of the precipitation regimes. Further, our study shows a differential sensitivity to changes in the soil water regime of the microbial community and associated N- (for example, nitrification, denitrification) and C-cycling related (for example, soil:atmosphere CH₄ and CO₂ exchange) processes of the different ecotypes. This different sensitivity could lead to a decoupling between soil C and N cycles in the Cerrado, with likely important but hard to anticipate effects on these ecosystems (Peñuelas and others 2012; Delgado-Baquerizo and others 2013; Durán and others 2017).

Our study also shows multiple direct and indirect (via soil microbes and abiotic properties) effects of an intensification of the precipitation regime on soil C- and N-related processes, but that these effects may vary depending on the type of Cerrado ecotype in which they occur. Thus, whereas our structural equation model shows that open Cerrado ecotypes are associated with lower N₂O soil emissions, this effect is at least partially compensated if the same soils experience an intensification of the hydrological regime. Our results suggest that the increases in potential N₂O emissions linked to the intensification of the hydrological regime are likely to be driven by microbial turnover and associated increases in N availability (Stark and Firestone 1995; Durán and others 2013b), and that the observed lower potential N₂O emissions in open savannas as compared to more closed ecotypes might be at least partially driven by changes in soil features such as bacterial diversity and silt and C concentrations. Similarly, while increases in the intensity of the hydrological regime were strongly linked to decreases in CO₂ fluxes, changes from the closest formations to other ecotypes might compensate this effect not only directly, but also indi-

rectly through increases in soil pH (Malik and others 2018), and through decreases in soil C and silt concentrations (Cleveland and others 2006; Cable and others 2008). In the case of soil CH₄ uptake, it is important to note that our analyses suggest that an intensification of the hydrological regime would likely decrease the capacity of these soils to fix atmospheric CH₄, both directly and indirectly through decreases in bacterial abundance. Thus, this study adds experimental evidence of a mechanism that might help to explain the observed decrease in CH₄ uptake from forest soils worldwide (Ni and Groffman 2018). Interestingly, our results also show that this effect would be at least partially compensated in more open ecotypes, and particularly in intermediate ecotypes, due to the inherently higher soil pH and bacterial abundance in these more open ecotypes (Sitaula and others 1995; Li and others 2017). Finally, our SEM also confirms that the lower N mineralization rates observed in more open physiognomies can also be indirectly mediated via cascade effects on soil pH, C and silt concentrations, and bacterial diversity (Liu and others 2017; Geisseler and others 2019; Li and others 2019). Remarkably, our results do not show any clear direct or indirect significant compensatory or intensifying effect of a potential intensification of the hydrological regime, which suggests an unexpected tolerance to changes in soil water regimes and highlights the particularly strong links between the different ecotypes and their ability to mineralize soil organic nitrogen (Mueller and others 2013; Morillas and others 2015).

In summary, we show that the existence of different ecotypes, even in the same area and climate, have a strong potential to modulate the responses of soil microbial properties and functions to the intensification of the hydrological regime. These results suggest that any attempt to forecast the fate of the Cerrado in coming decades will necessarily need to consider how the effects of climate change operate in the different ecotypes of the Cerrado. This is of particular relevance considering that the Cerrado is one of the most important hotspots of land use change worldwide (Siqueira-Neto and others 2021). However, we acknowledge that soil laboratory incubations, where the influence of plant roots is missing, hardly enable forecasting actual ecosystem responses to changes in environmental conditions. Thus, although the controlled nature of our manipulations can provide powerful mechanistic insights about Cerrado functioning and its response to climate change, further experimental studies that consider the effect of vegetation are necessary to validate our results.

ACKNOWLEDGEMENTS

We would like to thank Glauca Tolentino and Jan Lehmann for their help with the selection of the sampling areas and fieldwork. J.D. and A.R. acknowledge support from the FCT (IF/00950/2014 and SFRH/BDP/108913/2015, respectively), as well as from the MCTES, FSE, UE, and the CFE (UIDB/04004/2020) research unit financed by FCT/MCTES through national funds (PIDDAC). M.D-B. acknowledges support from the Marie Skłodowska-Curie Actions of the Horizon 2020 Framework Programme H2020-MSCA-IF-2016 under REA Grant agreement no. 702057 (CLIMIFUN) and the BES Grant agreement n° LRA17\1193 (MUSG-ONET). The authors declare no conflict of interest.

FUNDING

Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

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DATA AVAILABILITY

Data are available at <https://figshare.com/s/87e1405b03d8faef0691>.

REFERENCES

- Anderson DW. 1988. The effect of parent material and soil development on nutrient cycling in temperate ecosystems. *Biogeochemistry* 5:71–97. <https://doi.org/10.1007/BF02180318>. Last accessed 25/01/2023
- Anderson MJ, Gorley RN, Clarke RK. 2008. *Permanova + for Primer: guide to software and statistical methods*. Plymouth, UK: PRIMER-E Ltd.
- de Assis ACC, Coelho RM, da Pinheiro E, S, Durigan G. 2011. Water availability determines physiognomic gradient in an

- area of low-fertility soils under Cerrado vegetation. *Plant Ecol* 212:1135–1147.
- Barel JM, Mouliá V, Hamard S, Sytiuk A, Jassey VEJ. 2021. Come rain, come shine: peatland carbon dynamics shift under extreme precipitation. *Front Environ Sci* 9:233.
- Blazewicz SJ, Schwartz E, Firestone MK. 2014. Growth and death of bacteria and fungi underlie rainfall-induced carbon dioxide pulses from seasonally dried soil. *Ecology* 95:1162–72. <https://doi.org/10.1890/13-1031.1>. Last accessed 19/11/2019
- Bonanomi J, Tortato FR, de Gomes R, SR, Penha JM, Bueno AS, Peres CA. 2019. Protecting forests at the expense of native grasslands: Land-use policy encourages open-habitat loss in the Brazilian cerrado biome. *Perspect Ecol Conserv* 17:26–31.
- Bustamante M, Nardoto G, Pinto A, Resende J, Takahashi F, Vieira L. 2012. Potential impacts of climate change on biogeochemical functioning of Cerrado ecosystems. *Brazilian Journal of Biology* 72:655–71. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1519-69842012000400005&lng=en&tlng=en. Last accessed 19/11/2019
- Cable JM, Ogle K, Williams DG, Weltzin JF, Huxman TE. 2008. Soil texture drives responses of soil respiration to precipitation pulses in the sonoran desert: Implications for climate change. *Ecosystems* 11:961–979.
- Cleveland CC, Nemergut DR, Schmidt SK, Townsend AR. 2006. Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* 82:229–240.
- Coutinho LM. 2006. O conceito de bioma. *Acta Bot Brasílica* 20:13–23.
- Dane JH, Topp GClarke, Campbell GS. 2002. Methods of soil analysis. Part 4, Physical methods. :1692.
- da Peixoto K, S, Marimon-Junior BH, Cavalheiro KA, Silva NA, das Neves EC, Freitag R, Mews HA, Valadão MBX, Marimon e. BS. 2018. Assessing the effects of rainfall reduction on litterfall and the litter layer in phytophysiologicals of the Amazonia-Cerrado transition. *Revista Brasileira De Botanica* 41:589–600.
- da Silva CJ, Sanches L, Bleich ME, Lobo FDA, Nogueira JDS. 2007. Produção de serrapilheira no Cerrado e Floresta de Transição Amazônia-Cerrado do Centro-Oeste Brasileiro. *Acta Amazon* 37:543–548.
- da Silva RJAB, da Silva YJAB, van Straaten P, do Nascimento CWA, Biondi CM, da Silva YJAB, de Araújo Filho JC. 2022. Influence of parent material on soil chemical characteristics in a semi-arid tropical region of Northeast Brazil. *Environ Monit Assess* 194. <https://pubmed.ncbi.nlm.nih.gov/35386016/>. Last accessed 25/01/2023
- de Campos ÉP, Duarte TG, Neri AV, da Silva AF, Meira-Neto JAA, Valente GE. 2006. Composição florística de um trecho de Cerradão e Cerrado sensu stricto e sua relação com o solo na Floresta Nacional (FLONA) de Paraopeba, MG, Brasil. *Revista Árvore* 30:471–479.
- de Castro AP, da Silva MRSS, Quirino BF, da Cunha Bustamante MM, Krüger RH. 2016. Microbial diversity in cerrado biome (neotropical savanna) soils. *PLoS One* 11.
- de Oliveira VA, de Mello CR, Beskow S, Viola MR, Srinivasan R. 2019. Modeling the effects of climate change on hydrology and sediment load in a headwater basin in the Brazilian Cerrado biome. *Ecol Eng* 133:20–31.
- de Souza PB, Junior AWS, Soares MP, Viana RHO, de Camargos VL, Neto JAAM. 2010. Floristic of an area of Cerradão within the national forest of Paraopeba - Minas Gerais. *Cerne*, Lavras 16:86–93.
- Delgado-Baquerizo M, Maestre FT, Gallardo A, Bowker MA, Wallenstein MD, Quero JL, Ochoa V, Gozalo B, García-Gómez M, Soliveres S, García-Palacios P, Berdugo M, Valencia E, Escolar C, Arredondo T, Barraza-Zepeda C, Bran D, Carreira JA, Chaieb M, Conceição AA, Derak M, Eldridge DJ, Escudero A, Espinosa CI, Gaitán J, Gatica MG, Gómez-González S, Guzman E, Gutiérrez JR, Florentino A, Hepper E, Hernández RM, Huber-Sannwald E, Jankju M, Liu J, Mau RL, Miriti M, Moneris J, Naseri K, Noumi Z, Polo V, Prina A, Pucheta E, Ramírez E, Ramírez-Collantes DA, Romão R, Tighe M, Torres D, Torres-Díaz C, Ungar ED, Val J, Wamiti W, Wang D, Zaady E. 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature* 502:672–676.
- Dijkstra FA, Augustine DJ, Brewer P, von Fischer JC. 2012. Nitrogen cycling and water pulses in semiarid grasslands: Are microbial and plant processes temporally asynchronous? *Oecologia* 170:799–808.
- Durán J, Morse JL, Rodríguez A, Campbell JL, Christenson LM, Driscoll CT, Fahey TJ, Fisk MC, Mitchell MJ, Templer PH, Groffman PM. 2017. Differential sensitivity to climate change of C and N cycling processes across soil horizons in a northern hardwood forest. *Soil Biol Biochem* 107:77–84.
- Durán J, Rodríguez A, Fernández-Palacios JM, Gallardo A. 2009. Changes in net N mineralization rates and soil N and P pools in a pine forest wildfire chronosequence. *Biol Fertil Soils* 45:781–788.
- Durán J, Rodríguez A, Morse JL, Groffman PM. 2013. Winter climate change effects on soil C and N cycles in urban grasslands. *Glob Chang Biol* 19:2826–2837.
- Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10:996–998.
- Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194–2200.
- Eiten G. 1972. The cerrado vegetation of Brazil. *The Botanical Review* 38:201–341.
- Gallardo A, Rodríguez-Saucedo JJ, Covelo F, Fernández-Alés R. 2000. Soil nitrogen heterogeneity in a Dehesa ecosystem. *Plant Soil* 222:71–82.
- Geisseler D, Miller KS, Aegerter BJ, Clark NE, Miyao EM. 2019. Estimation of annual soil nitrogen mineralization rates using an organic-nitrogen budget approach. *Soil Science Society of America Journal* 83:1227–1235.
- Giácomo RG, Pereira MG, Guareschi RF, Machado DL. 2015. Chemical and physical attributes of soil, and carbon, and nitrogen stock and humic fractions in different plant formations. *Ciência Florestal* 25:617–631.
- Gomes LC, Faria RM, de Souza E, Veloso GV, Schaefer CEGR, Filho EIF. 2019. Modelling and mapping soil organic carbon stocks in Brazil. *Geoderma* 340:337–350.
- Grace J, Jose JS, Meir P, Miranda HS, Montes RA. 2006. Productivity and carbon fluxes of tropical savannas. *J Biogeogr* 33:387–400. <https://doi.org/10.1111/j.1365-2699.2005.01448.x>. Last accessed 19/11/2019
- Grace JB. 2006. Structural equation modeling and natural systems. Cambridge University Press.
- Gregorich EG, Rochette P, Hopkins DW, McKim UF, St-Georges P. 2006. Tillage-induced environmental conditions in soil and substrate limitation determine biogenic gas production. *Soil Biol Biochem* 38:2614–2628.
- Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF. 2011. Transitions in bacterial communities

- along the 2000 km salinity gradient of the Baltic Sea. *ISME J* 5:1571–1579.
- Hooper D, Coughlan J, Mullen M. 2008. Structural Equation Modelling: Guidelines for Determining Model Fit. *Articles*.
- Huntington TG. 2006. Evidence for intensification of the global water cycle: Review and synthesis. *J Hydrol (amst)* 319:83–95.
- Jenkinson DS, Powelson DS. 1980. Measurement of microbial biomass in intact soil cores and in sieved soil. *Soil Biol Biochem* 12:579–581.
- Kline RB. 2011. Principles and Practice of Structural Equation Modeling, 3rd edn Guilford, 3rd edn. New York: Guilford Press.
- Lacerda F. 2019. Estoques atuais e futuros de carbono, nitrogênio e fósforo do solo no Cerrado.
- Li S, Song L, Gao X, Jin Y, Liu S, Shen Q, Zou J. 2017. Microbial Abundances Predict Methane and Nitrous Oxide Fluxes from a Windrow Composting System. *Front Microbiol* 8.
- Li Z, Tian D, Wang B, Wang J, Wang S, Chen HYH, Xu X, Wang C, He N, Niu S. 2019. Microbes drive global soil nitrogen mineralization and availability. *Glob Chang Biol* 25:1078–1088.
- Liu Y-R, Delgado-Baquerizo M, Trivedi P, He J-Z, Wang J-T, Singh BK. 2017. Identity of biocrust species and microbial communities drive the response of soil multifunctionality to simulated global change. *Soil Biol Biochem* 107:208–217.
- Maestre FT, Quero JL, Gotelli NJ, Escudero A, Ochoa V, Delgado-Baquerizo M, Garcia-Gomez M, Bowker MA, Soliveres S, Escolar C, Garcia-Palacios P, Berdugo M, Valencia E, Gozalo B, Gallardo A, Aguilera L, Arredondo T, Blones J, Boeken B, Bran D, Conceicao AA, Cabrera O, Chaieb M, Derak M, Eldridge DJ, Espinosa CI, Florentino A, Gaitan J, Gatica MG, Ghiloufi W, Gomez-Gonzalez S, Gutierrez JR, Hernandez RM, Huang X, Huber-Sannwald E, Jankju M, Miriti M, Moneris J, Mau RL, Morici E, Naseri K, Ospina A, Polo V, Prina A, Pucheta E, Ramirez-Collantes DA, Romao R, Tighe M, Torres-Diaz C, Val J, Veiga JP, Wang D, Zaady E. 2012. Plant Species Richness and Ecosystem Multifunctionality in Global Drylands. *Science* (1979) 335:214–8. <http://www.ncbi.nlm.nih.gov/pubmed/22246775>. Last accessed 27/09/2019
- Maksic J, Venancio IM, Shimizu MH, Chiessi CM, Piacsek P, Sampaio G, Cruz FW, Alexandre FF. 2022. Brazilian biomes distribution: Past and future. *Palaeogeogr Palaeoclimatol Palaeoecol* 585.
- Malghani S, Reim A, von Fischer J, Conrad R, Kuebler K, Trumbore SE. 2016. Soil methanotroph abundance and community composition are not influenced by substrate availability in laboratory incubations. *Soil Biol Biochem* 101:184–194.
- Malik AA, Puissant J, Buckeridge KM, Goodall T, Jehmlich N, Chowdhury S, Gweon HS, Peyton JM, Mason KE, van Agtmaal M, Blaud A, Clark IM, Whitaker J, Pywell RF, Ostle N, Gleixner G, Griffiths RI. 2018. Land use driven change in soil pH affects microbial carbon cycling processes. *Nat Commun* 9.
- Marengo JA, Ambrizzi T, da Rocha RP, Alves LM, Cuadra SV, Valverde MC, Torres RR, Santos DC, Ferraz SET. 2010. Future change of climate in South America in the late twenty-first century: intercomparison of scenarios from three regional climate models. *Clim Dyn* 35:1073–1097.
- Marimon Junior BH, Haridasan M. 2005. Comparação da vegetação arbórea e características edáficas de um cerrado e um cerrado sensu stricto em áreas adjacentes sobre solo distrófico no leste de Mato Grosso, Brasil. *Acta Bot Brasílica* 19:913–926.
- Martins CSC, Nazaries L, Macdonald CA, Anderson IC, Singh BK. 2015. Water availability and abundance of microbial groups are key determinants of greenhouse gas fluxes in a dryland forest ecosystem. *Soil Biol Biochem* 86:5–16.
- Masson-Delmotte V, Zhai P, Chen Y, Goldfarb L, Gomis MI, Matthews JBR, Berger S, Huang M, Yelekçi O, Yu R, Zhou B, Lonnoy E, Maycock TK, Waterfield T, Leitzell K, Caud N. 2021. Working Group I Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change Edited by. www.ipcc.ch. Last accessed 20/07/2022
- Medeiros MDODN, De Oliveira FHT, Preston W, Paiva MRDFC, Góis HMDMN. 2021. Comparison of methods for extracting available phosphorus from soils of the semi-arid. *Revista Ciência Agronômica* 52:1–14.
- Meira-Neto JAA, Tolentino GS, da Silva MCNA, Neri AV, Gastauer M, Magnago LFS, Yuste JC, Valladares F. 2017. Functional antagonism between nitrogen-fixing leguminous trees and calcicole-drought-tolerant trees in the Cerrado. *Acta Bot Brasílica* 31:11–8. <http://www.scielo.br/j/abb/a/NKw3DGBcBCWYsVyR3JDdqXD/?lang=en>. Last accessed 21/07/2022
- Mendonça RC, Felfili JM, Walter BMT, Silva Júnior MC, Rezende AV, Filgueiras TS, Nogueira PE, Fagg CW. 2008. Flora vascular do Bioma Cerrado: checklist com 12.356 espécies. In: Sano SM, Almeida SP, Ribeiro JF, editors. *Cerrado: ecologia e flora*. Brasília: Embrapa Cerrados. pp 423–1279.
- Miranda AC, Miranda HS, Lloyd J, Grace J, Francey RJ, McIntyre JA, Meir P, Riggan P, Lockwood R, Brass J. 1997. Fluxes of carbon, water and energy over Brazilian cerrado: an analysis using eddy covariance and stable isotopes. *Plant, Cell and Environment* 20(3): 315–328 20:315–28.
- Morillas L, Durán J, Rodríguez A, Roales J, Gallardo A, Lovett GM, Groffman PM. 2015. Nitrogen supply modulates the effect of changes in drying-rewetting frequency on soil C and N cycling and greenhouse gas exchange. *Glob Chang Biol* 21:3854–63. <http://www.ncbi.nlm.nih.gov/pubmed/25916277>. Last accessed 23/04/2020
- Morse JL, Durán J, Groffman PM. 2015. Soil Denitrification Fluxes in a northern hardwood forest: the importance of snowmelt and implications for ecosystem N budgets. *Ecosystems* 18:520–532.
- Moyano FE, Manzoni S, Chenu C. 2013. Responses of soil heterotrophic respiration to moisture availability: An exploration of processes and models. *Soil Biol Biochem* 59:72–85.
- Mueller KE, Hobbie SE, Tilman D, Reich PB. 2013. Effects of plant diversity, N fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term experiment. *Glob Chang Biol* 19:1249–1261.
- Nelson D, Summers L. 1996. Total carbon, organic carbon and organic matter. In: Sparks D, editor. *Methods of Soil Analyses. Part 3. Chemical Methods*. Madison, WI: American Society of Agronomy-Soil Science Society of America. pp 539–94.
- Neri AV, Schaefer CEGR, Silva AF, Souza AL, Ferreira-Junior WG, Meira-Neto JAA. 2012a. The influence of soils on the floristic composition and community structure of an area of Brazilian Cerrado. *Edinb J Bot* 69:1–27.
- Neri AV, Schaefer CEGR, Silva AF, Souza AL, Ferreira-Junior WG, Meira-Neto JAA. 2012b. The influence of soils on the floristic composition and community structure of an area of Brazilian cerrado vegetation. *Edinb J Bot* 69:1–27.
- Neri AV, Schaefer CEGR, Souza AL, Ferreira-Junior WG, Meira-Neto JAA. 2013. Pedology and plant physiognomies in the cerrado, Brazil. *An Acad Bras Cienc* 85:87–102.

- Neto MS, de Piccolo M, C, Junior CC, Cerri CC, Bernoux M. 2011. Greenhouse gas emission caused by different land-uses in Brazilian savannah. *Rev Bras Cienc Solo* 35:63–76.
- Ni X, Groffman PM. 2018. Declines in methane uptake in forest soils. *Proc Natl Acad Sci U S A* 115:8587–8590.
- Peñuelas J, Sardans J, Rivas-ubach A, Janssens IA. 2012. The human-induced imbalance between C, N and P in Earth's life system. *Glob Chang Biol* 18:3–6.
- Radu DD, Duval TP. 2018. Precipitation frequency alters peatland ecosystem structure and CO₂ exchange: Contrasting effects on moss, sedge, and shrub communities. *Glob Chang Biol* 24:2051–65. <https://onlinelibrary.wiley.com/doi/full/https://doi.org/10.1111/gcb.14057>. Last accessed 24/01/2023
- Rengasamy P, Churchman G. 1999. Cation exchange capacity, exchangeable cations and sodicity. In: Peverill K, Sparrow L, Reuter D, Eds. *Soil analysis: an interpretation manual*. Collingwood, Vic: CSIRO Publishing. pp 147–157.
- Rey A, Jarvis P. 2006. Modelling the effect of temperature on carbon mineralization rates across a network of European forest sites (FORCAST). *Glob Chang Biol* 12:1894–1908.
- Ribeiro JF, Walter BMT. 2008. *As principais fitofisionomias do bioma Cerrado*, 1st edn. Brasília: Embrapa.
- Rodríguez A, Durán J, Gallardo A. 2007. Influence of legumes on N cycling in a heathland in northwest Spain. *Web Ecol* 7:87–93.
- Rodríguez A, Durán J, Rey A, Boudouris I, Valladares F, Gallardo A, Curiel J. 2019a. Geoderma Interactive effects of forest die-off and drying-rewetting cycles on C and N mineralization. *Geoderma* 333:81–89.
- Rodríguez A, Durán J, Rey A, Boudouris I, Valladares F, Gallardo A, Yuste JC. 2019b. Interactive effects of forest die-off and drying-rewetting cycles on C and N mineralization. *Geoderma* 333:81–89.
- Santos AJB, Quesada CA, da Silva GT, Maia JF, Miranda HS, Carlos Miranda A, Lloyd J. 2004. High rates of net ecosystem carbon assimilation by *Brachiaria* pasture in the Brazilian Cerrado. *Glob Chang Biol* 10:877–885.
- Schermelleh-Engel K, Moosbrugger H, Müller HH. 2003. Evaluating the fit of structural equation models: tests of significance and descriptive goodness-of-fit measures.
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75:7537–7541.
- Seddon AWR, Macias-Fauria M, Long PR, Benz D, Willis KJ. 2016. Sensitivity of global terrestrial ecosystems to climate variability. *Nature* 531:229–232.
- Silveira Sartori Silva MR, Pereira de Castro A, Krüger RH, Bustamante M. 2019. Soil bacterial communities in the Brazilian Cerrado: Response to vegetation type and management. *Acta Oecologica* 100.
- Siqueira-Neto M, Popin GV, Piccolo MC, Corbeels M, Scopel E, Camargo PB, Bernoux M. 2021. Impacts of land use and cropland management on soil organic matter and greenhouse gas emissions in the Brazilian Cerrado. *Eur J Soil Sci* 72:1431–1446.
- Sitaula BK, Bakken LR, Abrahamsen G. 1995. CH₄ uptake by temperate forest soil: effect of N input and soil acidification. *Soil Biol Biochem* 27:871–880.
- Soliveres S, Maestre FT, Eldridge DJ, Delgado-Baquerizo M, Quero JL, Bowker MA, Gallardo A. 2014. Plant diversity and ecosystem multifunctionality peak at intermediate levels of woody cover in global drylands. *Global Ecology and Biogeography* 23:1408–1416.
- Song X, Zhu J, He N, Huang J, Tian J, Zhao X, Liu Y, Wang C. 2017. Asynchronous pulse responses of soil carbon and nitrogen mineralization to rewetting events at a short-term: Regulation by microbes. *Sci Rep* 7:7492.
- Souza JP, Melo NMJ, Pereira EG, Halfeld AD, Gomes IN, Prado CHBA. 2016. Responses of woody Cerrado species to rising atmospheric CO₂ concentration and water stress: gains and losses. *Functional Plant Biology* 43:1183–1193.
- Stark JM, Firestone MK. 1995. Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Appl Environ Microbiol* 61:218–221.
- Tang J, Baldocchi DD. 2005. Spatial-temporal variation in soil respiration in an oak-grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. *Biogeochemistry* 73:183–207.
- Varella RF, Bustamante MMC, Pinto AS, Kisselle KW, Santos R v., Burke RA, Zepp RG, Viana LT. 2004. Soil fluxes of CO₂, CO, NO, and N₂O from an old pasture and from native Savanna in Brazil. *Ecological Applications* 14.
- Wu GL, Liu YF, Cui Z, Liu Y, Shi ZH, Yin R, Kardol P. 2020. Trade-off between vegetation type, soil erosion control and surface water in global semi-arid regions: A meta-analysis. *Journal of Applied Ecology* 57:875–85. <https://doi.org/10.1111/1365-2664.13597>. Last accessed 25/01/2023
- Xue P-P, Carrillo Y, Pino V, Minasny B, AlexB McBratney. 2018. Soil properties drive microbial community structure in a large scale transect in South Eastern Australia. *Sci Rep* 8:11725.
- You Y, Wang J, Huang X, Tang Z, Liu S, Sun OJ. 2014. Relating microbial community structure to functioning in forest soil organic carbon transformation and turnover. *Ecol Evol* 4:633–47. <https://pubmed.ncbi.nlm.nih.gov/25035803/>. Last accessed 21/07/2022
- Zheng Q, Hu Y, Zhang S, Noll L, Böckle T, Richter A, Wanek W. 2019. Growth explains microbial carbon use efficiency across soils differing in land use and geology. *Soil Biol Biochem* 128:45. [/pmc/articles/PMC6774786/](https://pubmed.ncbi.nlm.nih.gov/3174786/). Last accessed 20/07/2022
- Zuazo VHD, Pleguezuelo CRR. 2008. Soil-erosion and runoff prevention by plant covers. A review. *Agronomy for Sustainable Development* 28:1 28:65–86. <https://doi.org/10.1051/agro:2007062>. Last accessed 25/01/2023