GABRIELA SIEWERDING MEIRELLES

SOIL MICROBIAL ATTRIBUTES AND FUNCTION ACROSS MULTIPLE SCALE IN NEOTROPICAL SAVANNA

LAVRAS-MG
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Thesis submitted for the degree of Doctor of Philosophy as a Dual PhD between the Lancaster Environment Centre, Lancaster University, United Kingdom and the Applied Ecology Postgraduate Program, Universidade Federal de Lavras, Brazil.

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ATRIBUTOS E FUNÇÃO MICROBIANA DO SOLO EM ESCALA MÚLTIPLA NA SAVANNA NEOTROPICAL

Thesis submitted for the degree of Doctor of Philosophy as a Dual PhD between the Lancaster Environment Centre, Lancaster University, United Kingdom and the Applied Ecology Postgraduate Program, Universidade Federal de Lavras, Brazil.

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Para a minha mãe e para meu pai.

Dedico

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“Adianta querer saber muita coisa? O senhor sabia, lá para cima – me disseram. Mas, de repente, chegou neste sertão, viu tudo diverso diferente, o que nunca tinha visto. Sabença aprendida não adiantou para nada.”

“A gente tem de sair do sertão! Mas só se sai do sertão é tomando conta dele a dentro.”

“Sertão sempre. Sertão é isto: o senhor empurra para trás, mas de repente ele volta a rodear o senhor dos lados. Sertão é quando menos se espera: digo.”

“Sertão, - se diz -, o senhor querendo procurar, nunca não encontra. De repente, por si, quando a gente não espera, o sertão vem.”

“[...]O sertão aceita todos os nomes: aqui é o Gerais, lá é o Chapadão, lá acolá é a caatinga[...]”

“O senhor vê aonde é o sertão? Beira dele, meio dele?... Tudo sai mesmo de escuros buracos, tirando o que vem do céu.”

Trechos do livro *Grande Sertão: Veredas* de João Guimarães Rosa

RESUMO

As savanas são os ecossistemas mais sensíveis às futuras mudanças no uso da terra e no clima. Portanto, a compreensão dos fatores que sustentam a função ecossistemica da savana é urgentemente necessária para a conservação e o manejo ecologicamente relevantes. Esta tese tem como objetivo abordar essa lacuna do conhecimento, investigando como as condições locais e as características da paisagem influenciam os atributos microbianos do solo, e a resistência e resiliência do funcionamento do solo dentro dos três tipos de vegetação do bioma Cerrado: Floresta de galeria, Veredas e Cerrado *sensu stricto*. Primeiramente, avaliei como as características da vegetação local e as propriedades físico-químicas do solo interagem e influenciam os atributos microbianos do solo (Capítulo 2). Depois, investiguei como o distúrbio antropogênico e a topografia em escala de paisagem afetam a estrutura da comunidade microbiana do solo e a atividade enzimática (Capítulo 3). Por fim, avaliei como as emissões de CO_2 do solo estão relacionadas aos atributos microbianos e a topografia, e avaliei a resistência e resiliência dos solos (respiração) dos diferentes tipos de vegetação em resposta à seca e a reumidificação (Capítulo 4). Em escala local, encontrei diferentes padrões nas interações acima e abaixo do solo nos três tipos de vegetação. As propriedades físico-químicas do solo afetaram fortemente os atributos microbianos do solo em florestas de galerias e Veredas, enquanto as características vegetação foram os principais determinantes no Cerrado *sensu stricto*. Na escala de paisagem, encontrei que a topografía é o principal preditor dos atributos microbianos em todos os tipos de vegetação e que o distúrbio antropogênico influencia as comunidades microbianas do solo tanto positiva quanto negativamente. Os solos das Veredas foram os mais resistentes à seca, enquanto que os solos das Florestas de Galeria foram os mais resilientes em resposta a reumidificação após a seca. Com base nesses resultados, discuto a importância dos regimes de inundação nos tipos de vegetação de várzea (florestas de galeria e Veredas) para o funcionamento do ecossistema e implicações para o futuro uso da terra e mudanças climáticas no bioma Cerrado (Capítulo 5), e ressalto a necessidade de a legislação ambiental brasileira priorizar a conservação integral das áreas periodicamente inundadas.

Palavras-chave: Savana neotropical. Bioma Cerrado. Floresta de galeria. Veredas. Cerrado *sensu stricto.* Distúrbio antropogênico. Topografia. Alagamento. Propriedades do solo. Resistência e resiliência dos solos.

ABSTRACT

Savannas are the most sensitive ecosystems to future changes in land use and climate. Therefore, understanding of the factors underpinning savanna ecosystem function is urgently required for ecologically-relevant conservation and management. This thesis aims to address this knowledge gap, investigating how local conditions and landscape features influence soil microbial attributes, and the resistance and resilience of soil functioning within the three distinct vegetation types of the Brazillian Cerrado biome: Gallery forest, Veredas and Cerrado *sensu stricto*. Firstly, I evaluated how local vegetation characteristics and soil physico-chemical properties interact and influence soil microbial attributes (Chapter 2). Secondly, I investigated how landscape-scale anthropogenic disturbance and topography affect soil microbial community structure and enzyme activity (Chapter 3). Lastly, I assessed how soil CO_2 emissions are related to microbial attributes and topography, and the resistance and resilience of soil functioning (respiration) of the vegetation types in response to drought and drying-rewetting (Chapter 4). At the local scale, I found different patterns in above- and below-ground interactions within the three vegetation types. Soil physico-chemical properties strongly affected soil microbial attributes in Gallery forests and Veredas, whereas vegetation characteristics were the main determinant in Cerrado *sensu stricto*. At the landscape scale, I found topography to be the major predictor of microbial attributes in all vegetation types, and that anthropogenic disturbance influences soil microbial communities both positively and negatively. Veredas soils were the most resistant to drought, while Gallery forests soils were the most resilient in their reponse to rewetting after drought. Based on these findings, I discuss the importance of flooding regimes in the wetland vegetation types (Gallery forest and Veredas) for ecosystem functioning, and implications for future land-use and climate change in the Cerrado biome (Chapter 5), and I emphasize the need for Brazilian environmental legislation to prioritize the integral conservation of periodically flooded areas.

Keywords: Neotropical savana. Cerrado biome. Gallery forest. Veredas. Cerrado *sensu stricto*. Anthopogenic disturbance. Topography. Flooding. Soil properties. Soil resistance and resilience.

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FIRST PART

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1 GENERAL INTRODUCTION

1.1 The Neotropical Savanna: Cerrado biome

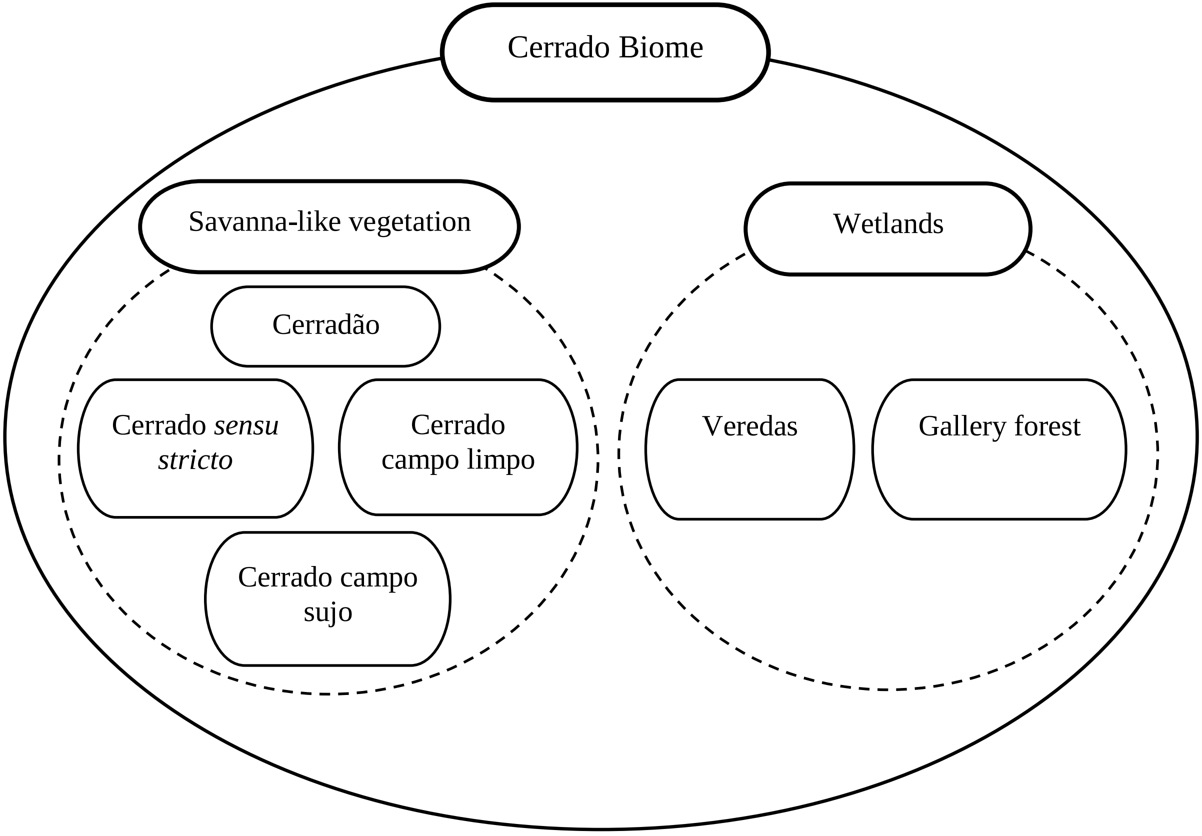
Savannas occupy nearly one fifth of the earth's land surface (LEHMANN et al., 2014; SCHOLES; ARCHER, 1997) and are located in Africa (65%), South America (45%) and Asia (10%) (ARCHIBOLD, 1995; SCHOLES; ARCHER, 1997; WERNER, 1991). In South America, the savannas are found mainly in Brazil where they cover approximately 200 million hectares (Mha), while Colombian savannas occupy around 20 Mha and Venezuelan around 12 Mha (FISHER et al., 1994). Savannas provide ecosystem services that sustain about 20% of humans on the globe, have high levels of plant and animal species diversity (SCHOLES; ARCHER, 1997), and are home to most of the world's remaining megafauna (MALHI et al., 2016).

The Cerrado biome is the largest savanna in South America and the second biggest biome in Brazil, covering approximately 24% of the country's total area (HUGHES, 2017; LAPOLA et al., 2013). Cerrado is found in central Brazil, located between other important biomes such as the Amazon, Atlantic forest, Caatinga and Chaco (SILVA; BATES, 2002). It contains three of the most important hydrological basins of South America: i.e. Tocantins-Araguaia, Paraná-Prata and São Francisco basins (FELFILI; SILVA JUNIOR, 2005). It is the most diverse and species rich savanna in the world (LEWINSOHN; PRADO, 2005), with high levels of endemism for several groups of animals and plants (MÜLLER, 1973). Due to losses of endemic species through land conversion to cropland and managed pastures, this biome is now one of the most threatened biodiversity hotspots in the world (MITTERMEIER et al., 2005). The natural vegetation and soil of the Cerrado biome has been shown to provide a significant carbon (C) sink, with stocks previously estimated to be 265 Mg ha^{-1} (down to 1 m soil depth) with soil organic matter (SOM) contributing approximately 70% of this value (ABDALA, 1993). Despite its importance in providing ecosystem services to humanity, the natural vegetation cover of the Cerrado has been reduced by 50% in the last 30 years (HUNKE et al, 2015). Continued deforestation is expected to significantly alter the biogeochemical functioning of the

Cerrado, compromising C storage potential and intensifying C emissions to the atmosphere (BRASIL, 2010). It is estimated that the combined effects of land use intensification and climate change will cause large-scale eutrophication, pesticide contamination of water sources, reduced ecosystem stability, with feedbacks to agricultural productivity (HUNKE et al., 2015).

The Cerrado biome is composed of a wide range of savanna-like vegetation types, ranging from grasslands to woodlands (BUSTAMANTE, 2012) including: Cerrado campo limpo (grassland); Cerrado campo sujo (grassland with scattered scrub and trees); Cerrado *sensu stricto* (woodland with closed scrub and sparse trees); and Cerradão (dense woodland with a closed canopy) (COUTINHO, 1978). In addition to these, annual flooding, during the rainy seasons, from rivers and overflow from water tables promotes the development of a mosaic of evergreen forests (Gallery Forests) and grasslands swamps (Veredas), which possess different vegetation compositions, soil conditions and higher organic matter accumulation compared to the savanna-like vegetation types described above (OLIVEIRA-FILHO, 1989). A schematic diagram of the vegetation type nomenclature is presented in Figure 1.1, in which the ‘Savanna-like vegetation’ group comprises vegetation types with Cerrado physiognomies, while Gallery forest and Veredas are included in the ‘Wetlands’ group.

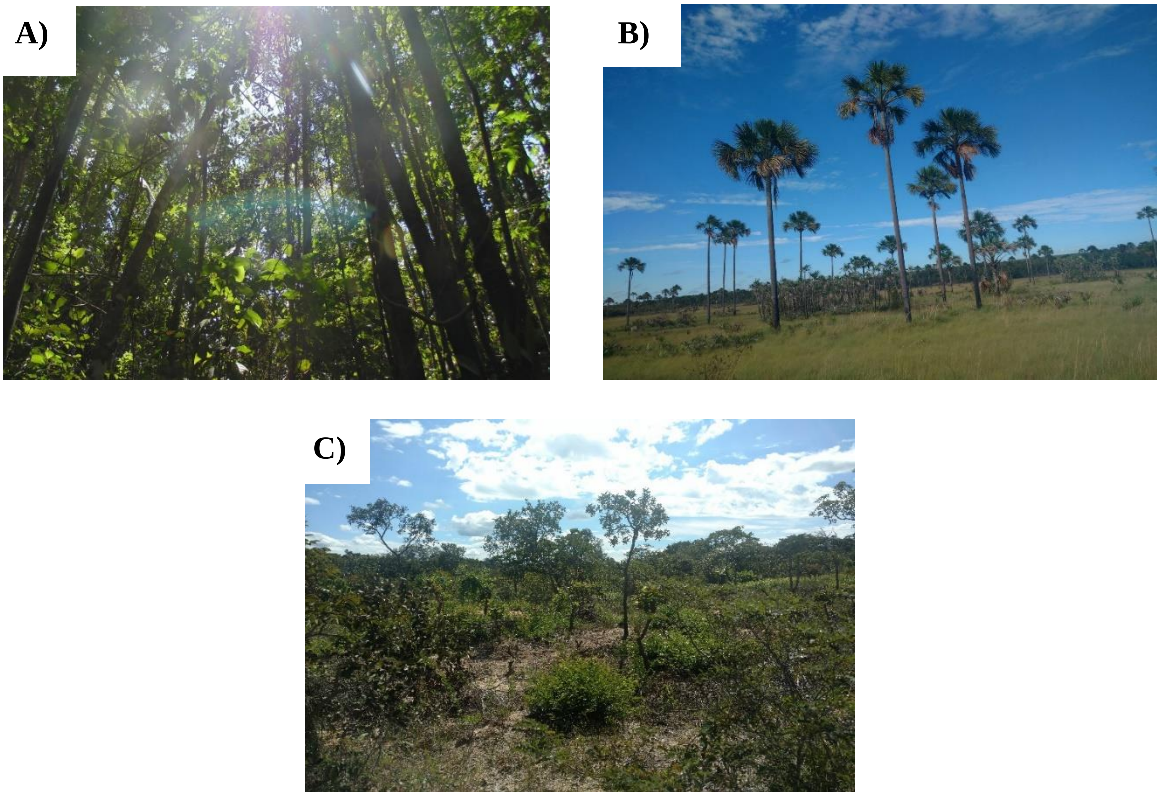
Figure 1.1 – Schematic diagram of the different vegetation types found in Cerrado biome and their classification with respect to the 'Savanna-like vegetation' and 'Wetland' groups



Source: From the author (2019)

Although Gallery forest and Veredas are defined here as wetlands, it is important to highlight that these ecosystems are distinct not only from the Savanna-like vegetation, but also from one another. Figures 1.2 A, B, and C illustrate the differences in vegetation structure of these vegetation types (Cerrado *sensu stricto* here representing the Savanna-like vegetation group), taken within the field sites chosen for study in this thesis.

Figure 1.2 – A) Gallery forest vegetation structure: high tree density and closed canopy. B) Veredas: grasslands with sparse scrub and palm trees (*Mauritia flexuosa* L.). C) Cerrado *sensu stricto*: savanna woodland with sparse scrubs and trees.



Photos taken by the author (2016).

The topography and hydrology determine the formation of these different vegetation types. The Savanna-like vegetation occupies the upper parts of valleys on well-drained soil, while Gallery forest forms in the bottom of valleys and Veredas, the marshy fields, lie in the transition between these two ecosystem types (Fig 1.3).

Figure 1.3 – Spatial location of Gallery forest, Vereda and Cerrado *sensu stricto* in the landscape of Cerrado biome of the Pandeiros river basin, Minas Gerais, Brazil.

Image from Google Earth (2016)

It is known that climate, landscape topography, impoverished nutrient status, water availability and human influence are the main factors influencing vegetation community composition (OLIVEIRA-FILHO, 1989). Although these basic factors are established, the main drivers of ecosystem functioning remain poorly understood within this biome and its respective vegetation types – particularly in the context of anthropogenic disturbance and climate change. The Cerrado, as well as all other savannas, are the most sensitive ecosystems to changes in land use and climate (ZHANG et al., 2019). Therefore, improved understanding of the factors affecting savanna functioning is urgently required to informing ecologically-relevant conservation and management decisions now and in the future (SANKARAN et al., 2005; SALA et al., 2000).

Soil microorganisms play a crucial role in ecosystem functioning. They are vital for the breakdown of organic matter, nutrient cycling, providing plant access to essential nutrients (MAILLARD et al., 2019; CAMENZIND et al., 2018; WARDLE et al., 2004), and are responsible for most of the carbon dioxide (CO_2) emissions from the soil - one of the largest fluxes of the global C cycle (SCHLESINGER; ANDREWS, 2000). As the Cerrado is heavily used for monoculture crop plantations (i.e. soya, cotton, maize, planted pastures) most studies of soil microorganisms have focused on enhancing crop productivity (BRESOLIN et al., 2010). Cerrado soil microbial community structure and activity have been reported in order to establish bio-indicators of soil quality for agriculture (MENDES et al., 2019), soil recovery from the effects of mining degradation (SILVA et al., 2018). Only a few studies have evaluated soil CO_2 emissions in native Cerrado vegetation types (BUTTLER et al, 2012; ARRUDA et al, 2016). As such there is a great lack of studies investigating the factors influencing soil microbial attributes at both landscape and local scales, as well as the resistance and resilience of soils to anthropogenic disturbance in native Cerrado ecosystems.

1.2 Interactions between vegetation and soil microbes

Below- (soil microbial) and above-ground (vegetation) ecology have traditionally been studied independently of one another (WARDLE et al., 2004). There is, however, an increasing recognition of the importance of studying the interactions between soil microorganisms and plants and their joint role in ecosystem functioning (VAN DER PUTTEN et al., 2013) especially in the context of rapidly changing human-modified landscapes and climate change (MAILLARD et al., 2019; CAMENZIND et al., 2018; BROCKET et al., 2012). Vegetation and soil microbiota are linked through a wide range of reciprocal plant-soil feedback processes (FUJII et al., 2018). In tropical soils in particular, where the high abundance of aluminum and iron can decrease nutrient availability to both plants and microorganisms (SOLINS et al., 1988), extracellular enzymes (synthesised by plants and soil microbes) are released in the soil to breakdown organic matter and overcome nutrient deficiency. Also, symbiotic associations between certain groups of fungi and tree roots have developed through co-evolution in order to facilitate nutrient absorption by the plant while also benefiting fungal partners (JONES, 1998).

In forest ecosystems, canopy cover is an important structural vegetation attribute that provides different microclimatic conditions compared to outside the forest, while also indicative of litter production (GATTI et al., 2015, VEENENDAAL et al., 2015; VEENENDAAL et al., 2018). Photosynthetically-assimilated C is transferred to the rhizosphere from the trees via canopy leaves that are deposited as litter (HOPKINS et al., 2013), the quantity and quality of which have been shown to alter soil microbial community structure (LAMMEL et al., 2015). Changes in canopy cover, e.g. through creation of canopy gaps, can alter soil temperature, physiochemical and microbiological properties (BAUHUS, 1996), and stimulate soil organic C retention by microorganisms, affecting nutrient cycling processes (LIU et al 2018).

Tree structural attributes such as basal area (or biomass) and height, are found to be related to soil physico-chemical properties in tropical forests as well as canopy cover (SANTIAGO-GARCÍA; FINEGAN, BOSQUE-PÉREZ, 2019). Some studies found that increases in tree biomass is usually related to decreases in soil pH and exchangeable bases, and increases in aluminum content. This can be due to the high rates of primary production in tropical forests (FUJII et al., 2010), in which the excess of cation uptake by plants leads to an increase in soil acidification in these regions (FUJII, 2014). Trees with larger basal areas can indicate not only a larger canopy structure and consequent higher litter production, but also a larger root system and an enhanced C flux to the soil through root exudates that affect soil microbial biomass and respiration (BRÉCHET et al., 2009).

Open ecosystems, such as grasslands, are absent of canopy closure and therefore the contribution to SOM by litter via tree leaves is not significant. Most of the organic matter is located in the superficial layer of the soil in the grass rhizosphere (DENNIS; MILLER, HIRSCH, 2010). The substrates released by grass roots is readily available for soil microorganisms, and is crucial for stimulating the microbial cycling of all elements (PAUSCH; KUZYAKOV, 2018). Grasses release labile organic C compounds through exudation, which tends to select bacterial over fungal communities (KAISERMANN et al., 2015), while recalcitrant C compounds in organic matter derived from forest litter requires a greater fungal abundance for decomposition (LAUBER; STRICKLAND; BRADFORD, 2008).

Above- and belowground C allocation by plants is strongly dependent on soil nutrient availability (LEFF et al., 2015). Grasslands used for pasture usually receive fertilizer additions in order to promote plant productivity. N enrichment alters plant inputs to soils by increasing acidity and changing the concentrations of base cations

(ROUSK et al., 2010), having both negative and positive effects on soil microbial C and N cycling (VOURLITIS; ZORBA, 2007).

The periodical flooding of a grass-dominant ecosystems, as is the case for the Veredas vegetation type in Neotropical savannas (OLIVEIRA-FILHO, 1986), is another factor that interferes on soil function as microbial metabolisms, growth rate and composition are intrinsically linked with soil moisture levels (BARNARD; OSBORNE; FIRESTONE, 2015). Associated drying-rewetting regimes have recently been found to select for adapted microbial communities that are tolerant to these types of disturbances (EVANS; WALLENSTEIN, 2012).

As the Cerrado biome comprises a wide range of vegetation types, from woodlands with large variation in tree structure, to grasslands that flood periodically and often used as pasture, we aim to assess the effects of vegetation characteristics on soil microbial attributes in different ecosystems of the Cerrado biome.

1.3 Interactions between soil physico-chemical properties and soil microbial attributes

Due to the close relationship between soil microbial physiology and the environmental conditions of the soil matrix they occupy, soil physical and chemical properties are found to strongly influence and structure soil microbial communities (BÅÅTH, ANDERSON, 2003). The interaction between soil particles and microorganisms is bidirectional; while particles control the environment in which microorganisms live, microorganisms can modify particle arrangement through aggregation (CHENU et al., 2002). More specifically, soil particles affect microbes by determining soil pores sizes, affecting aggregate distribution and physical and chemical conditions - shaping the soil environment (MOREIRA; SIQUEIRA, 2006). Small pores provide refuges for bacteria to protect against protozoas and bacterivorous nematodes. Due to their body sizes, bacteria are usually more likely to be found in soil micropores or microaggregates rather than fungi (KILHAM, 1994). A greater content of larger particles such as sand can lead to destabilization of soil aggregates, creating a different microenvironment for bacteria which tend to be more easily washed away from sandy soils during flooding (MOREIRA; SIQUEIRA, 2006).

Soil pH has also been found to affect both bacterial and fungal communities (BÅÅTH; ANDERSON, 2003), but with a stronger influence on bacteria (LAUBER et al., 2008). An increase in acidity negatively affects the amount of exchangeable bases and subsequent nutrient availability for plants and microorganisms (KIN et al., 2011; FACELLI; PICKETT, 1991). Furthermore, greater acidity can also lead to accumulation of aluminium (Al) and manganese (Mn) content and therefore increase toxicity in soils (MALAVOLTA et al., 1997), affecting both and plants and soil microorganisms due to intrinsic feedbacks between them (KUNITO et al., 2016). Levels of toxicity and nutrient deficiencies are well defined for cultivated crops plants, however Haridasan (2008) highlight that these should not be extended indiscriminately to natural ecosystems as native plants have developed biological mechanisms to deal with adverse environmental conditions. Although aluminum and acidity is reported to suppress microbial biomass in native forest soils (KUNITO et al., 2016), other studies show that microorganisms that interact with plants to facilitate their own nutrient accessibility can present adapted mechanisms to deal with high Al content in the soils (PANDEY; YARZÁBAL, 2019). Mn is also known to cause toxicity in cultivated plants under acidic conditions (HARIDASAN, 2008), but in native ecosystems such as riparian forests for example, the accumulation of trace elements through flooding seems not to limit plant growth as these forests are found to have advanced tree structure and density even in acidic soils. Moreover, soil microorganisms were also reported to be adapted to these conditions by utilising Mn oxy-hydroxides as alternative electron acceptors in response to the reduced supply of oxygen in flooded soils (OLIVIE-LAUQUET et al., 2001). As there are contradictory findings regarding effects of soil toxicity on microorganisms, and considering the high Al content and trace elements present in Cerrado soils, more studies are needed to understand how soil microbial attributes respond to soil natural conditions in this biome. In the Cerrado, due to the poor nutrient status of soils, organic inputs from vegetation and deposition of sediments transported through river catchments can greatly enhance soil fertility and determine soil microbial characteristics and function (HUNKE et al., 2015).

1.4 Landscape features influencing ecosystem functioning

1.4.1 Anthropogenic disturbance

The increasing intensification of agriculture and exploitation of natural resources to support the expansion of human populations is leading to changes in landscapes worldwide (PETERS et al., 2019). Native ecosystems have been transformed by human land use (hereafter referred to as ‘anthropogenic disturbance’), exerting negative effects on global biodiversity and ecosystem functioning (NEWBOLD et al., 2015). Ongoing anthropogenic disturbance through land use change and the conversion of natural ecosystems, including deforestation, is resulting in soil erosion, environmental degradation, and the reduced provision of ecosystem services to humanity (WANG et al., 2015). Furthermore, there is a global concern that the negative impacts of agricultural intensification on soil quality could irreversible (MGANGA; RAZAVI; KUZYAKOV, 2016).

The reduction of natural vegetation cover through anthropogenic disturbance directly affects the quality of tropical soils (QUESADA; LLOYD, 2016), which are known to be old, deep, highly weathered and poor in nutrients (VITOUSEK; SANFORD, 1986). The plant communities that are evolved and adapted to these conditions are subsequently essential to the conservation of critical soil properties including organic matter and nutrients (VITOUSEK, 1984). Plant litter is generally decomposed faster in tropical soils than in soils found in the colder climatic biomes of the globe. Therefore, comparatively higher nutrient cycling and C turnover rates are found in tropical regions (CHAMBERS et al., 2004). At the same time changes in land use and intensive land management are known to alter the amount and quality of plant inputs to the soil (QUESADA; LLOYD, 2016). For example, deforestation has been shown to affect vegetation inputs leading to decreases in SOM, on which soil microbial communities depend (MAILLARD et al., 2019). As such, soil microorganisms themselves and the ecosystem functions they underpin are also reported to be sensitive to anthropogenic disturbances (PABST et al., 2016). Vegetation is also required for developing soil structure throughout the root system by aggregation, enhancing aeration, water infiltration, porosity and C sequestration (LENKA et al., 2012; TANG et al., 2010). Loss of soil protection by plants leads to changes in soil physico-chemical characteristics and reduced C stocks that are intensified by land management and repeated disturbance (BRUUN et al., 2013). The destruction of soil structure directly affects microbial communities dependent upon aggregates for physical protection, including fungal hyphae (DEMENOIS et al., 2018) and bacteria, while soil aggregates also hold on to organic substrates used as a microbial food source through breakdown by extracelular enzymes (GUPTA; GERMIDA, 2015). Some studies have shown disturbance may increase soil microbial stress and negatively affect energy use efficiency through evaluation of metabolic quotients (qCO_2) (INSAM; HASELWANDTER, 1989), with consequences for C and nutrient cycling (ANDERSON; DOMSCH, 1993; WARDLE; GHANI, 1995).

1.4.2 Topography

Topography is a key abiotic factor shaping environmental heterogeneity as it dictates ground-water regimes, soil physical and chemical properties, and consequent determination of aboveground floristic composition and differentiation between vegetation types (PINTO et al., 2005). As soil microbial biomass and enzyme activities are related to soil properties such as water content (BARNARD; OSBORNE; FIRESTONE, 2015), pH (STARK et al. 2014), and organic C (BHATT et al. 2011), heterogeneity in soil conditions created by topographical variation is also intrinsically linked to soil microbial communities and processes (OSBORNE et al., 2017).

Gradient of slope is reported to control the accumulation of plant litter, impacting on the quantity of substrate available for soil enzymatic activities (PAN et al., 2018). Sloped areas usually haver higher soil infiltration capacities than gentler gradients, indicating that the soil nutrients are more easily leached (ZHONG et al., 2018). Also, the potential for movement of nutrients in soil pores on slopes can promote accumulation of organic matter and create C and nitrogen (N) mineralization pools on gentler gradients and valley bottoms (HOOKE; BURKER, 2000).

Altitude (or elevation) is related to climatic regimes, and although changes in precipitation and temperature along large altitudinal gradients in montane ecosystems are found to determine soil microbial community structure and function, studies considering altitude in different ecosystems and biomes around the globe remain limited (ZHANG et al., 2013; WHITAKER et al., 2014). Known mechanisms affecting soil microbial community attributes are: decreases in fungal and bacterial biomass with increasing altitude on tropical hillslopes, as organic matter tends to accumulate in greater quantities at lower altitudes compared to higher elevations (GIRI et al., 2007); more gram-negative relative to gram-positive bacteria may be found at higher altitudes, as this group is more tolerant to lower soil pH often associated with increasing altitudes (MARGESIN et al 2009); increases in soil humidity and decreases in C:N ratios associated with precipitation patterns at higher altitudes may result in reductions in fungi:bacteria ratios (ZHAO et al., 2019); exposed windy conditions and shallow soils at the tops of mountains limit the establishment of trees, reducing abundances of root-associated fungi compared to lower altitudes (MARIAN et al., 2019).

Soil moisture content influences microbial metabolism and community structure to a great extent (BARNARD; OSBORNE; FIRESTONE, 2015), itself strongly determined by topography. Higher soil moisture contents are commonly found in the bottom of valleys and at lower altitudes, where both water and organic matter accumulate. In flooded soils anoxic microbial communities can develop, and organic matter decomposition is usually slower than in aerobic soils. For this reason, topographic areas that are more susceptible to water accumulation need to be evaluated separately to assess to what extent ecosystem functioning differs from upper parts of the terrain. Wetness indices can be derived from topographic metrics and usually provide a high explanation of the variation in soil properties and vegetation characteristics (DROVER et al., 2015), and therefore can potentially be used as predictors of microbial attributes and processes.

All the studies cited above that have evaluated the influence of topography on soil microbial attributes were carried in montane environments or on hillslopes. The Cerrado biome itself possesses a comparatively flat topography although slopes and hills exist. Nevertheless, the characteristics of this biome provides an opportunity to understand how small variations in topography such as elevation, slope and wetness indices influence important microbial attributes and nutrient cycling in the Cerrado landscape.

1.5 Soil resistance and resilience

CO_2 emissions from soil constitute one of the largest fluxes of the global C cycle (SCHLESINGER; ANDREWS, 2000). CO_2 is emitted from soil as a result of the respiration of soil microorganisms during decomposition of SOM (TRUMBORE, 2000) and C compounds released by plants. Even small changes in temperature (RAICH; SCHLESINGER, 1992) and precipitation (UNGER et al., 2010) can cause alterations in the magnitude of soil respiration and consequent atmospheric CO_2 concentrations (SCHLESINGER; ANDREWS, 2000). For this reason, soil respiration has been studied under different regimes of water availability and drought cycles (MORENO et al., 2019) and temperature (BARBA et al., 2018; CHEN et al., 2018), as it is expected that alterations in precipitation patterns and temperature are likely to occur with global climate change (DAI; ZHAO; CHEN, 2018; SCHLESINGER; ANDREWS, 2000). As a result of climate change, it is predicted that increases in temperature in the Cerrado biome will lead to changes in the precipitation regimes, with more intense and longer drought seasons, that therefore will interfere in soil functioning (BUSTAMANTE et al., 2012).

Soil resistance can be defined as is the ability of the soil microbial community and its functions to remain unchanged facing a specific disturbance, for example drought. Soil resilience is the ability to recovery from this disturbance (e.g. after soil rewetting following a drought period) (DE VRIES; SHADE, 2013; PIMM, 1984). As a response to repeated drought events, soil microbial communities may become adapted to drought and demonstrate more resistant responses to dry conditions (GRIFFITHS et al., 2000), while repeated rewetting may select more resilient microbial communities with faster recovery times (EVANS; WALLENSTEIN, 2012). This has important implications for the C budget of these communities (NIJS et al., 2019), and consequently for soil C storage. Faster growth rates and recovery of microbial biomass after drought is related to higher microbial C use efficiency (CUE), the proportion of C used for microbial growth relative to the total amount of C consumed and related to respiraton of CO_2 to the atmosphere (NIJS et al., 2019). Therefore, it is crucial to improve understanding of resistance and resilience of soil microbes in order to predict soil CO_2 emissions and soil C storage capability in the context of drought and rewetting events.

However, despite the global importance of soil respiration as part of climate change, studies focusing on the effects of drought/rewetting cycles and temperature remain insufficient (MORENO et al., 2019), as soil respiration is a complex process affected by many factors (LAI et al., 2012). Therefore, further research is required to understand these factors for developing more accurate predictions for future climate change scenarios, and supporting effective management and conservation strategies for mitigation of the negative effects climate change.

1.6 Thesis objectives

Savannas are highly threatened by land use and climate change, yet understanding about the form and function of Neotropical savanna is lacking. As soil microorganisms and their interactions with aboveground components are vital for ecosystem functioning, the overall objective of this thesis is to assess how local environmental vegetation and soil characteristics and landscape affect soil microbial attributes and function (Figure 1.4). The understanding of factors influencing these attributes across multiple scales is essential for promoting ecologically-relevant conservation and management efforts in this globally important biome. For that, I chose typical and distinct ecosystems of the Neotropical Cerrado biome in Brasil - two wetland ecosystems: Gallery forest and Vereda as they are distinct from one another, and the Cerrado *sensu stricto*, as it is the predominant savanna-like vegetation in the study region.

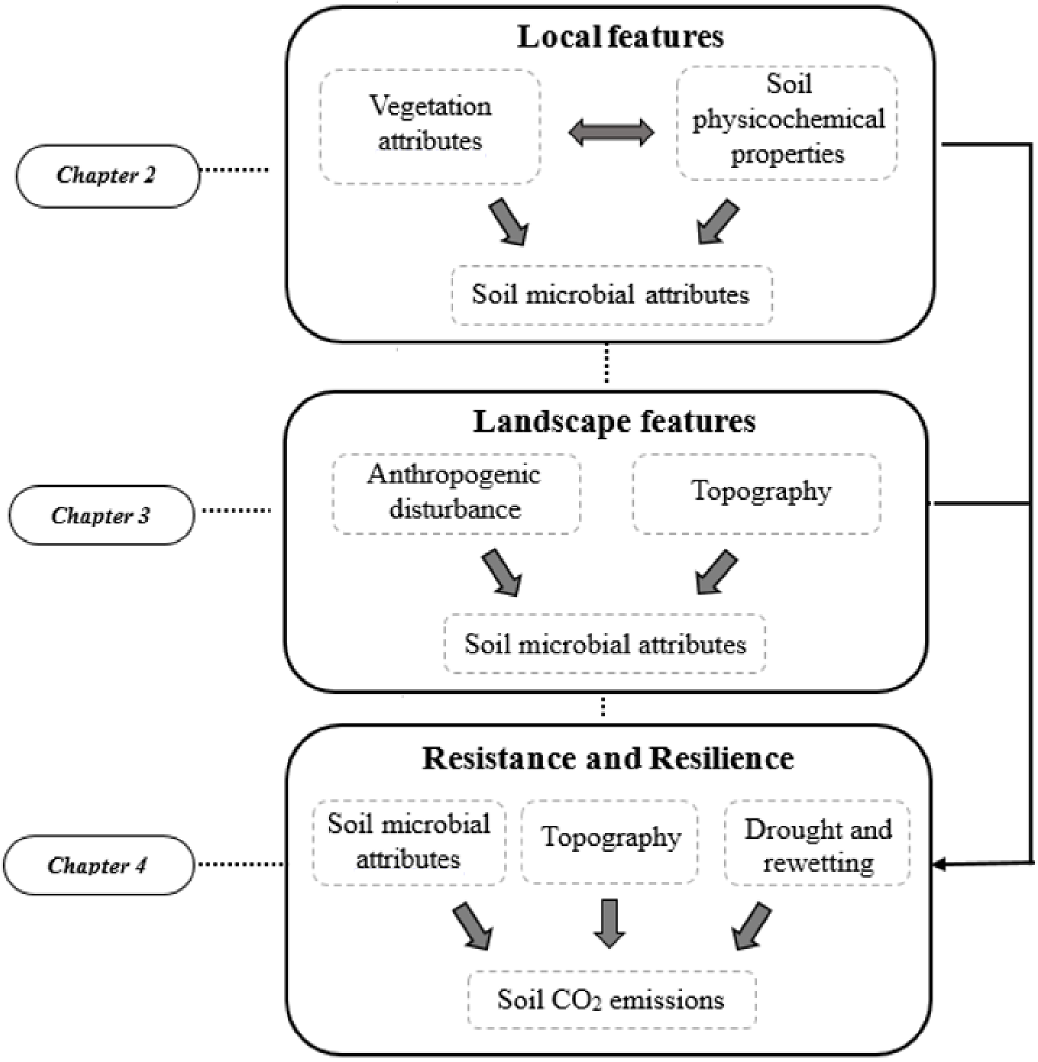
The work presented in this thesis aims to answer the following questions:

1) How soil microbial community structure and enzyme activity are influenced by local vegetation and soil physiochemical properties across and within the three Cerrado vegetation types? (Chapter 2).

2) How do landscape scale anthropogenic disturbance and topographical aspects such as slope, altitude, soil moisture index, and the presence of lakes and rivers influence soil microbial community structure and enzyme activities in the three typical Cerrado vegetation types (Chapter 3).

3) How does drought/rewetting disturbance, soil microbial attributes and topography affect soil CO_2 emissions in the three Cerrado vegetation types? (Chapter 4).

Figure 1.4 – Overall thesis structure and links between chapters.



Source: From the author (2019)

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SECOND PART - MANUSCRIPTS

MANUSCRIPT 1

LOCAL CONTROLS ON SOIL MICROBIAL ATTRIBUTES IN NEOTROPICAL SAVANNA

(Preliminary version prepared for submission to Ecological Indicators)

ABSTRACT

Soil microorganisms play a central role in nutrient cycling, organic matter decomposition and are crucial for ecological processes. The interactions between vegetation and soil physico-chemical properties and soil microorganisms determine ecosystem functioning and maintenance. The Neotropical savanna biome is globally important for agriculture and biodiversity and yet its soils are poorly understood. In this study we wanted to examine the influence of local plant-soil properties on soil microbial attributes in samples collected from Cerrado *sensu stricto*, Gallery forest and Veredas at 10 sites within the Pandeiros river basin. We conducted a statistical Factor Analysis to identify the most sensitive (variables) of microbial attributes, soil physico-chemical properties, and vegetation characteristics. A Structural Equation Modelling (SEM) approach was used to assess the relationships between soil properties, vegetation and microbial community attributes. Results from the SEM showed that soil physico-chemical properties explained more of the variance in soil microbial attributes than vegetation attributes. However vegetation attributes were strongly correlated with soil properties across all Cerrado vegetation types. Although vegetation characteristics had no direct influences on microbial attributes, the overall model demonstrated its influence on soil properties. In Gallery forests, soil physico-chemical properties showed direct influence on microbial attributes and vegetation characteristics were strongly correlated with soil properties. In Veredas, soil microbial attributes were influenced for both soil physico-chemical properties and vegetation, with grass cover showing a positive and direct effect. Cerrado *sensu stricto* had a peculiar result with soil physico-chemical properties having no direct influence while vegetation characteristics showed direct and positive affect on microbial attributes. This study offers new information regarding the role of soil physico-chemical properties and vegetation attributes as local determinants of soil microbial communities in Neotropical savanna ecosystems. Rapid land-use and climate change across the Cerrado biome is likely to affect local characteristics in its different ecosystems, resultant microbial community structure and function with consequences for biogeochemical cycling.

Key words: Cerrado *stricto sensu*, Gallery Forest, Veredas, soil enzymes, PLFAs.

1 INTRODUCTION

Soil microorganisms play a central role in nutrient cycling and organic matter decomposition, and are crucial for soil ecological processes and stabilization (Brockett, Prescott and Grayston 2012; Mora and Lázaro, 2013; Zhao et al., 2019). Soil microbial community structure and activity are influenced by both vegetation and soil properties (Nottingham et al., 2018; Kaschuk et al., 2011). Interactions between these above-below ground properties are important regulators of ecosystem functioning and maintenance (Mora and Lázaro, 2013).

Local scale Soil physico-chemical properties are known to influence soil microbial communities (Castro et al., 2016) with bacteria affected by soil texture (Girvan et al., 2003), pH (Malik et al., 2018), nitrogen content and the abundance of labile organic C compounds, while fungi are affected by soil water content, nutrient availability (Kaisermann et al., 2015) and the availability of recalcitrant C compounds (Lauber, Strickland and Bradford, 2008). Local scale vegetation attributes including structure, and relative cover of different functional groups are also known to influence soil microorganisms (Mendes et al., 2012; Silva and Batalha, 2008). Trees with higher basal area and greater height tend to produce greater quantities of litter (Turnbull and Madden, 1983), and greater root biomass leads to enhanced exudate release through the roots (Eisenhauer et al., 2017). These factors are drivers of greater soil microbial biomass and enzyme activity (Morales-Londoño et al., 2019; Xiao et al., 2019), and shape soil microbial structure by selecting for specific functional groups (Awad et al., 2019). For example, grass cover has been shown to be important in shaping soil microbial structure through selecting root-associated bacterial communities that benefit from the labile carbon compounds released in the form of root exudates (Guerrero et al., 2005; Araujo et al., 2012).

Although studies have assessed relationships between vegetation characteristics, soil physico-chemical properties and soil microbial attributes in temperate, mediterranean and tropical rainforest global biomes (Liu et al., 2008; Zechmeister-Boltenstern et al., 2011; Tian et al., 2017; Medvigy et al., 2019), but such studies in tropical savanna ecosystems such as the Neotropical Cerrado remains insufficient. Due to the high diversity and productivity in plant communities and variation of soil properties in the tropics (Fujii et al., 2018), these relationships are likely to be equally varied. A clearer knowledge of the interactions between soil properties and microorganisms under different vegetation types is needed to understand the resistance and resilience of terrestrial ecosystems (Zhao et al., 2019). Despite this, understanding of these interactions in the Neotropical savanna (Cerrado biome), and across its range of different vegetation types, is still unclear.

The soils of the Neotropical savanna, the Cerrado biome, are generally old, well drained, nutrient poor with a high aluminum content (Gibbs, Leitao Filho and Shephered., 1983). The vegetation comprises a range of savanna–like vegetation (Cerradão - dense woodland with canopy but not completely closed; Cerrado *sensu stricto* - woodland with scattered trees and shrubs; Campo limpo – grassland; Campo sujo - grasslands with the presence of shrubs) (Castro et al., 2016; Mendes et al., 2012). This biome also includes vegetation types that are strongly influenced by the presence and availability of water. While the typical savanna-like vegetation types tend to occupy the well-drained areas on the hill-tops and laterals, Gallery forests and Veredas are usually found in the valley bottoms, where the water table is closer to the surface and the soils are poorly drained. The Veredas are marshy fields often surrounding Gallery Forests, occupying the transition between Gallery Forests and other Cerrado vegetation types (Oliveira-Filho et al., 1989). The Veredas and Gallery Forest vegetation types comprise different plant communities and soil conditions compared to the savanna-like vegetation due to the influences of water accumulation and higher organic matter inputs and content (Oliveira Filho et al., 1989; Delitti and Burger, 2000).

The Brazilian Cerrado is one of the most threated biomes in the world (Sano et al., 2010), as the expansion of agriculture over the last 30 years has replaced native vegetation with crops and pasture for cattle (Hunke et al., 2015). More than 50% of the natural Cerrado cover has already been lost and the remaining part is partially modified by extensive cattle use or extracting activities (e.g. coal, plants, fruits, craftwork) (Brazilian Ministry of the Environment 2010). This degradation has and will continue to contribute to significantly altered biogechemical functioning whilst intensifying greenhouse gases emissions to the atmosphere. Considering the ongoing conversion of native vegetation of the Brazilian savanna and the need to understand how vegetation and soil properties are related to soil microbial community form and function (Castro et al., 2016; Bardgett, Freeman and Ostle, 2008; Wardle et al., 2004; Camenzind et al., 2018), our aim was to assess the relationships between 'local' vegetation characteristics, soil properties and soil microbial attributes under typical vegetation types of the Cerrado biome: Cerrado *sensu stricto*, Gallery forest and Veredas. We hypothesised that there would be both direct and indirect effects of vegetation characteristics and soil physico-chemical properties on soil microbial attributes (Fig 2.1) and that interactions between these would be influenced by Cerrado vegetation type. We measured soil microbial biomass, extracellular enzyme activities, as well as the abundances of bacteria, gram positive bacteria, gram negative bacteria, and of fungi. Soil physico-chemical properties included pH, macro and micronutrients, carbon and nitrogen content and soil texture, and vegetation attributes were tree basal area, density and height, canopy cover, grass and shrub cover and litter quantity. We expected that there would also be a significant correlation between vegetation attributes and soil properties, as they are usually linked to plant-soil feedback processes (Jenny 1994; Fujii et al., 2018).

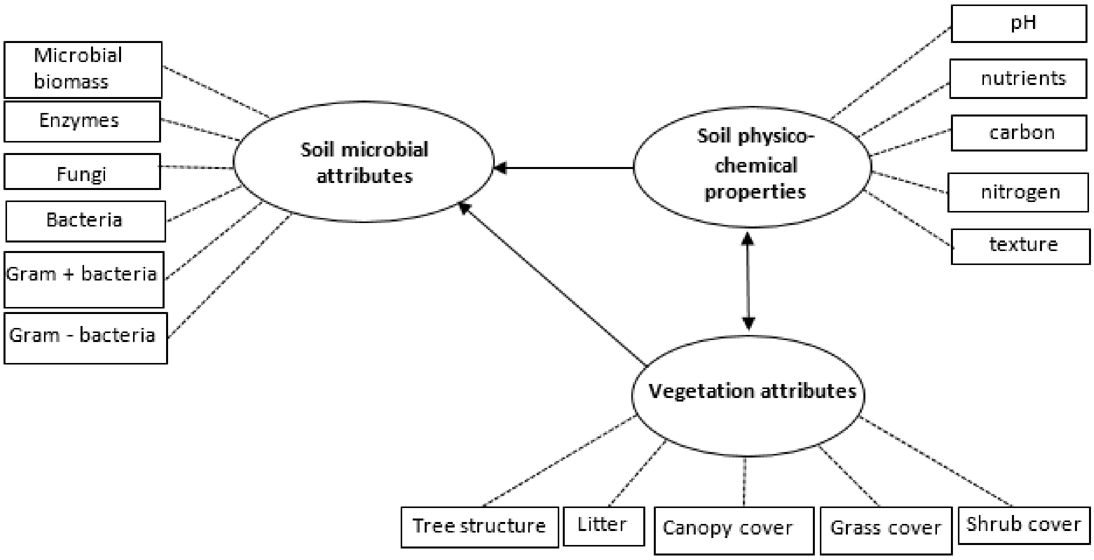

Figure 2.1. Diagram of hypothetical model proposed to assess the relationships between soil microbial attributes, soil physico-chemical properties and vegetation characteristics within different vegetation types of the Cerrado biome: Cerrado *sensu stricto*, Gallery forest and Veredas.

Based on the results of statistical analyses including Structural Equation Modelling, we aimed to develop a descriptive and conceptual model with the key soil properties and vegetation characteristics influencing soil microbial attributes across and within the different vegetation types of the Cerrado biome. This framework is crucial to improve understanding plant-soil interactions under global environmental change, including conversion of native vegetation to crops and pasture, and subsequent modification of nutrient cycling (e.g carbon and nitrogen) and soil properties in the context of climate change.

2 METHODS

2.1 Study Area

We carried out this study in the Cerrado biome, in the Rio Pandeiros basin, north Minas Gerais State, located between the south latitude meridians 14º00 to 16º30 and the longitude meridians 43º00 to 46º00. We selected 10 sites for sampling along the basin, following the rivers, each one including the three ecosystems: Gallery Forest, Veredas and Cerrado *sensu stricto* (Fig 2). The soil of the sampled areas of all vegetation types are classified as Oxisols and Entisols.

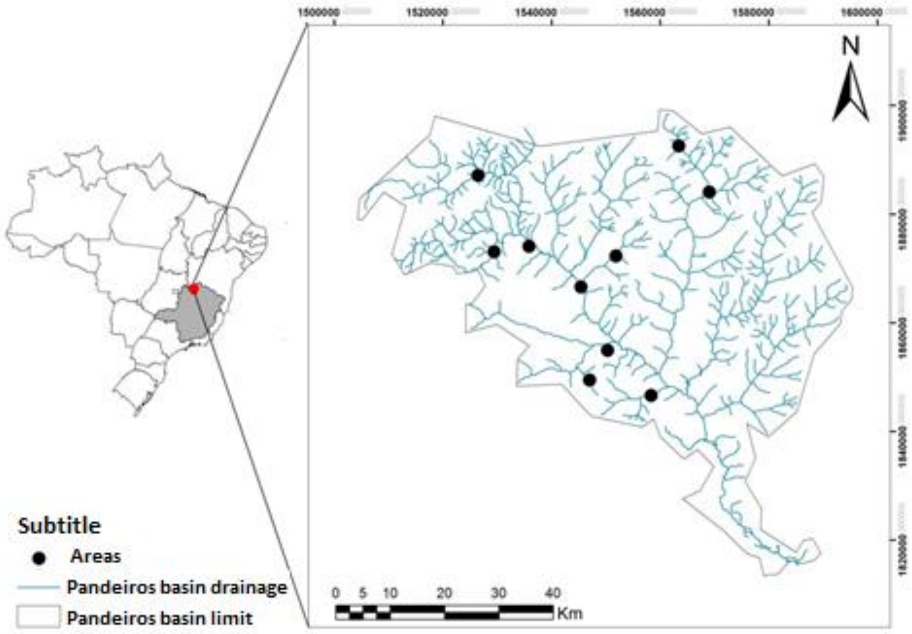

Figure 2.2. The Pandeiros river basin, in the northern region of Minas Gerais state, Brazil. Black dots show the sampled sites, each one comprising the three vegetation types: Gallery Forest, Veredas and Cerrado *sensu stricto*.

2.2 Data collection

In each one of the 10 sites, we recorded vegetation measurements and collected soil samples at 9 sampling points (totaling 90 samples overall): three in Cerrado *sensu stricto*, three in Vereda and three in the Gallery forest. In each vegetation type, sampling points were located 30 m apart. These spatial pseudoreplicates were taken in order to assess and account for spatial variation within the vegetation types at each site.

At each sampling point, we established a circular plot with a 3 m radius for vegetation measurements. We measured all trees with a diameter at breast height (DBH at 1.30 cm) ≥ 5 cm, and for trees with multiple trunks we measured each stem to calculate a single DBH for the tree. The relative cover of graminaceous-herbaceous, non-graminaceous-herbaceous and shrub was estimated visually as a percentage, as was relative cover of exposed soil.

At each sampling point we took soil cores following a cross design where a first sample was collected in center of the cross (also the center of the circular vegetation plot), followed by four other soil cores taken 1.5 m from the center at each cross arm, resulting in a total of five samples that were then mixed together to form one composite sample. For each soil core, we removed the litter layer and collected soil at a depth of 0-10 cm. Subsamples were taken and kept at environmental temperature for physicochemical analysis, and the remainder was kept in a freezer (-20 ºC) and taken to a laboratory for microbiological analysis. At field conditions, Gallery forests and Veredas soils can be found completely flooded in the wet season, therefore we carried our sampling during the dry season (March, 2016).

2.3 Soil data

Soil samples were taken to the soil laboratory for physiochemical analyses at Universidade Federal de Lavras to evaluate: pH measured by suspension in water; content of potassium (K^+), phosphorus (P), sodium (Na^{2+}), boron (B), zinc (Zn), manganese (Mn^{2+}), iron (Fe^{2+}) and copper (Cu^{2+}) extracted by the Mehlich-1 solution (Mehlich, 1953), calcium (Ca^{2+}), magnesium (Mg^{2+}), aluminium (Al^{3+}) extracted by 1 mol L -1 KCL (McLean et al., 1958) and sulfur (S) extracted by monocalcium phosphate in acetic acid (Walkley and Black, 1934); potential acidity (H+Al) by SMP extractor (Shoemaker et al., 1961); sum of bases (SB); cation exchange capacity at pH 7.0 (CEC), extracted by Ca 0.5 mol L-1 acetate;; V% (Bases saturation); m% (Aluminum saturation). The soil texture was evaluated by the Bouyoucos method (Bouyoucos, 1951). Carbon (C) and Nitrogen were measured in the Plant-soil laboratory at Lancaster Environment Centre, Lancaster University, UK, using an Elemental Analyzer – Elementar Vario EL III run in C:N mode.

Soil enzymes related to carbon (β-glucosidase), phosphorus (acid and alkaline phosphatase), nitrogen (urease) and sulfur (arylsulfatase) cycling were evaluated. For each enzyme assay, we used soil samples of 1 g. β-glucosidase activity was incubated in 1 mL of ρ-nitrophenyl-β-D-glucoside substrate and determined according to Eivazi and Tabatabai (1988) assay. Acid and alkaline phosphatase activities were incubated in 1 mL of ρ-nitrophenyl-phosphate substrate and determined according to Eivazi and Tabatabai (1977) method. Urease activity was determined using the method of Tabatabai and Bremner (1972). Arylsulfatase activity was incubated in 1 mL of ρ-nitrophenyl sulfate substrate and determined by the method of Tabatabai and Bremner (1970). We carried out analysis of fluorescein diacetate hydrolysis (FDA) using the method of Dick et al. (1996), which has been used to determine amounts of different enzymes including proteases, lipases and esterases (Schnürer and Rosswall, 1982). The supernatant was filtered and read in a spectrophotometer at 490 nm to determine optical density difference of each sample.

For total soil microbial biomass carbon, we firstly irradiated soils in a microwave (Islam and Weil, 1998) and extracted following Vance, Brooks and Jenkinson (1987).

Soil microbial community structure was assessed by phospholipid fatty acid (PLFA) analysis (Bardgett, Hobbs and Frostegård., 1996) extracted from 1.5 g freeze-dried soil using an Agilent 6890 Gas Chromatograph (detector FID and column 60m Agilent RTx-1 capillary column - 60m x 0.32mm ID, 0.25um film thickness). The fatty acid nomenclature were based on Petersen and Klug (1994). The Gram positive bacteria were evaluated considering the ester-linked branched-chain fatty acids: 15:0i, 15:0a, 16:0i, 17:0i, 17:0a; while gram negative bacteria were evaluated by considering the cyclopropyl saturated and monosaturated fatty acids: 16:1ω7, 7,cy-17:0, 18:1ω7,7,8cy-19:0 (Rinnan and Bååth, 2009). The biomarkers used for fungi were: 18:2 ω6,9 and 18: 1ω9 (De Deyn et al., 2011). Total PLFA was calculated as the sum of all the PLFAs cited above, plus all other identified biomarkers: 14:0, 15:0, 16:1, 16:1ω5, 16:0,17:1ω8, 7Me-17:0, br17:0, br18:0, 18:1ω5, 18:0, 19:1. Fungi:Bacteria and Gram positive : Gram negative bacteria (gram+:gram-) ratios were also calculated.

2.4 Statistical Analyses

Firstly, to evaluate differences in all soil properties and microbial attributes between the vegetation types, we averaged the replications within site in each vegetation type and ran an analysis of variance (ANOVA) with Tukey's test with pairwise comparison for normal data, and Kruskal-Wallis followed by Mann-Whitney pairwise comparison for non-normal data. We carried analyses out using the Palaeontological Statistics software package PAST (Hammer, Harper and Ryan, 2001).

To reduce the number of soil variables used for modelling, we ran a Principal Component Analysis (PCA) on all soil enzyme data and used axis 1 as a new variable called "enzymes" (Appendix Table 2.1). Another PCA was run on the soil chemical and physical dataset, variables with the largest eigenvalues in axis 1 and 2 (Individual eigenvalue > 0.6) were selected for subsequent analysis (Appendix Table 2.2). In order to select the most expressive variables and form the groups of "Soil microbial attributes", "Vegetation attributes", and "Soil properties", we the carried out a Factor Analysis using maximum likelihood as the extraction method with varimax rotation. The selected variables for soil properties, vegetation characteristics and soil microbial attributes were evaluated in separated groups. Two factors for each group were extracted to compose the latent variables used in the Structural Equation Modeling. Only the variables with the highest individual explanation (communalities > 0.3) were extracted. The poorest explanatory variables were excluded from the analysis until the p-value of the Goodness-of-fit test show a non-significant result, meaning the null hypothesis – (i.e. that the number of selected factors are enough to explain the proportion of variance) of the test was accepted. These analyses were run in IBM SPSS Statistics version 23.

We used Structural Equation Modeling to evaluate the relationship between soil microbial attributes, soil physicochemical properties and vegetation characteristics, constructiing path diagrams using the latent variables representing multiple existent variables previously selected by Factor Analysis. Three latent variables were created: 1) Soil microbial attributes (comprising axis 1 of the PCA of the evaluated enzymes activity that we called “enzymes”, microbial biomass carbon, soil respiration, total PLFAs, total fungi, total bacteria, fungi:bacteria ratio and gram+:gram- ratio); 2) Soil Properties (Sand, Clay, Carbon, Nitrogen, pH in water, K, P, Ca, Mg, Al, V%, m%, Zn, Fe, Mn, Cu, B and S); and 3) Vegetation attributes (basal area, tree density, canopy cover, litter, exposed soil cover, grass cover and shrub cover). The multivariate normality test was carried out, and non-normal data was transformed in order to improve normality. We used non-parametric bootstrapping with 1000 bootstrap samples and bias-corrected confidence intervals of 90% for models that showed non-normal distribution. The good indicators of model fit were chosen according to Kline (1998), recommending a $\chi2$ / d.F ratio of 3 or less. The Goodness-of-fit index (GFI), adjusted goodness-of-fit (AGFI) and root mean square error of approximation (RMSEA) were chosen as suggested by Schreiber et al (2008). Finally the most parsimonious model was selected as recommended by Schreiber et al. (2008). Structural equation modelling analysis was conducted in IBM SPSS Amos version 24.0. (Pseudoreplicated) sample data were not averaged by site for these analysis, in order to assess comprise the variation both between and within sites. For all analyses we considered $p \geq 0.05$ to be significant.

3 RESULTS

The results of comparisons between vegetation types for soil microbial attributes, soil physico-chemical properties and vegetation characteristics are summarised in Table 2.3. The pH was similar across the vegetation types. The bases K^+, Na^{2+}, Ca^{2+}, Mg^{2+} as well as Sum of bases (SB), Cation exchange Capacity (CEC), Zn^{2+}, Mn^{2+}, B and S were significantly higher in the Gallery Forest compared to Veredas and Cerrado *sensu stricto*. Na^{2+}, Potential Acidity (H+Al) and Fe^{2+} were significantly higher in Gallery Forest and Veredas. Al^{3+}, m% and Cu^{2+} were significantly higher in Veredas, and Remaining phosphorus (Rem-P) and Sand were significantly higher in Cerrado *sensu stricto.* Soil microbial respiration, total PLFA, total fungi, total bacteria, total gram positive bacteria and total gram negative bacteria were significantly larger in Gallery forests, while microbial biomass carbon and enzymes were significantly larger in Gallery Forests and Veredas and Gram + : Gram- ratio were significantly larger in Cerrado *sensu stricto* and Veredas.

Table 2.3. Summary of means of all soil properties and soil microbial attributes and in the three studied Cerrado vegetation types. Statistically similar groups at the $P \leq 0.05$ level are indicated with with similar lower-case letters, as tested through ANOVA with Tukey's pairwise comparisons and Krukal-Wallis with Mann-Whtney pairwise comparisons

	Measurements	**Cerrado *sensu stricto***	**Veredas**	**Gallery Forest**
Soil Properties	Carbon (ug C. g^{-1} dry soil)	6,352 **c**	63,427 **b**	141,417 **a**
	Nitrogen (ug C. g^{-1} dry soil)	569 **c**	4,620 **b**	9,164 **a**
	C:N Ratio (%)	12,40	12,61	14,13
	pH	4.7	4.6	5.0
	K (mg/dm^3)	20.0 **c**	35.5 **b**	87.8 **a**
	P (mg/dm^3)	2.7 **b**	3.5 **ab**	5.7 **a**
	Na (mg/dm^3)	1.9 **b**	5.4 **a**	9.5 **a**
	Ca ($cmol/dm^3$)	0.3 **b**	0.8 **b**	5.7 **a**
	Mg ($cmol/dm^3$)	0.1 **b**	0.2 **b**	2.2 **a**
	Al ($cmol/dm^3$)	0.5 **b**	1.7 **a**	1.0 **b**
	H+Al ($cmol/dm^3$)	2.3 **b**	11.4 **a**	13.6 **a**
	SB ($cmolc/dm^3$)	0.5 **b**	1.6 **b**	8.1 **a**
	CEC ($cmolc/dm^3$)	1.0 **c**	2.9 **b**	9.1 **a**
	V%	17.9 **a**	12.7 **b**	37.4 **a**
	m%	53.3 **b**	64.6 **a**	25.3 **c**
	Rem-P (mg/L)	41.8 **a**	18.2 **b**	21.9 **b**
	Zn (mg/dm^3)	0.3 **c**	1.4 **b**	4.7 **a**
	Fe (mg/dm^3)	38.2 **b**	325.9 **a**	413.4 **a**
	Mn (mg/dm^3)	3.9 **b**	5.7 **b**	13.7 **a**
	Cu (mg/dm^3)	0.18 **b**	0.62 **a**	0.70 **ab**
	B (mg/dm^3)	3.9 **b**	5.7 **b**	13.7 **a**
	S (mg/dm^3)	5.5 **b**	3.7 **b**	17.0 **a**
	Sand %	93.1 **a**	49.1 **b**	24.9 **b**
Microbial Community attributes	Microbial Biomass C (ug C. g^{-1} dry soil)	80.1 **b**	422.3 **a**	471.1 **a**
	Respiration C-CO2 (mg. kg^{-1}C dry soil)	8.6 **c**	21.4 **b**	45.7 **a**
	Enzymes (Axis 1 of PCA)	-1.1 **b**	0.2 **a**	0.9 **a**
	total PLFA (nmol. g^{-1} dry soil)	5.0 **b**	17.2 **b**	36.5 **a**
	total Fungi (nmol. g^{-1} dry soil)	0.9 **c**	2.9 **b**	6.9 **a**
	total Bacteria (nmol. g^{-1} dry soil)	2.3 **c**	6.9 **b**	15.1 **a**
	Fungi:Bacteria (nmol. g^{-1} dry soil)	0.6	0.4	0.5
	Gram + bacteria (nmol. g^{-1} dry soil)	1.4 **c**	4.2 **b**	8.5 **a**
	Gram - bacteria (nmol. g^{-1} dry soil)	0.9 **c**	2.4 **b**	6.3 **a**
	Gram+:Gram- (nmol. g^{-1} dry soil)	1.5 **a**	1.8 **a**	1.4 **b**

The overall structural equation model including all the vegetation types showed that soil microbial attributes were influenced more by soil properties than by vegetation characteristics. Vegetation characteristics were positively correlated with soil properties, and exposed soil had a negative influence on soil microbial attributes (Table 2.4, Figure 2.3). The variables that composed each latent variable in the best model were the soil microbial attributes of microbial biomass carbon, enzymes, total fungi, total bacteria; the soil properties of pH, SEB, Zn^{2+}, Mn^{2+} and C; and the vegetation characteristics of tree density, basal area, canopy cover and litter. Exposed soil showed an isolated influence on the soil microbial latent variable.

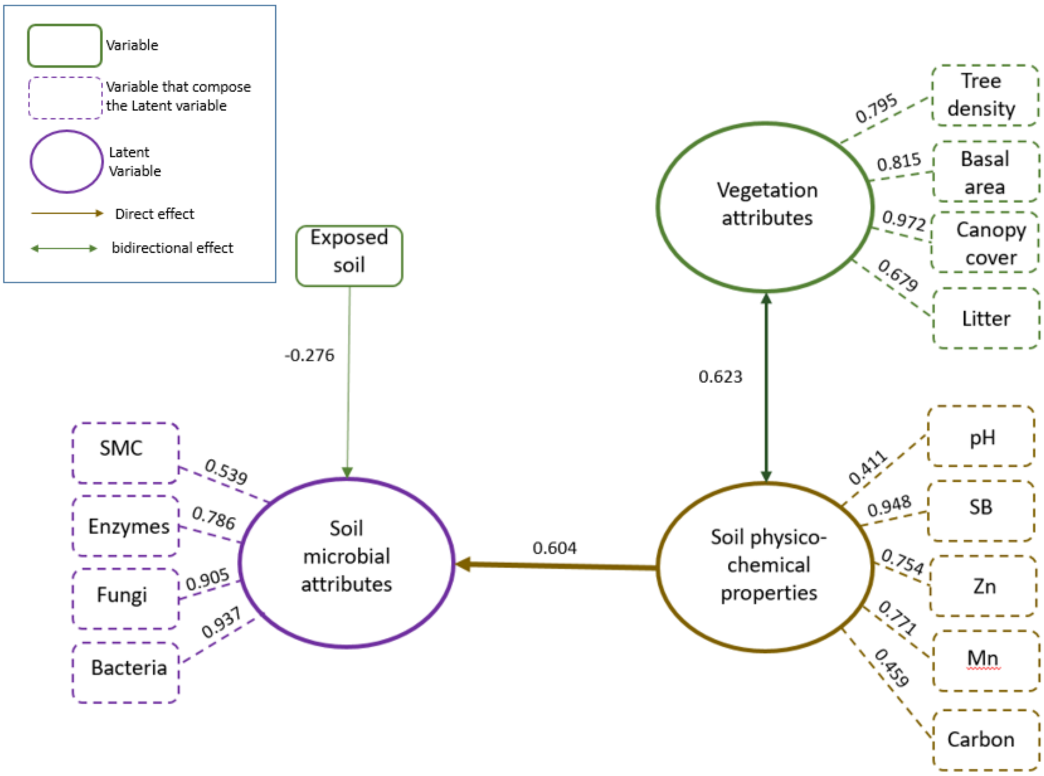

Figure 2.3. Structural equation model path diagram showing the patterns influence of soil properties on soil microbial attributes, the correlation between vegetation characteristics and soil properties, and the influence of vegetation attributes and grass cover on soil microbial attributes across all sampled Cerrado vegetation types in the Pandeiros river basin. Circles represent latent variables and squares the observed variables that compose the latent variables selected through factor analysis. Exposed soil was an observed variable that directly and independently explained soil microbial

properties. The strength of these relationships, Estimates and Bias-corrected confidence values of the Bootstrapping method are shown in Table 2.4.

Table 2.4. Values of Estimate, Lower, Upper and *P* of the Structural Equation Modelling results for non-normal distribution with 1000 Bootstrap samples and bias-corrected confidence intervals of 90% for the global model of all Cerrado vegetation types.

Regression Weights: Bias-corrected percentile method - Bootstrapping					
Parameter		**Estimate**	**Lower**	**Upper**	***P***
Microbial attributes	Soil properties	161	86	397	0.002*
Microbial attributes	Exposed soil	-0.079	-0.125	-0.032	0.016*
Bacteria	Microbial attributes	1000	1000	1000	…
Fungi	Microbial attributes	0.455	0.401	0.539	0.002*
Enzymes	Microbial attributes	0.106	0.081	0.136	0.002*
SMB	Microbial attributes	23.806	17.581	30.818	0.002*
pH	Soil properties	1.000	1.000	1.000	…
SB	Soil properties	21.323	14.443	49.370	0.002*
Zn	Soil properties	16.958	10.630	43.259	0.002*
Mn	Soil properties	20.083	12.734	53.642	0.002*
Carbon	Soil properties	1.561	728	5.767	0.002*
Litter	Vegetation attributes	1.000	1.000	1.000	…
Canopy cover	Vegetation attributes	37.584	34.541	40.673	0.001*
Basal area	Vegetation attributes	0.078	0.066	0.092	0.001*
Tree density	Vegetation attributes	6.393	5.352	7.683	0.001*
Soil properties	Vegetation attributes	0.623	0.484	0.751	0.002*

*significant values considering $P \leq 0.05$. Microbial attributes: soil microbial attributes; SMB: soil microbial biomass; SB: Sum of bases; Zn: zinc; Mn: Manganese.

In the Gallery forest vegetation type alone, the results showed that soil microbial attributes were positively influenced by soil properties and that vegetation characteristics were negatively correlated with soil properties (Table 2.5, Figure 2.4).

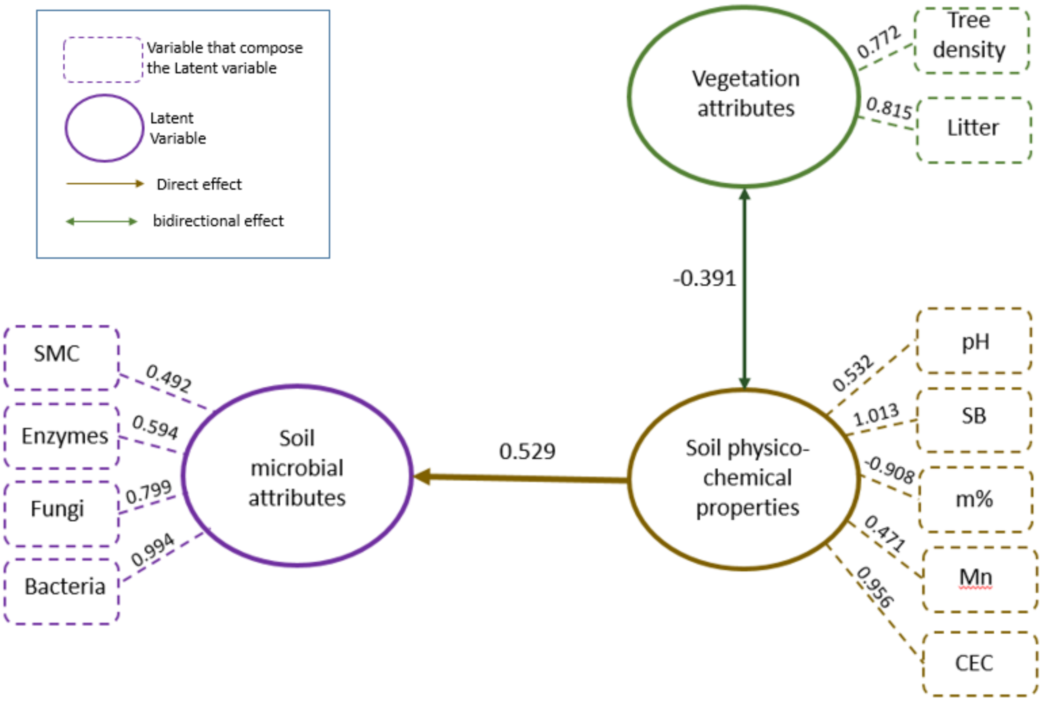

Figure 2.4. Structural equation model path diagram showing the influence of soil properties on soil microbial attributes, and the correlation between vegetation characteristics and soil properties within the Gallery Forest vegetation type. Circles represent latent variables and squares the observed variables that compose the latent variables selected through factor analysis. The Goodness-of-fit of the final model, the strength of these relationships Standardized Estimates and *P* values are shown in Table 2.5.

Table 2.5. Goodness-of-fit values of the most parsimonious model and standardized regression weights of the Structural Equation Modelling results for normal distribution data of the Gallery forest vegetation type.

Goodness-of-fit of the final model				
Model	**χ2 /d.F**	**GFI**	**AGFI**	**RMSEA**
Final model (default)	2.154	0.67	0.500	0.199

Standardized Regression Weights			
Parameter		**Estimate**	**P**
Microbial attributes	Soil properties	0.529	0.035*
pH	Soil properties	0.532	0.002*
m%	Soil properties	-0.908	0.000*
Mn	Soil properties	0.471	0.006*
CEC	Soil properties	0.956	0.000*
Sum of bases	Soil properties	1.013	0.000*
SMB	Microbial attributes	0.492	0.000*
Fungi	Microbial attributes	0.799	0.005*
Bacteria	Microbial attributes	0.994	0.004*
Enzymes	Microbial attributes	0.594	0.016*
Tree density	Vegetation attributes	0.772	0.001*
Litter	Vegetation attributes	0.815	0.000*
Soil properties	Vegetation attributes	-0.391	0.029*

*significant values considering $P \leq 0.05$.

In the Veredas vegetation type, soil microbial attributes were influenced most strongly and positively by soil properties, and also positively by relative grass cover, and negatively by other vegetation attributes. The variables that best composed the latent variables and the best model fit are shown in Table 2.6 and Figure 2.5.

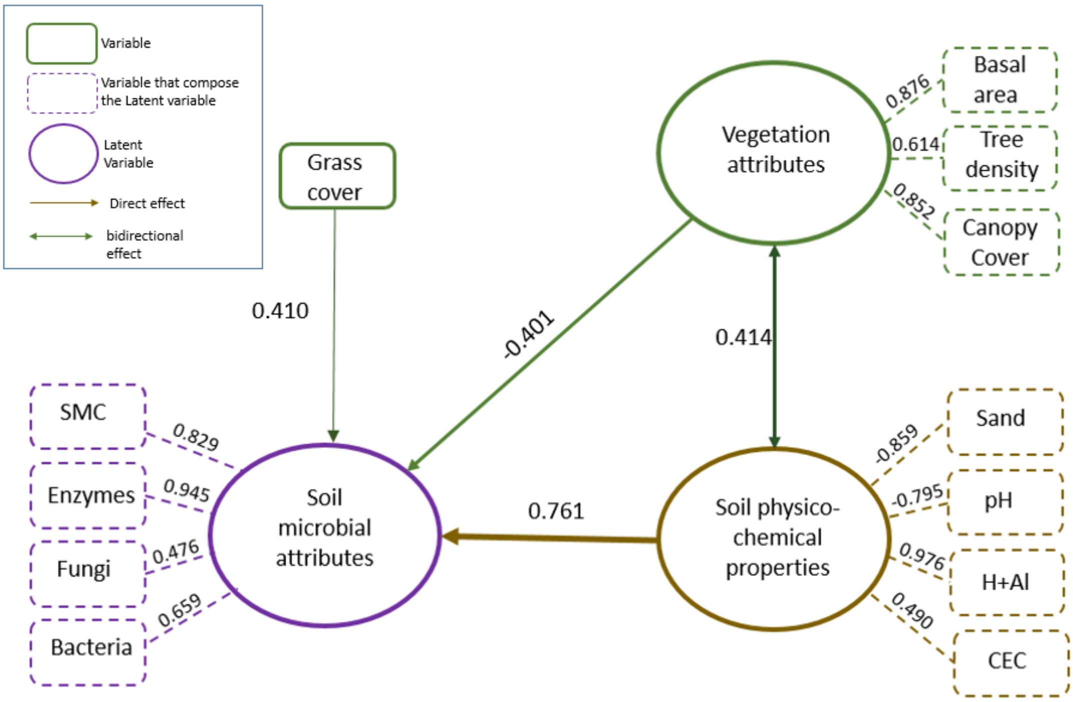

Figure 2.5. Diagram of structural equation modelling showing the direct effect of soil properties on soil microbial attributes, the correlation between vegetation attributes and soil properties, and direct effects of vegetation attributes and grass cover on the soil microbial attributes within the Veredas vegetation type. Circles represent latent variables and squares the observed variables that compose the latent variables selected through factor analysis. The Goodness-of-fit of the final model, the strength of these relationships Standardized Estimates and *P* values are shown in Table 2.6.

Table 2.6. Goodness-of-fit values of the most parsimonious model and the standardized regression weights of the Structural Equation Modelling results for normal distribution data of the Veredas vegetation type.

Goodness-of-fit of the final model

Model	**χ2 /d.F**	**GFI**	**AGFI**	**RMSEA**
Final model (default)	1.660	0.730	0.588	0.151

Standardized Regression Weights

Parameter		**Estimate**	**P**
Microbial attributes	Soil properties	0.761	0.000*
Microbial attributes	Vegetation attributes	-0.401	0.018*
Microbial attributes	Grass cover	0.410	0.003*
Sand	Soil properties	-0.859	0.000*
pH	Soil properties	-0.795	0.000*
H+Al	Soil properties	0.976	0.000*
CEC	Soil properties	0.490	0.006*
SMB	Microbial attributes	0.829	0.000*
Enzymes	Microbial attributes	0.945	0.000*
Bacteria	Microbial attributes	0.659	0.000*
Fungi	Microbial attributes	0.476	0.008*
Tree density	Vegetation attributes	0.614	0.000*
Basal area	Vegetation attributes	0.876	0.000*
Canopy cover	Vegetation attributes	0.852	0.000*
Soil properties	Vegetation attributes	0.414	0.016*

*significant values considering $p \leq 0.05$.

In Cerrado *sensu stricto* the soil microbial attributes were influenced positively by vegetation attributes, showing no influence of soil properties. The variables that best composed the latent variables and the best model fit are shown in Table 2.7 and Figure 2.6.

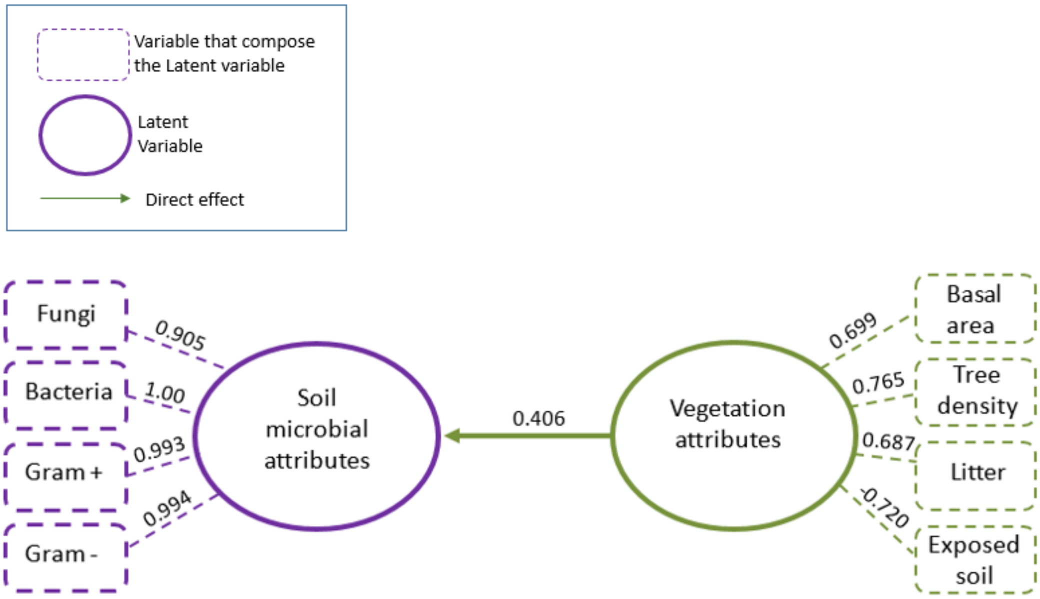

Figure 2.6. Diagram of structural equation modelling showing the direct effects of vegetation attributes on soil microbial community attributes within the Cerrado *sensu stricto* vegetation type. Circles represent latent variables and squares the observed variables that compose the latent variables selected through factor analysis. The Goodness-of-fit of the final model, the strength of these relationships Standardized Estimates and *P* values are shown in Table 5.

Table 2.7. Goodness-of-fit values of the most parsimonious model and regression weights of the Structural Equation Modelling results for normal distribution data of the Cerrado *sensu stricto* vegetation type.

Goodness-of-fit of the final model

Model	**χ2 /d.F**	**GFI**	**AGFI**	**RMSEA**
Final model (default)		21,093	,865	,745

Standardized Regression Weights

Parameter		**Estimate**	**P**
Microbial attributes	Vegetation attributes	0.406	0.056*
Basal area	Vegetation attributes	0.699	0.002*
Tree density	Vegetation attributes	0.765	0.006*
Litter	Vegetation attributes	0.687	0.000*
Fungi	Microbial attributes	0.905	0.000*
Bacteria	Microbial attributes	1.002	0.000*
Gram +	Microbial attributes	0.993	0.000*
Gram -	Microbial attributes	0.994	0.000*
Exposed soil	Vegetation attributes	-0.720	0.000*

*significant values considering $p \leq 0.05$.

4 DISCUSSION

4.1 Differences in soil properties and soil microbial attributes among vegetation types

The evaluation of the soil physico-chemical properties showed no differences in pH between vegetation types, with higher values of soil bases K, Na, Ca, Mg and S in the Gallery Forest that are most likely due to conservative nutrient cycling in a system with high organic matter inputs and stocks. Although it should be noted the larger amount of bases and water availability in riparian zones allows the development of forest and not savanna in the first place (Oliveira-Filho, Ratter and Shepherd, 1990), once the forest is established however, the litter and organic matter (OM) input by the trees contributes to increase the conservation of bases in these ecosystems (Oliveira-Filho et al., 1994). Gallery Forest and Veredas showed greater quantities of Na^+, a possible result of the shallow water table and restricted drainage in these ecosystems (Thomas, Fitzpatrick and Heinson et al., 2011). The redox potential of hydromorphic environments found in the soils of Gallery forest and Vereda vegetation types can influence the availability and mobility of elements, and retain metals such as Cu and Fe (found to be greater in Veredas and Gallery forests) and Mn (found to be greater in Gallery forest) (Nascimento et al., 2018; Junk and Piedade, 2010). The soil texture of the Cerrado *sensu stricto* is predominantly sand, with an average content of 93%, while Veredas showed an average of sand content of 49 % and Gallery forest 24%. Carbon and nitrogen contents were found to be greater in Gallery forest, secondly in Veredas, and lowest in Cerrado *sensu stricto*. The different proportions of sand reflect the effects of plant inputs in the three vegetation types, which suggest that the amount of OM deposition is altering the soil texture by creating a top layer of more organic soils in Gallery forests and Veredas, more sand and less organic in Cerrado *sensu stricto*. On the other hand, differences in texture are also likely to have significant effects on the retention of organic matter, nutrients and water with feedbacks to plant productivity.

The larger amount of soil microbial respiration, total PLFA, total fungi, total bacteria, total gram positive bacteria and total gram negative bacteria in Gallery forests suggest that the carbon inputs (through litter, soil organic matter deposition and roots exudates) (Crow et al., 2009; Brant et al., 2006) are controlling most aspects of microbial community in this vegetation type. The greater amount of microbial biomass carbon and enzymes in Gallery Forests and Veredas suggests that these ecosystems can accumulate more substrate to support productivity. Although there is lower litter deposition in Veredas compared to Gallery forests, these soils can retain organic substrates due to cyclical drying/rewetting events that result in the accumulation of organic matter during dry periods, followed by flooding from the rivers and more transportation of OM to these areas in rainy seasons (Rezende et al., 2016). The greater Gram + : Gram- bacteria ratio found in Cerrado *sensu stricto* and in Veredas suggest that the drought and dry/rewetting regimes of these ecosystems are selecting gram-positive over gram-negative bacteria (Hueso, Garcia and Hernandez, 2012), as the cellular membrane of this group is thicker and more resistant to drought and to osmotic stress caused by changes in the soil moisture conditions (Fierer, Schimel and Holden, 2003).

4.2 Relationships between soil microbial attributes, soil properties and vegetation attributes

We found soil microbial attributes were directly related to soil physical and chemical properties, which in turn affected and were affected by vegetation characteristics.

In the Cerrado biome, waterlogging in the wetlands along the rivers strongly alters soil physical and chemical properties, conditioning riparian vegetation to be different from the surrounding savanna as well as affecting organic matter deposition and nutrient cycling (Junk et al., 2014). In the present study, the main drivers of soil properties positively influencing soil microbial attributes were pH, SB, carbon, Zn and Mn. The Mn selected in our model reflects the mechanisms within the wetland vegetation types (Gallery forest and Veredas) in this study. Wetlands are usually associated with accumulation of trace elements (Abd-Elfattah and Wada, 1981) and reduction of soil Mn oxy-hydroxides (Olivie-Lauquet et al., 2001). The selection of this element suggests the soil microorganisms may be using Mn oxy-hydroxides as alternative electron acceptors in response to the reduced supply of oxygen in these flooded soils (Olivie-Lauquet et al., 2001). The Zn found here as an important driver of soil microbial attributes may be due to the positive correlation usually found between Zn^{2+}, pH and bases in Brazilian soils (Matos et al., 2001). Silva et al. (2018), found enhanced soil enzymatic activities in soils with higher pH and organic matter content in the Cerrado biome. This corresponds to our finding that pH, SB and carbon are positively influencing soil microbial attributes, which also reflects the influence of litter inputs.

Soil properties were shown to be positively correlated to the vegetation characteristics of tree density, basal area, canopy cover and amount of litter. There is a reciprocal relationship where vegetation influences soil conditions, while soil properties themselves affect plant physiological processes (Jenny, 1994), which may also include tree community structural attributes. Litter quantity was selected in the overall model due to the importance of organic matter accumulation on soil fertility in the Cerrado biome (Lammel et al., 2015), in which higher tree density, greater basal area and canopy cover produce greater quantities of litter, enhancing soil OM accumulation, and positively affecting soil microbial attributes and activity (Lammel et al., 2015).

The overall SEM, including all the vegetation types, was run in order to assess the main relationships and factors driving these interactions irrespective of vegetation type. Here, soil properties positively influenced the soil microbial attributes of soil microbial biomass, enzyme activities, and the abundances of fungi and bacteria. The soil physico-chemical factors of Zn and Mn reflect trace element accumulation as a result of waterlogging with consequences for microbial attributes. Carbon, base ions and soil pH reflect soil fertility status that is potentially related to vegetation OM inputs. Although overall vegetation characteristics showed no direct effect on soil microbial attributes, they did show their importance in shaping the soil physico-chemical properties that in turn affected soil microbial attributes.

4.3 Relationships between vegetation characteristics, soil physico-chemical properties and soil microbial attributes in the Gallery forest

In Gallery forest, our results showed soil physico-chemical properties to directly and positively influence soil microbial attributes, and that vegetation characteristics were correlated with soil physico-chemical properties. No direct influence of vegetation on soil microbial attributes was found (Fig 2.4).

Although plant litter inputs are an important resource for soil microbial communities, its influence was shown to be indirect through the correlation with soil properties in our study. Some aspects of soil properties including acidity, bases and exchangeable cations had a negative correlation with increases in vegetation structural characteristics. The high input of litter can produce humic acids, decrease soil pH and increase Al that also normally reflects a reduction in the amount of exchangeable bases (Facelli and Pickett, 1991, Kin et al., 2011, Malavolta et al., 1997). This may explain the negative correlation of tree density and amount of litter in the vegetation attributes group with pH, cation exchange capacity, Sum of bases, and the negative contribution of aluminium saturation of the soil properties group influencing soil microbial properties.Mn was selected as part of the "soil properties" group of the Gallery Forest vegetation type as the anoxic conditions of these wet environments mobilize this soluble reduced ion (Naiman and and Décamps, 1997), and therefore it will be used alternatively to oxygen by microorganisms as electron acceptors (Olivie-Lauquet et al., 2001).

The vegetation is not the only factor altering soil conditions in these ecosystems. Waterlogging along the rivers strongly influences soil physico-chemical properties (Junk et al., 2014) including OM content, substrate availability for microbial communities and nutrient cycling in riparian zones. Silva et al (2011) found that riparian forests in the Cerrado biome tend to absorb nutrients carried through the streams that are derive from agricultural fertilizer additions, and therefore, they recommend the conservation of these forests in order to improve the quality of water delivered to downstream ecosystems. As we found indicators of soil fertility such as pH, bases and exchangeable cations to enhance microbial biomass, enzyme activities, total fungi and total bacteria, our results reinforce the importance of Gallery Forests in balancing nutrient cycling by retaining and conserving nutrients carried by streams.

4.4 Relationships between soil microbial community attributes, soil properties and vegetation attributes in the Veredas vegetation type

The Veredas vegetation type had the most variable soil microbial attributes (microbial biomass, enzymes and bacteria) which were positively related to soil physico-chemical properties (pH, H+Al and CEC with negative contribution of sand in the latent variable) and grass cover, and negatively related to vegetation characteristics (tree density, canopy cover and basal area) and soil properties. The factor analysis did not select grass cover for the latent variable "Vegetation attributes", due to its effects being being strongly unrelated to those of tree density, canopy cover and basal area. Veredas are open ecosystems, in which soil water saturation and poor water leaching induce a grass-dominated plant community (Filgueiras, 2002), and therefore, microbial communities that are potentially better adapted in terms of biomass, fungi, bacteria and extracellular enzyme activity to the substrate released by a rhizosphere dominated by grass roots, (Garcia et al., 2005; Singh et al., 2007). This may explain the positive influence of grass cover on soil microbial attributes found here (Fig 2.5). The negative influence of vegetation attributes (tree density, canopy cover and basal area) on microbial attributes in this context may be due to the fact that greater amount of trees, basal area and canopy cover reduce the area of grass cover. In addition, it is important to highlight that our study evaluated soils at 0-10 cm depth, and thus, the microbial activity and community attributes represent the surface horizon where the grass rhizosphere is influential. Trees and shrubs have deeper roots than grasses, and therefore, the microbial community associated with their rhizosphere may be located in deeper soil layers. In forests, the larger microbial activity found in 0-10 cm depth is probably a result higher plant litter inputs from the trees (McGee et al., 2019). The Veredas vegetation does possess sparse shrubs, trees, and the *Mauritia flexuosa* palm tree, although these produce less litter than other trees found in the Gallery forest systems. Consequently the microbial community of the upper soil layers are likely responding more to the grass rhizhosphere exudates than to tree-community derived organic matter deposition from litter inputs.

Soil properties were shown to strongly influence soil microbial attributes. Soil pH negatively contributed to the soil properties group, whereas potential toxicity

(H+Al) contributed positively (Fig 2.5). This result suggests that the high acidity associated with aluminum toxicity may be a limiting factor for crops, but not for native plant species that may possess adaptive mechanisms in Brazilian savannas (Haridasan 2008). Our results corroborate that vegetation and microbial communities are adapted to acidity and aluminum toxicity in Veredas soils. Sand content had a negative influence on soil microbial attributes. Due to the lesser aggregation and stability of sandy soils (Moreira and Siqueira, 2006), soil microorganisms can be more easily washed from the system during the rainy season, while the size of the particles can also affect enzyme adsorption (Datta et al., 2017). The positive influence of cation exchange capacity on soil microbial attributes may be due the accumulation of organic matter, due to mechanisms associated with periodic flooding as described above, that consequently increase bases and nutrient availability and enhances primary production (Pant, Rechcigl and Adjei 2003).

4.5 Relationships between soil microbial community attributes, soil properties and vegetation attributes in the Cerrado sensu stricto vegetation type

In Cerrado *sensu stricto*, the vegetation characteristics of basal area, tree density and litter positively influenced soil microbial attributes, whereas soil physico-chemical properties had no influence and were not correlated with vegetation. Microbial biomass carbon and enzymes were not selected (i.e. Factor Analysis) for inclusion in the soil microbial attributes group, reflecting the low capacity of the sandy Cerrado *sensu stricto* soil to retain substrates required for microbial biomass and enzyme activity (Datta et al., 2017). Instead, other components of microbial community structure (i.e. total fungi, total bacteria, gram positive and gram negative) were selected for this group (Fig 2.6). This finding suggests that higher tree densities and basal are resulting in greater litter inputs, and that the quantity and potentially the quality of this organic matter is crucial in shaping microbial community structure in this vegetation type. Lammel et al. (2015) showed the importance of Cerrado *sensu stricto* vegetation in shaping bacterial community composition as a result of litter quality, with recalcitrant compounds such as lignin expected to be found in this vegetation type. Our results evidence that fungal communities are key in the decomposition of leaf litter derived organic matter, due to their capacity to degrade recalcitrant compounds (Brant et al., 2006) and persist in dry environments

(Kaisermann et al., 2015). Considering the importance of the relationship between soil microorganisms and vegetation structure in ecosystem functioning as shown in our study, we suggest that future studies focus on bacterial and fungal community compositions based on Next-Generation Sequencing techniques, and their role in the litter decomposition of Cerrado *sensu stricto*.

5 CONCLUSION

The overall Structural Equation Model that included all 3 Cerrado vegetation types showed how local soil physico-chemical properties influence soil microbial attributes, and how they are correlated with vegetation characteristics. The influence of flooding in shaping soil conditions is reflected in the soil properties with respect to the presence of trace elements and other indicators of fertility, while the amount of carbon and bases in the soil reflects organic matter inputs from vegetation. Although vegetation characteristics showed few direct effects on microbial attributes, the overall statistical model demonstrated the importance of the indirect effects of vegetation cover on soil microbial attributes *via* influences on soil physico-chemical properties. This is corroborated by evidence of a direct negative influence of exposed soil on microbial attributes.

In the Gallery forest, close to watercourses, the strong and direct relationship between soil properties and microbial attributes might be a consequence of water shaping soil conditions. In Veredas flooding and soil water saturation is inducing a grass dominated plant community and a grass-associated soil microbial community, as well as enhancing organic matter deposition and cation concentrations in the soil. Finally, in Cerrado *sensu stricto*, the prevalence of sand showed that these soils have very little capability to support or retain microbial communities, resulting in an adaptation and strong dependence on the quantity and quality of the litter produced by the vegetation. Therefore, we recommend that further studies assess the soil microorganisms based on Next-Generation Sequencing techniques for a deeper identification of community composition, and evaluate how it relates with litter decomposition process in all vegetation types.

This study offers important advancement of understanding regarding the local vegetation and soil controls on soil microbial attributes and functions across typical

Neotropical savanna ecosystems. Even though these three ecosystems are spatially so close to one another, they have completely different above- below-ground interactions patterns. It means that any generalization of any kind for Cerrado biome should to take into account of these differences.

Considering the rapid conversion of native Cerrado vegetation to croplands and pastures during recent decades, this knowledge is crucial to predict how changes in vegetation characteristics and soil properties will alter important ecological processes in the different vegetation types, as well as the maintenance and stabilization of these ecosystems in response to future climate change.

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APPENDIX CHAPTER 2 (A2)

Table A2.1. Axis 1 and Axis 2 of the Principal Component Analysis of the enzymes: Arylsulphatase, β-Glucosidase, Alkaline and Acid phosphatase, Urease and FDA. Axis 1 was used for the Structural Equation Modell Analysis as a component "Enzymes". Site and Vegetation type are shown: VE = Veredas; CE= Cerrado sensu stricto; GA=Gallery forests.

Sites/Vegetation	Axis 1	Axis 2
Site1VE	4652,2	1569
Site2VE	-1845,6	282,65
Site3VE	11181	-1591,1
Site4VE	-2048,2	1550,4
Site5VE	-1757,8	1336,6
Site6VE	-1505,7	336,28
Site7VE	-1376,9	1036,7
Site8VE	-1747,9	-483,26
Site9VE	-1656,5	411,15
Site10VE	-2104,2	-323,17
Site1CE	-2701,3	-1768,4
Site2CE	-2830,2	-1672,3
Site3CE	-2988,9	-1852,8
Site4CE	-2817,6	-1783,4
Site5CE	-2652	-1371,4
Site6CE	-3157,5	-1726,5
Site7CE	-3104,6	-1868,5
Site8CE	-2755,8	-1568,6
Site9CE	-2585,1	-1704,9
Site10CE	-2423,2	-1773,9
Site1GA	18025	171,23
Site2GA	12667	-2281,3
Site3GA	2409	-798,3
Site4GA	-3305,6	1939,5
Site5GA	1804,9	5052,8
Site6GA	-1711,4	1222,5
Site7GA	86,883	3745
Site8GA	-1478	735,75
Site9GA	-548,98	2547,7
Site10GA	-1722,3	630,76

Table A2.2. Axis 1 and Axis 2 of the Principal Component Analysis of the soil physico-chemical properties: Nitrogen (N), Carbon (C), pH, potassium (K), phosphorus (P), sodium (Na), calcium (Ca), Magnesium (Mg), Aluminum (Al), potential acidity (H+Al), sum of bases (SB), cation exchange capacity (CEC), base saturation (V%), aluminum saturation (m%), remaining phosphorus (P-rem), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), boron (B), sulphur (S).

	Axis 1	Axis 2
N	0,576	0,7312
C	0,5059	0,7551
pH	0,5922	-0,6541
K	0,7663	0,2087
P	0,2361	0,1963
Na	0,6739	0,3401
Ca	0,9195	-0,2427
Mg	0,8753	-0,2524
Al	-0,1998	0,8543
H+AL	0,1427	0,9012
SB	0,9262	-0,2445
CEC	0,9305	-0,1149
V%	0,7919	-0,5331
m%	-0,7381	0,3977
P-rem	-0,2202	-0,675
Zn	0,6535	0,09269
Fe	0,2464	0,4267
Mn	0,6556	-0,2232
Cu	0,0917	0,4487
B	0,5874	0,5488
S	0,8624	0,177

MANUSCRIPT 2

LANDSCAPE CONTROLS ON SOIL MICROBIAL ATTRIBUTES IN NEOTROPICAL SAVANNA

(Preliminary version prepared for submission to Science of the Total Environment)

ABSTRACT

Soil microorganisms and soil enzymes play a central role in controlling ecosystem processes such as organic matter decomposition and nutrient cycling. Although the microbial community and its functionality are good indicators of soil quality in different ecosystems, there is still much to understand about how anthropic disturbance and environmental factors govern microbial community structure and nutrient cycling. In this study we evaluated how anthropic disturbance, topography and soil properties relate to microbial community structure and soil enzyme activities in three typical Cerrado vegetation types: Gallery forest, Veredas and Cerrado *sensu stricto*. We collected soils (0-10 cm depth) from 10 sites of each vegetation type along the Pandeiros river basin, Minas Gerais, Brazil. The soil microbial biomass, respiration PLFAs, β-Glucosidase, Arylsulfatase, Urease, Acid and Alkaline Phosphatases, Fluorescein diacetate (FDA) hydrolysis, and soil physicochemical properties were assessed. A land-use classification was made across scales of 500 meters, 1 kilometer and 2 kilometer radius in order to determine the most significant scale of influence on the soil microbial properties. Topography was assessed using a digital elevation model (DEM) that determined the elevation within pixels of 12.5 m of spatial resolution. From this DEM, the topographic wetness index, and slope gradient were calculated and their values, along with the elevation, were extracted at each sampled point. Using a GLM approach we found that human disturbance was related to soil microbial biomass carbon and PLFAs, while aspects of soil property and topography were important predictors of soil respiration, metabolic quotient, total PLFAs, fungi, bacteria, gram positive and negative bacteria abundances. Topography was a strong predictor of soil enzyme activities and all other soil microbial attributes with the exception of the soil microbial biomass. This suggests the influence of soil moisture content that is driven by topography. We found that the intensity and direction of the relationships between human disturbance and topography varied according to the vegetation type. This study shows the potential for increases in landscape scale human disturbances to change soil properties and that topography regulates moisture. I discuss the significance of this for soil microbial attributes that contribute to Cerrado functionality.

Key words: Cerrado, soil extracellular enzymes, PLFAs, Anthropogenic disturbance, topography, soil moisture.

1 INTRODUCTION

Microorganisms are known to play a central role in soil ecological processes and nutrient cycling (Brockett et al., 2012). Due to its importance, it is crucial to understand how landscape features determines its processes in order to support conservation of ecosystems and services they provide to humanity (Hooper et al., 2000). The ongoing anthropogenic disturbance is leading to an increase in deforestation and consequent soil erosion, environment degradation and ecosystems services provision to humanity (Wang et al., 2015). Becase vegetation and microorganisms are usually linked in plant-soil feedback processes (Fujii et al., 2018), the reduction of vegetation cover by the anthropogenic disturbance interfere directly on the soil microorganisms communities and soil functioning (Quesada and Lloyd, 2016).

Soil microbial communities and activity have been shown to change rapidly with anthropogenic disturbance or any other biotic and abiotic perturbations (Rodríguez-Valdecantos et al., 2017). Changes in the environment conditions alter soil properties and microhabitat of living organisms (Gömöryová et al., 2017; Kooch et al., 2014). The legacy of the perturbation can last many years as the soil microbial communities respond along the whole successional regeneration process of the natural ecosystems (Gömöryová et al., 2017). In Brazil, anthropogenic disturbance is shown to affect soil functioning of the different biomes, as the reduction in vegetation also decline the organic carbon input with consequent changes in nutrients mineralization (Kaschuk et al., 2011).

Besides anthropogenic disturbance, the topography is also an important factor influencing soil microbial community and its activities. The slope gradient has been found to contribute to the litter and nutrient collection, and consequently increasing the soil microbial biomass and enzymes activities (Pan et al., 2018). Sloping areas themselves were also found to be sensitive to nutrient mineralization (Zhong et al., 2018) as the soil infiltration and nutrients leaching are higher in this areas (Hook and Burke, 2000). Also, the soil moisture can vary with topography (Osborne et al., 2017). More specifically, aspects such as elevation, slope and features such as depressions can reflect on the soil moisture content that affects soil physico-chemial properties, microbial attributes and nutrient mineralization (Pan et al., 2018; Zhong et al., 2018).

In the Cerrado biome, the topography selects for distinct vegetation types: cerrado savanna woodlands are found in interfluvial areas, Veredas (waterlogged grasslands) occur in valley bottoms, and gallery forests border rivers and commonly possess waterlogged soils (Oliveira-Filho et al., 1990). The aim of this study was to understand how landscape scale anthropogenic disturbance and topography relate to soil microbial attributes and whether this varies across across typical Cerrado vegetation types. This was achieved with a landscape-classification approach using satellite imagery and landscape digital elevation models (DEM). The DEM represents the altitude (elevation) of an area as a raster-based and continuous type of data, providing an overview of the topography. Moreover, from a DEM, digital terrain data can be extracted, such as slope gradient, topographic wetness index, curvatures, etc. Some research has examined the relationship of landscape scale data with soil and vegetation properties (Su and Bork, 2006; Pfeffer, Pebesma and Burrough 2003; Luo et al., 2015), but the studies evaluating their relationship with soil microbial attributes remains insufficient, especially in the Cerrado biome.

We hypothesize that: 1) Soil microbial attributes will be affected by the area of landscape scale anthropogenic disturbance in all Cerrado vegetation types. We expect that, regardless of vegetation type, anthropogenic disturbance acts mainly by reducing natural vegetation cover, decreasing plant derived organic matter inputs and consequently altering soil microbial communities and nutrient cycling. 2) Soil microbial attributes will be affected by topography in different ways between the vegetation types: a) Attributes will be affected by topographic wetness index in the wetter vegetation types of Gallery forest and Veredas, in different directions than in Cerrado *sensu stricto.*; b) Slope will negatively affect soil microbial attributes regardless of the vegetation type; c) Elevation will positively affect soil microbial attributes in the Gallery forests and Veredas and negatively in Cerrado *sensu stricto*, as increases in elevation in wetter areas in this generally flat biome may increase the proportion of the air to water in soil pores, creating better conditions for soil microbial processes, wheres drier areas will undergo the opposite effect.

In this study we assessed the following soil microbial attributes related to microbial community structure: microbial biomass carbon (MBC), respiration, metabolic quoeficient (qCO_2), total PLFAs, total fungi, total bacteria, gram positive bacteria and gram negative bacteria. We also analyzed microbial enzymes activities: β-glucosidase, that acts on the organic matter cycling participating on the degradation of cellulose in the soil producing glucose (Adetunji et al., 2017; Knight and Dick, 2004); alkaline phosphatase, that is related to the soil phosphorus availability as it catalyzes the hydrolysis of H_3PO_4 (Bhatt et al., 2011); urease, that hydrolyses urea producing CO_2 and NH_3 (Adetunji et al., 2017; Das and Varma, 2010); arylsulfatase, that mineralizes aromatic sulfate esters ($RO\text{-}SO_3$) releasing inorganic sulfate (SO_4), the available sulfate form for plants uptake (Karaca et al., 2010). These enzymes are linked to carbon (C), phosphorus (P), nitrogen (N) and sulphur (S) cycling respectively. With these measures of microbial community and activity we intended to assess responses to landscape scale features.

2 METHODS

2.1 Study area

This study was conducted in the Cerrado biome, in the northern region of Minas Gerais state, along the Rio Pandeiros hydrological basin. The basin is located between the south latitude meridians 14º00 to 16º30 and the west longitude meridians 43º00 to 46º00. The main savanna woodland vegetation type found in the region is the Cerrado *sensu stricto* found on the drier interfluvial areas. On the valley bottoms another two distinct vegetation types exist, gallery forests found bordering the rivers, and the Veredas vegetation type can be found in depressions and valley bottoms, where the topography allows water to form marshy fields covered mainly by grasses and shrubs , with the palm tree *Mauritia flexuosa* LF. We selected 10 sites that included the three typical and distinct Cerrado vegetation types: Gallery Forest (riparian Forest), Veredas (marshy fields) and Cerrado *sensu stricto* (savanna woodland) (Fig 3.1).

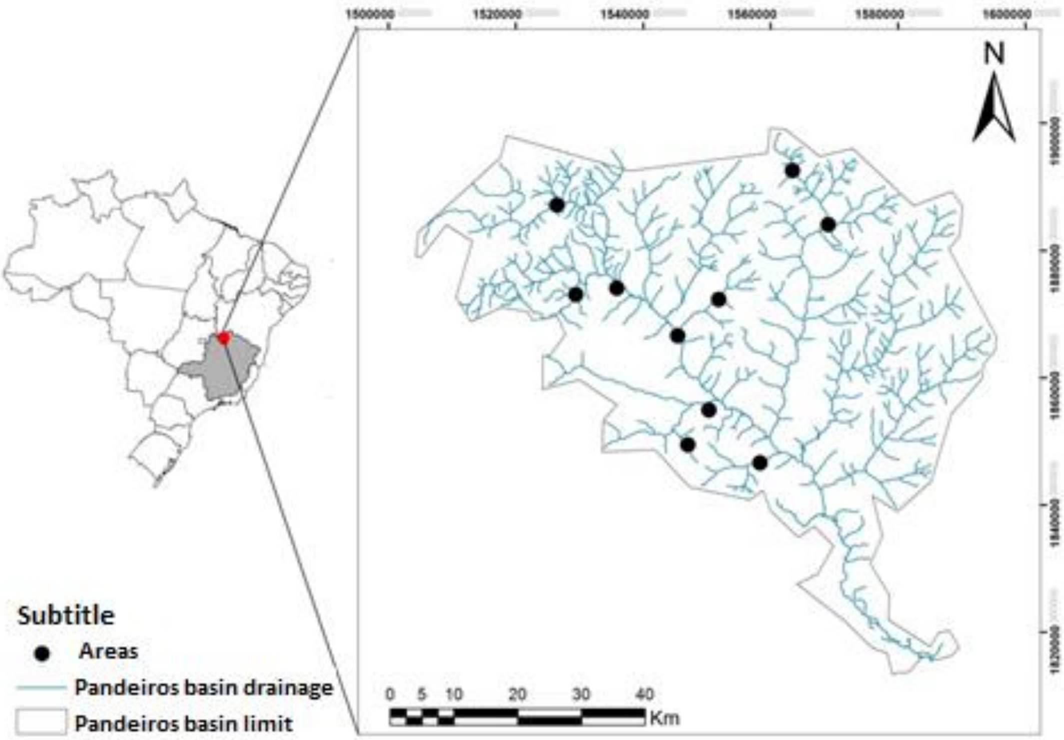

Figure 3.1. Pandeiros river basin in the north region of Minas Gerais state, Brazil. (• sampled sites that included: Gallery Forest, Veredas and Cerrado *sensu stricto*).

2.2 Land cover classification

We classified the land cover using RapidEye images with 5 meter resolution, freely available through the Brazilian Ministry of the Environment on the Geocatálogo platform (www.geocatalogo.mma.gov.br). For each site, we worked with a buffer of 2 km radius centred in the centre point of all our sample sites (located within middle the Veredas vegetation type at each location). For each buffer we classified and determinated the relative cover of Cerrado *sensu stricto*, Vereda, Vereda/grassland, Gallery forest, roads, pasture, exposed soil, human settlements, lakes and rivers, forest and agriculture (i.e. planted cropland). We used the object based image analysis approach (Blaschke, 2010) with segmentation procedure and manual classification to improve the classification accuracy for the scene. The classification was carried in the eCognition Developer® software (Trimble, 2012). The 2 km radius buffer was further subdivided into 1 km and 500 m radius buffers. We carried out analysis using these three buffers sizes to be able to select the best scale for predicting the soil microbial attributes (Figure 3.2). The variable called “Disturbance” was created based on the sum cover (hectares) of the classes: roads, pasture, exposed soil, human settlements and agriculture within each buffer.

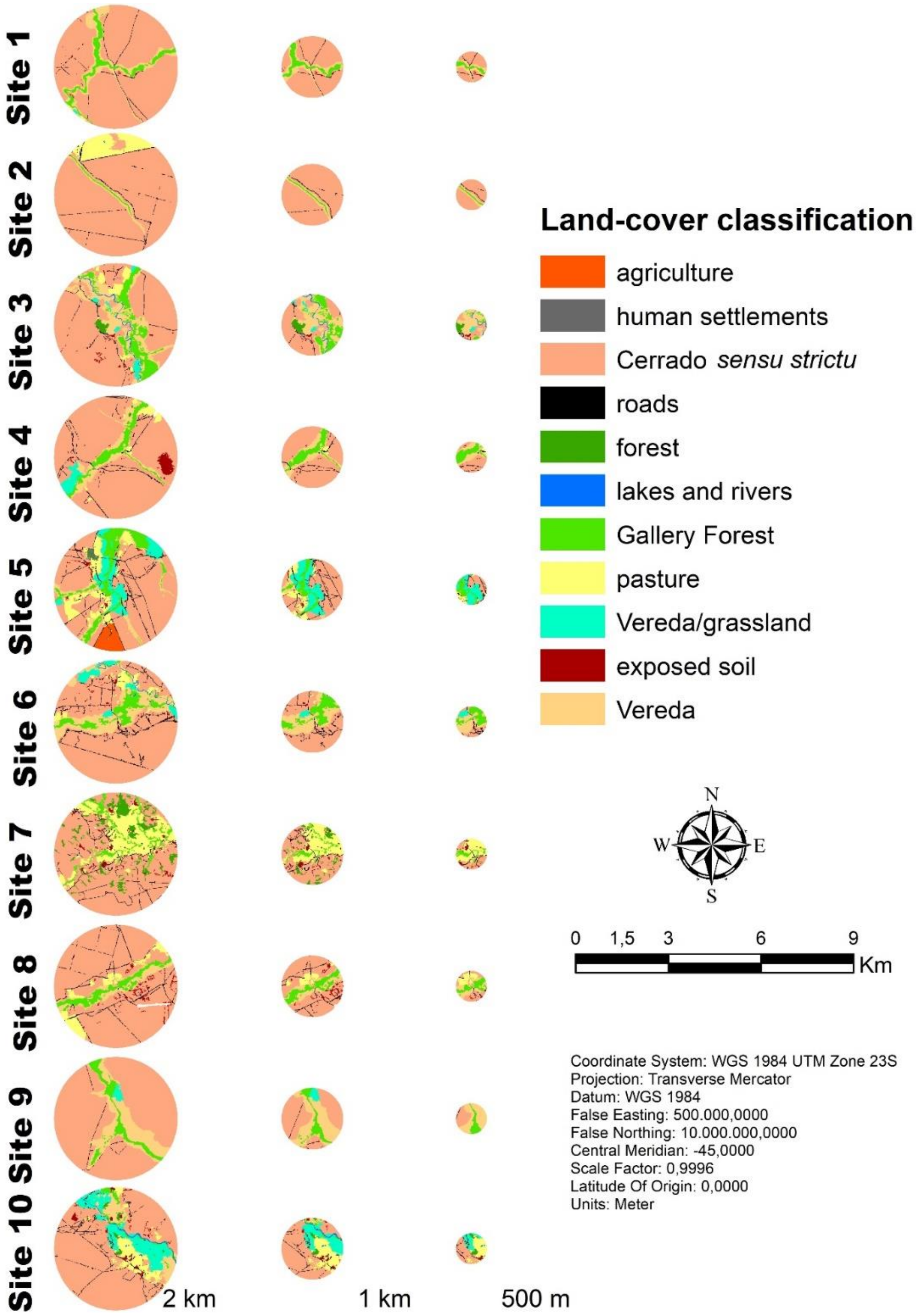

Figure 3.2. Land cover classification within different radius buffer scales: 2 km, 1 km and 500 m of the 10 selected sites in the Pandeiros river basin in north region of Minas Gerais state, Brazil.

2.3 Topography

We used a digital elevation model (DEM) Alos PALSAR with 12.5 m of spatial resolution, downloaded from www.asf.alaska.edu, to create slope and topographic wetness index through the software SAGA GIS (Conrad et al., 2015). Then, the values of elevation, slope and topographic wetness index, which shows the areas more likely to accumulate water due to topographic features (Beven and Kirkby, 1979), were extracted from each sampling location and used for statistical analyses. Fig. 2.3 shows the elevation and the terrain models used in this work.

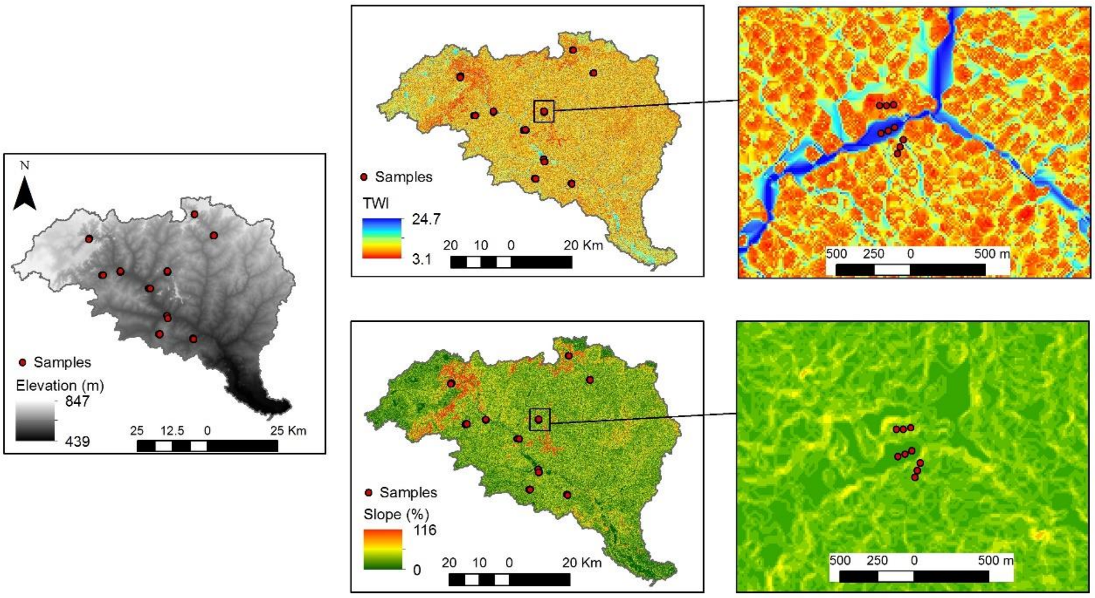

Figure 3.3. Elevation, slope and topographic wetness index (TWI) from Digital Elevation Models (DEM) used in the sampled sites of Pandeiros river basin in the north region of Minas Gerais state, Brazil. Red balls are the sampled points.

2.4 Soil collection

Within each site, we had the three distinct vegetation types: Cerrado *sensu stricto,* Veredas and Gallery forest. Inside each vegetation type at each site we collected three composite soil samples totaling 90 samples considering all the sites and all the vegetation types. The spatial (pseudo) replication (three composite samples) within each vegetation type of each site was designed in order to capture the variation within the sites. The composite samples were collected 30 m apart, comprising five samples collected in a cross shape (in a distance of 1.5 m between each single sample, including the central sample resulting in five soil cores) which were mixed to form one unique composite sample. The litter layer was removed before sampling soil at 0-10 cm depth. Part of these samples were kept in environment temperature for the physicochemical analysis and the other part were kept in sterile pots, in a freezer (-20ºC), and taken into the laboratory for microbiological analysis.

2.5 Soil properties

Soil samples were taken to the soil laboratory for physicochemicals analyses at Universidade Federal de Lavras. We evaluated pH, measured by suspension in water (1: 2.5), available contents of potassion (K^+), phosphorus (P), sodium Na^{2+}, boron (B), Zn (zinc), manganese (Mn^{2+}), iron (Fe^{2+}) and copper (Cu^{2+}) extracted by the Mehlich-1 solution (Mehlich, 1953); exchangeable calcium (Ca^{2+}), magnesium (Mg^{2+}) and aluminium (Al^{3+}) extracted by 1 mol L^{-1} KCl (McLean et al., 1958); potential acidity (H+ Al) by SMP extractor (Shoemaker et al., 1961); sum of bases (SB), cation exchange capacity at ph 7.0 (CEC), extracted by Ca 0.5 mol L^{-1} acetate; and available sulfur (S) extracted by monocalcium phosphate in extracting acetic acid (Walkley and Black, 1934). We calculated Base saturation (V%) and Aluminum saturation (m%). The texture (sand, silt and clay contents) was evaluated by the Bouyoucos method (Bouyoucos, 1951). Carbon (C) and Nitrogen (N) were measured in the Elemental Analyzer machine - Elementar Vario EL III run in C:N mode.

The enzymes activity of fluorescein diacetate hydrolysis (FDA) (Dick et al., 1996), β-glucosidase (Eivazi and Tabatabai, 1988), alkaline phosphatase (Eivazi and Tabatabai, 1977), urease (Tabatabai and Bremner, 1972) , arylsulfatase (Tabatabai and Bremner, 1970). For that, soil samples of 1 g were incubated with 1 mL of substrate: ρ-nitrophenyl-β-D-glucoside, ρ-nitrophenyl-phosphate and ρ-nitrophenyl sulfate were used for β-glucosidase, alkaline phosphatase, and arylsulfatase respectively. The supernatant was filtered and read in a spectrophotometer at 490 nm to determine optical density difference of each sample.

For the total microbial biomass carbon, we firstly irradiated the material in a microwave (Islam and Weil, 1998) and then extracted following Vance et al. (1987), and also measured the soil basal respiration (Anderson and Domsch, 1993). The microbial community structure was assessed using the phospholipid fatty acid (PLFA) analysis (Bardgett et al., 1996) extracted from 1,5 g soil freeze dried weight using an Agilent 6890 Gas Chromatograph (detector FID and column 60m Agilent RTx-1 capillary column - 60m x 0.32mm ID, 0.25um film thickness). The fatty acid nomenclature were based on Petersen and Klug (1994). Gram positive bacteria were the ester-linked branched-chain fatty acids: 15:0i, 15:0a, 16:0i, 17:0i, 17:0a; the gram negative bacteria were the cyclopropyl saturated and monosaturated fatty acids: 16:1ω7, 7,cy-17:0, 18:1ω7,7,8cy-19:0 (Rinnan and Bååth, 2009); and the biomarker for fungi were: 18:2 ω6,9 and 18: 1ω9 (De Deyn et al., 2011). The total PLFA considered the sum of all the PLFAs cited above, plus all the other identified biomarkers: 14:0, 15:0, 16:1, 16:1ω5, 16:0,17:1ω8, 7Me-17:0, br17:0, br18:0, 18:1ω5, 18:0, 19:1. The Fungi:Bacteria and Gram positive: Gram negative bacteria (gram+:gram-) ratios were calculated.

2.6 Statistical analysis

We ran a Principal Component Analysis (PCA) with the soil properties selecting those ones with coefficients ≤ 0.6 (+ or -) in the first two components. The most expressive variables were: N, C, pH, Na^{2+}, H+Al, SB and B. To sumarize all of these important attributes and create the “soil properties” variable, we ran another PCA considering only these selected variables and extracted the principal factor based on the first axis of the Principal Component with varimax rotation. The percentage of variation of axis 1 and axis 2 and the coefficients values of the factor of the component matrix are shown in Appendix Table 3.1. The PCA and the factor extraction were carried in IBM SPSS Statistics version 23.

To identify how landscape, topography and soil properties influenced the soil microbial attributes in the vegetation types, we used a Generalized Linear Model (Crawley, 2007) with a Multi Model Inference approach (Burnham & Anderson, 2002). The Gaussian distribution was used after testing for normality by Shapiro-Wilk test and the residuals were verified. Variables with non-normal distribution were log-transformed to fit in the Gaussian family. We used the ‘lme4’ (Bates et al., 2014), lmerTest (Kuznetsova et al., 2017), ‘MuMIn’ (Barton, 2016) and ggplot2 (Wickham and Chang, 2016) packages in R version 3.4.3 (R core Team 2017).

We assessed the Goodness-of-fit of predictor variables of the models through adjusted coefficients of determination (Nakagawa and Cuthill, 2007). We ran all possible combinations of the predictors variables and ranked the models from the best to the worst. We accepted the models with Aikaike's information criterion (ΔAICc) < 2 as equaly plausible (Burnham and Anderson, 2002). From the candidate models set (ΔAICc < 2), we averaged coefficients, thus taking into account a greater uncertainty (Vierling et al., 2013). The relative importance of each predictor was conferred by the sum of the Akaike weights (Σwi) of the candidates models set (Burnham and Anderson, 2002). The Multi Model Inference analyses were performed separately for the buffers of 500 m, 1 km and 2 km, avoiding then possible collinearity between factors measured at different scales (Carrara et al., 2015; Neter et al., 1996), with the same soil microbial attributes as response variables in all the analysis. The topography predictors (elevation, slope and topographic wetness index) and soil properties were the same for all analysis and scales. Finally, we selected the buffer size where models had larger R^2 values as the scale where landscape variables had greater effect on soil microbial properties.

3 RESULTS

Most of buffers had models well adjusted, showing in general $R^2 \geq 0.40$ (R^2 values are shown in Table 3.2). We adopted the 2 km radius resolution buffer because it gave the most adjusted model outputs (n=9) with the best potential to predict microbial attributes (1 km radius buffer = 4 models, 500 m radius buffer = 1 model. The subsequent results are for the 2km resolution statistical modelling scale.

Table 3.2: R^2 values for the models for each response variable considering the land-cover classification and topography as predictor variables for the 500 m, 1 km and 2 km ray buffers. The numbers in bold indicate which buffer had the best model for each response variable.

	Models R^2 for different Buffers		
Response variables	500m	1km	2km
Arylsulfatase	-	-	**0.853**
β-glucosidase	0.766	0.777	**0.802**
Alkaline phosphatase	0.687	0.727	**0.750**
Biomassa	0.744	**0.753**	0.658
FDA	0.785	**0.804**	0.778
Respiration	0.955	0.954	0.949
qCO_2	0.389	**0.459**	0.419
Urease	0.780	0.778	**0.799**
total PLFA	-	0.667	**0.799**
total Fungi	0.893	0.880	**0.903**
total bacteria	0.635	0.646	**0.681**
Fungi:bacteria ratio	0.422	**0.753**	0.384
Gram + bacterias	**0.638**	0.602	**0.643**
Gram - bacterias	0.674	0.634	**0.722**
Greater R^2 values	1	4	9

3.1 Anthropogenic disturbance and land-cover class effects on soil microbial attributes

Our results indicate that anthropogenic disturbance negatively affected SMB-C (relative importance value, RIV ≈ 51%; Fig. 3.4A) regardless of the vegetation type; positively affected total fungi (RIV ≈ 31%; Fig. 3.5B) and total bacteria (RIV ≈ 15%; Fig. 3.5C), and negatively affected the fungi:bacteria ratio (RIV ≈ 21%; Table 3.3) regardless of the vegetation type. Anthropogenic disturbance positively affected alkaline phosphatase activity (RIV ≈ 78%; Fig. 3.8A), showing an interaction with vegetation type (i.e. the interaction terms of the best model), indicating that this positive effect was concentrated in Gallery Forest (Fig. 3.12A). The relative importance values are not shown for the effect of the predictor variable on the response variable considering each vegetation type because the RIV is the sum of variable weights that are selected in the set of best models.

Table 3.3 The variable predictors considering the best models with ΔAICc < 2 to explain each response variable (enzyme activity); coefficient (Coef) and standard estimate (SE) are shown for each selected variable, relative importance value (RIV) is shown only for variables with no interation with vegetation type and R^2 is shown for the whole model.

Response variables	**Predictors**	***Coef***	***SE***	**RIV**	**R^2**
Arylsulfatase	topographic wetness index	-0.30889	0.13325	100%	0.853
	lakes and rivers	-0.12694	0.08221	32%	
	soil properties	0.21124	0.17113	100%	
	Cerrado *sensu stricto* x topographic wetness index	0.46495	0.27732		
	Veredas x topographic wetness index	0.45573	0.20129		
	Cerrado *sensu stricto* x soil properties	2.04339	3.95543		
	Veredas x soil properties	0.72033	0.25636		
β-glucosidase	slope	-0.3948	0.2532	75%	0.802
	topographic wetness index	-0.5141	0.1822	100%	
	Cerrado *sensu stricto* x slope	0.5973	0.308		
	Veredas x slope	-0.1237	0.336		
	Cerrado *sensu stricto* x topographic wetness index	0.9537	0.3332		
	Veredas x topographic wetness index	0.3087	0.2421		
Alkaline phosphatase	topographic wetness index	-0.40802	0.15444	100%	0.751
	lakes and rivers	-0.15063	0.11902	23%	
	anthropic disturbance	0.55963	0.19847	78%	
	Cerrado *sensu stricto* x anthropic disturbance	-0.48872	0.28048		
	Veredas x anthropic disturbance	-0.88939	0.30215		
Soil Microbial Biomass	anthropic disturbance	-0.1772	0.1053	51%	0.658
	soil properties	0.3412	0.1867	100%	
	Cerrado *sensu stricto* x soil properties	-0.9297	5.2061		
	Veredas x soil properties	1.1527	0.3501		
FDA	Elevation	0.32438	0.18716	75%	0.778
	topographic wetness index	-0.14912	0.1014	100%	

	slope	-0.14912	0.1014		
	Cerrado *sensu stricto* x elevation	-0.51692	0.19738	54%	
	Veredas x elevation	-0.3683	0.1954		
Respiration	elevation	0.68	0.11	100%	0.949
	soil properties	0.11	0.11	100%	
	Cerrado *sensu stricto* x elevation	-0.64	0.15		
	Veredas x elevation	-0.65	0.15		
	Cerrado *sensu stricto* x soil properties	-2.08	3.01		
	Veredas x soil properties	0.89	0.20		
qCO$_2$	slope	0.44884	0.17625	80%	0.420
	soil properties	-0.21163	0.17595	30%	
Urease	elevation	0.17514	0.09655	64%	0.800
	slope	0.16523	0.11626	15%	
	topographic wetness index	-0.26403	0.13936	66%	
	Cerrado *sensu stricto* x topographic wetness index	-0.04767	0.28867		
	Veredas x topographic wetness index	0.46334	0.21018		
total PLFAs	elevation	0.4994	0.1738	64%	0.799
	topographic wetness index	-0.2368	0.1231	27%	
	lakes and rivers	-0.1892	0.1061	60%	
	soil properties	0.3257	0.1773	68%	
	Cerrado *sensu stricto* x elevation	-0.6587	0.2357		
	Veredas x elevation	-0.6512	0.2331		
total fungi	elevation	0.66905	0.13577	100%	0.903
	lakes and rivers	-0.19987	0.08304	100%	
	anthropic disturbance	0.09943	0.07585	31%	
	Cerrado *sensu stricto* x elevation	-0.19987	0.08304		
	Veredas x elevation	-0.19987	0.08304		
total bacteria	topographic wetness index	-0.2128	0.121	39%	0.68
	lakes and rivers	-0.1495	0.1085	19%	
	anthropic disturbance	0.1328	0.1095	15%	
	soil properties	0.4642	0.1695	100%	
fungi:bacteria ratio	lakes and rivers	1.45E-01	1.95E-01	22%	0.38
	anthropic disturbance	-1.28E-01	1.96E-01	21%	
gram positive bacterias	topographic wetness index	-0.234	0.1287	61%	0.64
	soil properties	0.4409	0.1786	100%	

gram negative bacterias	elevation	-0.1087	0.10328	18%	0.72
	lakes and rivers	-0.22611	0.10001	100%	
	soil properties	0.25108	0.14462	64%	

Lakes and rivers showed a negative effect on total microbial PLFAs (RIV ≈ 60%; Fig. 3.5A), on total fungi (RIV ≈ 100%; Fig. 3.5B), on total bacteria (RIV ≈ 19%; Fig. 3.5C), on gram negative bacterias (RIV ≈ 100%; Fig. 3.6B), a positive effect on fungi:bacteria ratio (RIV ≈ 22%; Table 3.3); a negative effect on arylsulfatase (RIV ≈ 32%; Fig. 3.7C) and on alkaline phosphatase (RIV ≈ 23%; Fig. 3.8A) enzymes, regardless of the vegetation type. These results are presented in Table 3.3.

3.2 Soil property and soil microbial attributes

Soil physico-chemical properties showed influence on arylsulfatase (RIV ≈ 100%; Fig. 3.7C), SMB (RIV ≈ 100%; Fig. 3.4A) and respiration (RIV ≈ 100%; Fig. 3.4B) varying according to the vegetation type; showed negative effect on $q\mathrm{CO}_2$ (RIV ≈ 30%; Fig. 3.4C), positive on total PLFA (RIV ≈ 68%; Fig. 3.5A), on total bacteria (RIV ≈ 100%; Fig. 3.5C), on gram positive (RIV ≈ 100%; Fig. 3.6A) and on gram negative bacterias (RIV ≈ 64%; Fig. 3.6B) regardless the vegetation type. The results accounting for the interaction terms of the best models of each vegetation type showed that soil properties affected positively arylsulfatase in Gallery forest and Veredas (Fig. 3.11D), positively SMB in Gallery forest and Veredas (Fig. 3.9A) and positively respiration in Gallery forest and Veredas (Fig. 3.9B). These results are presented in Table 3.3

3.3 Effects of topography on soil microbial attributes

Elevation showed a positive influence on respiration (RIV ≈ 100%; Fig. 3.4B) regardless of the vegetation type (i.e. no interaction between the predictor variable and vegetation type was found). The elevation had a positive relationship with total PLFAs (RIV ≈ 64%; Fig. 3.5A) and total fungi (RIV ≈ 100%; Fig. 3.5B), however, in both cases, this relationship was restricted to the Gallery Forest (Fig. 3.10A and 3.10B, respectively). For both response variables, elevation showed a negative relationship in both Veredas and Cerrado *sensu stricto;* negative influence on gram negative bacteria (RIV ≈ 18%; Fig. 3.6B) regardless the vegetation type; and positive effect on urease activity (RIV ≈ 64%; Fig. 3.7B) regardless of the vegetation type. Slope showed influence on β-glucosidase activity (RIV ≈ 75%; Fig. 3.7A), affecting positively β-glucosidase in Gallery Forest and negatively in Veredas (Fig. 3.11A). Elevation affected FDA enzymes (RIV ≈ 75%; Fig. 3.8B), and positively in Gallery forest and Veredas (Fig. 3.12B). qCO_2 (RIV ≈ 80%; Fig. 3.4C) was affected by slope positively regardless the vegetation type. Topographic wetness was a strong predictor that showed influence on all the enzymes and the marjority of microbial attributes: arylsulfatase (RIV ≈ 100%; Fig. 3.7C), β-glucosidase (RIV ≈ 100%; Fig. 3.7A), alkaline phosphatase (RIV ≈ 100%; Fig. 3.8A), FDA (RIV ≈ 100%; Fig. 3.8B), urease (RIV ≈ 66%; Fig. 3.7B), total PLFA (RIV ≈ 27%; Fig. 3.5A), total bacteria (RIV ≈ 39%; Fig. 3.5C) and gram positive bacteria (RIV ≈ 64%; Fig. 3.6A). The topographic wetness index influenced all the enzymes negatively in Gallery forest: Arylsulfatase (Fig. 3.11E), β-glucosidase (Fig. 3.11B), FDA (Fig. 3.8B) and urease (Fig. 3.11C). Topographic wetness positively affected β-glucosidase activity in Cerrado *sensu stricto* (Fig. 3.11B). These results are presented in Table 3.3.

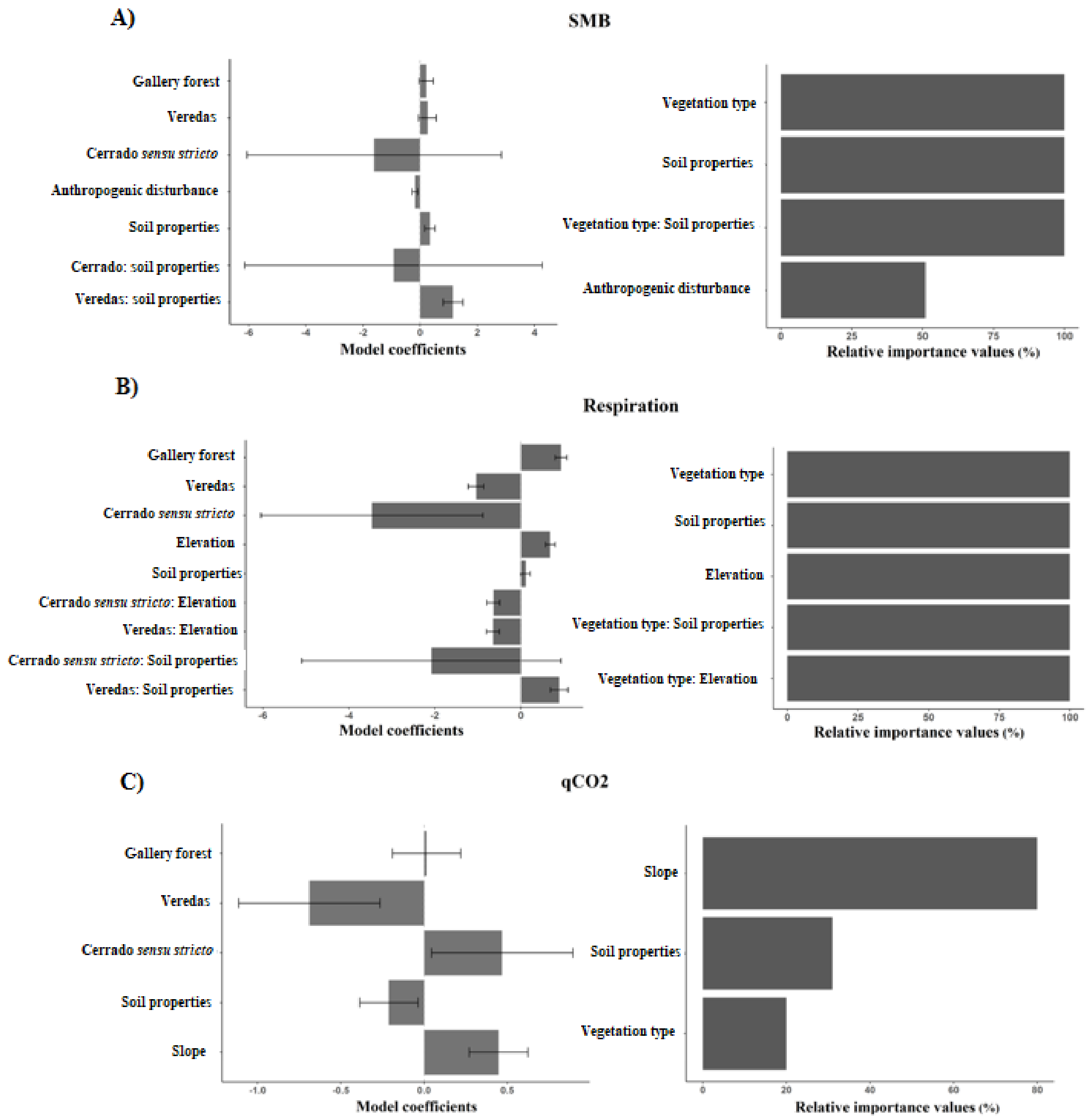

Figure 3.4. Model averaging of candidate models within ΔAICc < 2 for the response variables (A) SMB – Soil Microbial Biomass; (B) Soil Respiration; (C) Metabolic Quocient ($q\mathrm{CO_2}$). All averaged coefficients (±standard errors) are shown on the left side, relative importance values (RIV) are shown on the right side. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

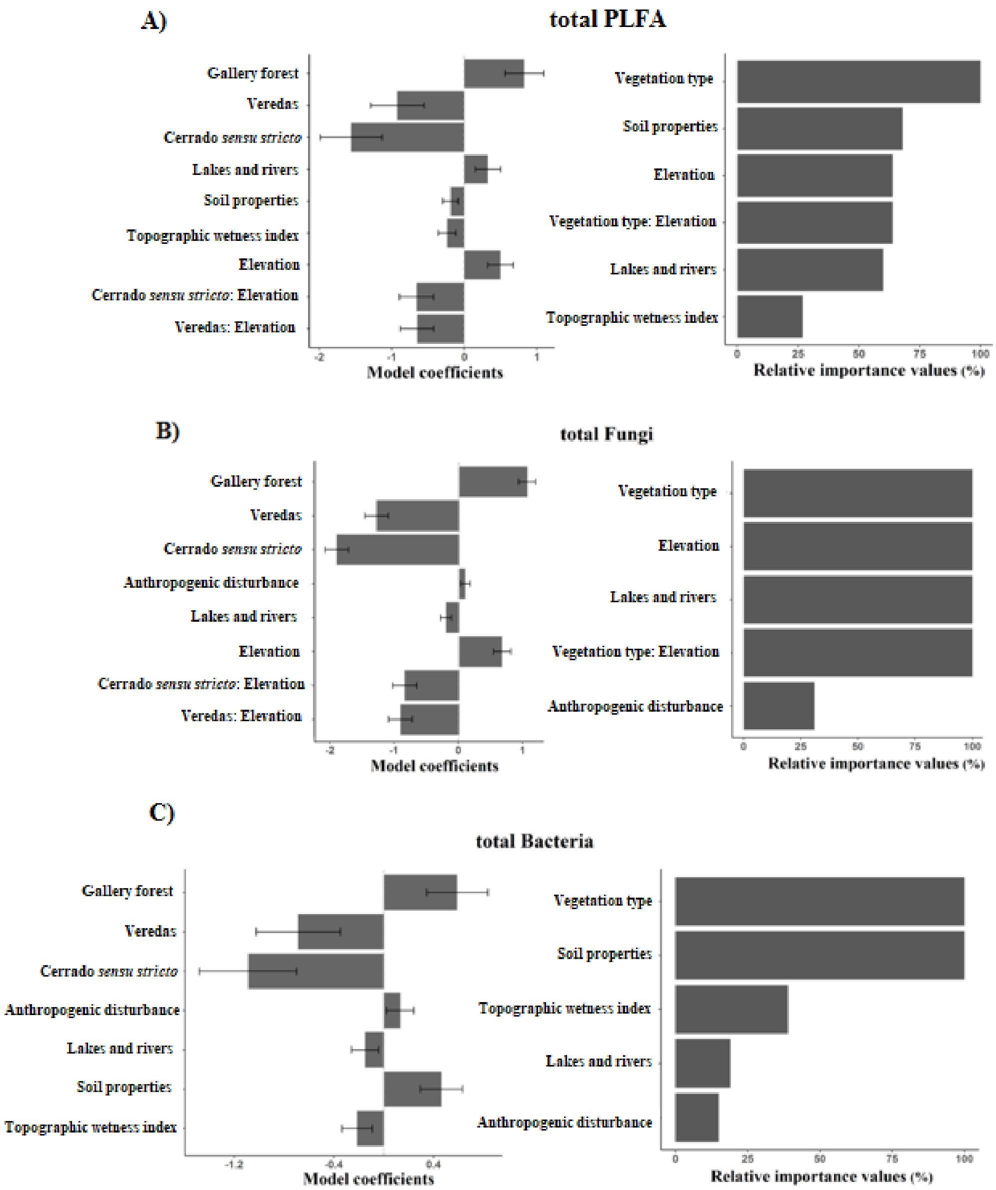

Figure 3.5. Model averaging of candidate models within ΔAICc < 2 for the response variables, (A) Total PLFA; (B) total fungi; (C) total bacteria. All averaged coefficients (±standard errors) are shown on the left side, relative importance values (RIV) are shown on the right side. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

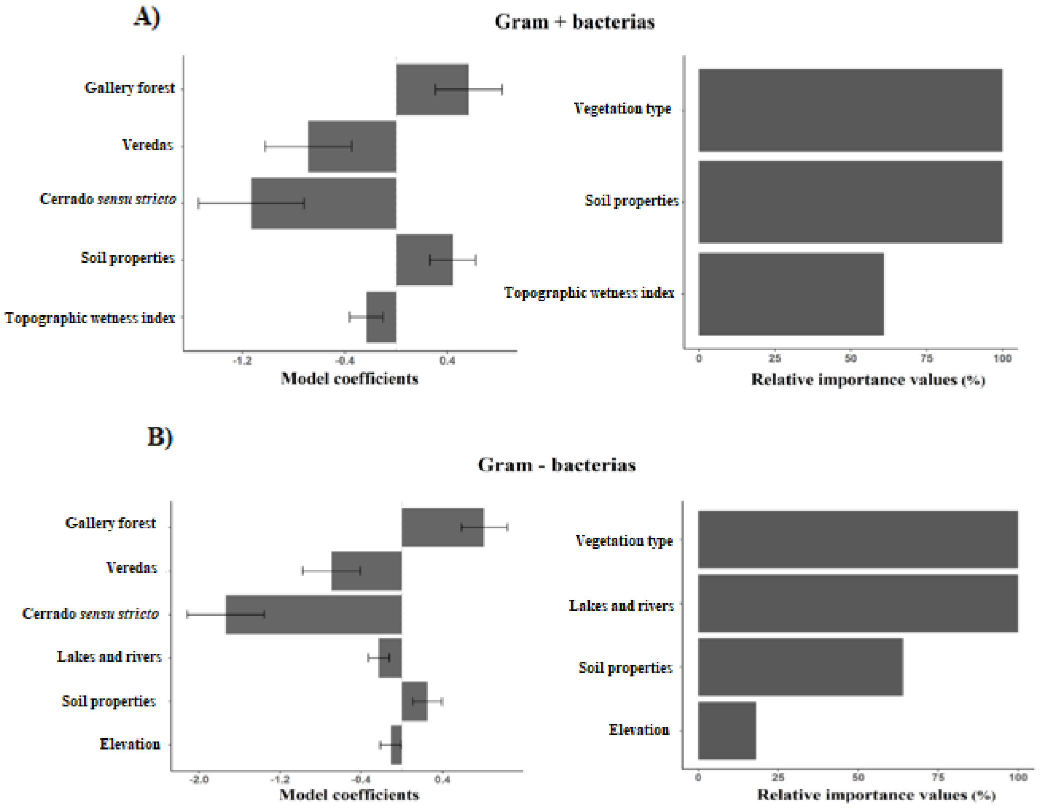

Figure 3.6: Model averaging of candidate models within ΔAICc < 2 for the response variables (A) Gram positive bacterias; (B) Gram negative bacterias. All averaged coefficients (±standard errors) are shown on the left side, relative importance values (RIV) are shown on the right side. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

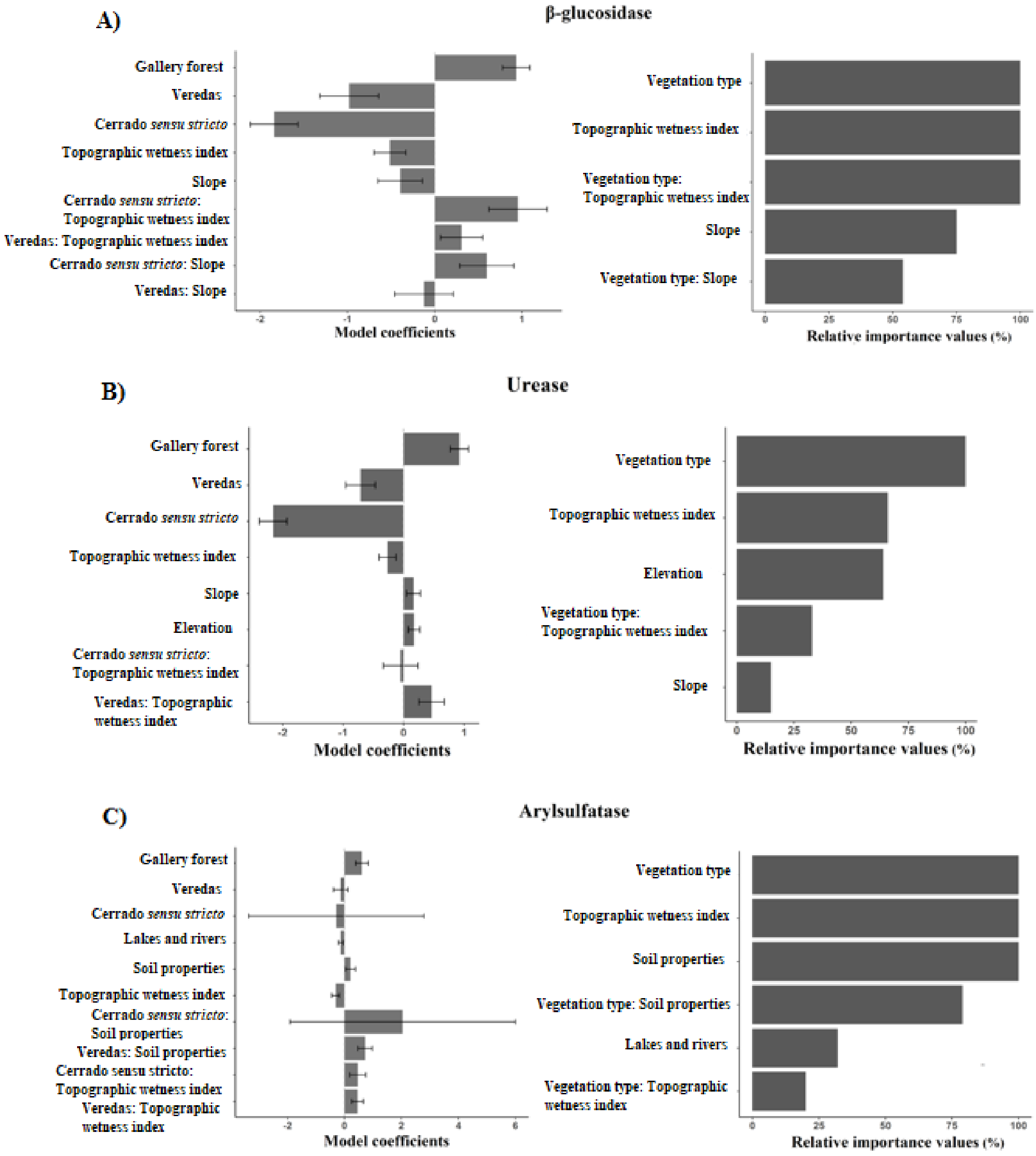

Figure 3.7. Model averaging of candidate models within ΔAICc < 2 for the response variables (A) β-glucosidase; (B) Urease; (C) Arylsulfatase. All averaged coefficients (±standard errors) are shown on the left side, relative importance values (RIV) are shown on the right side. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

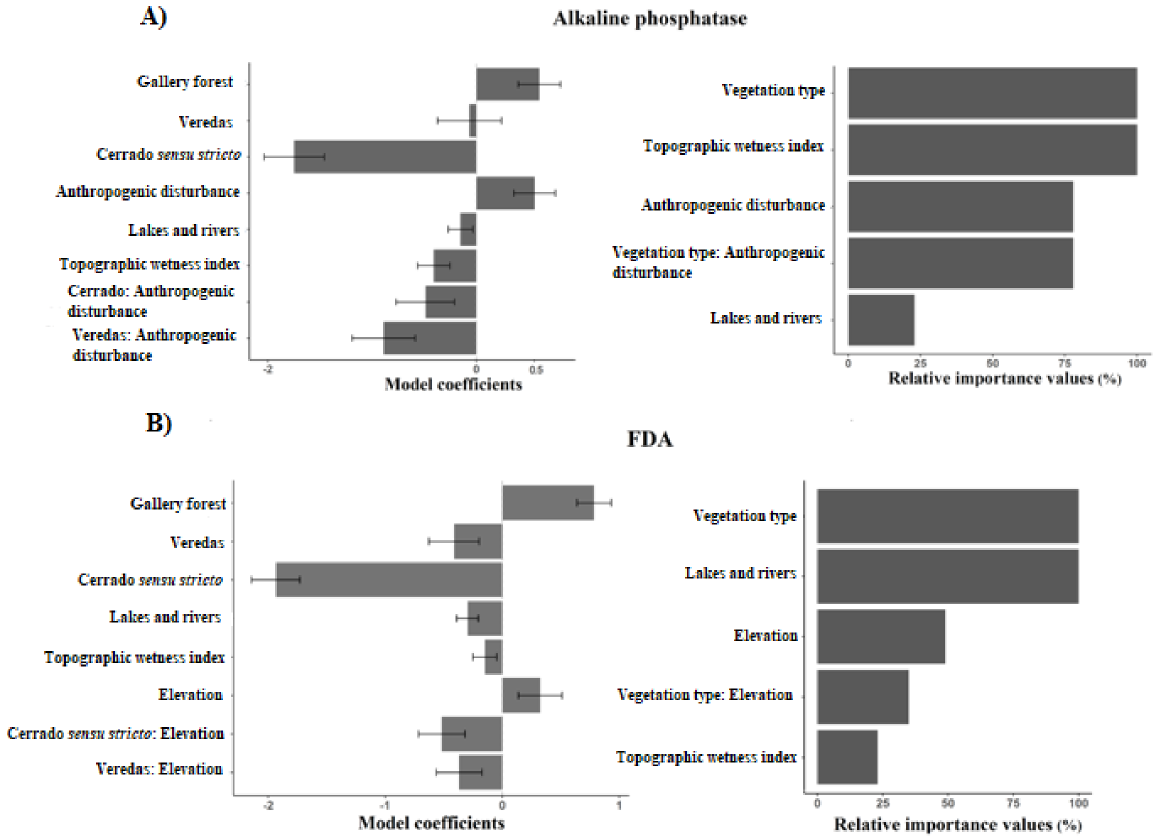

Figure 3.8. Model averaging of candidate models within ΔAICc < 2 for the response variables (A) Alkaline phosphatase; (B) fluorescein diacetate hydrolysis. All averaged coefficients (±standard errors) are shown on the left side, relative importance values (RIV) are shown on the right side. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

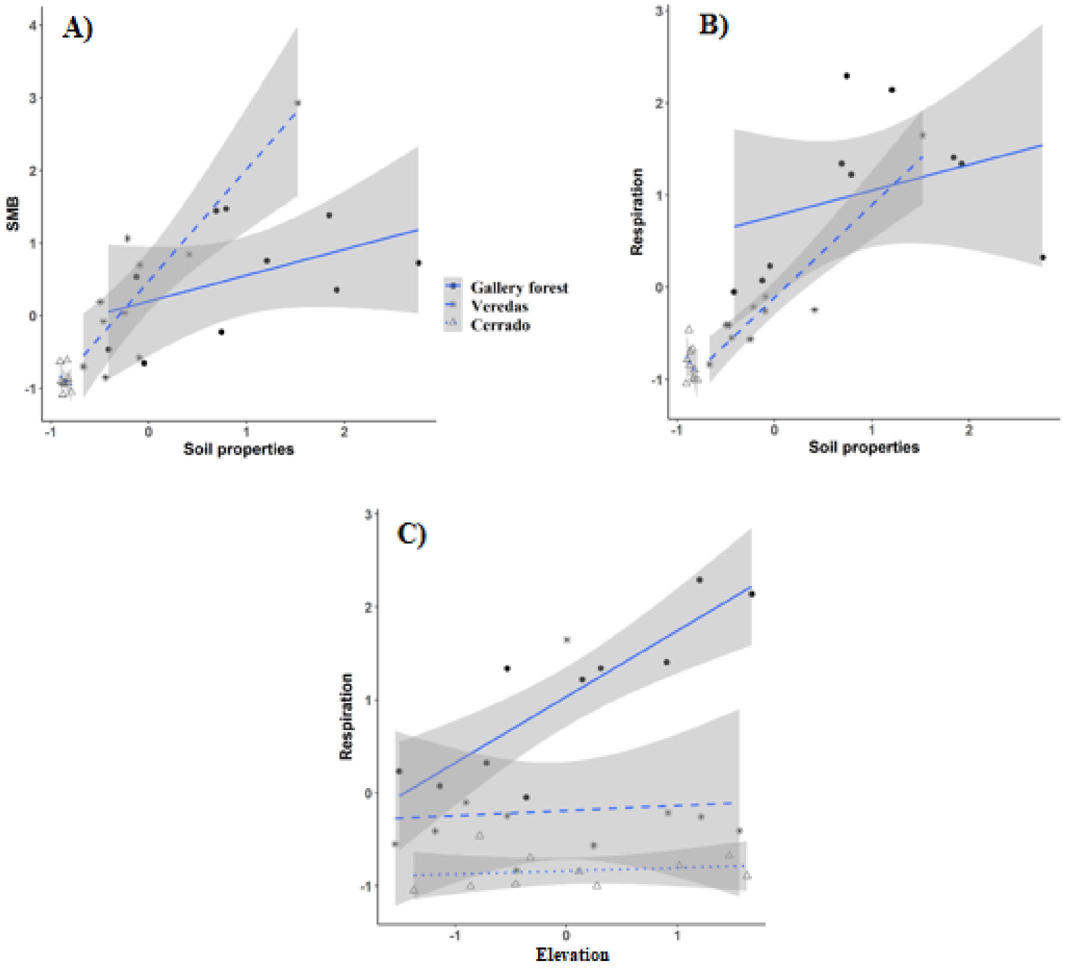

Figure 3.9. Response variables affected by predictors at different ways according to vegetation type. Only variables selected by the best interaction models with vegetation type. (A) Soil microbial biomass (SMB) is positively affected by soil properties in Gallery forests and Veredas. (B) Respiration is positively affected by soil physico-chemical properties in Gallery Forest and Veredas and weakly affected in Cerrado *sensu stricto*. (C) Respiration is positively affected by elevation in Gallery forests. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

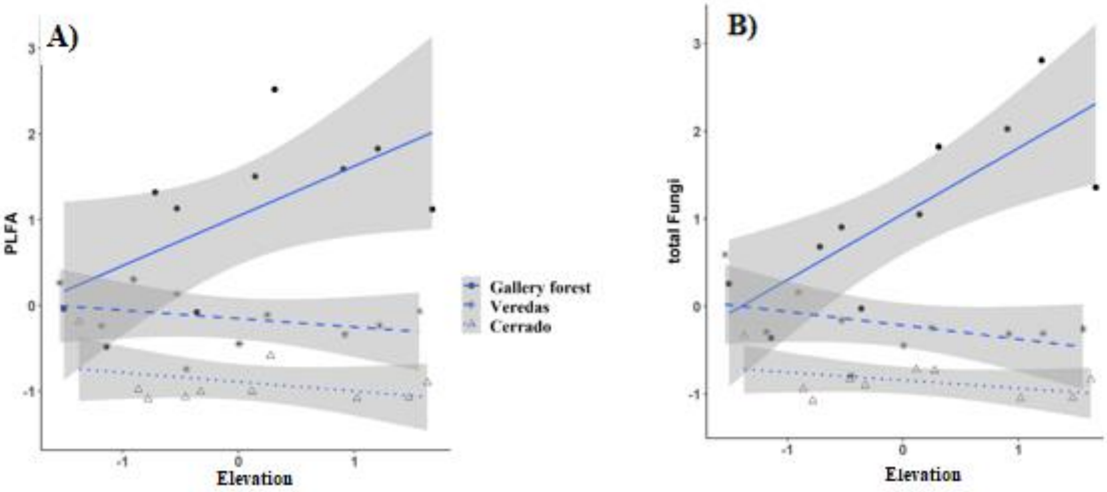

Figure 3.10. Response variables affected by predictors at different ways according to vegetation type. Only variables selected by the best interaction models with vegetation type. (A) total PLFA is positively affected by elevation in Gallery forests and negatively affected in Veredas and Cerrado *sensu stricto*. (B) total fungi is positively affected by elevation in Gallery Forest and negatively in Veredas andin Cerrado *sensu stricto*. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

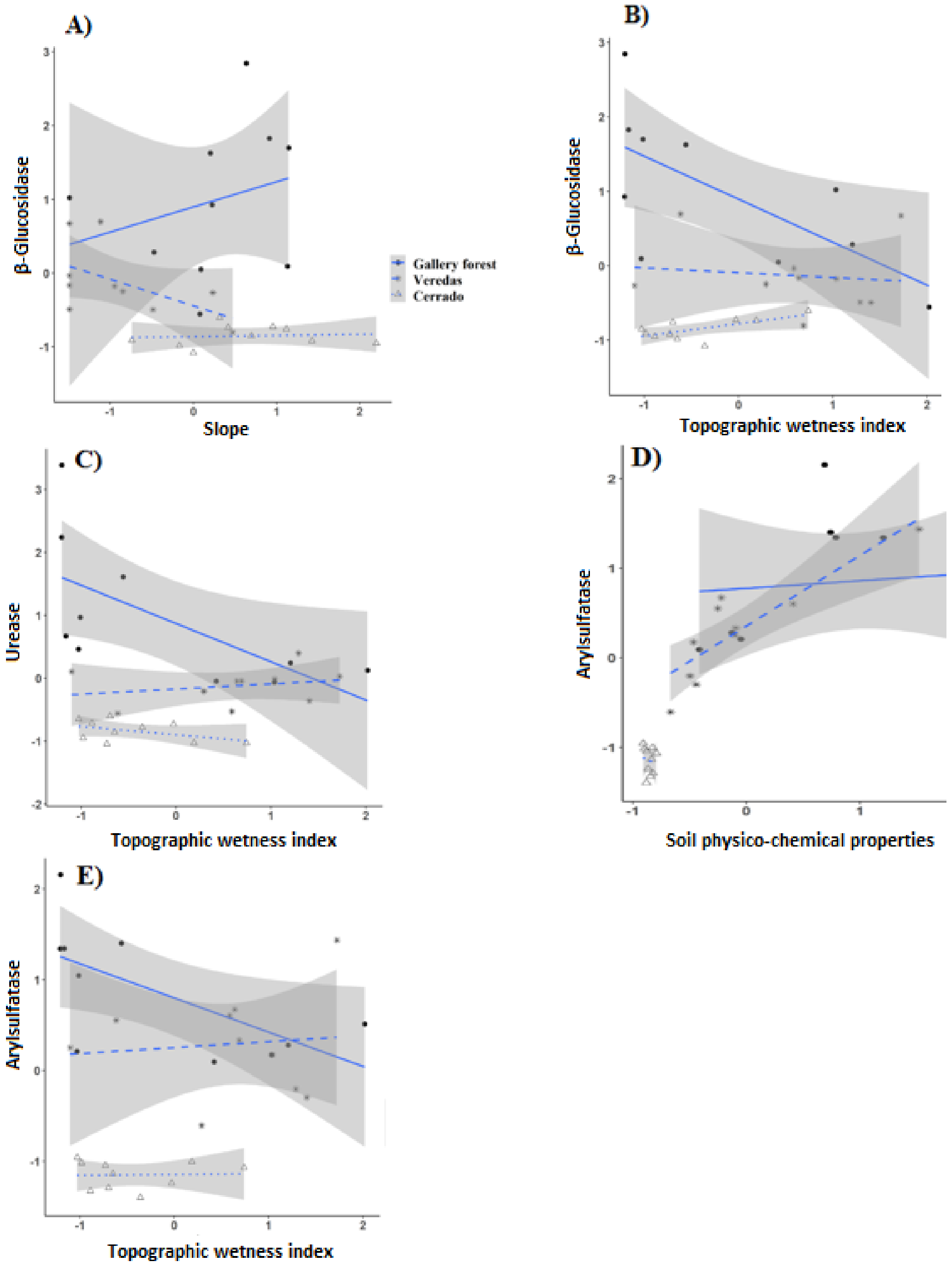

Figure 3.11. Response variables affected by predictors at different ways according to vegetation type. Only variables selected by the best interaction models with vegetation type. (A) B-Glucosidase is positively affected by slope in Gallery forests and negatively in Veredas. (B) B-Glucosidase is negatively affected by topographic wetness index in Gallery Forest, positively in Veredas and in Cerrado *sensu stricto*. (C) Urease is negatively affected by topographic wetness index in Gallery forests and positively in Veredas. (D) Arylsulfatase is positively affected by soil properties in Gallery Forests and in Veredas and negatively in Cerrado *sensu stricto*. (E)

Arylsulfatase is negatively affected by topographic wetness index in Gallery forest and positively in Veredas and in Cerrado *sensu stricto*. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

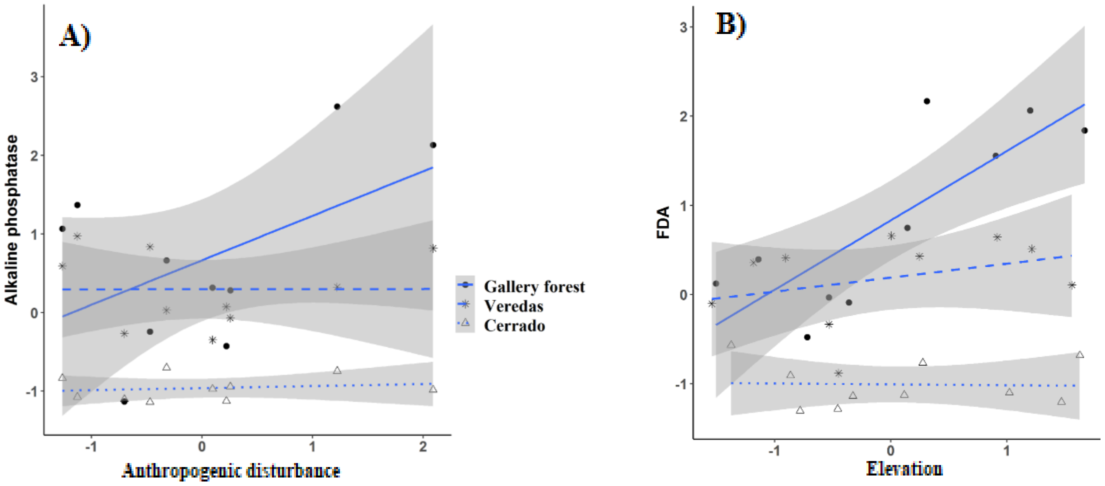

Figure 3.12. Response variables affected by predictors at different ways according to vegetation type. Only variables selected by the best interaction models with vegetation type. (A) Alkaline phosphatase is positively affected by antropic disturbance in Gallery Forest. (B) FDA is positively affected by elevation in Gallery forests and Veredas. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.

4 DISCUSSION

Our objectives were to assess the effects of anthropogenic disturbance, topography and soil properties on soil microbial attributes within and between distinct vegetation types in Brazilian neotropical savanna, and to identify the best predictors of microbial attributes in order to provide novel insights into environmental and landscape drivers in this biome. We evaluated the land-use classification in 500 m, 1 km and 2 km radius buffers and selected the 2km radius buffer size based on the best model adjustment (R^2) of 'anthropogenic disturbance' variable in predicting microbial attributes. Therefore, all the results and discussion about anthropogenic disturbance and lakes/river area are presented here considering at the 2km buffer size.

4.1 Anthropogenic disturbance influencing soil microbial attributes

We hyphothesized that soil microbial attributes would be negatively affected by anthropogenic disturbance in all vegetation types. We found Anthropogenic disturbance to negatively affect soil microbial biomass (SMB) regardless of vegetation type. The conversion of natural ecosystems into other land-uses has been found in previous studies to alter SMB. Some studies have shown that deforestation is expected to cause soil properties and SMB alterations (Singh et al., 2010; Vimal et al., 2017). This can occur as plant community itself has been found to be an important factor driving SMB (Thakur et al., 2015), and consequently, any disturbance in plant communities might affect SMB (Singh and Gupta, 2018). In the context of our study area, Kaschuk et al. (2011) found that Cerrado biome is the most sensitive of all Brazilian biomes when evaluating the effect of disturbance on SMB, which implies that soils in this biome may be less resistant to disturbance than other tropical biomes. Our results reinforce that this is true for the different ecosystems within the Cerrado biome.

Anthropogenic disturbance positively affected fungal and bacterial abundances regardless of the vegetation type, and related positively to alkaline phosphatase in Gallery forest. The influences of anthropogenic disturbance on fungi and bacteria was not as strong as topographic aspects (discussed below), but the positive influence on these microbial attributes may be due to the inclusion of "pasture" as a land cover classification, it had the greatest contribution of land cover to "Anthropogenic disturbance" (Fig 3.2). The impact of Cerrado pasture management (i.e. fertilizer use, pH regulators) for cattle are likely to be positively affecting some of these soil microbial attributes. Alkaline phosphatase was positively affected by anthropogenic disturbance in Gallery Forest. This is possibly connected to the use of orthophosphate fertilizer in pasture and agricultural areas (Fanin et al., 2015; Fraser et al., 2015). Nutrients from these managed soils are likely be leached into the rivers, and then absorbed by the Gallery forest soils affecting alkaline phosphatase activity in this vegetation type more strongly than in the others. Topographic wetness index and the presence of lakes and rivers negatively affected the activity of this enzyme regardless of vegetation type, suggesting the moisture sensitivity of this hydrolytic enzyme in the natural environment.

4.2 Soil physico-chemical properties influencing microbial attributes

We hypothesized that soil physico-chemical properties would affect soil microbial attributes in all vegetation types. When evaluating soil properties (PCA axis 1 representing mainly nitrogen, carbon, H+Al and boron) it is shown to positively affect SMB in Gallery forests and in Veredas, and negatively in Cerrado *sensu stricto* (i.e. the interaction terms of the best model). This relationship between soil properties and SMB is consistent with some studies that found SMB to respond to soil chemical and physical properties and nutrient availability status. The negative relationship between soil physico-chemical properties and SMB in Cerrado *sensu stricto* could be due to the poor fertility and low soil microorganism abundances in this vegetation type.

Soil respiration was found to be positively related to soil physico-chemical properties in Gallery Forest and Veredas, and negatively in Cerrado *sensu stricto*. Soil respiration constitutes the respiration contributions of soil microorganisms and plant roots (Hanson et al., 2000). Considering that our measures were made in the laboratory and not *in situ*, we expect most of our soil respiration to be a result of the microbial respiration. As soil microbial biomass is strongly related with soil properties in this study, as the main predictor of soil respiration, soil respiration is also shown to be regulated by soil physico-chemical properties. The strength and direction of this relationship varies according to the vegetation types: positive effects in Gallery forests and Veredas, and a weak relationship in Cerrado *sensu stricto*. This could be a reflection of lower nutrient availability in Cerrado *sensu stricto* in comparison to Gallery forests and Veredas (Haridasan 1998).

The metabolic quotient (qCO_2) was affected negatively by soil properties regardless of the vegetation type. The qCO_2 represents the quantity of CO_2 released by soil relative to the soil microbial biomass over a certain period of time. An increase in respiration and not in biomass increases qCO_2, which suggests microbial metabolic stress. It is therefore used to explain lower efficiency in C metabolism (Kaschuk et al., 2011). The soil physico-chemical properties index presented here (axis 1 of Principal Component Analysis) reflects mainly C and N and nutrient inputs, meaning an increase in nutrients may increase metabolic efficiency of soil microorganisms.

Our results showed that total PLFA, total bacteria, gram+ and gram - bacteria are positively related to soil physico-chemical properties in all vegetation types. Some studies have already shown patterns of PLFAs and FAMEs to respond strongly to edaphic properties (Bath and Anderson, 2003; Girvan et al., 2003; Lauber et al., 2008) as nutrient requirements of the microbial community is an important factor shaping its structure. For example: the C:N ratio, or organic matter input quality shape microbial community, as bacteria are usually more correlated with N and more labile organic C, texture (Girvan et al, 2003) and pH (Lauber et al., 2008). While fungi are responsible for breaking down more recalcitrante plant detritus (Högberg et al., 2007; Lauber et al., 2008). Our findings reinforce the pattern that soil physico-chemical properties are crucial in shaping soil microbial communities in Brazilian Cerrado biome and its vegetation types.

Arylsulfatase in Gallery forest and in Veredas had a positive relationship with soil properties, but weak relationship in Cerrado *sensu stricto*. This enzyme has been reported to have a correlation with soil organic carbon as well as with other hydrolases (Balota et al., 2014) and to be conditioned by the influences of vegetation type on soil nutrient availability (Štursová and Baldrian, 2011).

4.3 Topography influencing soil microbial attributes

We hypothesized that topography (i.e. elevation, slope and wetness index) would affect soil microbial attributes in distinct ways in the different vegetation types. We found that topography was the strongest predictor in this study, as it was found to influence all the microbial attributes evaluated here, except SMB.

Changes in elevation have been found to affect soil respiration, which suggest that elevation is altering soil water content (Rodeghiero and Cescatti, 2005; Wang et al., 2011). Similarly to the respiration response, our results showed that total PLFA is positively related with elevation in Gallery Forests, and negatively in Veredas and Cerrado *sensu stricto*. Total PLFA was also negatively related to the relative cover of lakes and rivers and topographic wetness index in all vegetation types. Some studies found that the range of the quality and quantity of aboveground and belowground litter and soil organic matter along altitudinal gradients is mainly responsible for shaping soil microbial community structure (Alburquerque et al., 2011; Hamman et al., 2007; Huang et al., 2014; Xiang et al., 2016). Moreover, elevation also alters soil temperature and water content (Fierer and Jackson, 2006; Meier et al., 2010) and therefore, the soil microbial community (Xue et al., 2009). The Cerrado biome, especially in the study area, comprises a comparatively flat topography, where changes in elevation are generally visually imperceptible and not enough to change vegetation. However, our results suggest that the effect of elevation is associated to soil water content. Further evidence that water is a driver of PLFA concentrations is the negative relationship with lake and river cover (area within a 2 km ray buffer) and topographic wetness. Finally, we also have to consider that PLFA markers are mostly good indicators of aerobic conditions (this can also explain the negative response to soil moisture) (Bossio and Scow, 1998). Our results also showed elevation affected total PLFAs between the vegetation types in distinct ways: total PLFA in Cerrado *sensu stricto* and Veredas were affected negatively by elevation, but positively within Gallery forest. These findings suggest that for the Cerrado *sensu stricto* and Veredas, elevation may reduce soil water content in soil pores in such a way that microbial community survival decreases, while in Gallery forest, the reduction of water saturation in the soil with elevation may favour microbial abundance.

Total fungi was found to be negatively affected by elevation in Cerrado *sensu stricto* and Veredas and positively in Gallery forest, negatively by relative cover of lakes and rivers regardless of vegetation type. Studies have found that for certain types of fungi, e.g. mycorrhizal, growth is reduced in soil with higher water content (Theodorou, 1978) and may even die under waterlogged conditions (Coutts and Nicoll, 1990), showing general reductions in both diversity and growth under extreme moisture conditions (Barnes et al., 2018). Soil water content influences fungal communities through impacts on oxygen concentrations and nutrient availability (Drenovsky et al., 2004), as flooding reduces the oxigen levels, while the decrease in soil moisture caused by increases with elevation, can lead to environmental desiccation that boosts fungi spore production (Sylvia et al., 2005). Additionally, fungal communities generally demonstrate better adaptation to drying than bacteria, as a drier environment stimulates growth in hyphal networks which facilitates access to water and nutrients (Kaisermann et al., 2015). This may explain differences in the effects within vegetation types, where in the most flooded vegetation type (Gallery forest) total fungi increase with elevation and in Cerrado *sensu stricto* and Veredas, drier ecosystems compared to Gallery forest, the total fungi showed a weaker relationship with elevation. Kaisermann et al. (2015) found that fungal communities depend on non-extreme moisture conditions for better development, and this suggests that the elevation might play an important role in these three vegetation types with different soil moisture regimes and natural extremes.

Total bacterial responded in a similar way to total PLFAs and fungi, nevertheless, it responded more strongly to soil physico-chemical properties than topographic aspects. Soil properties link strongly with bacteria because bacteria act directly in nutrient immobilization and release (Wardle and Nicholson, 1996). Total gram positive bacteria were affected positively by soil properties and negatively by topographic wetness index, with the effect of soil properties being more important (RIV = 100%) followed by topographic wetness index (RIV = 64%), while gram negative bacterias were affected more strongly and negatively by lakes and rivers (RIV = 100%), followed by soil properties (RIV = 64%) and elevation (RIV = 18%). The differences of these groups in responding to predictors might be explained by the differences in the cellular membrane permeability of these different functional groups and its capability in absorbing nutrients in soil and being affected by the water content. Although topographic wetness affected both groups, it showed greater importance for gram negative bacteria, possibly due to their thinner celular membrane that possess reduced capability in dealing with water stress caused by rewetting events (Schimmel et al., 2007), commonly found in regions with greater topographic wetness index in the present study.

The metabolic quotient (qCO_2) was affected positively by slope regardless of the vegetation type. The qCO_2 represents the quantity of CO_2 released by soil relative to the soil microbial biomass over a certain period of time. The increases in qCO_2 in steeper sloping areas may be related to adverse environmental conditions such as leaching of nutrients through increased soil water movement, consequently reducing the efficiency in the use of the soil C by the microorganisms. Also, sloped areas retain less water, and this could be causing greater stress in well-drained soils.

The effect of the topographic wetness index and slope on β-glucosidase enzyme activity was clearly dependent of the vegetation type. β-glucosidase is an important enzyme that acts in the last stage of breaking down cellulose by hydrolyzing cellobiose, releasing glucose as a final product and labile compounds ready to be used by microorganisms (Adetunji et al., 2017; Gil-Sotres et al., 2005; Merino et al., 2016). Therefore, changes in this enzyme might affect the activity of the other enzymes as a result of effects on glucose availability (Sardans and Peñuelas, 2005). Soil water content has been found to be important for increasing β-glucosidase activity, as it can increase the movement of the enzyme and corresponding substrate between the pores, enhancing dissolution and translocation (Dilly and Munch, 1996). On the other hand, β-glucosidase activity can decrease if the soil water content is too high, altering the enzyme kinectics (Zhang et al., 2011). These findings suggest that this enzyme activity is possibly limited in Cerrado *sensu stricto* and Veredas in the dry season when water is limited, whereas, in the Gallery Forest soil becomes saturated in the wet season. Flatter areas in Veredas and in Cerrado *sensu stricto* with higher topographic wetness index and lower slope, possibly increase β-glucosidase activity for increasing water soil content during the dry season. On the other hand, because Gallery forest areas receives more flooding influences from the river, an increase in the wetness can reduce this enzyme activity. Similarly to β-glucosidase activity, urease, arylsulfatase and Fluorescin diacetate (FDA) were strongly affected by topographic aspects in distinct ways in the different vegetation types. This result suggests that the enzymes studied here may have an optimum soil moisture content for operation (Borowik and Wyszkowska, 2016).

5 CONCLUSION

Our assessment of soil microbial responses to landscape scale anthropic disturbance, topography and to soil properties has provided novel insights about patterns and drivers of soil microbial attributes in the Cerrado biome and its vegetation types.

The results show that anthropogenic disturbance negatively affected soil microbial biomass carbon, fungi:bacteria ratio and that some soil physico-chemical properties and topographical variables were important predictors of soil respiration, metabolic quotient, total PLFAs, total fungi, total bacteria and total gram positive and negative bacteria. The intensity and direction of these drivers varied according to the vegetation type. Soil enzyme activities were mostly related to topography, specifically topographic wetness index, and elevation and slope. The intensity and direction of these relationships varied according to vegetation type, which suggests that enzyme activities were more sensitive to soil moisture content than to the surrounding land cover and other soil properties. In addition to the importance of topography for all enzymes, arylsulfatase activity was also found to be related with soil chemical properties, suggesting that the quality of organic matter input may be more important for this enzyme. Alkaline phosphatase was shown to have a positive relationship with anthropic disturbance in Gallery Forests, and we believe that the managed pasture and agriculture in the study area is affecting this vegetation type through absorption of nutrients leached into Gallery Forest soils.

Although the anthropogenic disturbance assessed here was shown to negatively affect soil microbial biomass carbon, it positively influenced other structural attributes of the soil microbial community, evaluated through fungi and bacteria abundances, and Alkaline phosphatase activity due to the possible impact of agriculture and managed pasture. We also highlight that the Pandeiros river basin, is located in a region classified as “Environmentally Protected Area of Sustainable Use” by the Brazilian government, in which only sustainable activities are allowed. In this study we did not find a strong negative effect of anthropogenic disturbance on soil microbial properties which is likely due to low intensity farming and the integration of farming into the natural landscape.

Overall we conclude that topography factors were the strongest predictors of soil microbial attributes at a landscape scale, and it reflects the importance of soil moisture content in underlying ecosystem functioning in the different vegetation types of Cerrado biome.

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APPENDIX CHAPTER 3 (A3)

Appendix Table 3.1. Total variance and component matrix of axis 1 and axis 2 of the Principal Componet Analysis of the soil chemical and physical properties.

Total Variance explained		
Axis 1 % variance	55.21%	
Axis 2 % variance	30.18%	
Component Matrix		
Soil properties	**Axis 1**	**Axis 2**
N	.984	-.003
C	.979	-.097
pH	-.240	.941
Na	.549	.545
H+Al	.860	-.464
Sum of Bases	.387	.831
B	.830	.123

MANUSCRIPT 3

DROUGHT RESILIENCE OF CERRADO SOIL CO_2 EMISSIONS

(Preliminary version prepared for submission to Science of the Total Environment)

ABSTRACT

Climate change is altering global biogeochemical cycles including the microbially mediated release of CO_2 from soils. Savanna ecosystems occupy 20% of the Earth's land surface and are globally important for biodiversity, agriculture, hydrology and biogeochemical carbon cycling. Brazilian Cerrado savanna ecosystems cover >2 million km^2 of Brazil where the length and intensity of the dry season is predicted to increase. There is uncertainty regarding the effect of drought and rewetting cycles on the release of CO_2 from poorly researched Cerrado soils. This study examined, 1) the influence of local topography/human disturbance and soil microbial properties on baseline CO_2 emissions, 2) the effects of drought-rewetting on soil CO_2 emissions from three Cerrado vegetation types: Gallery forest, Vereda wetlands and Cerrado *sensu stricto*. Soils were sampled from seven sites of each vegetation type within the Pandeiros river basin, Minas Gerais, Brazil for inclusion in a controlled microcosmo drought-rewetting experiment. Soil respiration (CO_2) was measured from control, drought and drought-rewetting treatments on 3 occassions over 26 days. Microbial biomass carbon, microbial biomarkers and enzyme activities were also determined for each initial soil type. We found that in Gallery forests, soil respiration was significantly related to soil carbon, enzyme activity, elevation, slope and topographic wetness index. In Veredas soil respiration was linked to total bacteria, total fungi and elevation. Whereas soil respiration in Cerrado *sensu stricto* was influenced by total fungi, enzyme activity, bacteria abundance and slope. We found that the drought- rewetting affected absolute amounts and response ratios of soil respiration in Gallery forest and Veredas wetlands vegetation types. We concluded that Veredas soils are the most resistant to drought as soil respiration remained unchanged for longer than that of Gallery forests. The rapid recovery of respiration in Gallery forests soils after rewetting suggests that they are the most resilient to drought. Neither drought nor rewetting significantly affected Cerrado grassland soil respiration, which may reflect the poor organic matter content in these soils. We discuss the potential implications for future climate change scenarios in the Cerrado biome.

Key words: Veredas, Gallery Forests, soil organic matter, soil respiration, resistance, resilience.

1 INTRODUCTION

Globally savannas occupy 20% of the Earth’s land surface and are located in Africa (65%), South America (45%) and Asia (10%) (Archibold. 1995; Scholes and Archer, 1997; Werner, 1991). Savannas contain a range of ecosystems (e.g. grasslands, riparian forests, swamps, savannic woodland) and are important for biodiversity, agriculture, hydrology and biogeochemical carbon cycling (Silva and Bates, 2002; Scholes and Archer, 1997; Malhi et al., 2016). The Cerrado biome is the largest savanna in South America and the second most extensive biome in Brazil, covering approximately 2 million km2 (24% of the country's total area) (Hughes, 2017, Lapola et al., 2013). It is the richest and the most diverse savanna in the world (Lewinsohn and Prado, 2005), and its high level of endemism classifies this biome as a global hotspot of biodiversity (Mittermeier et al., 2005). The Brazilian Cerrado also comprises the major river basins of South America: Tocantins-Araguaia, Paraná-Prata and São Francisco (Felfili and Silva Junior, 2005). Despite its importance in providing ecosystem services, the Cerrado is one of the most threatened biomes in the world (Sano et al., 2010), as the expansion of mechanized agriculture has replaced natural vegetation cover with monoculture crops (Hunke et al, 2015), resulting in aa 50% loss of the total area since 2010.

The Cerrado biome is composed of a wide-range of savana-like vegetation ranging from grasslands to woodlands (Bustamante et al, 2012) as: Cerrado campo limpo (grassland), Cerrado campo sujo (grassland with scattered shrubs and trees), Cerrado *sensu stricto* (woodland with sparsed scrubs and trees - typical cerrado), and Cerradão (woodland) (Ribeiro and Walter, 1998). Besides the savanna vegetation, the flooding from the rivers and the overflow of the water table in Cerrado also promote a mosaic of ever green forests (Gallery Forests) and grasslands swamps (Veredas) which possess different vegetation composition, soil conditions and higher organic matter accumulation than the savanna-like vegetations (Oliveira-Filho 1989) cited above. Due to the dense tree stems in Gallery Forests, it is reported that this vegetation type possess higher organic matter content in the vegetation biomass and belowground than other Cerrado savanic ecosystems (Delliti and Burger, 2000).

The natural vegetation and soil of the Cerrado biome has been shown to function as a significant carbon sink, with stocks estimated to be 265 Mg ha^{-1} (to 1 m soil depth) with soil organic matter (SOM) accounting for approximately 70% (Abdala, 1993). Therefore, deforestation is expected to significantly alter the biogeochemical functioning of Cerrado, compromising carbon storage and intensifying emissions to the atmosphere (Brasil, 2010). At the same time climate change is expected to lead to changes in precipitation regimes, with more intense and longer drought seasons in the Cerrado biome (Bustamante et al, 2012). Droughts are known to reduce soil respiration, by decreasing substrate accessibility by microorganisms, and causing dormancy or death of soil microbes (Pulleman and Tietema, 1999). In contrast, the rewetting of soils after drought can reactivate microbial activity, stimulating the rapid breakdown of SOM accumulated during dry conditons, and lead to large pulses of CO_2 emission known as the “Birch effect” (Birch, 1958; Kim et al., 2012).

The capacity of the soil microbial community to tolerate and adapt to changes in soil moisture conditions is a key determinant of soil respiration (Nijs et al., 2019). Research has shown that the ability of the soil microbial community and its functions to resist disturbance such as drought and show resilience with rapid recovery will have a strong influence on resultant ecosystem processes (de Vries and Shade, 2013; Pimm, 1984). There is also evidence, in grassland ecosystems, that soil microbial communities may become adapted to drought and develop increased resistance dry conditions (Griffiths et al., 2000), while rewetting may select for more resilient microbial communities with faster recovery times (Evans and Wallenstein, 2012).

Recent studies in the Cerrado biome have made initial evaluations of soil CO_2 emissions under field conditions (Buttler et al, 2012; Arruda et al, 2016). However, there are no experimental studies controlling the drought-rewetting on soil CO_2 emissions and microbial resistance/resilience across a wider range of vegetation types typical of the Cerrado biome, particularly in the wetlands (Gallery forest and Veredas) that pass throght periodic annually flooding regimes. The overarching aim of our study was to improve understanding of the factors regulating the resistance and resilience of soil microbial CO_2 emissions to drought in three important Cerrado vegetation types (i.e. Cerrado *sensu stricto*, Gallery Forests and Veredas). Specifically we wanted to address the following objectives to determine 1) the influence of local topography and soil microbial properties on baseline CO_2 emissions, and 2) the effects of drought-rewetting on soil CO_2 emissions from three Cerrado vegetation types: Gallery forest, Vereda wetland and Cerrado *sensu stricto*. We hypothesised that, 1) soil microbial properties and topography are significant predictors of baseline CO_2 emissions, and 2) there are significant diferences in resistance and resilience of microbial CO_2 emissions to drought across different Cerrado ecosystems, 3) resistance and resilience of soil respiration to drought is strongly influenced by organic matter content. This was achieved by sampling intact soil cores across Cerrado ecosystems in the Pandeiros river basin, Minas Gerais, Brasil that were then studied in a controlled drought resistance/resilience experiment.

2 METHODS

2.1 Study Area

Soil core sampling was conducted in the Cerrado biome, in the northern region of Minas Gerais state, along the Rio Pandeiros basin, Brazil. The basin is located between the south latitude meridians 14º00 to 16º30 and the longitude meridians 43º00 to 46º00. The vegetation types studied were: Cerrado *sensu stricto* (savanna woodland), Gallery Forests (forests bordering the rivers); and Veredas (swamp/marshy fields that flood periodically). We selected seven sites along the tributaries of the Pandeiros river (selected from the 10 sites described in Chapters 2 and 3) that included all three vegetation types.

2.2 Topography indexes and classification of anthropogenic disturbance

We used a digital elevation model (DEM) Alos PALSAR with 12.5 m of spatial resolution, downloaded from www.asf.alaska.edu, to create slope and topographic wetness index through the software SAGA GIS (Conrad et al., 2015). Then, the values of elevation, slope and topographic wetness index, which shows the areas more likely to accumulate water due to topographic features (Beven and Kirkby, 1979), were extracted from each sampling location and used for statistical analyses.

The anthropogenic disturbance was determined using land cover classification based on RapidEye images with 5 meter resolution, freely available through the Brazilian Ministry of the Environment in the Geocatálogo platform (www.geocatalogo.mma.gov.br). For each sampling site, we established a buffer of 2 km radius in which land-cover was classified to calculate the relative cover of roads, pasture, exposed soil, human settlements and agriculture. These areas were then summed to generate the overall “Anthropogenic Disturbance” variable. We used an object-based image analysis approach (Blaschke, 2010) with segmentation and manual classification to improve classification accuracy. Classification was carried out using eCognition Developer® software (Trimble, 2012).

2.3 Soil microbial attributes

We measured the following microbial attributes: microbial biomass, soil extracellular enzymes (FDA) and microbial community stricture through fungi and bacteria abundances (PLFA). See chapter 2 for detailed description of soil sampling and soil microbial attributes.

2.4 Collection of soil cores and experimental design

At each of the seven sampling locations we collected three soil cores 0-10 cm depth and 7.5 cm in diameter in each of the three vegetation types, totalizing 9 cores per site. The cores were sent to Centre for Ecology and Hydrology (CEH) in Lancaster, UK. There, we performed a controlled experiment in an incubator room with the temperature maintained at 27 ºC, the average temperature of Cerrado biome. Each soil core was placed in a chamber of 1,515.00 cm^3. All the soil cores were kept wet at 100% water holding capacity (WHC) for 5 days for the stabilisation of soil respiration before the begining of the experiment. Cores from each vegetation type were randomly allocated to the three treatments in equal groups totalling seven replicates of each vegetation type per treatment and 63 cores. ‘Wet’ soil cores were kept at 100% WHC during the 26 day incubation period), ‘Drought’ soil cores were allowed to dry withought further water additions, and Drought/Rewet soil cores were allowed to dry over 26 days and then rewet to 100% WHC.

2.5 CO2 sampling

All treatments were sampled after 5 days of soil wet stabilization and after 15 and 26 days. For the drought-rewetting (D/RW) treatment the final sampling was made two hours post rewetting on day 26. For the ‘Wet treatment’, control, the soil cores were maintained at 100% WHC during the entire experimental period.

At each sampling point, chambers were closed using a lid with a rubber seal, and soil respiration (Rs) was determined by calculating the rate of CO_2 accumulated in the headspace of the chamber over 30 minutes. Gas was collected using a sterile syringe inserted through a rubber septum (to avoid gas leaking during sampling) at 10 minute intervals: time 0 (t0 – first collection), time 1, (t1 – 10 minutes after the first collection), time 2 (t2 – 20 minutes after the first collection), time 3 (t3 – 30 minutes after the first collection). 9 ml of gas was collected at each interval and injected into evacuated 3.5 ml vials. Cores were also weighed for calculating % WHC on each sampling occasion.

Concentration of CO_2 measurements were measured using a PerkinElmer (PerkinElmer, USA) Autosystem Gas Chromatograph (GC) fitted with two flame ionization detectors (FID) operating at 130 (FID) and 300 C (FID with methaniser) respectively. Results were calibrated against certified gas standards (Air products, UK)

CO_2 fluxes were calculated using linear regressions of CO_2 concentrations (ppm) against time (0, 10, 20, 30 min). CO_2 flux expressed as µgCO_2-C/g/ soil dry weight $^{-1}$/hr-^{1} was calculated according to the formula:

$$\mathrm{\mu gCO2 - C/g/\ soil\ dry\ wt\ - 1/hr - 1} = \frac{60 \times (rate\ of\ change\ in\ ppm) \times (volume\ of\ chamber) \times massC\ \times 1}{\mathrm{Soil\ dry\ weight} \times R\ \ \times (T) \times 1000}$$

where mass$C$ is the molecular mass of Carbon; R is the universal gas constant; T is the temperature expressed in kelvin; volume of chamber accounts for the soil core in m^3.

To evaluate resistance and resilience we based our interpretations on absolute change and response ratio results. Absolute change in CO_2 flux was calculated as the difference in CO_2 flux between a soil core from the drought treatment (or drought/rewet) and a soil core from the control Wet treatment (an equivalent soil core from the same site and vegetation type). Similarly, the response ratio was calculated to express CO_2 flux from a soil core from the dry (or dry/rewet) treatment as a proportion of CO_2 flux from a soil core from the wet control treatment, i.e. dry (or drought-rewet) treatment/wet treatment × 100.

2.6 Statistical analysis

To assess which factors are related to soil CO_2 baseline respiration in each vegetation type, we ran Generalized Linear Mixed Models (GLMM) using CO_2 fluxes of the first sampling point (after stabilization) as response variable. Two separated models were run, one just with microbial attributes and soil total carbon, and other with landscape features. The microbial and carbon explanatory variables were:

microbial biomass carbon (MBC), total phospholipid fatty acids (total PLFA), total fungi, total bacteria, total soil carbon, enzymatic activity of fluorescein diacetate hydrolysis (FDA). Topographical characteristics (altitude, slope and moisture index) and anthropogenic disturbance were also evaluated as explanatory variables in separate models. Before running the GLMMs, we conducted Pearson correlation analyses to identify strong correlations (<0.60) between explanatory variables and select the most parsimonious variables. The baseline CO_2 fluxes from all cores (considering the first day of measurement after the stabilization) were used in GLMM analysis, where site was included as a random effect to account for spatial pseudoreplication. GLMMs were run using the function lmer with Gaussian family within the following packages: lme4 (Bates et al. 2014), lmerTest (Kuznetsova et al. 2016), MuMIn (Barton 2016), and relationships were visualised using ggplot2 (Wickham and Chang 2016). We assessed the Goodness-of-fit of predictor variables of the models through adjusted coefficients of determination (Nakagawa and Cuthill, 2007). We ran all possible combinations of the predictor variables and ranked models from the best to the worst according to the Aikaike's information criterion (AIC), and averaged the set of models with a difference in AIC (ΔAICc) < 2 from the best (lowest AIC) model due to their equal plausibility (Burnham and Anderson, 2002).

We used two-way ANOVA for each treatment nested with sampling points to evaluate if CO_2 respiration rates differed between vegetation along the experiment. To evaluate the effect of drought and drought/rewetting along the experiment in terms of soil respiration absolute changes, response ratios and water holding capacity (WHC), we used one-way ANOVA. These analyses were followed by pairwise Tukey's HSD post-hoc test for each vegetation type and treatment for normal data distributions, and Kruskal-Wallis followed by the pairwise Wilcoxon rank post-hoc test with Bonferroni correction for non-normal data distributions. All statistical analyses were conducted in R version 3.5.2 (R core Team 2018).

3 RESULTS

3.1 Factors influencing soil respiration in the different vegetation types

For Gallery Forests, our results indicate that total carbon (relative importance value, RIV ≈ 29%; Fig. 4.1A), FDA (RIV ≈ 71%; Fig 4.1B) and elevation (RIV ≈ 50%; Fig 4.1C) influenced positively and slope (RIV ≈ 50%; Fig. 4.1D) and topographic wetness index (RIV ≈ 50%; Fig. 4.1E) influenced negatively the soil respiration. For Veredas, total bacteria (RIV≈ 49%; Fig. 4.2A) influenced positively; total fungi (RIV ≈ 28%, Fig. 4.2B) influenced negatively; and altitude (RIV ≈ 100%, Fig. 4.2C) influenced positively the soil respiration. For Cerrado *sensu stricto*, total fungi (RIV≈36%, Fig. 4.3A) influenced positively; FDA (RIV≈18%; Fig. 4.3B) and total bacteria (RIV ≈ 36%, Fig. 4.3C) showed negative influence; and slope (RIV ≈ 100%, Fig. 4.3D) influenced positively the soil respiration. Coefficient (Coef), standard estimate (SE) and relative importance value (RIV) are shown for each selected variable for the factors related to soil respiration rates selected through model inference, and the R^2 of the models adjustment is shown for each model: microbial attributes and landscape features models in Table 4.1.

Table 4.1: Factors related to soil respiration rates, selected through model inference. Coefficient (Coef), standard estimate (SE) and relative importance value (RIV) are shown for each selected variable and R^2 of model adjustment is shown for each model. The response variable of all models is the soil respiration rate: µgCO$_2$-C/g/ soil dry wt -^{1}/hr-^{1}.

	Vegetation type	**Predictors**	***Coef***	***SE***	**RIV**	**R^2**
Response variable: soil respiration rates	Gallery Forest	Microbial				0.70
		FDA	2,467	0.331	71%	
		total Carbon	2,228	0.135	29%	
		Landscape				0.80
		slope	-4,245	0.36	50%	
		topographic wetness index	-4,907	0.36	50%	
		altitude	2,567	0.353	50%	
	Veredas	Microbial				0.62
		total Bacteria	0.954	0.172	49%	
		total Fungi	-0.748	0.172	28%	
		Landscape				
		elevation	0.5322	0.479	100%	0.45
	Cerrado *sensu stricto*	Microbial				0.55
		total Fungi	0.1434	0.301	36%	
		total Bacterias	-0.1696	0.232	36%	
		FDA	-0.04309	0.114	18%	
		Landscape				
		slope	0.05691	0.35	100%	0.43

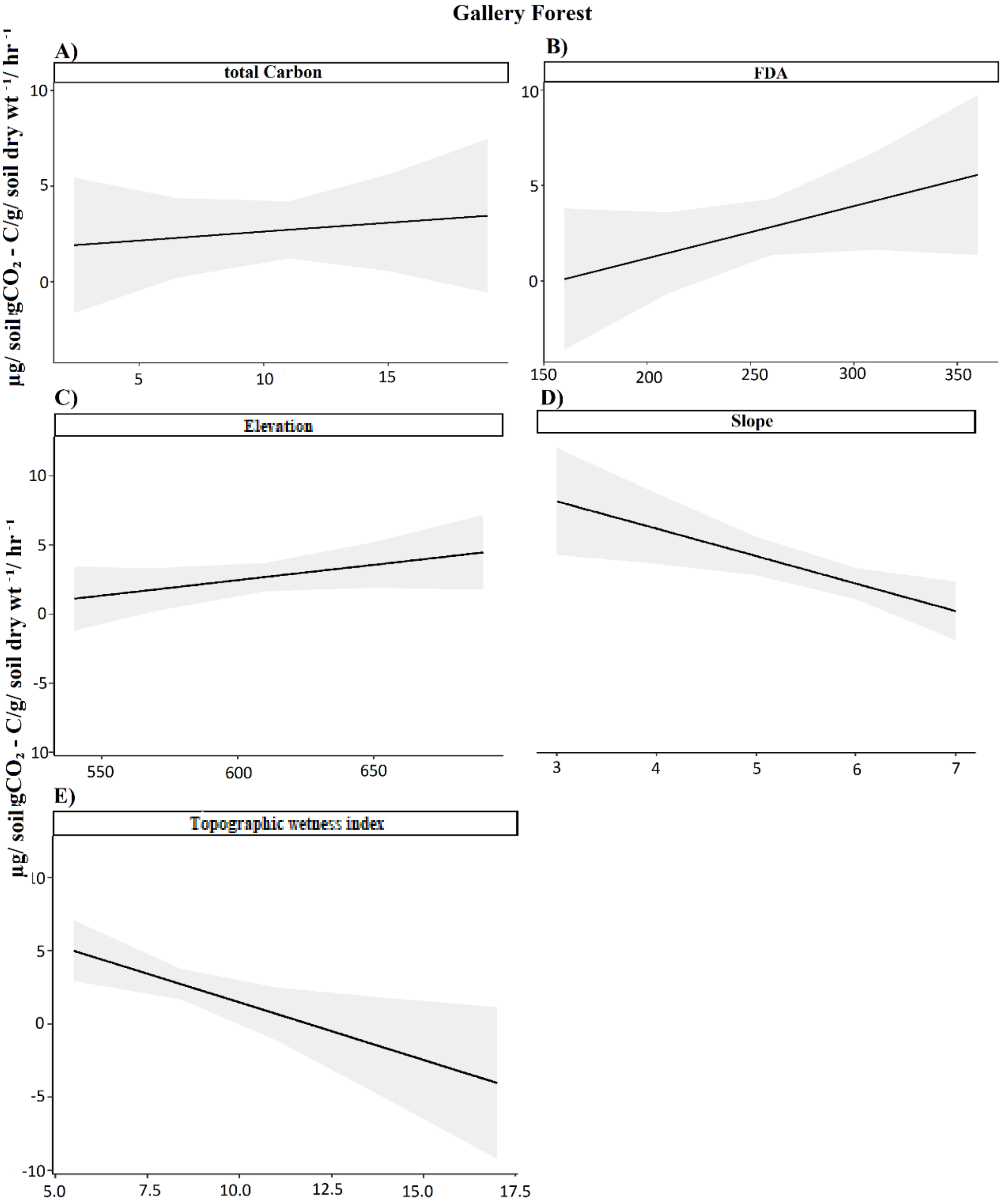

Figure 4.1. Factors influencing soil respiration (µgCO$_2$-C/g/ soil dry wt -^{1}/hr-^{1}) in Gallery forests: (A) total carbon; (B) fluorescein diacetate hydrolysis (FDA); (C) altitude; (D slope and; and (E) moisture index.

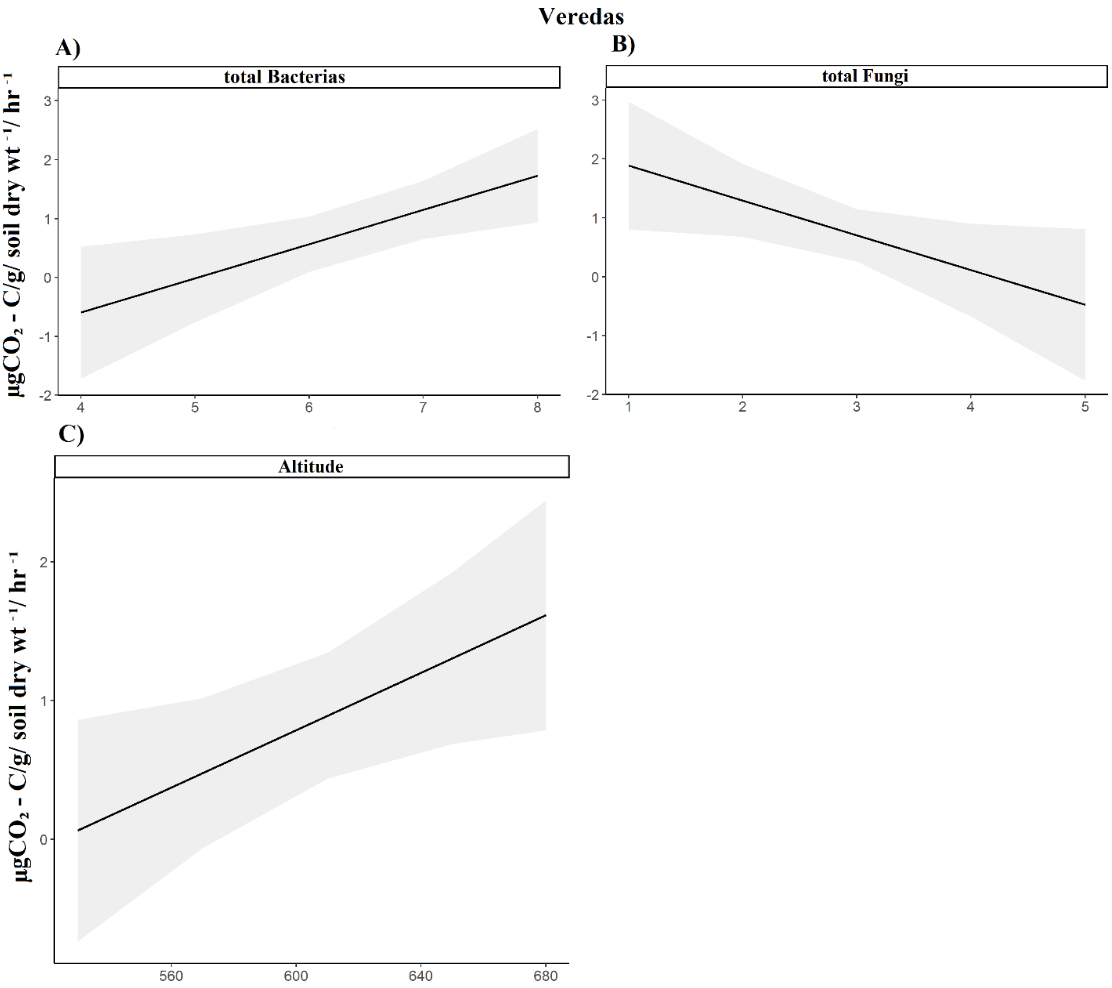

Figure 4.2. Factors influencing soil respiration (µgCO_2-C/g/ soil dry wt -^{1}/hr-^{1}) in Veredas. (A) Relationship of total bacteria with soil respiration; (B) relationship of total fungi and soil respiration; (C) relationship of altitude and soil respiration.

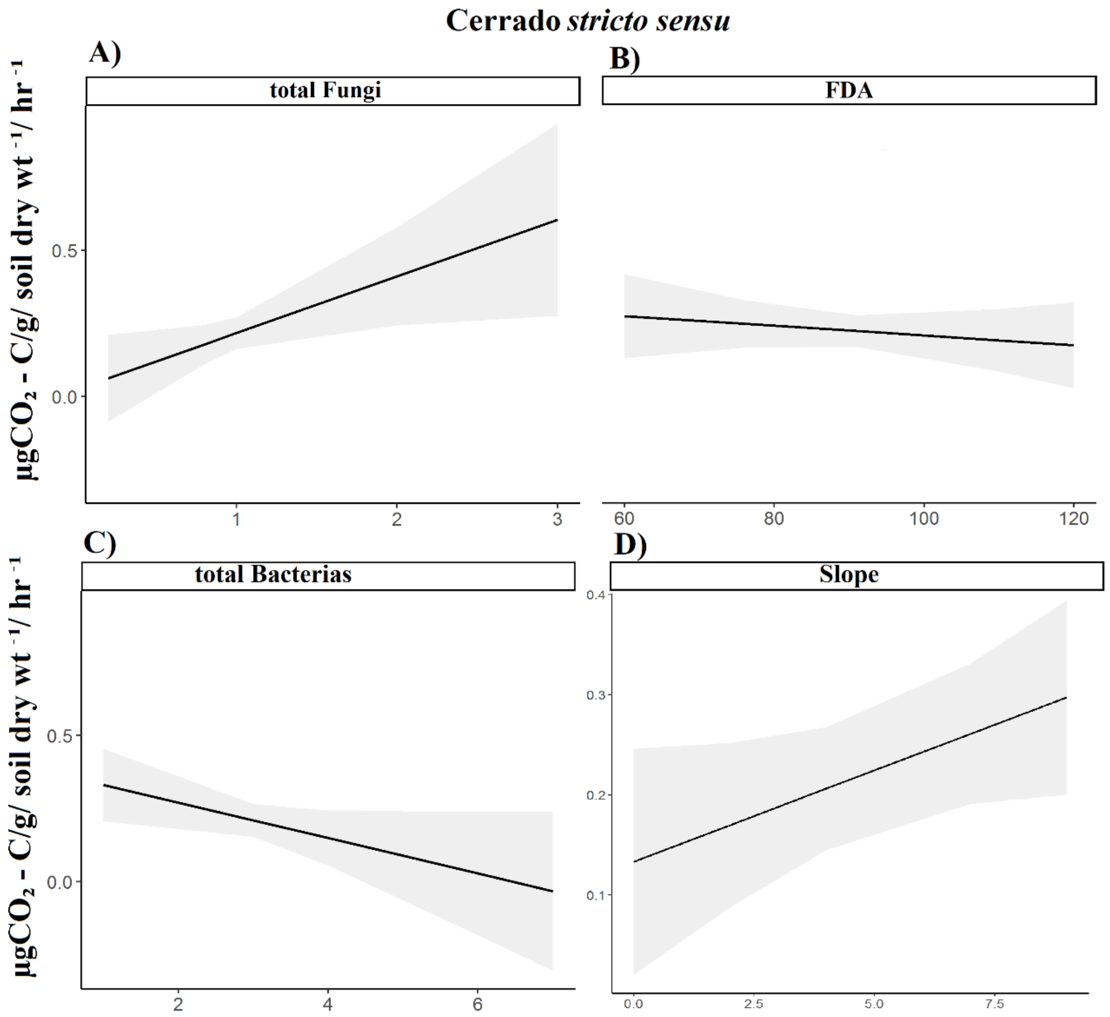

Figure 4.3. Factors influencing soil respiration (μgCO_2-C/g/ soil dry wt -^{1}/hr-^{1}) in Cerrado *sensu stricto*. (A) relationship of total fungi with soil respiration; (B) relationship of FDA and soil respiration; (C) relationship of total bacterias and soil respiration; (D) relationship of slope with soil respiration.

3.2 Differences in soil respiration between vegetation types

We found differences in soil CO_2 respiration in the Wet treatment between vegetation types ($P \leq 0.05$; *F*-value = 11.249) (Fig 4.4A; 4.4C) but not between sampling points ($P > 0.05$; *F*-value = 0.325) (Fig 4.4A; 4.4B). The drought treatment showed differences between vegetation types (*P*≤0.05; *F*-value = 5.263) (Fig 4.5A; 4.5C) with no differences between sampling points (*P*>0.05; *F*-value = 0.242) (Fig 4.5A; 4.5B). The drought-rewet treatment showed differences between vegetation types (*P*≤0.05; *F*-value = 5.417) (Fig 4.6A; 4.6C) and differences between sampling points (*P*≤0.05; *F*-value = 4.604) (Fig 4.6A; 4.6B).

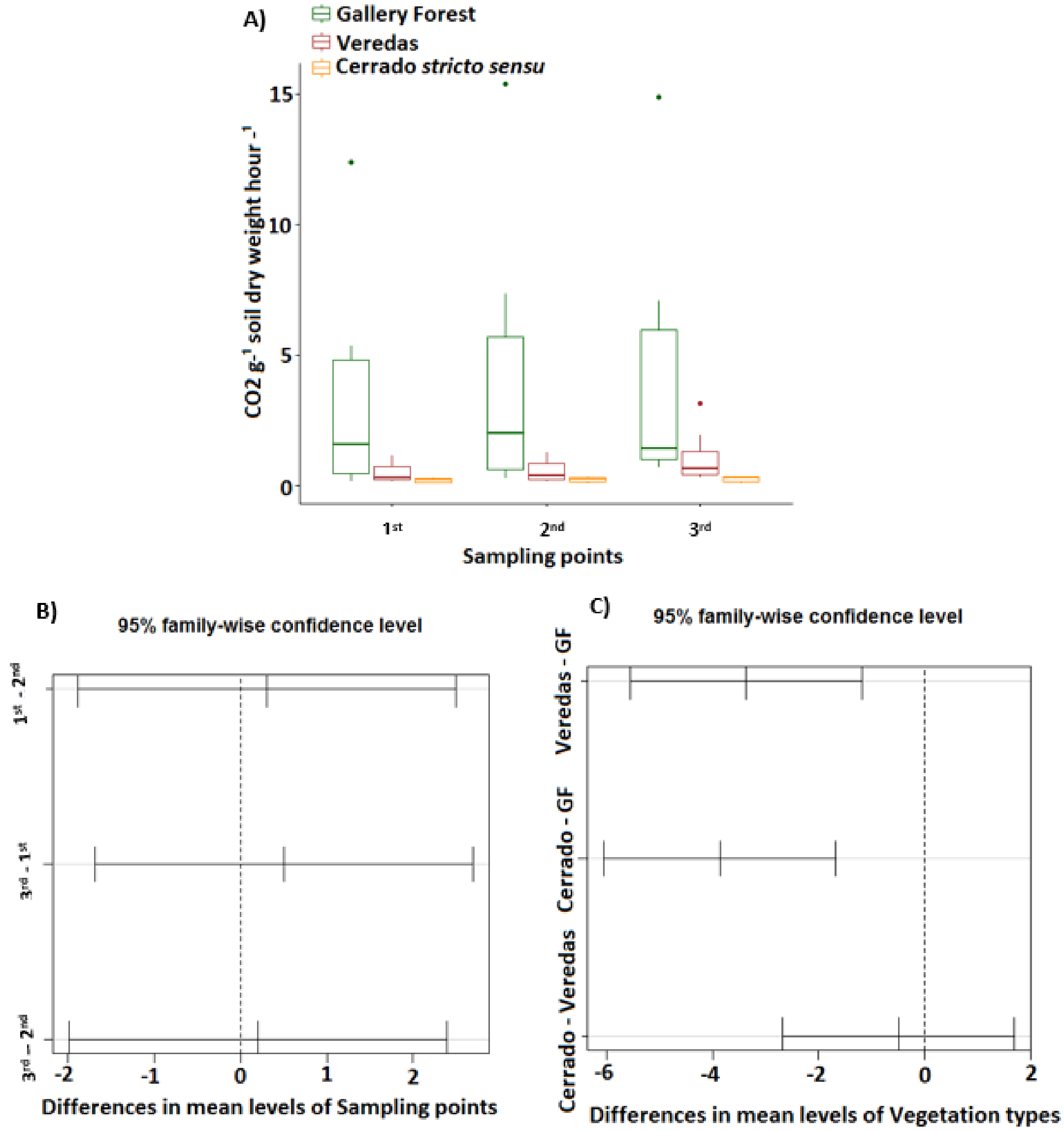

Figure 4.4. (A) Soil CO_2 respiration of wet treatment: μgCO_2-C/g/ soil dry wt $-^1$/hr-^{1}, soils under 100% water holding capacity along the sampling points: 1st sampling point - first day of soil respiration measurement; 2nd sampling point - after 15 days of experiment; 3rd sampling point - after 26 days of experiment between the different

vegetation types. Values are mean ±SE. In (B) the differences in mean levels of sampling points for all vegetation types. In (C) the differences in mean levels of vegetation types. No significant differences if the 95 % confidence interval overpass zero, and significant differences if the 95% confidence interval do not overpass zero.

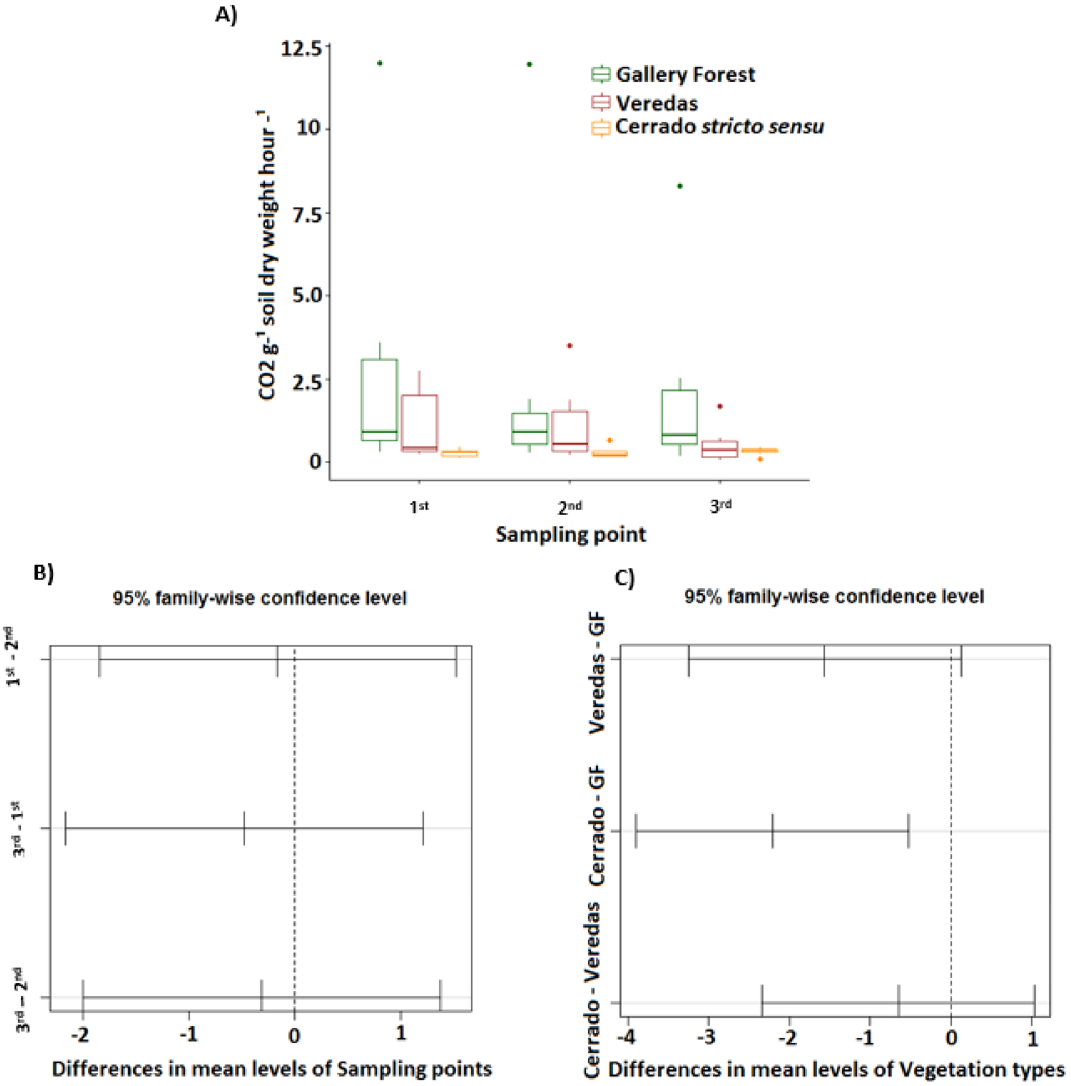

Figure 4.5. (A) Soil CO_2 respiration, µgCO_2-C/g/ soil dry wt -^{1}/hr-^{1} of drought treatment, : 1st sampling point - first day of soil respiration measurement; 2nd sampling point - after 15 days of experiment; 3rd sampling point - after 26 days of experiment between the different vegetation types. Values are mean ±SE. In (B) the differences in mean levels of sampling points for all vegetation types. In (C) the differences in mean levels of vegetation types. No significant differences if the 95 % confidence interval overpass zero, and significant differences if the 95% confidence interval do not overpass zero.

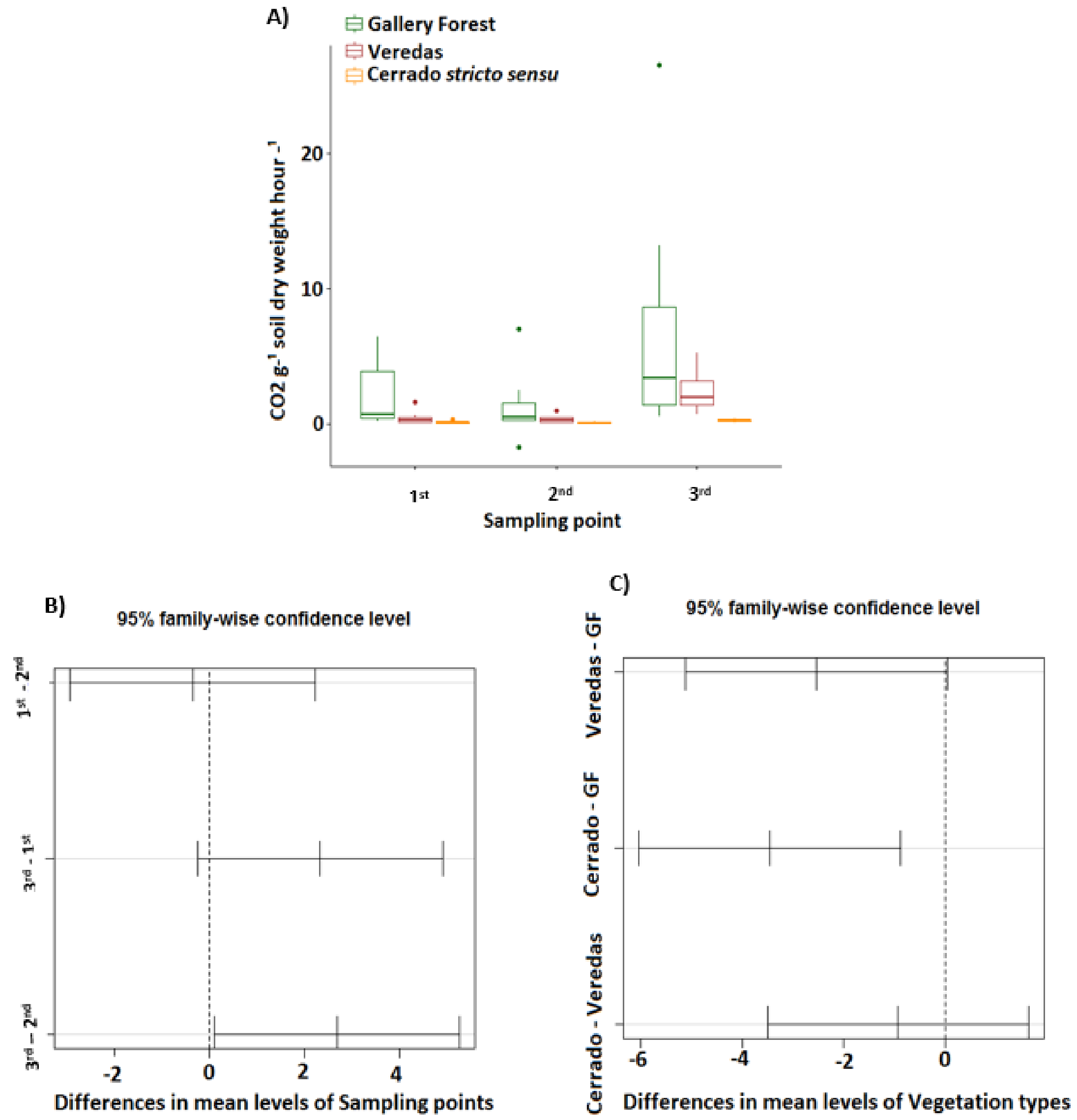

Figure 4.6. (A) Soil CO$_2$ respiration, µgCO$_2$-C/g/ soil dry wt -^{1}/hr-^{1} of drying/rewet (D/RW) treatment: 1st sampling point - first day of soil respiration measurement; 2nd sampling point - after 15 days of experiment; 3rd sampling point - after 26 days of experiment, but after two hours after rewetting. Values shown for the different vegetation types. Values are mean ±SE. In (B) the differences in mean levels of sampling points for all vegetation types. In (C) the differences in mean levels of vegetation types. No significant differences if the 95 % confidence interval overpass zero, and significant differences if the 95% confidence interval do not overpass zero.

3.3 Resistance of soil respiration to drought

For Gallery Forest, our results indicate no effect of the drought treatment on absolute change in soil CO_2 emission (Kruskal-Wallis $P>0.05$; Chi-squared = 2.961) (Fig 4.7A), but affected the response ratio in the Gallery Forests (ANOVA $P\leq0.05$; F-value = 6.346) (Fig 4.7B) between 1st and 3rd sampling points (TukeyHSD $P\leq0.05$). The % WHC was different (Kruskal-Wallis $P\leq0.05$; Chi-squared = 16.186) between 1st and 2nd, between 2nd and 3rd and also between 1st and 3rd sampling points (Wilcoxon rank $P\leq0.05$) (Fig 4.7C).

For the Veredas vegetation type, the drought treatment affected the absolute change in soil CO_2 respiration (Kruskal-Wallis $P\leq0.05$; Chi-squared = 9.024) between 1st and 3rd and between 2nd and 3rd sampling points (Wilcoxon rank $P\leq0.05$) (Fig 4.7D). We found differences in response ratios (Kruskal-Wallis $P\leq0.05$; Chi-squared = 8.972) between 1st and 3rd and between 2nd and 3rd sampling points (Wilcoxon rank $P\leq0.05$)(Fig 4.7E). The % WHC was different (Kruskal-Wallis $P\leq0.05$; Chi-squared = 14.033) between 1st and 2nd and between 1st and 3rd sampling points (Wilcoxon rank $P\leq0.05$)(Fig 4.7F).

For the Cerrado *sensu stricto* vegetation type, the drought treatment showed no effect on absolute change in soil CO_2 respiration (ANOVA $P>0.05$; F-value = 2.511) (Fig 4.7G) and no effect on response ratios (Kruskal-Wallis $P>0.05$; Chi-squared = 0.385) (Fig 4.7H). The % WHC was different (Kruskal-Wallis $P\leq0.05$; Chi-squared = 17.818) between 1st and 2nd, and between 1st and 3rd sampling points (Wilcoxon rank $P\leq0.05$) (Fig 4.7I).

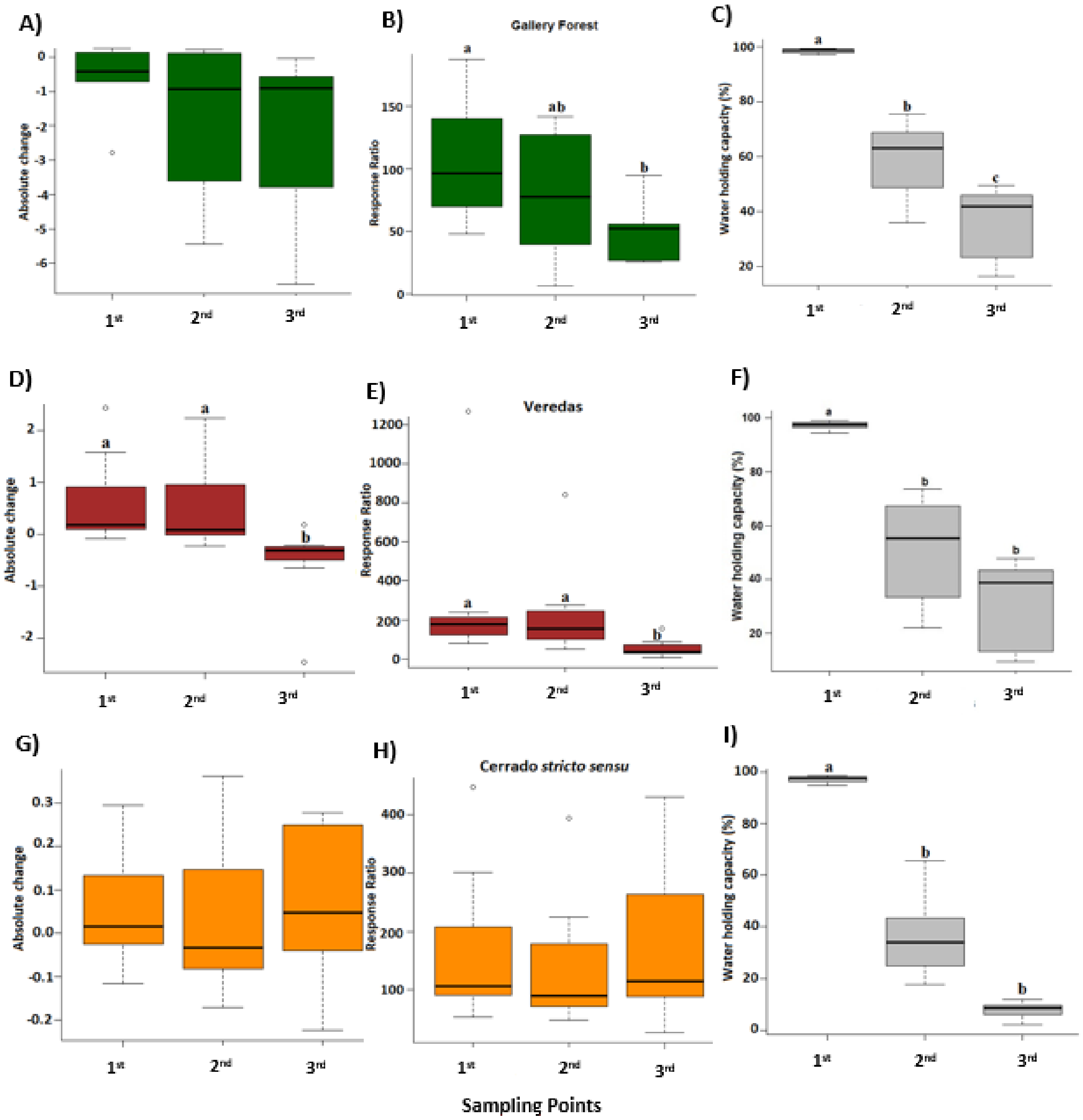

Figure 4.7. The absolute change, response ratio of soil CO_2 emissions and water holding capacity along the drought experiment of the Gallery forest, Veredas and Cerrado *sensu stricto* vegetation types. (A) Absolute changes of soil respiration in Gallery Forest; (B) Response ratio of soil respiration in Gallery forests; (C) water holding capacity in Gallery forests. (D) Absolute changes of soil respiration in Veredas; (E) Response ratio of soil respiration in Veredas; (F) water holding capacity in Veredas. (G) Absolute changes of soil respiration in Cerrado *sensu stricto*; (H) Response ratio of soil respiration in Cerrado *sensu stricto*; (I) water holding capacity in Cerrado *sensu stricto*.

3.4 Resilience of soil respiration to drought-rewetting

The soil CO2 emissions in Gallery forest were affected by the D/RW treatment: the absolute change was different (Kruskal-Wallis $P \leq 0.05$; Chi-squared = 7.413) between 1st and the 3rd sampling points (Wilcoxon rank $P \leq 0.05$) (Fig 4.8A). The response ratio was different (Kruskal-Wallis $P \leq 0.05$; Chi-squared = 6.033) between 2nd and 3rd sampling points (Wilcoxon rank $P \leq 0.05$) (Fig 4.8B). The water holding capacity (% WHC) was shown to be different (Kruskal-Wallis $P \leq 0.05$; Chi-squared = 14.182), between the 1st and 2nd sampling points and between 2nd and 3rd sampling points (Wilcoxon rank $P \leq 0.05$)(Fig 4.8C).

The CO_2 emission in the soils of Veredas vegetation type was also affected by the D/RW treatment: the absolute change was different (Kruskal-Wallis $P \leq 0.05$; Chi-squared = 13.299) between 2nd and 3rd, and 1st and 3rd sampling points (Wilcoxon rank $P \leq 0.05$)(Fig 4.8D). The response ratio was different (Kruskal-Wallis $P \leq 0.05$; Chi-squared = 11.317) between 1st and 3rd and between 2nd and 3rd sampling points (Wilcoxon rank $P \leq 0.05$)(Fig 4.8E).The % WHC was shown to be different (Kruskal-Wallis $P \leq 0.05$; Chi-squared = 11.288) between 1st and 2nd and between 2nd and 3rd sampling points (Wilcoxon rank $P \leq 0.05$) (Fig 4.8F).

The soil CO2 emissions in Cerrado *sensu stricto* showed to not be affected by D/RW (Kruskal-Wallis $P > 0.05$; Chi-squared = 3.094) (Fig 4.8G; Fig 4.8H). The % WHC showed differences (Kruskal-Wallis $P \leq 0.05$; Chi-squared = 13.455) between 1st and 2nd and between 2nd and 3rd sampling points (Wilcoxon rank $P \leq 0.05$) (Fig 4.8I).

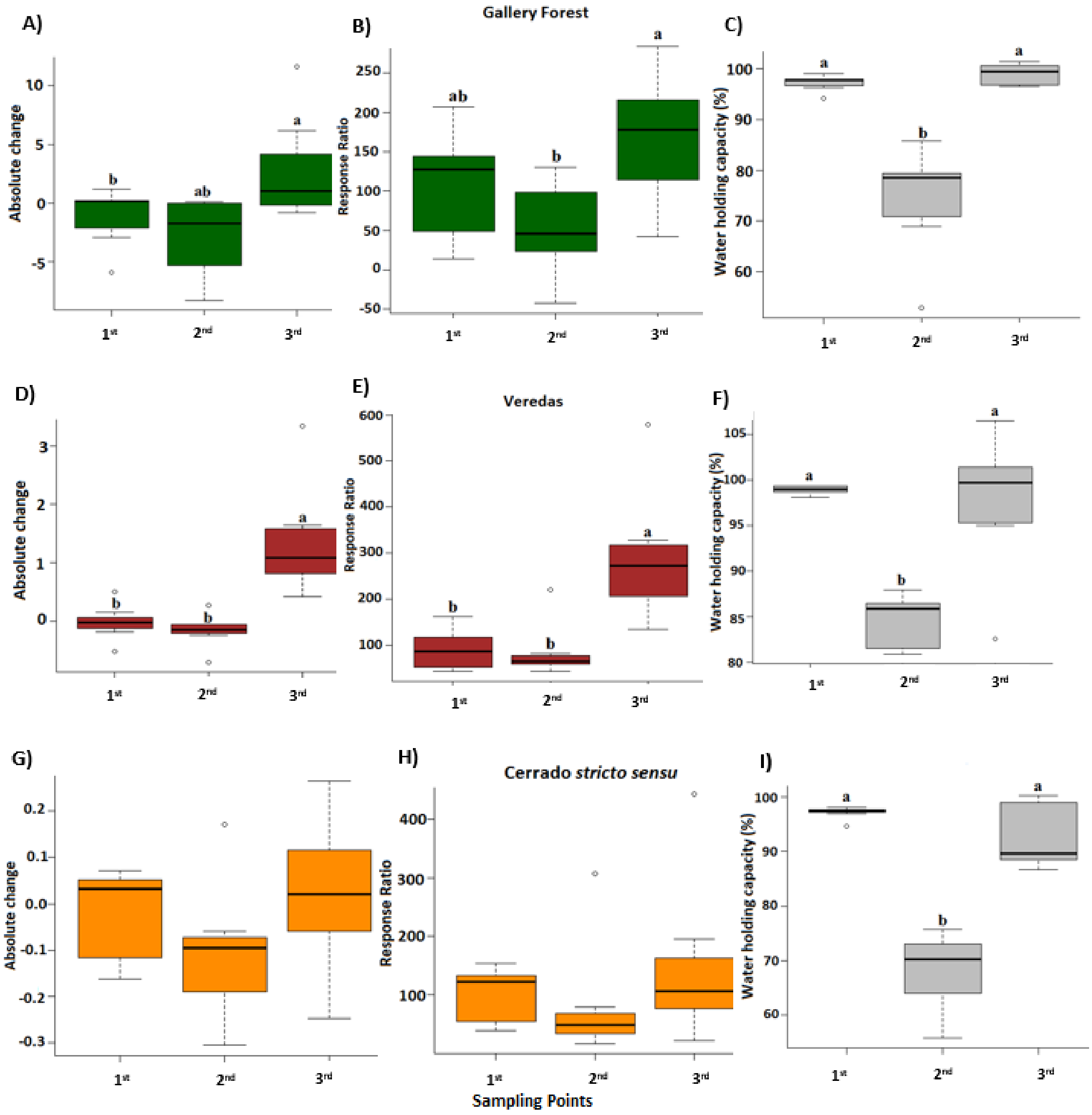

Figure 4.8. The absolute change, response ratio of soil CO_2 emissions and water holding capacity along the drying and rewetting experiment of the Gallery forest, Veredas and Cerrado *sensu stricto* vegetation types. (A) Absolute changes of soil respiration in Gallery Forest; (B) Response ratio of soil respiration in Gallery forests; (C) water holding capacity in Gallery forests. (D) Absolute changes of soil respiration in Veredas; (E) Response ratio of soil respiration in Veredas; (F) water holding capacity in Veredas. (G) Absolute changes of soil respiration in Cerrado *sensu stricto*; (H) Response ratio of soil respiration in Cerrado *sensu stricto*; (I) water holding capacity in Cerrado *sensu stricto*.

4 DISCUSSION

Our study shows how microbial community and topography attributes are related to the baseline respiration and its resilience in savanna ecosystems with contrasting soil moisture conditions (Cerrado *sensu stricto*, Veredas and Gallery Forests). We also showed that soil respiration in those ecosystems responded differently to drought and rewetting.

4.1 Factors related to baseline soil respiration rates

In the Gallery Forests, soil respiration rates were positively related to total soil carbon, soil extracellular enzyme activity (FDA) and elevation, and negatively related to slope and topographic wetness index. Some authors found that soil respiration is positively related to soil organic matter input in temperate and tropical riparian zones (Audet et al., 2013; Oelbermann and Raimbault, 2015; Zanchi et al., 2014) with relationships to stem density within these forests (Hopfensprenger et al., 2009). This suggests the importance of the relationship between vegetation structure and soil microbial respiration. In our study sites, the Gallery Forests have a complex vegetation structure (e.g. high canopies and high tree density), which may promote litter input and accumulation of organic matter on the forest floor, increasing carbon sequestration (Delliti and Burger, 2000). The flooding that gallery forests are often submitted to might suppress soil microbial activity, as soil respiration showed a negative relation with the soil topographic wetness index. Although some studies found a positive relationship between soil topographic wetness index and soil respiration (Pacific et al., 2011), our study showed the opposite effect. We suggest this may be the result of too much water in low-lying areas that remain flooded for longer, hampering microbial activity and aerobic respiration.

There was a strong positive relationship between hydrolytic enzyme activity and soil respiration rates in the Gallery Forests. Ali et al. (2015) found that enzymes have a non-linear relationship with soil CO_2 flux. The authors attribute this non-linearity to differences in substrate quality and nutrient availability, e.g. N and P, and the season of sampling. We sampled in only one season (i.e. the dry season) and in a vegetation type high litter accumulation. Our results indicate that extracelular enzyme hydrolysis is related to soil respiration rates in Gallery forests ecosystems. This can be a result of the high substrate (from organic matter) availability in these soils (Allison and Vitousek, 2005) that stimulate enzyme activity and nutrient mineralization (i.e. C, P, S and N) (Bárta et al., 2014), therefore enhancing microbial biomass and respiration.

Soil respiration was related positively to altitude and negatively to slope in Gallery Forests. Pacific et al (2011) found that landscape position, slope for example, can affect soil surface CO_2 fluxes in transitions between hillslopes and riparian forests. Although the slope in their study was also evaluated through a DEM, the authors considered the slope gradient along the hillslope and not the local microtopography as in our study. Our study shows that the effect of topography can be even more local, as we were able to measure altitude and slope within a pixel at a resolution of 12.5 m^2, showing that even small-scale variation in topography can affect soil respiration. The negative effect of slope on soil respiration rates may be related to increased substrate leaching on steeper gradients. Furthermore, local differences in altitude were positively related to variation in soil respiration (whereas in Gallery Forests variation in altitude can range from 532 to 688 meters – data not shown). Higher local elevation positively affected soil respiration in gallery forests. This could be the result of increased elevation may be reducing soil water content, and therefore facilitatating increased aerobic microbial activity in the forest floor.

In the Veredas vegetation type, soil respiration rates were influenced by total bacteria, total fungi (PLFAs) and elevation. The positive influence of bacteria and negative influence of fungi on CO_2 emissions in this vegetation type suggests that bacterial communities are a major contributor to soil respiration, with a higher bacterial abundance in this ecosystem. This reflects the specific decompositional processes of this environment, as for example, fungi often use more recalcitrant C sources (Brant et al., 2006) while bacteria are known to quickly colonize labile C resources (Reischke et al., 2014). Forests possess higher quantities of recalcitrant organic matter from leaf and wood inputs compared to grasslands, and consequently usually have higher fungi:bacteria ratios (Ingham and Thies, 1996). Grasslands, however, possess root-associated bacterial communities that are fed by readily decomposable exudates released in the rhizhosphere. Also strong bacterial components generally occur in N-rich soils (Wang et al., 2019), and most of Veredas areas are amended with fertilizers to be used as grasslands for cattle production. Moreover, a study conducted in the Brazilian savanna by Oliveira et al. (2002) suggested that grasses have allelopathic effects that inhibit the development of fungal populations. The positive effect of altitude on soil respiration in Veredas is likely due to corresponding drier conditions in wetlands that enhance microbial activity rates.

In the Cerrado *sensu stricto* vegetation type, soil respiration was affected by fungi, bacteria, soil extracellular enzyme activity and slope. Fungi was related positively while bacteria negatively to soil respiration rates. These results suggest that in Cerrado *sensu stricto*, fungi dominate the microbial community and contribute more to soil respiration compared to bacteria. Due to the greater ability of fungi to break down structural compounds such as lignin and cellulose, this group of microorganisms are usually more abundant than bacteria in woodlands (Ingham and Thies, 1996) as this vegetation produces more recalcitrant compounds in their leaves (Brant et al., 2006). Cerrado *sensu stricto* has previously been reported to produce litter with high lignin and cellulose contents due to the xeromorphic vegetation (Lammel et al., 2015). In addition to the influence of organic matter quality on which microbial group are contributing to soil respiration, soil texture might be another factor underpinning the negative relationship between bacteria and soil CO_2 emissions in the Cerrado. Soil texture and mineralogy are known to affect pore size within soil agregates and consequently affect fungal and bacteria communities (Six et al., 2006). Small pores provide refuges for bacteria against attack from protozoans and bacterivorous nematodes, and due to their body sizes, bacteria are usually more likely to be found in micropores or microaggregates than fungi (Killham, 1994). The Cerrado *sensu stricto* vegetation type in our study showed soils with an average of 93% sand, which suggests that these soils do not provide beneficial microenvironments for bacterial community development and protection. Also fungi is known to be more resistant to drought than bacteria (Kaisermann et al., 2015), which may also explain the positive relationship between fungi and soil respiration in Cerrado *sensu stricto*, in contrast with the positive relationship between to bacteria and soil respiration in the periodically flood Vereda ecosystems.

Soil extracellular enzyme activity evaluated with the FDA assay showed a negative relationship with soil respiration in the Cerrado *sensu stricto* vegetation type.

Gallo et al (2006) found that increases in soil water content increased enzyme activity and drought decreased enzymes in arid ecosystems. This might explain the different directions of extracellular enzymes relationships on soil respiration within the dry soils of Cerrado *sensu stricto* and the periodically flooded soils of Gallery Forest. Finally, this result suggests that the fungal community that promote the respiration is probably not the major producer of extracellular enzymes.

The topography was also important for soil respiration in the Cerrado *sensu stricto* vegetation type. Slope showed a positive effect on soil CO_2 flux, demonstrating the opposite relationship found in Gallery Forest. In Gallery Forest, the topography determines how the water flows within soil pores, and increases in slope may contribute to more OM leaching/moving within the pores. Our results suggest that the slope in Cerrado *sensu stricto* vegetation type is possibly contributing to accumulation of OM by the downward transport of particulates and creating areas of high soil CO_2 efflux (Lecki and Creed, 2016).

Intensity of anthropogenic disturbance was not selected as an important variable influencing soil CO_2 efflux for any of the vegetation types. However, land use has been found to affect soil respiration in other studies (Inubushi et al., 2003; Santos et al., 2019; Wanyama et al., 2018). Our study area, the Pandeiros river basin, is included in an environmental protection area (Área de Proteção Ambiental– APA), in which only sustainable agricultural practices are permitted, mainly for local subsistance. This may have contributed to a low variation in the "anthropogenic disturbance" variable and its limited effect on soil attributes. Moreover, although we showed previously that microbiological attributes respond significantly to the disturbance in a buffer of 2 km of radius, soil respiration could be responding more to local and topographic aspects than to the land-use within the buffer size chosen for this study. Due to the importance of understanding the effects of land-use and climate on soil respiration we recommend that future studies consider evaluating the effectiveness of buffer sizes to assess the effect of land-use on soil CO_2 fluxes in the neotropical savanna and its different vegetation types.

4.2 Differences in soil respiration rates between vegetation types

Overall we found that Gallery Forests have higher soil CO_2 emissions than Veredas and Cerrado *sensu stricto* vegetation types, which were similar. Higher CO_2 emissions in the Gallery Forests may result from higher organic matter inputs through litter deposition (Pinto et al., 2018), and possibly more accumulation of nutrients from the catchment area due to the filtering capacity of this riparian vegetation type (Hunke et al. 2015; Parron et al., 2010). Although Veredas and Cerrado *sensu stricto* were shown to have similar CO_2 fluxes, Veredas soils had higher variation. This is possibly because it occupies an intermediate position between Cerrado *sensu stricto* and Gallery forests, and it has a more heterogeneous pattern of OM accumulation due to flooding.

The differences in soil respiration rates between vegetation types were consistent regardless of drought and rewet treatment. A more detailed discussion about the effects of these treatments is made further on.

4.3 Resistance of soil respiration to drought

Our results showed that the drought treatment caused a decrease in soil respiration rates in Gallery Forests and in Veredas, although respiration rates in soils of Cerrado *sensu stricto* were unaffected.

Absolute change represents the amplitude of the differences of soil CO_2 emissions responses to the drought considering the control treatment (Ågren and Bosatta, 2002). Furthermore, response ratios detect relative changes by standardizing values and enabling overall comparisons of soil respiration dynamics across different vegetation types. No differences were found in absolute change with the drought treatment for gallery forests. Despite this, the response ratio values showed that the drought had an effect on CO_2 emissions in Gallery Forest after 26 days of drought. It is important to highlight that the sampling point after 15 days of drought is statistically similar to the sampling point of 26 days after drought which suggest that part of the soil microbial community might start responding to the drought after 15 days of drought.

The soils of the Vereda vegetation type showed differences in soil moisture when compared the first to the 15th day of experiment, and no differences between the 15th and the 26th day of drought. The absolute change in soil respiration rates and response ratios were shown to be affected by drought after 26 days, which suggests that these soils are more resistant than Gallery Forests because the CO_2 (Pimm, 1984). Soil microbial resistance reflects microbial tolerance to lower soil moisture (Nijs et al., 2019) with studies showing that drought events result in a shift in microbial communities towards more stress tolerant taxa (Evans and Wallenstein, 2012; Griffiths and Philippot, 2013; Nijs et al., 2019). This adaptation occurs because microbial processes are intrinsically linked with moisture levels that influence metabolism, growth rate, composition and size (Barnard, Osborne and Firestone, 2015). Thus environmental conditions and the legacy of drought events are crucial in shaping stress tolerant soil microbial communities (Hawkes et al., 2017). Although Vereda vegetation types are periodically flooded, these ecosystems are also subject to more intense and frequent drought events than Gallery Forests, as these open ecosystems have no canopy cover. Consequently, soils are more susceptible to drying through evaporation. Also, in the Veredas the level of the water table rises to the soil surface during the wet season and falls during the dry season (Eiten, 1982) causing periodic cycles of flooding and drying (i.e. anaerobic and aerobic conditions). In contrast, in the Gallery Forests, most of the soils are hydromorphic and remain wet even during the dry season because of the constant flood from the rivers (Oliveira-Filho, 1989; Oliveira-Filho and Martins, 1986; Ratter, 1980). Our results suggests that the soil microbial communities in Veredas are potentially more tolerant to drought than the in Gallery forests (Evans and Wallenstein, 2012).

Soil respiration of Cerrado *sensu stricto* remained unchanged during the drought in terms of absolute changes and response ratios, even though these soils showed the greatest decrease in soil moisture as a result of drought. This result could suggest that these soils are more resistant or tolerant to lower soil moisture content (Nijs et al., 2019), but probably reflects their low microbial activity rather than resistance or resilience. It is also important to highlight that the baseline soil respiration rates are by far the lowest of all the vegetation types evaluated in the present study, reflecting poor organic matter content. Kaschuk et al (2011) found that, due poor fertility, the Cerrados savana-like vegetation conversely possess the least resistant soils compared to other brazilians ecosystems, with an inherent low capacity to support soil microbial activity and resist the effects of disturbance on C stocks and soil microbial biomass. This supporting capacity is low in Cerrado *sensu stricto* because of the high sand content (> 90%).

4.4 Resilience of soil respiration to drought-rewetting

Our results showed that drought promoted a decrease followed by an increase of soil respiration rates after rewetting in Gallery Forests and in Veredas, while the soils of Cerrado *sensu stricto* were not affected by either the drought or the rewetting. We recorded soil respiration rates two hours after rewetting soils after 26 days of drought. We recognise that this study is restricted regarding analyses of soil resilience as more days of measurements after the rewetting would be required for a better evaluation of the exact moment of soil respiration recovery (Hueso, Hernández and García, 2011; Nijs et al., 2019). However, our results allow us to infer which soils recover faster from drought disturbance. Respiration rates of soil from Gallery Forest were more similar to their respective controls than the soils from Veredas after rewetting, therefore, we suggest that Gallery forests soils were more resilient than Veredas. Some studies have found that ecosystems with more frequent drying and rewetting cycles tend to select microbial communities adapted to the rewetting perturbation (Griffiths and Philippot, 2013; Nijs et al., 2019). Gallery Forests border rivers and thus are more likely to experience more rewetting cycles than Veredas (Oliveira-Filho and Martins, 1986; Ratter, 1980), which might explain the greater resilience of soil respiration in this vegetation type.

In ecosystems that are not subjected to flooding regimes, where long dry seasons and droughts are expected, like in Cerrado *sensu stricto* for example, soil organic matter content is essential to sustain microbial functions and their resilience (Hueso, Hernández and García, 2011; Sardans and Peñuelas, 2005). Nevertheless, the soils of Cerrado *sensu stricto* showed no effects of drying and rewetting on soil respiration, which suggest a small microbial activity probably due to the low organic matter content in these soils, making them the least resilient.

4.5 Implications for future Climate Change scenarios

Our results suggest that Veredas possess the most resistant soils, Gallery Forests the most resilient, while the Cerrado has low microbial activity. Therefore, the potential implications for more intense droughts associated with predicted climate scenarios in the Cerrado biome may be a slower recovery of soil functioning after rewetting events in Veredas soils compared to Gallery Forests, meaning soils under Veredas vegetation may release CO_2 for longer than Gallery Forests.

This was the first study evaluating the effect of a cycle of drought and rewetting on the soils functioning in the different vegetation types of Cerrado. We recommend that more experimental studies should be done in order to understand how the different drought intensities and different frequencies of rewetting cycles affect soil functioning in the distinct vegetation types in the Cerrado, and thus, predict more robust scenarios for this biome.

5 CONCLUSION

Our results provide new insights into the effect of local and landscape climate on soil respiration in the main Cerrado biome vegetation types: Gallery Forests, Veredas and Cerrado *sensu stricto*. We evaluated how drought and drought/rewetting affect soil CO_2 fluxes and compared soil resistance and resilience of these three different ecosystems.

Overall, higher soil CO_2 fluxes were found in Gallery Forest soils when compared to Veredas and Cerrado *sensu stricto*. We identified some factors including soil microbial attributes, total carbon, and topographic characteristics related to soil respiration in these vegetation types. For Gallery Forests, the positive effect of total soil carbon on soil respiration might reflect the complexity of vegetation structure and its organic matter inputs; the positive relationship between hydrolytic enzyme activity and soil respiration may be a result of the higher nutrient availability in these soils; and the positive effects of altitude on soil respiration suggests that the decrease of water content within the soil pores of this frequently flooded ecosystem at higher elevations might increase the metabolism of the microbial community. For Veredas, the positive effect of bacterial abundance and negative effect of fungi suggests that the major contribution of soil respiration derives from root- associated bacterial communities within these grasslands, and the increase in elevation also might increase the soil microbial metabolism in wetland conditions.

For Cerrado *sensu stricto*, the positive relationship between fungal abundance and soil respiration may reflect the quality of litter in terms of high lignin and cellulose content, and a greater resistance of fungi to drought compared to bacteria. The negative relationship between bacteria and soil respiration may reflect the inhibition of bacterial development from the adverse conditions assocated with sandy soils. The positive effect of slope on soil respiration suggests that the downward trasport of OM and nutrients may be an underlying mechanism influencing soil CO_2 fluxes.

We showed that drought affected soil respiration in Gallery Forest and Vereda soils, but not Cerrado *sensu stricto* soils. We concluded that Veredas had the most resistant soil since respiration remained unchanged for longer than that of Gallery

Forests. Rewetting increased soil CO_2 fluxes in both Veredas and Gallery Forests after drought. We found soil respiration rates of Gallery Forest to be most similar to controls two hours after rewetting, suggesting a rapid recovery of function in Gallery Forest soils which we concluded to be the most resilient.

Neither drought nor rewetting significantly affected Cerrado *sensu stricto* soil respiration absolute changes or response ratios, and the baseline soil respiration rates in these soils were the lowest of the vegetation types evaluated in the present study, reflecting the poor organic matter content and low microbial activity in these soils.

The potential implications for more frequent and intense droughts associated with predicted climate scenarios in the Cerrado biome may be a slower recovery of soil functioning after rewetting events in Veredas soils compared to Gallery Forests, meaning soils under Veredas vegetation may release extra CO_2 for longer than Gallery Forests. This was the first study evaluating soil respiration in a drought and rewetting cycle in the distinct vegetation types of the Cerrado biome. Results confirm that varied properties of the range of Cerrado ecosystems will determine their resilience to local and landscape climate change.

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GENERAL DISCUSSION

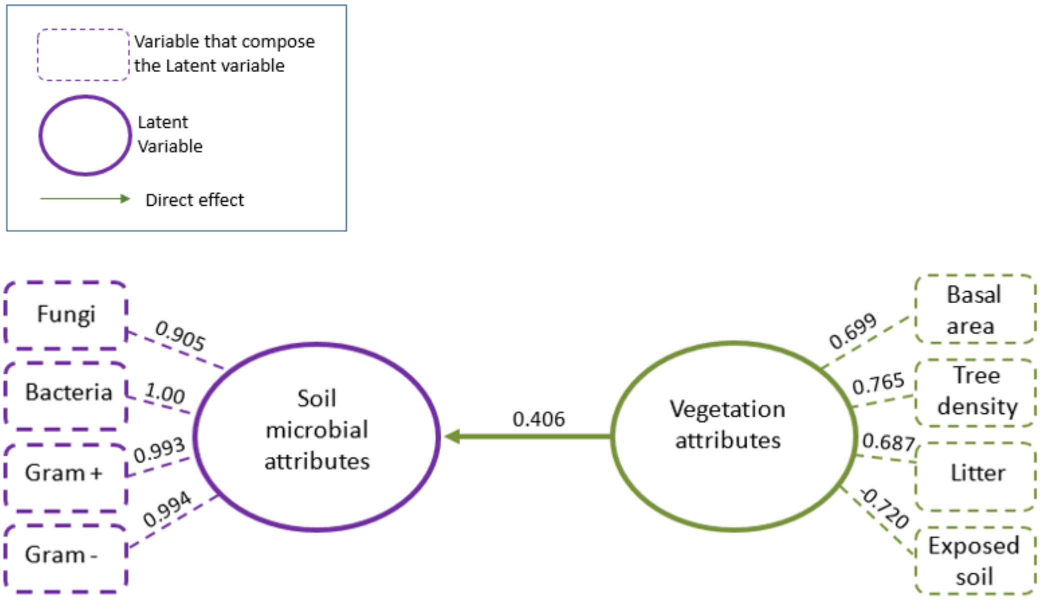
Savannas are important for providing crucial ecosystem services for humanity, and sustaining high levels of plant and animal species diversity and endemism (SCHOLES; ARCHER, 1997). Despite this importance, these ecosystems are highly threatened worldwide by being most sensitive to land use and climate change (ZHANG et al., 2019). More than half of the natural vegetation cover of Neotropical savannas has already been lost due to land conversion to crop plantations and pasture, and yet understanding about ecosystem functioning within this biome and the effects of degradation is lacking. As soil microorganisms are vital for ecosystem maintenance and nutrient cycling, the overall objective of this thesis was to assess how environmental, vegetation and soil characteristics, and landscape features affect soil microbial attributes and function within three typical vegetation types of Brazilian Neotropical Savanna, the Cerrado biome. This understanding is essential for informing ecologically-relevant conservation and management efforts in this globally important biome. This chapter discusses the key findings of this thesis, as well as implications for land use and climate change, and conclusions and recommendations for future studies.

Below- (soil microbial and physiochemical properties) and above-ground (vegetation) ecological systems have traditionally been studied independently of one another. Nevertheless, a coupled approach provides valuable information about the detailed mechanisms underpinning ecosystem functioning. In the study presented in Chapter 2, I investigated how local-scale vegetation and soil physiochemical properties are related to soil microbial attributes in Gallery forest, Veredas and

Cerrado *sensu stricto*. In Gallery forest, the direct effect of soil physicochemical properties on soil microbial attributes, particularly with the contribution of trace elements, suggests that flooding is important in shaping soil abiotic and biotic conditions in this ecosystem. Vegetation characteristics were related to soil physiochemical properties in a bidirectional way, suggesting potential indirect influences on soil microbial attributes through feedbacks with soil environment. In Veredas, soil physicochemical properties had the strongest influence on soil microbial attributes, although grass cover also had a direct independent effect. This suggests that the flooding and soil water saturation that these systems periodically experience is enhancing organic matter deposition and cation concentrations in the soil, possibly inducing a grass-dominated plant community and grass-associated soil microbial community. The soil environment, moisture conditions and grass dominance in this vegetation type is mainly affecting the quantity of microbial biomass, enzymes and bacteria, which may also be due to soil organic matter accumulation in this ecosystem (PANT; RECHCIGL, ADJEI 2003, JUNK et al., 2014), providing substrate for overall soil microorganisms and enzymes. Bacteria were selected as an important microbial attribute, possibly due to the ability of bacteria to break down labile organic C compounds released in the rhizosphere of grasses (KAISERMANN et al., 2015), and in dealing with high soil moisture contents and drying-rewetting regimes characteristic of these ecosystems (SCHIMMEL et al., 2007).

In Cerrado *sensu stricto*, soil physico-chemical properties had no effect on soil microbial attributes, with vegetation being the key predictor of soil microbial community structure, affecting relative abundances of total fungi, total bacteria, gram-positive and gram-negative bacteria. The prevalence of sand in these soils means little capability to support or retain microbial communities, resulting in highly adapted communities that are strongly dependent on the quantity and quality of the litter produced by the vegetation (Figure 2.6).

Figure 2.6. - Diagram of structural equation modelling showing the direct effects of vegetation attributes on soil microbial community attributes within the Cerrado *sensu stricto* vegetation type.

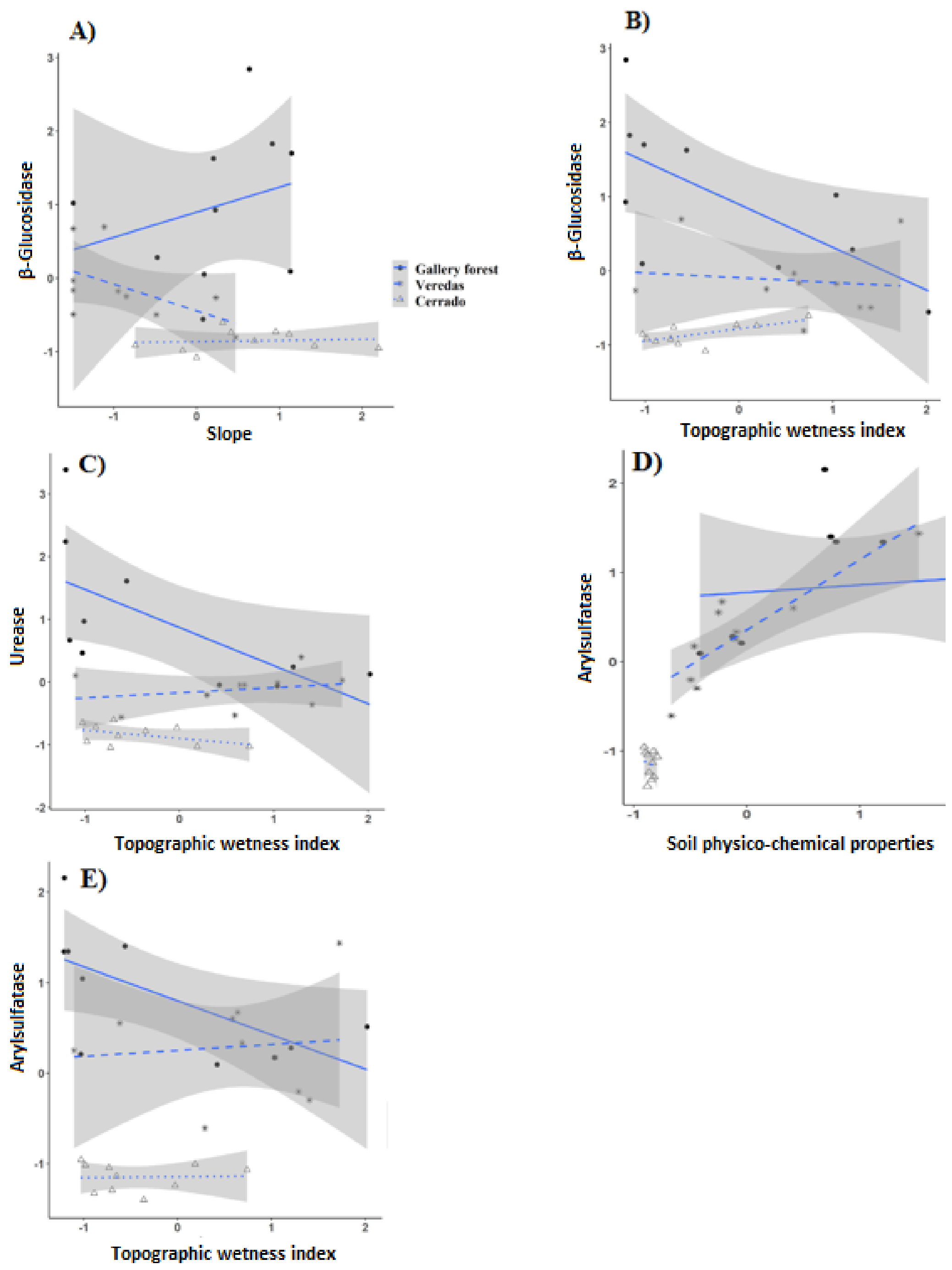


I recommend further studies to identify soil microbial communities and their relationship with soil organic matter quality within the three vegetation types through Next-Generation Sequencing (NGS) techniques, with an emphasis on litter decomposition in Cerrado *sensu stricto*. Although bacteria have already been reported to be strongly influenced by litter quality under savanna-like vegetation in the Cerrado biome (LAMMEL et al., 2015), I recommend that the fungal community should be studied more carefully, as my results have shown that it could be a potentially important component in dealing with the recalcitrant litter produced by this vegetation type. In this study, I demonstrated that even though these vegetation types are in close proximity to one another, they possess completely distinct patterns of above- and below-ground interactions at the local-scale. Changes in these identified factors through land management will alter important ecological processes, as well as the maintenance and stabilization of these ecosystems in response to future climate change.

After establishing a conceptual model with the key attributes underlying ecosystem functioning for the three typical Cerrado vegetation types at the local scale, I asked how do landscape-scale anthropogenic disturbances and topographical aspects influence soil microbial community structure and enzyme activities in these different ecosystems (Chapter 3). Anthropogenic disturbance through land use such as agricultural intensification is causing large-scale alterations to ecosystem functioning in Neotropical savannas (HUNKE et al., 2015), while topographical landscape characteristics such as elevation and slope are determinants of vegetation type and nutrient cycling (OLIVEIRA-FILHO et al., 1989). I found a decrease in soil microbial biomass and fungi:bacteria ratio with increasing anthropogenic disturbance in all vegetation types, reflecting alterations to the quantity and quality of litter inputs due to a reduction in native vegetation cover. Conversely, anthropogenic disturbance was found to increase alkaline phosphatase activity in Gallery forests, and increase fungal and bacterial relative abundances, with a stronger effect on bacteria. The positive influence of anthropogenic disturbance on these aspects is possibly due to the impact of agriculture and amended soils for managed pasture in the study region. The Pandeiros river basin is located in a region classified as an “Environmentally Protected Area of Sustainable Use” by the Brazilian government, in which only sustainable activities are permitted. Although anthropogenic disturbance did negatively affect some soil microbial attributes, overall a strong negative effect on most of soil microbial attributes was not seen, likely due to the low intensity of farming practices and the integration of farming into the natural landscape.

Topographical characteristics were the most important predictors for all soil microbial attributes, with the exception of soil microbial biomass. All soil enzymes were particularly strongly influenced by topography (Figure 3.11).

Figure 3.11. - Response variables affected by predictors at different ways according to vegetation type. Only variables selected by the best interaction models with vegetation type. (A) β-Glucosidase is positively affected by slope in Gallery forests and negatively in Veredas. (B) β-Glucosidase is negatively affected by topographic wetness index in Gallery Forest, positively in Veredas and in Cerrado *sensu stricto*. (C) Urease is negatively affected by topographic wetness index in Gallery forests and positively in Veredas. (D) Arylsulfatase is positively affected by soil properties in Gallery Forests and in Veredas and negatively in Cerrado *sensu stricto*. (E) Arylsulfatase is negatively affected by topographic wetness index in Gallery forest and positively in Veredas and in Cerrado *sensu stricto*. Sampling replicates n=30 at the three vegetation types of Gallery forest, Veredas, Cerrado *sensu stricto* along the Pandeiros river basin, Minas Gerais, Brazil.



The intensity and direction of these relationships varied according to vegetation type, which suggests that soil microbial attributes are strongly dependent on soil moisture content; enzyme activity especially may have an optimum soil moisture content for operation in natural ecosystems. Overall, I demonstrated that anthropogenic disturbance and topography affect soil microbial attributes, with topography being the strongest predictor through determining soil moisture content. At a landscape scale, water availability is therefore the key differentiator for ecosystem functioning between the three vegetation types.

After considering the influences of landscape features on soil microbial community structure and enzyme activity, I finally explored key factors underlying baseline soil CO_2 emissions and evaluated the resistance and resilience of soil functioning (here using soil respiration) in the different vegetation types (Chapter 4). I used the previously studied datasets of soil, vegetation and topographical characteristics and anthropogenic disturbance at both local and landscape scales to identify factors related to baseline CO_2 emissions. Controlled drought and drying-rewetting microcosm experiments were conducted to evaluate the resistance and resilience of soil functioning. I found that in Gallery forest, soil CO_2 emissions were positively related to total soil carbon, hydrolytic enzyme activity and elevation. This indicates that soil microbial enzymes are efficient in mineralizing organic matter inputs from vegetation, while water content in soil pores may decrease with elevation in these flooded ecosystems, allowing an increase in soil microbial metabolism. For Veredas, the positive effect of bacterial abundance and negative effect of fungi suggests that the major contribution to soil respiration is derived from root- associated bacterial communities within these grasslands. Here raised elevations may also be important for increasing soil microbial metabolism in wetland conditions. For Cerrado *sensu stricto*, the positive relationship between fungal abundance and soil respiration may reflect the quality of litter inputs in terms of high lignin and cellulose contents, and a greater resistance of fungi to drought compared to bacteria. The negative relationship between bacteria and soil respiration may be a response to the inhibition of bacterial development due to adverse conditions associated with sandy soils. The positive effect of slope on soil respiration suggests that the downward transport of OM is creating pools of C mineralization along a gradient towards gentler inclines.

The results of the controlled drought experiment showed drought affected soil respiration in Gallery Forest and Veredas soils, but not Cerrado *sensu stricto* soils. Veredas had the most resistant soils, as soil respiration remained unchanged for a longer period of time compared to Gallery Forests under increasingly dry conditions. Rewetting increased soil CO_2 fluxes in both Veredas and Gallery Forests significantly after the drought. Soil respiration rates from Gallery Forest were found to be most similar to controls two hours after rewetting, suggesting a rapid recovery of function in Gallery Forests soils which were therefore concluded to be the most resilient (Fig 3.8)

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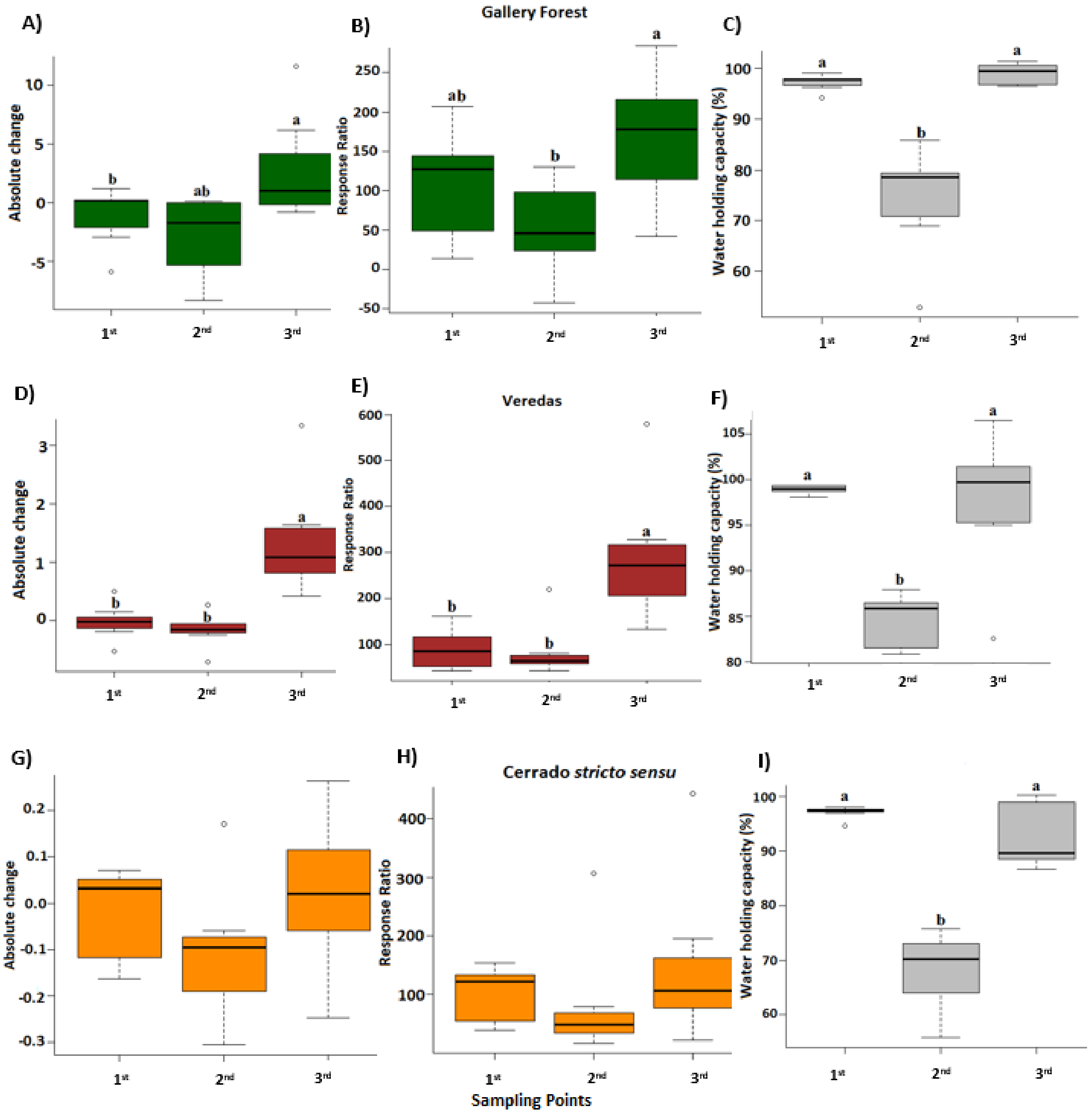
Figure 3.8. - The absolute change, response ratio of soil CO_2 emissions and water holding capacity along the drying and rewetting experiment of the Gallery forest, Veredas and Cerrado *sensu stricto* vegetation types. (A) Absolute changes of soil respiration in Gallery Forest; (B) Response ratio of soil respiration in Gallery forests; (C) water holding capacity in Gallery forests. (D) Absolute changes of soil respiration in Veredas; (E) Response ratio of soil respiration in Veredas; (F) water holding capacity in Veredas. (G) Absolute changes of soil respiration in Cerrado *sensu stricto*; (H) Response ratio of soil respiration in Cerrado *sensu stricto*; (I) water holding capacity in Cerrado *sensu stricto*.



Neither drought nor rewetting significantly affected Cerrado *sensu stricto* soil respiration tested by absolute change and response ratios. Baseline respiration rates for these soils were also by far the lowest of the three vegetation types evaluated in the present study, reflecting the poor organic matter content and low microbial activity in these soils.

The potential implications for more intense droughts associated with predicted climate change scenarios in the Cerrado biome may be a slower recovery of soil functioning after rewetting events in Veredas soils compared to Gallery forests, meaning soils under Veredas vegetation may release CO_2 for longer than Gallery Forests. In addition, as this was the first study evaluating soil respiration in a drought and rewetting cycle in these distinct vegetation types, I recommend that more studies should be carried out with different drought intensities and rewetting frequencies in order for more robust prediction of soil CO_2 release to the atmosphere under future climate change scenarios in the Cerrado biome.

In the Gallery forest and Veredas wetland vegetation types, I found that water content is an important factor in shaping local (Chapter 2) and landscape scale (Chapter 3) soil conditions, an important determinant of ecosystem functioning in terms of nutrient cycling and CO_2 emissions, and adaptation of soil microbial communities to be resistant and resilient to future climate scenarios (Chapter 4). In light of this, I would like to highlight the importance of these wetlands in the global and Brazilian environmental legislative context. The Ramsar Intergovernmental Convention recommend the integral preservation of wetland ecosystems worldwide, considering wetlands as all regions that periodically flood. Wetlands are globally vital for human survival, biodiversity, ecosystem services, productivity, carbon storage, groundwater recharge and climate change mitigation (RAMSAR, 2015). Due to their importance, the Brazilian Environmental Legislation classify Gallery forests and Veredas as Areas of Permanent Protection (APP). Current legislation (BRASIL, 2012) states criteria for protecting areas of Gallery forest and Veredas: the minimum width of Gallery forest riparian buffer zones (i.e. the distance from the river) to be protected is calculated relative to the ‘regular’ (non-flooded) river width (i.e. during the dry season); Veredas wetland within a minimum buffer of 50 meters around a ‘permanent’ flooded swamp (which also suggest to be measured during the dry season) is required to be protected. For wetlands already degraded before 2008, the area required to be restored is dependent upon the size of individual farms/properties. However, designating areas of protected wetland based on ‘regular’ dry season river and swamp widths ignores their real size, as these ecosystems are strongly influenced by the maximum periodical flooding potential (JUNK et al., 2014). Because of the influence of soil water content and flooding regimes on soil abiotic and biotic properties and implications for ecosystem function in Gallery forest and Veredas found in this thesis, I recommend protected wetland areas in Brazilian Cerrado to be designated in a more ecologically-relevant way according to maximum periodical flooding potential of rivers and swamps, rather than being relative to ‘regular’ water body extents based on dry season measures.

In conclusion, evaluation of local- and landscape-scale factors affecting soil microbial attributes identified important mechanisms underlying ecosystem functioning in the three typical vegetation types of Neotropical savanna. For both wetland vegetation types, soil moisture content is particularly important in shaping soil properties and subsequent soil microbial attributes, with vegetation interacting in a bidirectional relationship with the soil environment, therefore also an important component nutrient conservation and cycling in these ecosystems. For Cerrado *sensu stricto*, vegetation characteristics are particularly important for soil ecosystem functioning, especially in the sandy soils of the study region. Alterations in soil microbial attributes caused by anthropogenic disturbance were reported for all vegetation types at the landscape scale. Veredas was found to be the vegetation type most resistant to drought, whereas Gallery forest were the most resilient. Future research should aim to identify soil microbial communities in Cerrado ecosystems using Next-Generation Sequencing techniques, and their relation to organic matter decomposition and litter quality in the different vegetation types. Furthermore, more controlled climate manipulation studies should be undertaken with different drought intensities and rewetting frequencies to improve predictions of soil CO_2 fluxes to the atmosphere under future climate change scenarios. As flooding regimes are a hugely important mechanism underlying ecosystem functioning in Gallery forest and Veredas, I recommend that Brazilian Environmental Legislation should consider maximum periodical flooding potentials of rivers and swamps for a more ecologically-relevant designation of protected wetland areas, considering integral conservation of these vital ecosystems in order to mitigate against the negative impacts of land use and climate change.

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