UNIVERSIDADE DE BRASÍLIA - UnB

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

PÓS-GRADUAÇÃO EM BIOLOGIA MICROBIANA

Diversidade microbiana do solo de área nativa do Cerrado com adição de nutrientes a longo prazo.

Áurea Christie Vasconcelos Santos

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Dissertação apresentada como requisito parcial para a obtenção do título de Mestre em Biologia Microbiana pelo Programa de Pós-Graduação em Biologia Microbiana da Universidade de Brasília.

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“Come and join me in my rotation
We won't be held by no bars
Our reunion was tagged the celestial
The very moment stars got us baptized
Stars

Wave your hand from parallel universe
Hidden in a folded palm
After twenty-eight light-years
My research must be ended somehow”

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A adição de nutrientes ao solo é uma prática comum em sistemas agrícolas, mas seus efeitos em ecossistemas naturais, como o Cerrado brasileiro, ainda são pouco compreendidos. Este estudo busca preencher essa lacuna ao investigar os efeitos de longo prazo da adição de nutrientes sobre as comunidades microbianas do solo em uma área nativa de *Cerrado sensu stricto*, simulando um cenário de uso agrícola. O experimento contempla cinco tratamentos distintos: controle (sem adição de nutrientes), cálcio (Ca), nitrogênio (N), nitrogênio + fósforo (NP) e fósforo (P). Diversas abordagens foram utilizadas para essa avaliação. A qualidade nutricional do solo foi analisada por meio da determinação de macro e micronutrientes e da atividade de enzimas-chave dos ciclos do carbono, fósforo e enxofre (β-glicosidase, fosfatase ácida e arilsulfatase). A qualidade geral do solo foi estimada utilizando o índice BioAS, integrando dados físicos, químicos e biológicos. A estrutura e diversidade das comunidades microbianas foram avaliadas por meio de metagenômica, com foco na abundância relativa de fungos e bactérias. Os resultados revelaram que todos os tratamentos com adição de nutrientes promoveram alterações significativas no solo em comparação ao controle. A calagem (Ca) foi o tratamento que apresentou os efeitos mais abrangentes, principalmente no aumento da disponibilidade de nutrientes e da atividade enzimática, ao mesmo tempo em que favoreceu comunidades microbianas específicas, como Gammaproteobacteria, Acidobacteria subgrupo 2 e fungos da ordem Agaricales. Também foram observadas melhorias nos atributos físico-químicos do solo, com aumento do pH, redução do alumínio trocável e maior disponibilidade de nutrientes essenciais — fatores que podem favorecer o estabelecimento de plantas não nativas e alterar a dinâmica ecológica da vegetação local. Nos tratamentos com fósforo (P), nitrogênio (N) e sua combinação (NP), também foram observados impactos relevantes, especialmente na composição e diversidade das comunidades microbianas. Houve aumento da abundância de Myxococcales e de fungos celulolíticos como Pleosporales, além de alterações nos padrões de diversidade fúngica e bacteriana. Esses tratamentos também modificaram parâmetros bioquímicos do solo, como a atividade enzimática diferenciada entre os ciclos biogeoquímicos e os índices de qualidade do solo BioAS, com destaque para as funções de ciclagem e armazenamento de nutrientes. Esses resultados indicam que, embora a calagem seja fundamental para a correção da acidez e melhoria dos aspectos físico-químicos do solo, a adição de N e P contribui de forma complementar, promovendo mudanças na estrutura e função microbiana que refletem diretamente na sustentabilidade e funcionamento ecológico do solo do Cerrado.

Palavras-chave: adição de nutrientes, calagem, qualidade do solo, diversidade microbiana, Cerrado

4. ABSTRACT

The addition of nutrients to soil is a common practice in agricultural systems, but its effects on natural ecosystems, such as the Brazilian Cerrado, remain poorly understood. This study aims to fill this gap by investigating the long-term effects of nutrient addition on soil microbial communities in a native Cerrado *sensu stricto* area, simulating an agricultural use scenario. The experiment includes five distinct treatments: control (no nutrient addition), calcium (Ca), nitrogen (N), nitrogen + phosphorus (NP), and phosphorus (P). Multiple approaches were used for this assessment. Soil nutritional quality was analyzed through the determination of macro- and micronutrients and the activity of key enzymes involved in the carbon, phosphorus, and sulfur cycles (β-glucosidase, acid phosphatase, and arylsulfatase). Overall soil quality was estimated using the BioAS index, which integrates physical, chemical, and biological data. The structure and diversity of microbial communities were assessed using metagenomics, focusing on the relative abundance of fungi and bacteria. The results showed that all nutrient addition treatments led to significant changes in the soil compared to the control. Liming (Ca) was the treatment with the most comprehensive effects, notably increasing nutrient availability and enzymatic activity, while also favoring specific microbial communities such as Gammaproteobacteria, Acidobacteria subgroup 2, and fungi from the order Agaricales. Improvements in the soil's physico-chemical attributes were also observed, including increased pH, reduced exchangeable aluminum, and greater availability of essential nutrients — factors that may favor the establishment of non-native plant species and alter the ecological dynamics of local vegetation. The phosphorus (P), nitrogen (N), and combined (NP) treatments also produced relevant impacts, particularly on the composition and diversity of microbial communities. There was an increase in the abundance of Myxococcales and cellulose-degrading fungi such as Pleosporales, along with shifts in fungal and bacterial diversity patterns. These treatments also altered soil biochemical parameters, such as enzyme activity across biogeochemical cycles and BioAS soil quality indices, with emphasis on nutrient cycling and storage functions. These results indicate that, although liming is essential for correcting soil acidity and improving its physico-chemical properties, the addition of N and P nutrients plays a complementary role by promoting changes in microbial structure and function that directly affect the ecological sustainability and functioning of Cerrado soils.

Key words: nutrient addition, liming, soil quality, microbial diversity, Cerrado.

INTRODUÇÃO GERAL

O Cerrado, segundo maior bioma do Brasil, ocupa cerca de um quarto do território nacional e é reconhecido globalmente como *hotspot* de biodiversidade. Além de abrigar milhares de espécies e uma alta heterogeneidade fisionômica, esse bioma presta serviços ecossistêmicos essenciais, como a conservação de recursos hídricos e a regulação climática regional. Em um contexto de rápidas mudanças no uso da terra, compreender os mecanismos que sustentam sua funcionalidade ecológica torna-se imperativo.

Os solos do Cerrado são, em geral, profundamente intemperizados, ácidos, com baixa disponibilidade de nutrientes e elevada porosidade. Apesar dessa limitação edáfica para a agricultura convencional, esses solos mantêm elevada diversidade biológica, sugerindo o papel crucial das comunidades microbianas na ciclagem de nutrientes e na manutenção da fertilidade natural. Microrganismos do solo conduzem processos como decomposição da matéria orgânica, mineralização, imobilização e solubilização de nutrientes — pilares da saúde e da qualidade do solo.

A expansão agropecuária sobre o Cerrado tem intensificado o uso de fertilizantes e corretivos (por exemplo, cálcio, nitrogênio e fósforo), alterando atributos físico-químicos e biológicos do solo e potencialmente erodindo a biodiversidade microbiana. Estudos prévios mostram que a adição de nutrientes modifica a composição da vegetação, favorece plantas exóticas e afeta ciclos biogeoquímicos. Entretanto, os efeitos de longo prazo dessas práticas sobre a estrutura e a função das comunidades microbianas em áreas naturais ou semi-naturais permanecem pouco elucidados.

Nesse cenário, a Reserva Ecológica do IBGE, inserida na APA Cabeça-de-Veado (DF) e integrante do Programa de Pesquisas Ecológicas de Longa Duração (PELD), constitui um laboratório natural único. Desde 1998, parcelas de Cerrado nativo recebem adição isolada e combinada de cálcio, nitrogênio e fósforo, além de parcelas controle, permitindo avaliar impactos cumulativos da fertilização. Contudo, a maior parte dos estudos anteriores baseou-se em microrganismos cultiváveis ou em poucos marcadores moleculares, o que limita a compreensão abrangente da diversidade e das funções microbianas. O advento do sequenciamento de nova geração (NGS) supera essas limitações ao revelar frações não cultiváveis, grupos raros e potenciais funcionais.

Diante disso, esta dissertação investiga como a adição de nutrientes afeta a qualidade do solo e a diversidade microbiana em um ecossistema de Cerrado sensu stricto. A pergunta central que orienta o trabalho é: em que medida a adição de cálcio, nitrogênio e fósforo — isoladamente ou em combinação — altera os atributos físico-químicos, bioquímicos e a estrutura das comunidades microbianas do solo em longo prazo? Para responde-la, integram-se análises físico-químicas, enzimáticas e metagenômicas, buscando conectar mudanças ambientais induzidas pela fertilização à composição e aos potenciais funcionais da microbiota.

Este estudo pretende preencher a lacuna sobre os efeitos de longo prazo da fertilização em solos de Cerrado nativo, oferecendo evidências que possam subsidiar estratégias de conservação do solo e da biodiversidade subterrânea, bem como orientar políticas de manejo em ecossistemas savânicos tropicais. A dissertação está organizada em capítulos: (i) um capítulo de fundamentação teórica sobre índices de qualidade do solo, enzimas, teor nutricional e principais grupos microbianos do Cerrado; e (ii) um capítulo em formato de artigo científico apresentando os principais resultados e implicações ecológicas da pesquisa.

5. OBJETIVOS E HIPÓTESES

		Objetivos	Hipóteses
Geral:		- Estimar a qualidade do solo ao mesmo tempo em que avalia a estrutura e composição das comunidades microbianas presentes no solo.	- A qualidade do solo nas áreas modificadas melhorou com a adição de nutrientes em relação ao controle; - As áreas com adição de nutrientes são mais ricas em relação as comunidades de fungos e bactérias comparadas com o controle.
Específico	**Capítulo 1**	Realizar um levantamento teórico do estado da arte sobre: **a)** a qualidade do solo; **b)** as principais enzimas do solo utilizadas como indicadoras de qualidade; **c)** os índices utilizados como avaliadores da qualidade; **d)** os microrganismos mais frequentes no solo do Cerrado; **e)** efeitos dos fatores bióticos e abióticos relacionados a adição de nutrientes em solos a longo prazo.	
	Capítulo 2	- Avaliar a qualidade do	- A qualidade do solo

	solo em relação as principais enzimas de ciclos importantes do solo;	aumenta com a adição de nutrientes se for comparado com o controle;
	- Estimar a qualidade do solo utilizando técnicas atuais frente as modificações que foram feitas no solo;	- As enzimas do solo aumentam com a adição de nutrientes quando comparadas com o controle;
	- Descrever as comunidades microbianas do Cerrado em relação as modificações realizadas na área;	- A comunidade microbiana do solo modifica sua abundância relativa com a adição de nutrientes aumentando seus índices de riqueza e abundância dos principais grupos microbianos quando comparados com os microrganismos da área controle;
	- Avaliar o quanto as modificações realizadas no solo alteraram sua estrutura tanto biótica quanto abiótica.	- As modificações realizadas no solo favorecem questões bióticas e abióticas no solo como sua qualidade e fatores nutricionais comparadas com o controle.

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6. CAPÍTULO 1 - REVISÃO DE LITERATURA

6.1. O bioma Cerrado

O Brasil possui vários biomas sendo que o maior deles é a Amazônia, seguido do Cerrado. O Cerrado se estende ao norte, pelas margens da Floresta Amazônica, até os estados do Paraná e São Paulo, no sul e sudeste, possui clima de savana com temperaturas médias entre 18 e 28°C, contém solo tipicamente com baixo pH e suas fitofisionomias variam desde matas de galeria acompanhando corpos d'água, áreas com arbustos e árvores baixas e até áreas somente de gramíneas (Ratter, 1997).

As diferenças nas fisionomias do Cerrado e seu regime de chuvas marcado por precipitações em abundância seguida de vários meses de estiagem é um grande seletor dos microrganismos. São encontrados mais microrganismos com genes relacionados ao estresse hídrico, captação de ferro, formação de parede celular e formação de esporos, ou dormência das células principalmente na época seca (De Castro et al., 2016). Também nesse estudo, os autores observaram que nos períodos chuvosos, os genes associados com aminoácidos, metabolismo de proteínas e ciclo celular aumentavam sua abundância relativa, indicando que quando a água está mais disponível, várias comunidades microbianas proliferam.

As chuvas também influenciam o ritmo em que ocorre a mineralização/imobilização de nitrogênio, tendo seu pico no início das chuvas com alto índice de imobilização, seguido de um período de mineralização do nutriente (Nardoto et al., 2003). Estudos avaliando a abundância e diversidade de microrganismos do solo fornecem informações importantes sobre a saúde do solo, seus componentes microbiológicos, as atividades que os microrganismos desempenham naquele ambiente e a existência ou não de patógenos importantes na área.

6.2. Adição de nutrientes

Os impactos da adição de nutrientes no solo em relação a comunidade microbiana estão sendo estudados em várias áreas do planeta. Na estação ecológica da província de Qianyanzhou, na China, fósforo, nitrogênio e potássio vem sendo adicionados no solo anualmente desde o ano 2000 (Wang et al., 2019). Nesse experimento de longo prazo, a adição de nutrientes, especialmente nos tratamentos com maiores doses de fertilizantes como N4P4K4 e N3P1K1, resultou em aumento da abundância do *Nitrospira* Clado A (73 bp T-RF). Em contrapartida, a abundância do Clado B (198 bp T-RF) diminuiu gradativamente com a intensificação dos tratamentos de fertilização, em comparação ao controle. O Clado A de *Nitrospira* é associado a ambientes com maior disponibilidade de amônia e nutrientes, enquanto o Clado B predomina em condições oligotróficas, com baixa concentração de substratos. Ambos são importantes no ciclo do nitrogênio por realizarem a oxidação completa da amônia a nitrato, influenciando a fertilidade do solo e a emissão de gases como N_2O (Li et al., 2023a).

Também sobre a adição de nutrientes, na mesma área do nosso estudo, foi investigada a abundância do gene *nif*H, presente no filo de Proteobactérias, que possui vários grupos de bactérias fixadoras de nitrogênio (Silveira et al., 2021b). Tal gene codifica o complexo enzimático nitrogenase redutase em Bacteria e Archaea capaz de catalisar as reações de redução do gás N_2 a NH_4^+ (Zehr et al., 2003). O resultado desse estudo apontou que o gene de interesse aumentava sua riqueza pela chegada da época das chuvas no Cerrado brasileiro, e diminuía em relação a adição de nutrientes, enfatizando o papel das chuvas nesse bioma.

Atualmente, as recomendações de adubação são baseadas em análises químicas do solo e nas necessidades nutricionais das culturas, buscando maximizar a produtividade sem comprometer a sustentabilidade (EMBRAPA, 2016; FAO, 2019). No entanto, estudos como o de Costa et al., (2021) que investigaram os efeitos de 20 anos de adição de N e P no Cerrado, mostram que o enriquecimento nutricional pode provocar mudanças profundas na anatomia e funcionamento hidráulico das plantas, na dinâmica da água e na composição da comunidade vegetal. Essas informações fornecem subsídios para ajustar as doses de fertilizantes, evitando a eutrofização e a perda de biodiversidade, e promovendo um manejo agrícola que respeite os limites ecológicos dos ecossistemas tropicais

Em estudo de 2011 na área de interesse, avaliando os efeitos da adição de nutrientes com a riqueza e uniformidade das espécies microbianas, foi elucidado que a adição de fósforo e nitrogênio concomitante era mais prejudicial para os parâmetros de riqueza e abundância se comparados com os mesmos parâmetros na área controle (Jacobson et al., 2011).

Em áreas agrícolas, a adição contínua de nutrientes, especialmente nitrogênio e fósforo, tem provocado alterações marcantes na comunidade microbiana do solo, reduzindo a diversidade de grupos funcionalmente importantes e promovendo o crescimento de microrganismos adaptados a ambientes enriquecidos em nutrientes (Liu et al., 2024; Zhang et al., 2025b). Dai et al., (2018) observaram que solos agrícolas submetidos à fertilização nitrogenada intensiva apresentaram uma diminuição significativa na diversidade de bactérias do filo Acidobacteria, consideradas oligotróficas, quando comparadas a solos nativos. De forma semelhante, (Byers et al., 2024) verificaram que a fertilização contínua em campos agrícolas reduziu a complexidade das redes de co-ocorrência microbiana, enquanto em áreas nativas essas redes apresentavam maior conectividade e resiliência frente a perturbações ambientais. Esses resultados reforçam que o manejo intensivo do solo com fertilizantes em áreas agrícolas pode comprometer a estabilidade da microbiota e afetar funções ecossistêmicas, ao passo que áreas nativas tendem a manter comunidades mais equilibradas e funcionais (Wang et al., 2024a).

A introdução de calcário altera o equilíbrio redox do solo, modificando a mobilidade de elementos como ferro e manganês, além de influenciar o pH e a toxicidade em solos ácidos. Alterações no potencial redox geralmente decorrem da combinação entre oxigenação, teor de matéria orgânica, nutrientes e pH — influenciando diretamente processos de oxidorredução e disponibilidade de micronutrientes (Camargo et al., 1999). Flutuações no potencial redox do solo promovem maior diversidade e atividade microbiana, enquanto condições redox estáticas (altamente oxidantes ou redutoras) selecionam comunidades menos diversas e menos dinâmicas (DeAngelis et al., 2010).

6.3. Fertilização do solo e ecologia microbiana

A área do presente estudo, está inserida no âmbito do projeto "Estudo dos efeitos das mudanças globais que determinam a estrutura e funcionamento de ecossistemas do Cerrado". Este programa foi inserido no escopo da iniciativa do Programa de Pesquisas Ecológicas de Longa Duração (PELD), inaugurado em 1996 (Ministério da Ciência Tecnologia e Inovação, 1996). O programa visa examinar e monitorar a evolução das relações ecológicas ao longo do tempo, com ênfase na preservação da biodiversidade e na utilização sustentável dos recursos naturais em diversas localidades do Brasil (https://www.gov.br/cnpq/pt-br/acesso-a-informacao/acoes-e-programas/programas/peld/sitios-peld-vigenteshttp://www.peld.unb.br/). Alguns dos trabalhos já realizados na área do nosso estudo estão demonstrados na Figura 1.

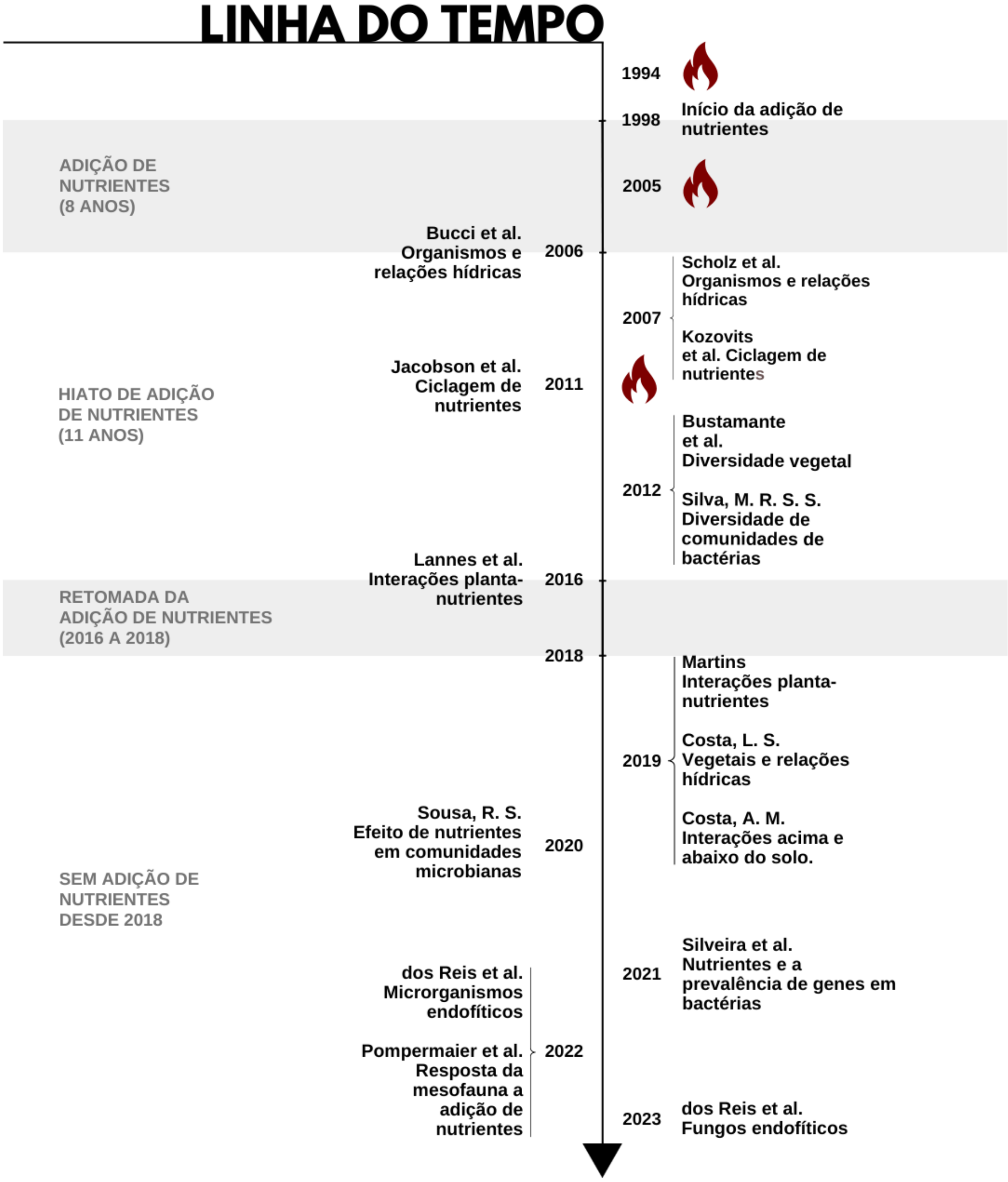

Figura 1: Esquema com a linha do tempo dos trabalhos realizados na área, os anos em que foram adicionados nutrientes e anos em que ocorreram queimadas na área (1994, 2005 e 2011). Fonte próprio autor.

Estudos direcionados para os efeitos das ações antropogênicas nos variados ambientes são imprescindíveis para identificar áreas em que essa perturbação causa um maior ou menor dano, visto que as ações humanas no meio ambiente não são homogêneas, e mesmo com os esforços existentes para proteger a biodiversidade, esses ambientes podem, ainda assim, se tornar bastante fragmentados (Bezerra et al., 2022). Já foi observado, em simulações, a substituição de áreas de floresta tropical por áreas de Cerrado devido às mudanças climáticas, associadas à padrões erráticos de precipitação, que são bastante influenciados por atividades antropogênicas (Maksic et al., 2022; Silva Junior et al., 2019). Várias modificações no uso da terra estão relacionadas à qualidade ambiental, em que a presença ou ausência de vegetação nativa modifica o grau de vulnerabilidade do ambiente, possui impactos na diversidade ali presente e, consequentemente, causa a perda de funções existentes anteriormente à perturbação (Trevisan et al., 2020).

O solo é um dos ecossistemas mais complexos e diversos do planeta, abrigando comunidades microbianas que desempenham papéis essenciais na evolução e adaptação das plantas. Estudos recentes demonstram que a associação entre microrganismos simbiontes e plantas, como as bactérias fixadoras de nitrogênio do gênero *Mesorhizobium*, tem raízes evolutivas profundas, sendo um mecanismo vantajoso que acompanha as plantas desde seus ancestrais primitivos, garantindo o acesso a nutrientes essenciais e contribuindo para a resiliência das espécies (Greenlon et al., 2019). A análise genômica em escala global realizada por Greenlon e colaboradores revelou que a diversidade e a distribuição geográfica desses simbiontes são fortemente moldadas por fatores edáficos e evolutivos, evidenciando que a simbiose foi uma força determinante na expansão e diversificação das plantas ao longo do tempo.

Dentre as características predominantes dos solos do Cerrado, estão a alta concentração de alumínio, pH ácido e baixa concentração de nutrientes (Aslam et al., 2022; Lira-Martins et al., 2022). O pH é um dos fatores que modifica várias funções dentro do solo, alterando comunidades microbianas, assim como a disponibilidade de alguns elementos (Lammel et al., 2018). Muitos representantes dos filos Proteobacteria, Actinobacteria e Firmicutes em solos do Cerrado exibem padrão oligotrófico — adaptados a solos ácidos e pobres em nutrientes, algumas linhagens conseguem crescer mesmo sob baixas concentrações de carbono e fósforo (default contaminação) — sugerindo estratégias metabólicas que favorecem sua persistência nesses ambientes de baixa fertilidade (De Castro et al., 2013; Souza et al., 2016). Além disso, diversos gêneros dentro desses filos isolados de solos do Cerrado, como *Burkholderia* (fixadores de nitrogênio) e *Bacillus* e *Pseudomonas* (solubilizadores de fósforo), além de endofíticos e simbiontes associados a leguminosas nativas como *Mimosa*, desempenham funções chave no ciclo de nutrientes em ecossistemas oligotróficos (Dos Reis Jr et al., 2010)

O pH é reconhecido como um dos principais preditores da diversidade microbiana do solo, pois valores mais ácidos estão associados a comunidades menos diversas e menor disponibilidade de nutrientes, enquanto a classificação do solo fornece informações adicionais que ajudam a explicar a variabilidade dessas comunidades além das variações de pH (Kaminsky et al., 2017). As comunidades microbianas são diversas tanto horizontalmente no solo, quanto em camadas mais profundas, sendo que alterações na carga nutricional, variações de pH, concentração de argila e silte influenciam a diversidade de microrganismos no ambiente com funções fisiológicas e ecológicas distintas (Liao et al., 2021).

Um dos macronutrientes essenciais para o desenvolvimento das plantas é o fósforo, sua deficiência modifica a arquitetura das raízes, inibe o crescimento de brotos e o desenvolvimento de sementes, limitando assim, o rendimento da plantação (Rajamanickam et al., 2024). Para uma melhor absorção do P, é necessário que exista água no ambiente para que ocorra processos de difusão, transporte e redistribuição do nutriente (Yao et al., 2021). A disponibilidade de água desempenha papel crucial na absorção de fósforo pelas plantas, uma vez que a mobilidade desse nutriente no solo ocorre predominantemente por difusão, processo severamente limitado em condições de seca, o que agrava a deficiência de P e compromete o crescimento e a produtividade vegetal (Aslam et al., 2022). Fungos micorrízicos arbusculares (AMF), como *Glomus intraradices*, desempenham papel destacado na absorção de fósforo por plantas em solos com alta capacidade de fixação de P, mesmo quando isso não resulta em aumento imediato de biomassa vegetal (Li et al., 2006). Em solos de Cerrado, espécies de AMF como *Acaulospora* e *Glomus* são comuns em raízes de gramíneas e contribuem significativamente para a aquisição de P em ambientes de baixa fertilidade (Dos Santos et al., 2022)

A fertilização química fornece bastante fósforo ao solo, porém a maior parte desse mineral pode ser adsorvida no solo, devido a alta afinidade desse nutriente com metais como o ferro e o alumínio (Wu et al., 2018). Esse mineral também pode ser incorporado às partículas do solo por difusão no estado sólido, o que torna esse mineral indisponível para absorção pelas plantas.

6.4. Microrganismos do solo

O Cerrado natural e a sua microbiota são bastante influenciados pelo pH e pelo conteúdo de alumínio no solo, já em áreas agrícolas, o perfil microbiano se correlaciona mais com níveis de nitrogênio, fósforo, cálcio e magnésio ali presentes (De Souza et al., 2021), de forma que o Cerrado natural apresenta maior diversidade funcional se comparado às áreas agrícolas (Reis et al., 2025).

Alguns microrganismos do solo, principalmente os dos gêneros *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Serratia*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter* e *Flavobacterium* (Rodríguez et al., 1999), possuem a capacidade de facilitar a absorção de fósforo pelas plantas. As estratégias utilizadas por esses microrganismos são variadas e incluem a excreção de íons hidrogênio, produção de sideróforos, produção de fosfatases (Mendes et al., 2003) e a liberação de ácidos como o citrato, que podem facilitar a disponibilidade do fósforo para as plantas. A presença desses solubilizadores melhora a captação desse mineral pelos vegetais, melhorando a nutrição e auxiliando no seu crescimento (Vargas Hoyos et al., 2021), além de serem ótimos biorremediadores de solo por reduzirem a eutrofização, aumentar o sequestro de carbono e aumentar a chance de plantas se estabelecerem em áreas degradadas (Wang et al., 2023b).

6.5. Enzimas do solo como bioindicadores

Enzimas extracelulares (como β-glucosidase, fosfatase, urease, desidrogenase) são importantes na decomposição da matéria orgânica, na ciclagem de carbono, nitrogênio, fósforo e enxofre. Essas, e outras enzimas, são altamente sensíveis a fatores ambientais (pH, umidade, temperatura) e, por isso, são ferramentas eficazes para monitoramento da qualidade do solo (Lee et al., 2020). A atividade enzimática do solo reflete mudanças rápidas por estresses ambientais ou práticas agrícolas, correlacionam-se bem com produtividade vegetal e atributos como matéria orgânica, biomassa microbiana e fertilidade do solo (Namli et al., 2010).

A fosfatase é uma enzima amplamente utilizada como indicador da atividade microbiana relacionada ao ciclo do fósforo no solo. Essa enzima catalisa a liberação de íons fosfato a partir de compostos orgânicos fosforados, tornando esse nutriente disponível para as plantas e outros organismos do solo. A atividade da fosfatase é fortemente influenciada pela presença de matéria orgânica, pela acidez do solo e pela demanda biológica por fósforo. Microrganismos como fungos micorrízicos e bactérias solubilizadoras de fósforo são os principais produtores dessa enzima, especialmente em solos com baixa disponibilidade de fósforo inorgânico (Tabatabai; Bremner, 1969; Thapa et al., 2021). A alta atividade da fosfatase é normalmente associada a solos mais férteis ou manejados com práticas que promovem a atividade biológica, como a adição de compostos orgânicos.

A β-glicosidase está associada ao ciclo do carbono e é responsável pela degradação de oligossacarídeos derivados da celulose, como celobiose, em glicose — uma fonte de energia para os microrganismos do solo. Essa enzima desempenha papel essencial na decomposição da matéria orgânica, especialmente da celulose vegetal, sendo secretada por bactérias, fungos e actinobactérias que colonizam resíduos vegetais. A β-glicosidase é considerada um indicador sensível das alterações causadas por diferentes usos da terra e práticas de manejo, devido à sua estreita ligação com a qualidade da matéria orgânica e à atividade microbiana ativa (Eivazi; Tabatabai, 1988; Fernández-Calviño et al., 2010). Solos com elevada atividade dessa enzima tendem a apresentar maior taxa de mineralização de carbono e maior fertilidade biológica.

A arilsulfatase é uma enzima chave no ciclo do enxofre no solo, atuando na mineralização de compostos orgânicos sulfurados e liberando íons sulfato (SO_4^{2-}) disponíveis para absorção pelas plantas. Sua atividade está ligada principalmente à presença de microrganismos heterotróficos do solo, como bactérias do gênero *Pseudomonas* e fungos decompositores, que participam da degradação da matéria orgânica sulfurada. A arilsulfatase é sensível a variações no teor de matéria orgânica, ao pH do solo e ao uso de fertilizantes, sendo um bom indicador da qualidade biológica em solos manejados de forma sustentável ou sob práticas conservacionistas (Dick, 2015; Tabatabai et al., 1970). A redução de sua atividade pode indicar empobrecimento biológico ou desequilíbrios no ciclo do enxofre.

Outras enzimas também são importantes para o solo como a urease. Produzida por microrganismos e raízes de plantas, a urease catalisa a conversão da ureia (um fertilizante comum) em amônia (NH_3) e dióxido de carbono (CO_2). Essa reação é essencial no ciclo do nitrogênio, pois transforma o nitrogênio da ureia em formas assimiláveis pelas plantas. No entanto, sua atividade excessiva pode levar à volatilização de amônia e perdas de nitrogênio no solo (Tabatabai et al., 1972). A enzima 2. N-acetil-glucosaminidase (NAGase) está envolvida na degradação da quitina, um polímero encontrado nas paredes celulares de fungos e nos exoesqueletos de insetos. Produzida principalmente por bactérias e fungos, a NAGase atua no ciclo do nitrogênio, liberando compostos nitrogenados da matéria orgânica microbiana em decomposição. Sua atividade é um indicador sensível da reciclagem de nutrientes em solos ricos em matéria orgânica (Guthrie et al., 2005). Fenoloxidases e Peroxidases são produzidas por fungos e alguns tipos de bactérias e estão envolvidas na decomposição de compostos recalcitrantes, como a lignina e os ácidos húmicos, presentes na matéria orgânica complexa. Essas enzimas são fundamentais no ciclo do carbono, pois ajudam a liberar carbono de estruturas resistentes à degradação. Além disso, desempenham papel na formação de húmus estável no solo (Sinsabaugh, 2010).

6.6. Índices de qualidade do solo

A qualidade do solo é um conceito amplo que envolve a capacidade do solo de funcionar como um sistema vivo, sustentando a produtividade das plantas tanto nativas quanto cultivadas, mantendo a qualidade ambiental e promovendo a saúde dos organismos que dele dependem. Para avaliar essa qualidade, utilizam-se os chamados Índices de Qualidade do Solo (IQS), que integram diferentes indicadores físicos, químicos e biológicos em uma medida única e interpretável. Esses índices permitem o diagnóstico do estado atual do solo, possibilitando intervenções no manejo para melhorar sua sustentabilidade (Andrews et al., 2004; Cherubinet al., 2017; Silva et al., 2019).

Entre os componentes dos IQS, destacam-se atributos como o pH, a matéria orgânica, a capacidade de troca de cátions (CTC), a densidade do solo, a estabilidade de agregados e indicadores biológicos, como a atividade enzimática e a respiração microbiana. A escolha e ponderação desses indicadores dependem do objetivo do estudo e do uso do solo, podendo variar entre ambientes agrícolas, florestais ou conservados. Além disso, a análise integrada desses parâmetros permite compreender melhor os efeitos do manejo agrícola, da fertilização e da compactação sobre a saúde do solo (Karlen et al., 2003).

No Brasil, um dos principais referenciais sobre a avaliação da fertilidade e qualidade do solo é o manual FERTBIO – Manual de Fertilidade do Solo, elaborado pela

Embrapa e pela Sociedade Brasileira de Ciência do Solo. Este documento fornece orientações técnicas para análise e interpretação de dados laboratoriais, com foco na nutrição de plantas e na conservação dos solos em sistemas tropicais. O FERTBIO reforça que a fertilidade não deve ser vista isoladamente, mas sim como parte de um conjunto maior de atributos que determinam a capacidade produtiva e ecológica do solo (Mendes et al., 2018). O índice FERTBIO, embora desenvolvido para áreas agrícolas, fornece uma abordagem integradora de atributos químicos, físicos e biológicos do solo, permitindo quantificar alterações funcionais causadas pela adição de nutrientes em solos nativos. Ao aplicar o FERTBIO em solos sob vegetação nativa, é possível avaliar o grau de antropização e a resposta do ecossistema a distúrbios que simulam práticas agrícolas, fornecendo uma métrica comparável entre sistemas naturais e manejados.

Além do FERTBIO, outros índices amplamente utilizados incluem o *Soil Management Assessment Framework* (SMAF), desenvolvido nos Estados Unidos, que propõe um sistema quantitativo de avaliação da qualidade do solo com base em diferentes usos e contextos ambientais (Andrews et al., 2004). O SMAF, assim como outras metodologias, utiliza uma abordagem de normalização dos dados, atribuição de pesos e integração dos resultados em um índice composto, permitindo comparações entre áreas ou ao longo do tempo. Ferramentas como essas vêm sendo adaptadas e aplicadas em solos tropicais para avaliar impactos de práticas agrícolas e políticas de uso do solo.

Diversos países têm desenvolvido indicadores de qualidade do solo adaptados às suas realidades ecológicas e sistemas de uso. Nos Estados Unidos, por exemplo, o programa *Soil Health Initiative*, promovido pelo USDA-NRCS (*United States Department of Agriculture – Natural Resources Conservation Service*), adota um conjunto de indicadores básicos e acessíveis para o monitoramento da saúde do solo. Esses indicadores incluem densidade aparente, estabilidade de agregados, infiltração de água, respiração basal do solo, teor de carbono orgânico e análise visual de perfil. Esses atributos são utilizados para construir um índice integrado que fornece uma avaliação geral da saúde edáfica, com o objetivo de orientar práticas agrícolas regenerativas e sustentáveis (Ye et al., 2021)

Na Europa, iniciativas como o *LANDMARK Project* e a *Soil Quality Indicator Set* da Comissão Europeia propõem uma abordagem funcional para avaliação da qualidade do solo, priorizando indicadores relacionados aos serviços ecossistêmicos. Entre os principais indicadores estão: capacidade de retenção de água, mineralização de nitrogênio, biodiversidade microbiana (avaliada via técnicas moleculares como metagenômica), emissão de gases do efeito estufa e resistência à compactação. Esses indicadores são integrados em sistemas multicritérios, com foco na resiliência do solo frente às mudanças climáticas e ao uso intensivo da terra (Valujeva et al., 2020). O uso de abordagens quantitativas e funcionais reflete uma tendência internacional de considerar o solo não apenas como suporte físico, mas como componente dinâmico de agroecossistemas.

6.7. Diversidade e função microbiana e NGS

É estimado que o planeta seja habitado por 10^{11} ou 10^{12} espécies diferentes de microrganismos no planeta, sendo que menos de 10^5 espécies são cultiváveis atualmente em condições de laboratório fazendo com que do total de espécies de microrganismos do planeta, ainda existem 99,999% que ainda não foram descritas e nem possuem suas funções caracterizadas (Locey et al., 2016). Para a avaliação da diversidade de microrganismos do solo, as ferramentas mais utilizadas envolvem o sequenciamento de genes ribossomais, que são genes conservados encontrados em archaea, bactéria e eucarioto e, por serem genes facilmente e rapidamente amplificados por reação em cadeia da polimerase (PCR) (Nkongolo et al., 2020). Com essas técnicas é possível elucidar as comunidades de microrganismos existentes no solo sem a necessidade de cultivo, de forma que sejam identificados. Os organismos do solo sofrem influência pela sua associação com o tipo de uso da terra, da mesma forma que as plantas influenciam no tipo de microrganismo que estará ali presente. Com as técnicas de sequenciamento em larga escala ou *Next Generation Sequencing* (NGS), é possível monitorar a estrutura das comunidades microbianas do solo observando variações em determinado solo submetido a alguma condição específica ou até mesmo ao longo do tempo naquele local (Gschwend et al., 2022).

As tecnologias de NGS revolucionaram a ecologia microbiana ao possibilitar o sequenciamento de DNA dos microrganismos que habitam ambientes complexos, como o solo (DeFord et al., 2024; Soliman et al., 2017), água (Hinlo et al., 2017), endofíticos (Akinsanya et al., 2015; Aleynova et al., 2023), de maneira rápida, precisa e com custos cada vez mais acessíveis. Diferentemente dos métodos tradicionais baseados em cultivo, que acessam apenas uma fração da diversidade microbiana presente no ambiente, o NGS permite aos pesquisadores obterem um panorama abrangente das comunidades microbianas por meio do sequenciamento de genes marcadores (como o 16S rRNA para bactérias e o ITS para fungos) ou até mesmo de metagenomas completos extraídos diretamente de amostras ambientais (Caporaso et al., 2012; Thompson et al., 2017).

Uma das principais vantagens do NGS é sua capacidade de detectar tanto os microrganismos mais abundantes quanto os menos frequentes dentro da comunidade microbiana (Ari et al., 2016). Isso é particularmente relevante em ecossistemas edáficos, que estão entre os habitats mais ricos em diversidade microbiana do planeta. Por meio do NGS, é possível identificar milhares de unidades taxonômicas operacionais (OTUs) ou variantes de sequência de amplicons (ASVs), fornecendo informações detalhadas sobre a riqueza e a estrutura da comunidade microbiana em uma amostra. Além disso, a profundidade do sequenciamento proporciona uma descrição mais precisa e representativa da microbiota, mesmo em amostras com alta complexidade biológica (Tedersoo et al., 2014).

A aplicação de metagenômica e *metabarcoding* transformou radicalmente os estudos sobre os impactos do manejo do solo nas comunidades microbianas, permitindo caracterizar grupos completos de microrganismos não cultiváveis e identificar tanto padrões taxonômicos quanto potenciais funcionalmente ativos diretamente do DNA ambiental. Por exemplo, Fierer, (2017) demonstra que abordagens de sequenciamento de alto desempenho revelam uma diversidade muito maior do solo e fornecem resolução detalhada sobre os fatores que moldam essas comunidades. De forma complementar, o trabalho de Mendes et al., (2011) utilizou metagenômica baseada em *microarray PhyloChip* na rizosfera para identificar mais de 33.000 espécies bacterianas e arqueanas associadas à supressão de patógenos, combinando os dados de *metabarcoding* com análises funcionais ligadas a genes específicos para elucidar mecanismos de controle biológico. Tais tecnologias permitem correlacionar alterações induzidas por práticas de manejo — como fertilização, rotação de culturas e calagem — com modificações na diversidade microbiana, abundância de genes funcionais e potenciais de ciclagem de nutrientes, oferecendo *insights* profundos para avaliação da saúde e resiliência do solo.

A tecnologia de NGS também permite a obtenção de informações funcionais por meio das abordagens de metagenômica e metatranscriptômica. Enquanto o sequenciamento de genes marcadores fornece uma identificação taxonômica das comunidades. Entre as plataformas NGS disponíveis, o sistema Illumina MiSeq tem sido amplamente utilizado em estudos metagenômicos de solo devido à sua alta acurácia, capacidade de gerar leituras pareadas de até 2 × 300 pb e custo relativamente acessível (Myrold et al., 2014; Pérez-Cobas et al., 2020). A tecnologia de sequenciamento por síntese empregada pelo MiSeq permite obter uma visão abrangente da estrutura e função das comunidades microbianas em diferentes contextos ambientais e agrícolas. Estudos recentes demonstram que o uso dessa ferramenta é eficaz para avaliar como práticas de manejo, como adubação e calagem, afetam a diversidade microbiana e os processos biogeoquímicos do solo (Pingel et al., 2023)

Caracterizar integralmente o microbioma do solo por meio do NGS é particularmente relevante nos contextos agrícola e ambiental. As comunidades microbianas do solo desempenham papéis fundamentais na ciclagem de nutrientes, decomposição da matéria orgânica e promoção da saúde vegetal. Ao identificar como a diversidade microbiana responde a práticas de uso do solo, fertilização ou estresses ambientais, o NGS fornece subsídios para a formulação de práticas agrícolas mais sustentáveis. Além disso, certos grupos microbianos podem atuar como bioindicadores da qualidade ou degradação do solo, representando uma poderosa ferramenta de monitoramento ecológico (Banerjee et al., 2019).

Com o sequenciamento em larga escala é possível observar parte da diversidade microbiana do solo, mesmo com a limitação de que sequências mais abundantes serão mais representadas, e que a escolha do iniciador (*primer*) acaba por limitar as sequências que serão amplificadas (Mantri et al., 2021). A técnica de NGS associada a outras técnicas de identificação de microrganismos são capazes de melhorar nosso entendimento da diversidade e estrutura existente no solo, e da dinâmica entre essas populações (Nkongolo et al., 2020). Alguns *primers* utilizados por técnicas de NGS para fungos e bactérias encontram-se na Tabela 1.

Os solos do Cerrado brasileiro abrigam uma comunidade microbiana diversa e complexa, onde bactérias dos filos Proteobacteria, Actinobacteria e Acidobacteria estão entre os mais abundantes (De Araujo et al., 2017). Essas bactérias desempenham papéis fundamentais na ciclagem de nutrientes, degradação da matéria orgânica e promoção do crescimento vegetal. As Proteobacteria são frequentemente associadas à fixação biológica de nitrogênio e à decomposição de compostos orgânicos, contribuindo para a disponibilidade de nitrogênio no solo (Li et al., 2023b). Já as Actinobacteria são conhecidas pela capacidade de decompor matéria orgânica complexa, como lignina e celulose, além de produzirem metabólitos com atividade antimicrobiana que influenciam a dinâmica da microbiota (Silva et al., 2022). As Acidobacteria são adaptadas a solos ácidos e contribuem para a ciclagem de carbono, embora muitas de suas funções ainda estejam sendo elucidadas (Kalam et al., 2020)

Além das bactérias, os fungos também são componentes importantes do microbioma do Cerrado, com gêneros do filo Ascomycota e Basidiomycota frequentemente relatados (Ferreira De Araujo et al., 2017). Esses fungos desempenham papel crucial na decomposição da matéria orgânica, formação de húmus e na simbiose micorrízica, que facilita a absorção de nutrientes pelas plantas em solos geralmente pobres em nutrientes (Ji et al., 2022). Os fungos micorrízicos arbusculares (FMA), em particular, são fundamentais para a adaptação das plantas do Cerrado ao estresse hídrico e baixa fertilidade, melhorando a eficiência na captação de fósforo e outros minerais essenciais (De Souza Buzo et al., 2023). Dessa forma, a diversidade e funcionalidade desses microrganismos são essenciais para a manutenção dos ciclos biogeoquímicos e a sustentabilidade dos ecossistemas do Cerrado.

Tabela 1. Descrição de primers existentes na literatura para NGS.

Primer (NGS)	**Sequência**	**Região**	**Microrganismos**	**Referência**
Bakt 341F	5′- CCTACGGGNGGCWGCAG-3′	V3-V4 do 16S rRNA	Bactérias	(Herlemann et al., 2011)
Bakt 805R	5′-GACTACNVGGGTATCTAATCC-3′			
341F	5′-CCTAYGGGDBGCWSCAG -3′	V3-V4 do 16S rRNA	Bactérias/ Arqueas	(Frey et al., 2016)
806R	5′-GGACTACNVGGGTHTCTAAT -3′			
ITS3	5′-CAHCGATGAAGAACGYRG -3′	ITS2	Fungos	(Frey et al., 2016)
ITS4	5′-TCCTSCGCTTATTGATATGC -3′			
515f	5′-GTGCCAGCMGCCGCGGTAA -3′	V4 do 16S rRNA	Bactérias	(Kozich et al., 2013)
806r	5′-GGACTACHVGGGTWTCTAAT -3′			
gITS7	5′-GTGARTCATCGARTCTTTG -3′	ITS2	Fungos	(Mašínová et al., 2017)
ITS4	5′-TCCTSCGCTTATTGATATGC -3′			
ITS1FI2	5′-GAACCWGCGGARGGATCA-3′	ITS1	Fungos	(Schmidt et al., 2013)
ITS2	5′-GCTGCGTTCTTCATCGATGC-3′			
ITS1	5′-XCTTGGTCATTTAGAGGAAGTAA-3′	ITS	Fungos	(Moussa et al., 2017)
ITS4	5′-YxxxxTCCTCCGCTTATTGATATGC-3′			
787F	5′-GNTACCTTGTTACGACTT-3′	16S rRNA	Bactérias	(Roesch et al., 2007)
1492R	5′-1492R ATTAGATACCCNGGTAG-3′			
EF4F	5′-GGAAGGG[G/A]TGTATTTATTAG-3′	18S rRNA	Fungos	(Smit et al., 1999)
Fung5R	5′-GTAAAAGTCCTGGTTCCCC-3′			

341F **785R**	5′-TCGTCGGCAGCGTCAGATGTGTAT AAGAGCAGCCTACGGGGGCWGCAG-3′ 5′-GTCTCGTGGGCTCGGAGATGT,GTATA AGAGACAGGACTACHVGGGTATCTAATCC-3′	V3-V4 do 16S rDNA	Bactérias	(Klindworth et al., 2013)
ITS1FI2 **5.8S**	5′-TCGTCGGCAGCGTCAGATGTGTATAA GAGACAGGAACCWGCGGARGGATCA-3′ 5′-GTCTCGTGGGCTCGGAGATGTGTATAA GAGACAGCGCTGCGTTCTTCATCG-3′	ITS1 rDNA	Fungos	(Schmidt et al., 2013)

6.8. CONSIDERAÇÕES FINAIS

O Brasil é um país com dimensões continentais e que possui vários biomas distribuídos em seu território. Sendo o Cerrado um dos seus maiores biomas, mesmo assim é uma área pouco estudada em relação a adição de nutrientes no solo, embora já existam muitas práticas agrícolas na área que façam correção nutricional. O solo do Cerrado é especial, por ter características próprias de acidez natural e quantidade de metais como o alumínio, que é tóxico a plantas, intrínseco.

As mudanças climáticas e outros tipos de modificações de uso do solo afetam tanto as características nutricionais do solo, como as comunidades de fungos e bactérias que habitam esse ambiente. Existe uma necessidade urgente de conhecer as comunidades microbianas do solo e saber suas funções e papeis ecológicos tanto no solo do Cerrado quanto em outras áreas do país ainda pouco exploradas.

Os microrganismos do solo realizam várias funções já conhecidas, mas ainda falta muito para que se conheça completamente a função de cada organismo do solo, principalmente os não cultiváveis que, por requererem formas não convencionais para o seu estudo, tornam essa tarefa mais difícil. Sabendo que vários fungos e bactérias participam de ciclos biogeoquímicos fundamentais para garantir a continuação da vida na Terra, a manutenção de seus habitats é fundamental para o equilíbrio ecológico. Esses microrganismos atuam na decomposição da matéria orgânica, na ciclagem de nutrientes como nitrogênio, fósforo e enxofre, na formação e estruturação dos agregados do solo e até mesmo na promoção do crescimento vegetal por meio de associações simbióticas. Assim, perturbações nesses sistemas, como o uso intensivo do solo, a aplicação excessiva de insumos químicos ou o desmatamento, podem causar impactos significativos e irreversíveis na biodiversidade microbiana e nos serviços ecossistêmicos que ela sustenta.

Estudos têm mostrado que os microrganismos do solo respondem de maneira sensível às mudanças nos manejos agrícolas e às alterações ambientais. Essa resposta pode ser observada por meio da abundância relativa de grupos microbianos, da diversidade funcional e da atividade de enzimas relacionadas à ciclagem de nutrientes. No entanto, o conhecimento sobre as dinâmicas microbianas ainda é limitado, especialmente em ecossistemas tropicais como o Cerrado, onde a biodiversidade é extremamente rica, mas pouco explorada. Com o avanço das técnicas de biologia molecular, como o NGS (*metabarcoding*) e a metagenômica, tornou-se possível acessar com maior profundidade os microrganismos não cultiváveis, permitindo uma compreensão mais abrangente de suas funções ecológicas.

A importância de se estudar a microbiota do solo vai além da curiosidade científica: compreender as interações biológicas nesse ambiente é essencial para o desenvolvimento de práticas agrícolas mais sustentáveis, para a recuperação de áreas degradadas e para o enfrentamento das mudanças climáticas. A diversidade microbiana do solo pode atuar como um indicador sensível de qualidade do solo, refletindo não apenas a fertilidade química, mas também a saúde biológica e estrutural do ecossistema. Avaliações baseadas em indicadores biológicos, como a atividade de enzimas do solo, são ferramentas promissoras para monitorar os impactos de práticas de manejo e propor soluções para a conservação e o uso racional do solo.

Além disso, os microrganismos desempenham papel-chave em interações com plantas, auxiliando na absorção de nutrientes, aumentando a resistência a estresses abióticos e bióticos, além de contribuir para a produtividade agrícola. Ao preservar e restaurar comunidades microbianas benéficas no solo, podemos favorecer uma agricultura mais resiliente e menos dependente de insumos externos. Isso destaca a urgência em integrar o conhecimento microbiológico nas políticas públicas e programas de manejo de solos, especialmente em áreas sensíveis e de alta biodiversidade como os biomas brasileiros.

Valorizar e expandir esse tipo de pesquisa é essencial para preservar a integridade dos ecossistemas naturais diante das mudanças ambientais em curso. Ao aprofundar o conhecimento sobre a microbiota do solo e suas funções ecológicas, torna-se possível compreender melhor os mecanismos que sustentam a resiliência dos ambientes naturais. Essa compreensão é especialmente relevante em um contexto de pressões antrópicas crescentes, onde decisões baseadas em evidências científicas são fundamentais para a conservação de paisagens naturais e da biodiversidade subterrânea que as sustenta.

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8.1. CAPÍTULO 2 – Long-term nutrient addition experiment affects enzymatic activity and microbial diversity in Brazilian savannah soil (Cerrado)

8.2. ABSTRACT

The Brazilian Cerrado, a biodiversity hotspot and an important agricultural frontier, is characterized by highly weathered, acidic soils with low natural fertility. To evaluate how nutrient management strategies affect soil health in this biome, we assessed the impacts of five treatments—control (C), liming (CA), nitrogen (N), phosphorus (P), and combined nitrogen and phosphorus (NP)—on soil chemical properties, enzymatic activity, and microbial community structure. Field treatments were applied to native Cerrado soil, and samples were analyzed for pH, nutrient availability, and enzymatic activities (β-glucosidase, acid phosphatase, and arylsulfatase). Soil microbial communities were characterized using amplicon-based metabarcoding of 16S rRNA genes for bacteria and ITS regions for fungi, and overall soil quality was integrated using a FertBio-based Soil Quality Index (IQS). Liming significantly increased pH and base saturation, while P and NP treatments improved phosphorus availability and stimulated β-glucosidase activity. Enzymatic profiles and microbial diversity shifted in response to nutrient additions, with copiotrophic taxa favored under fertilized conditions. The IQS effectively distinguished treatments, with CA and NP showing the highest scores, reflecting improvements across chemical, biological, and microbial dimensions. Bacterial communities showed notable shifts under different treatments, with copiotrophic taxa like Gammaproteobacteria enriched under N and NP, while acidophilic and oligotrophic groups such as *Acidobacteriota* and *Bryobacter* declined with liming (CA), reflecting sensitivity to changes in pH and nutrient availability. Fungal communities also shifted their community under different nutrient treatments. Liming (CA) markedly increased the relative abundance of *Hygrocybe* (28.8%) and *Trichoderma* (4.25%), compared to the control, reflecting sensitivity to changes in pH and nutrient availability. These findings underscore the value of integrated soil assessments in tropical systems and highlight the importance of balanced nutrient management to preserve microbial diversity and sustain the ecological integrity of the Cerrado.

8.3. KEY WORDS

soil quality, long term nutrient addition, soil enzimes, metabarconding, microbial community

8.4. INTRODUCTION

The Brazilian Cerrado, recognized as one of the world’s 36 biodiversity hotspots, spans approximately 2 million km², making it the second largest biome in South America after the Amazon (Da Silva Arruda et al., 2024). Characterized by a heterogeneous mosaic of savanna-like vegetation, the Cerrado harbors exceptional plant diversity and thousands of endemic species (Cardoso Da Silva et al., 2002). Despite its ecological significance, the biome is under increasing threat from agricultural expansion, deforestation, and soil degradation (Assis et al., 2021). These anthropogenic pressures significantly alter soil ecosystem structure and function, leading to cascading effects on biogeochemical cycles.

Soils in the Cerrado region are typically dystrophic with low inherent fertility and limited availability of key nutrients such as phosphorus (P), nitrogen (N), and sulfur (S) (Haridasan, 2008). The high capacity for phosphorus fixation, driven by abundant iron and aluminum oxides, further restricts nutrient availability for plants (Alovisi et al., 2020). To address these challenges and support sustainable land management, practices such as liming to adjust soil pH and nutrient fertilization are commonly applied (Brignoli et al., 2024). These soil amendments, while necessary to modify chemical properties, also impact soil microbial communities and enzymatic activities.

The addition of nutrients is a widespread agricultural strategy aimed at enhancing soil fertility, especially in nutrient-poor tropical and subtropical environments. In Brazil’s Cerrado, the combined use of liming and fertilization with N, P, and potassium (K) is routinely employed to improve soil chemical conditions (Camargo et al., 2024). However, these interventions may cause significant shifts in biological soil properties, including changes in microbial community structure, enzymatic functions, and nutrient cycling processes (Silveira et al., 2021a). Gaining a deeper understanding of these ecological effects is essential for developing land management practices that preserve soil health and ecosystem functioning.

Soil respiration, defined as the flux of carbon dioxide (CO_2) from the soil to the atmosphere, encompasses microbial decomposition of organic matter, root respiration, and other biological processes. It is widely recognized as a key indicator of soil health because it reflects the activity of the soil biota and the turnover of organic carbon (Adhikari et al., 2023).

Soil enzymes are key indicators of microbial activity and ecosystem function, as they mediate critical reactions involved in organic matter decomposition and nutrient cycling (Uwituze et al., 2022). Among them, β-glucosidase, acid phosphatase, and arylsulfatase are widely used as biological proxies for the C, P, and S cycles, respectively. β-glucosidase plays a central role in cellulose degradation, releasing glucose from plant residues and reflecting the microbial capacity for carbon mineralization.

Acid phosphatase activity reveals microbial and root ability to hydrolyze organic phosphorus compounds, a trait especially critical in P-deficient soils such as those in the Cerrado (Ferreira; Espíndola; Campos, 2016). Phosphatase activity typically increases under low P availability, indicating a biological strategy to access less available phosphorus pools. Arylsulfatase is involved in sulfur mineralization and is essential for degrading sulfate esters (Siwik-Ziomek et al., 2025). Since sulfur is vital for protein synthesis and enzymatic functions in both plants and microbes, its cycling is fundamental for maintaining soil fertility (Bayu, 2024). β-glucosidase is a key enzyme in soil carbon cycling, catalyzing the hydrolysis of cellobiose into glucose and thus facilitating the decomposition of organic matter (Mariscal-Sancho et al., 2018; Uwituze et al., 2022). Its activity is widely recognized as an important indicator of soil microbial function and health, responding sensitively to changes in soil management and environmental conditions.

To comprehensively evaluate how nutrient inputs affect soil health, we applied a Soil Quality Index (IQS) based on the FertBio framework, which integrates physical, chemical, and biological indicators (Mendes et al., 2019). The biological component includes enzymatic activity as a functional metric of microbial metabolism and nutrient cycling. This integrative tool allows for comparative analysis of soil conditions under different management regimes and offers insight into the multifunctionality of soils in the Cerrado (Liu et al., 2021).

In addition to enzymatic assays, microbial diversity and taxonomic composition were evaluated using a metagenomic approach based on high-throughput sequencing of environmental DNA (eDNA). Metagenomics enables culture-independent profiling of microbial communities, allowing identification of both dominant and rare taxa (Pérez-Cobas et al., 2020) This technique is particularly valuable in diverse and understudied ecosystems such as tropical soils, where traditional culturing methods underestimate microbial complexity.

Soil microbial communities, especially bacteria and fungi, are highly responsive to changes in nutrient availability and pH (Cui et al., 2021; Zhang et al., 2024). Nutrient enrichment can promote copiotrophic taxa adapted to nutrient-rich environments, such as members of the *Proteobacteria* and *Bacteroidetes* phyla, which play key roles in organic matter decomposition and nutrient cycling (Grant et al., 2022; Luo et al., 2023; Shu et al., 2023). Among fungi, saprotrophic genera like *Trichoderma* and mycorrhizal fungi such as Glomeromycota are critical for nutrient acquisition and soil structure maintenance (Yao et al., 2023). Conversely, oligotrophic groups such as *Acidobacteria* decline under nutrient enrichment, which may reduce microbial diversity and alter ecosystem functioning, leading to potential impacts on long-term soil sustainability (Kim et al., 2021; Zhang et al., 2024). The aim of this study is to evaluate the health and functionality of this native soil with these modifications, as well as to improve our understanding of the factors that determine the restructuring of microbial communities in terrestrial ecosystems.

8.5.METHODOLOGY

Site Description and Sampling Design

This study was conducted as part of a long-term nutrient addition experiment (Kozovits et al. 2007), located at the Recor Ecological Reserve (1,300 ha) of the Brazilian Institute of Geography and Statistics (Reserva Ecológica do IBGE, Recor IBGE) (15°56'S, 47°53'W). The reserve is located within the Cerrado biome. The experiment was conducted in a savanna (typical Cerrado), characterized by continuous herbaceous layer associated with a shrub-tree layer (Bustamante et al., 2012; Haridasan, 2008). The soil is classified as typic dystrophic Red Latosol, with a very clayey texture (LVdf - Oxisol) (Sousa, 2020). The climate is characterized as AW according to the Köppen classification, with a well-defined dry season (May to September) and a rainy season (October to April) when about 90% of the annual rainfall occurs (mean precipitation around 1,500 mm).

The fertilization experiment was initiated in 1998 and comprehended four treatments with three repetitions each with different addition of nutrient, and three control areas without addition of nutrients. The treatments were Control (C), Limmin (CA), Nitrogen plus Phosphorus (NP), Nitrogen (N) and Phosphorus (P). The description of the addition of nutrients and the annual quantities applied in each treatment are shown in Table 2. Each treatment consisted of four 15×15 m plots, randomly distributed with a minimum distance of 10 m between them. Each plot was subdivided into nine 5×5 m parcels to facilitate the application of the nutrient (Silveira et al., 2021a) as demonstrated in Figure 2. The addition of the nutrients was done in two periods: the beginning and the end of the rainy season.

There was a pause in nutrient additions between 2006 and 2017. After 11 years, in December 2017, nutrient additions were resumed and have continued to the present (Silveira et al., 2021a). In April 2018, a second round of nutrient addition was applied, except for the calcium treatment, which received only a single application due to strong residual effects of prior lime additions, as indicated by soil pH data. At the beginning of the experiment, there were no significant differences in soil nutrient concentrations among the plots, and the vegetation structure was also similar. The most recent nutrient addition occurred in 2018, and no further additions have been made since then.

The liming treatment comprised the annual application of 4 t/ha per year of 60% dolomitic limestone (CaO+MgO) + 40% agricultural gypsum ($CaSO_4.2H_2O$) until 2006 (Bustamante et al., 2012). During this period, the liming treatment was modifying the area significantly and it was decided to stop the addition for 11 years. The treatment was resumed in November 2017, with one more nutrient addition in 2018, with the application of 2t/ha of 60% dolomitic limestone (CaO+MgO) + 40% agricultural gypsum ($CaSO_4.2H_2O$). The other treatments consisted of ammonium sulphate $(NH_4)_2SO_4$; phosphorus, in the form of simple superphosphate $Ca(H_2PO_4)$ + $CaSO_4.2H_2O$ and ammonium sulphate and phosphorus (Jacobson et al., 2011), according to Table 2. An additional 1m area around the treated area was also fertilized and, finally, the control area has the same characteristics as the treated areas but without the addition of nutrients.

Soil samples (0-10 cm depth) were collected at the very end of the dry season, in November 2022. Topsoil samples were randomly collected in five different parcels per plot to reduce the effect of soil heterogeneity. Subsequently, samples were pooled (avoiding roots) into a single composite and a representative sample of each of the three plots per treatment. There were 15 samples collected, and they were identified as 1C, 5C and 21C (control treatment); 2N, 14N and 7N (nitrogen treatment); 4P, 9P and 15P (phosphorus treatment); 3CA, 12CA and 17CA (liming treatment); and 6NP, 13NP and 16NP (nitrogen + phosphorus treatment) as shown in Figure 2.

On November 16, 2023, the day we collected the samples, Brasília experienced an atypically hot day, marking a significant deviation from its usual mid-November climate (Figure 3). While the historical average high temperature for Brasília in November is around 29 °C, this particular day saw temperatures exceeding 32 °C. This spike occurred during Brazil's eighth major heatwave of 2023, a large-scale atmospheric event driven by a persistent heat dome and intensified by El Niño conditions (Marengo et al., 2025)

Tabela 2: Description of the addition of nutrients and their annual quantities.

Treatment	**Chemical name**	**Chemical phormula**	**Quantity (kg.ha^{-1} .year^{-1})**
Nitrogen	Ammonium sulfate	$(NH_4)_2SO_4$	100
Phosphorus	Single superphosphate 20%.	$Ca(H_2PO_4)_2$ + $CaSO_4.2H_2O$	100
Nitrogen + phosphorus	Ammonium sulfate + Single superphosphate 20%.	$(NH_4)_2SO_4$ + $Ca(H_2PO_4)_2$ + $CaSO_4.2H_2O$	200
Calcium	Dolomitic limestone 60% +Agricultural gypsum 40%	CaO+MgO + $CaSO_4.2H_2O$	4000

Control	-	-	-

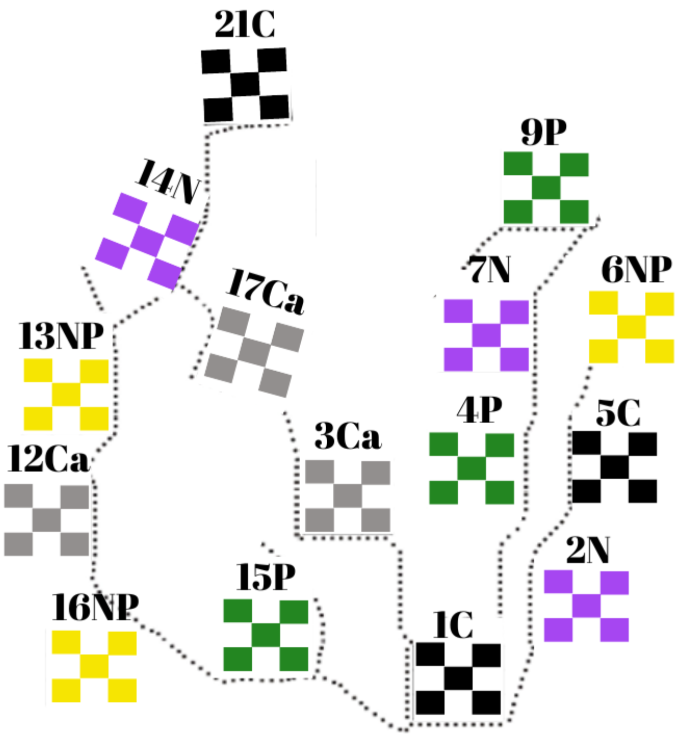

Figure 2: Diagram of the areas selected for composite soil sampling. The black areas represent the control plots, the gray areas indicate plots with calcium addition, the yellow areas correspond to plots with combined nitrogen and phosphorus addition, the green areas represent phosphorus addition, and the purple areas correspond to nitrogen addition.

The heatwave, which lasted from November 13 to 19, affected much of central Brazil. In Brasília, this resulted in a thermal sensation far higher than the actual temperature, with humidity levels dropping and solar radiation peaking at midday. Meteorologists characterized this episode as one of the most extreme for the season and pointed to broader climate change patterns (Marengo et al., 2025). Thus, November 14 stood out as an exceptionally hot day in Brasília, highlighting not only the local impact of short-term weather anomalies but also reinforcing long-term trends. In fact, 2023 was later confirmed to be the hottest year on record in Brazil (Bustamante, 2025). It is also important to note that the average temperature of the soil was 24°C, which typically falls within the normal range for the Cerrado biome. Therefore, while the temperature may not have been extreme, the hot streak during that period might still have had an impact on microbial processes, highlighting the importance of considering climatic conditions when interpreting soil data (Correia Filho et al., 2023; Oliveira et al., 2023)

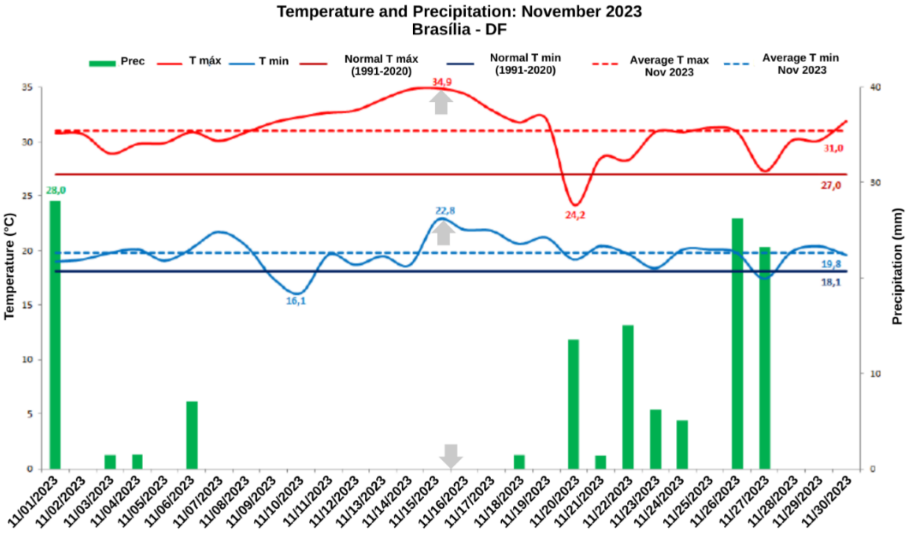

Figure 3. Graph with temperature (°C) and precipitation (mm) November 2023. The gray arrows indicate the hottest day that month. The red dotted line shows the average maximum temperature in November 2023; the solid red line, the daily maximum; and the dark red line, the normalized maximum (1991–2020). Similarly, the blue dotted line shows the average minimum in November 2023; the solid blue line, the daily minimum; and the dark blue line, the normalized minimum (1991–2020). Font: INMET (modified).

Soil physical-chemical analysis

Two subsamples were separated - one for physical-chemical analysis and one for molecular analyses - and immediately cooled in the field and later stored at -20°. The soil was then sent to VL *Agronegócios – Laboratório Agrícola* (Catalão, Goiás-GO) for physical-chemical analysis.

Soil analyses, including pH, macro- and micronutrients, and organic matter (OM), were carried out following the protocol described by Claessen (1996). Soil pH was determined by measuring with a calibrated potentiometer. Organic matter was quantified using the Walkley-Black method. Nutrients, such as phosphorus (P) and potassium (K), as well as micronutrients including copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), were extracted using the Mehlich-1 solution. Calcium (Ca^{2+}), magnesium (Mg^{2+}), and aluminum (Al^{3+}) were extracted with a 1 M KCl solution, while sulfur (S) was extracted using a monocalcium phosphate solution. The concentrations of macro- and micronutrients were determined by spectrophotometry, based on standard curves, as outlined by Cleassen, (1996).

Soil basal respiration analysis

Basal respiration was determined using the alkali absorption method as described by Anderson et al., (1992) with modifications. Briefly, 20 g of air-dried soil, free of coarse organic debris such as leaves and twigs, was weighed for each analysis. To account for variability, three subsamples (triplicates) of 20 g were prepared for each field-collected composite sample. The soil subsamples were placed in 500 mL airtight glass jars along with a small open container (e.g., test tube or beaker) containing 10 mL of 0.3 M potassium hydroxide (KOH) solution to capture CO_2 evolved from microbial respiration.

The jars were incubated in the dark at room temperature (approximately 25 °C) for seven days. After incubation, 3 mL of 20% barium chloride ($BaCl_2$) solution was added to the KOH solution to precipitate the captured CO_2 as barium carbonate ($BaCO_3$). Two drops of phenolphthalein indicator were added, and the remaining KOH was titrated with standardized 0.1 N hydrochloric acid (HCl) to determine the amount of CO_2 evolved. A set of blanks (jars with KOH but no soil) was included to correct for any background CO_2. The amount of CO_2 released was calculated and expressed as $\mu g\ CO_2\text{-}C\ g^{-1}$ soil day^{-1}.

Soil enzyme analysis

The method used to evaluate enzymes associated with phosphorus (acid phosphatases), β-glucosidase and arylsulfatase was based on the determination of p-nitrophenol released after incubating the soil with p-nitrophenyl glucoside, p-nitrophenyl phosphate p-nitrophenyl sulfate (for mesurement of β-glucosidase, acid phosphatase and arylsulfatase respecvely) for one hour at 37°C. One gram of soil was treated with 4 mL of modified universal buffer (pH 6.5 for acid phosphatase) and 1 mL of p-nitrophenyl phosphate solution prepared with the same modified universal buffer. The flasks were shaken and incubated in a rotary shaker for 1 hour at 37°C. After incubation, 1 mL of $CaCl_2$ (0.5M) and 4 mL of NaOH (0.5M) was added. The samples were mixed, and the soil suspension was filtered through a paper filter. The absorbance of the solution was measured at 400 nm, and the controls were performed with 1 mL of PNP solution with the addition of $CaCl_2$ (0.5M) and 4 mL of NaOH (0.5M) (Eivazi et al., 1988; Tabatabai et al., 1969; Tabatabai et al., 1970).

Integrated Assessment of Soil Quality Based on the Soil Quality Index (IQS)

Soil quality was determined using a methodology developed by EMBRAPA called BioAS (Pawlowski et al., 2024). A software that analyses two principal soil enzymes (arylsulfatase and β-glucosidase), along with the soil texture content, organic matter, and soil nutrients such as aluminum, magnesium, calcium, and potassium, as well as the determination of soil pH. The software is used primarily for cultivated and fertilized areas rather than native areas like the one in this study. However, the analysis was conducted to assess soil quality.

Based on the data generated by the Soil Quality Interpretation Module from Embrapa (BioAS), an evaluation of the Soil Quality Index (IQS) was conducted across the subjected areas. The analysis followed the guidelines proposed by which emphasize the importance of biological indicators in assessing soil health.

The indices used in this study include General Soil Quality Index (IQS); Biological IQS; Chemical IQS; Nutrient Cycling Index; Nutrient Storage Index and Nutrient Supply Index. These indices capture different facets of soil functioning, where biological indicators (enzyme activity, microbial biomass, organic C) are pivotal for nutrient cycling and system resilience. Chemical indicators (pH, exchangeable bases, available nutrients) reflect the soil's immediate fertility status.

Soil microbiota composition

Environmental DNA (eDNA) from the samples was extracted using MP Biomedicals FastDNA SPIN Kit for Soil (MP Biomedical, Santa Ana, CA, USA) according to the manufacturer’s instructions. The V3-V4 target sequence for bacteria was accessed by the primer pair 341F (5′-CCTAYGGGRBGCASCAG-′3) and 806R (5′-GGACTACNNGGGTATCTAAT′-3) and fungi by the primer pair ITS5-1737F (5’-GGAAGTAAAAGTCGTAACAAGG-3’) and ITS2-2043R (5’-GCTGCGTTCTTCATCGATGC-3’) Sequencing was performed on a MiSeq platform (Illumina, San Diego, CA, USA).

Bioinformatics

Bioinformatics analysis initially with the evaluation of the raw data quality from the 16S rRNA V3/V4 and ITS2 region sequences, performed using FastQC. Subsequently, low-quality sequences were filtered and trimmed, and primers were filtered using the Cutadapt 3.7 software (Martin, 2011). Next, the remaining high-quality sequences were subjected to dereplication, chimera removal, and clustering into amplicon sequence variants (ASVs) using the DADA2 software (Callahan et al., 2016). Finally, the obtained ASVs were taxonomically identified based on their similarity to sequences in the SILVA 138 16S rRNA bacterial database for 16S amplicons (Quast et al., 2012; YILMAZ et al., 2014) and UNITE v9 for ITS amplicons of fungi (Abarenkov et al., 2023). The raw sequences were mitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive.

Statistical analysis

All analyses were performed in triplicate, and results were expressed as mean ± standard deviation. Data were subjected to analysis of variance (ANOVA) to determine significant differences at a 5% significance level, followed by Tukey's test using XLSTAT 2014 software (Adinsoft®, Paris, France). PCA was performed with the software XLSTAT version 2015.2 (Adinsoft, Paris, France). Autoscaling of data was performed before the analysis to give the variables the same relevance. Better separation of the samples was obtained using Varimax rotation.

8.6.RESULTS

Soil physical-chemical analysis

In this study, we collected soil samples from 15 different sites across the RECOR (Ecological Reserve of the Brazilian Institute of Geography and Statistics), Brazil. The analysis of soil chemical properties revealed significant differences among the treatments. Soil pH varied from 4.33 in N treatment to 6.01 in CA treatment, the latter being significantly higher. This result confirms the corrective effect of calcium input on soil acidity, a typical limitation of Cerrado soils (Table 3 and Table S1).

The long-term nutrient addition treatments and liming significantly altered the soil chemical properties in the Cerrado ($p < 0.05$, Duncan test) (Table 3). Soil pH increased significantly under the liming treatment (6.01), compared to all other treatments, which remained acidic with pH values similar to the control (4.66) — including NP (4.42), N (4.33), and P (4.45) soils (Table 3 and Table S1). This highlights the effectiveness of liming in correcting soil acidity, whereas nutrient-only additions did not alter pH significantly (Enesi et al., 2023).

Phosphorus availability was significantly higher in the P (7.77) and NP (6.00) treatments relative to the control (1.27) and the other treatments, reflecting the direct input of phosphate. Sulfur content exhibited a strong response to nitrogen inputs, with significantly elevated values in NP (48.94) and N (45.39) compared to the control (3.96) and other treatments.

Calcium and magnesium were strongly affected by liming. The CA treatment presented significantly higher Ca^{+2} (5.83) and Mg (3.21) concentrations than all other treatments, which remained low and similar to the control (Ca: 0.06; Mg: 0.05). Exchangeable aluminum was completely eliminated in the CA treatment (0.00), contrasting sharply with the control (0.79) and nutrient addition treatments (NP: 1.29; N: 1.37; P: 1.15) soils (Table 3 and Table S1), where it remained high.

Among micronutrients, manganese (Mn) showed a significant increase in the CA treatment (16.67) compared to all other treatments, while copper (Cu) decreased significantly with liming (0.07) relative to the control (0.48), likely due to pH-induced precipitation. The sum of bases (SB) was highest in the CA treatment (9.04), significantly surpassing the control (0.11) and all nutrient-only treatments, which remained low (NP: 0.33; N: 0.18; P: 0.16) soils (Table 3 and Table S1). Organic matter (OM) and organic carbon (OC) did not differ significantly among treatments, with OM ranging from 1.81 to 3.45 dag kg^{-1} and OC from 1.05 to 2.00 dag kg^{-1}.

Tabela 3. Content of nutrients in soil (mean ± standard deviation) in a Cerrado sensu stricto area subjected to different long-term nutrient addition treatments.

Treatment	pH	P	S	Ca	Mg	Al	OM	OC	B	Cu	Fe	Mn	SB
C	4.66± 0.24^{b}	1.27± 0.8^{b}	3.96± 0.15^{d}	0.06± 0.02^{b}	0.05± 0.05^{b}	0.79± 0.06^{b}	2.44± 1.17^{a}	1.41± 0.68^{a}	0.42± 0.07^{a}	0.48± 0.24^{a}	73.07± 22.27^{ab}	3.76± 0.52^{b}	0.11± 0.07^{b}
CA	6.01± 0.13^{a}	1.11± 0.38^{b}	5.21± 1.05^{d}	5.83± 1.63^{a}	3.21± 0.72^{a}	0.00± 0.00^{c}	1.81± 1.32^{a}	1.05± 0.77^{a}	0.40± 0.10^{a}	0.07± 0.07^{b}	53.53± 9.43^{bc}	16.67± 11.98^{a}	9.04± 2.33^{a}
NP	4.42± 0.31^{b}	6.00± 1.65^{ab}	48.94± 0.35^{a}	0.27± 0.27^{b}	0.06± 0.06^{b}	1.29± 0.1^{a}	2.25± 0.83^{a}	1.31± 0.48^{a}	0.47± 0.13^{a}	0.17± 0.14^{b}	32.95± 21.82^{c}	2.94± 0.68^{b}	0.33± 0.33^{b}
N	4.33± 0.18^{b}	1.93± 1.19^{b}	45.39± 2.57^{b}	0.11± 0.06^{b}	0.06± 0.04^{b}	1.37± 0.14^{a}	3.45± 0.72^{a}	2.00± 0.42^{a}	0.37± 0.04^{a}	0.21± 0.05^{ab}	72.23± 5.23^{ab}	2.86± 0.31^{b}	0.18± 0.08^{b}
P	4.45± 0.10^{b}	7.77± 5.6^{a}	30.03± 2.32^{c}	0.12± 0.08^{b}	0.04± 0.01^{b}	1.15± 0.26^{ab}	2.88± 1.04^{a}	1.67± 0.60^{a}	0.35± 0.03^{a}	0.29± 0.15^{ab}	87.44± 13.8^{a}	3.12± 0.58^{b}	0.16± 0.09^{b}

Treatments include: C (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphate treatment), and P (phosphate treatment), The chemical variables measured are: Al 3+ (aluminum), B (boron), Ca $^{2+}$ (calcium), Cu (copper), Fe (iron), K + (potassium), Mg $^{2+}$ (magnesium), Mn (manganese), OC (organic carbon), P (phosphorus); S (sulphur) and Zn (zinc). Concentrations are expressed as follows: Al $^{3+}$, Ca $^{2+}$, K, and Mg $^{2+}$ in cmolc dm-^{3}; B, Cu, Fe, Mn, P, S, and Zn in meh-^{1} mg dm-^{3}; OC and OM in dag kg-^{1}. * Means followed by the same letter in the column do not differ according to Tukey, significance value of 0.05.

The Principal Coordinates Analysis (PCoA) shown in Figure 4 illustrates the differentiation among soil treatments based on chemical parameters, following nutrient additions in a Cerrado *sensu stricto* area. The dispersion of points in the biplot reflects how each treatment influences the soil's chemical composition. From the graph, it is evident that CA treatment clusters distinctly on the right side, showing strong associations with variables such as pH, Mn, Ca, Mg, and SB. In contrast, treatments involving N and P - either alone or together - are positioned in the left quadrant, suggesting influence on variables such as Aluminum (Al), Sulfur (S), Boron (B), and P. The combined NP treatment appears particularly associated with increases in S and B. Additionally, C, P and N treatments are closely aligned and positively associated with Copper (Cu) and Fe, suggesting that these micronutrients are more prominent in untreated or nitrogen-enriched soils without pH correction.

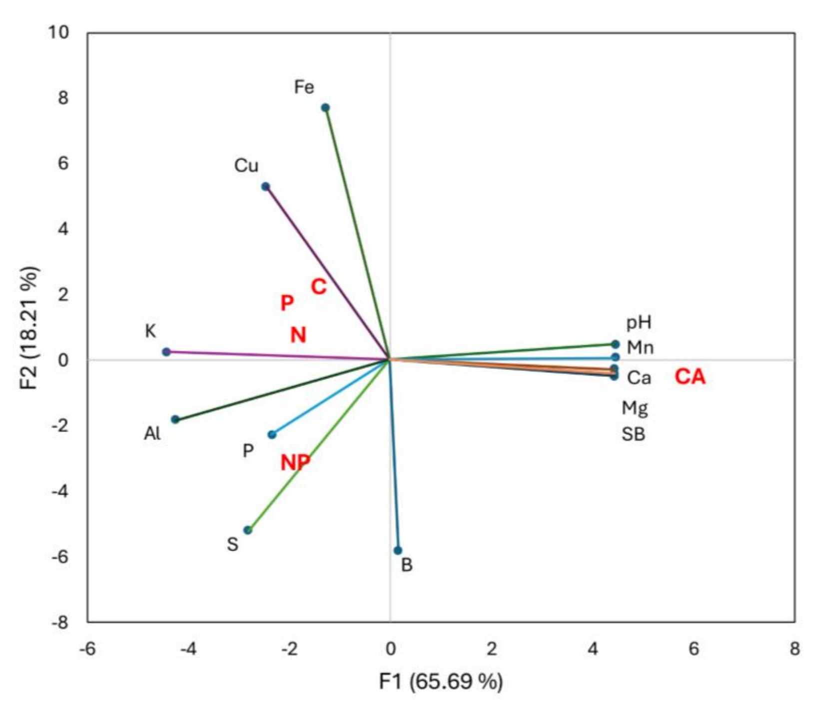

Figure 4. F1 (PC1) and F2 (PC2) represent the X and Y axes, respectively. Treatments: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphorus treatment), and; P (phosphorus treatment). The chemical variables measured are: Al 3+(aluminum), B (boron), Ca $^{2+}$ (calcium), Cu (copper), Fe (iron), K + (potassium), Mg 2+ (magnesium), Mn (manganese), P (phosphorus) and S (sulphur), and also the pH and the Sum of Bases.

Soil Basal Respiration analysis

Soil respiration rates in the Cerrado *sensu stricto* varied across long-term nutrient addition treatments but showed no statistically significant differences among them (p >

0.05). C treatment exhibited an average respiration rate of 23.71 mg CO_2/kg dry soil/day, while CA showed the highest value at 34.0 mg CO_2/kg/day. P addition also resulted in elevated respiration (33.88 mg CO_2/kg/day), followed by NP (25.31 mg CO_2/kg/day) and N, which had a similar rate of 24.56 mg CO_2/kg/day (Figure 5).

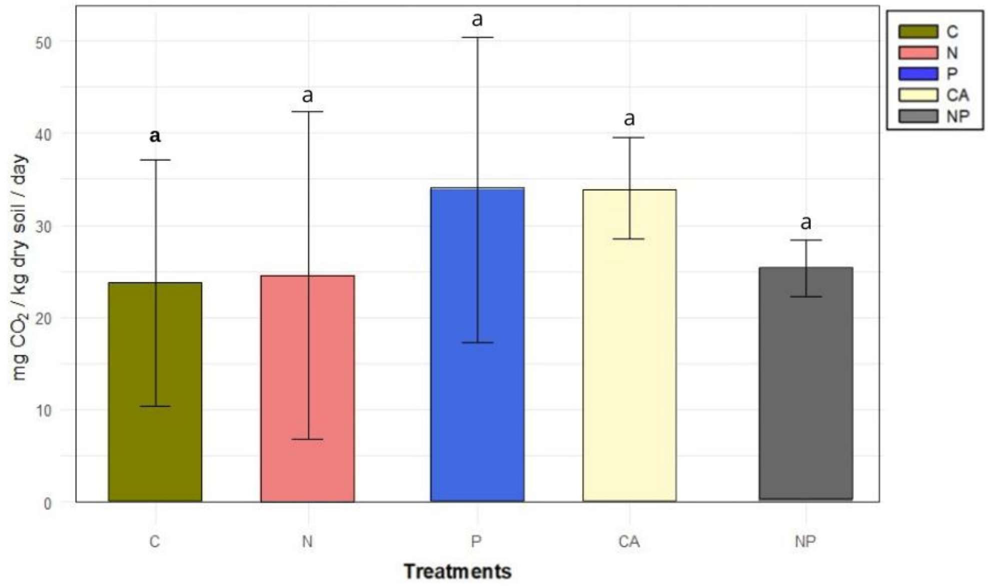

Figure 5 Values of soil respiration in a Cerrado sensu stricto area subjected to different long-term nutrient addition treatments. Treatments include: C (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphate treatment), and P (phosphate treatment). Means followed by the same letter in the column do not differ according to Tuckey, significance value of 0.05*

Soil enzyme analysis

β-glucosidase

The activity of the enzyme β-glucosidase, which is responsible for polysaccharide degradation and directly involved in the carbon cycle in soil (Zang et al., 2018), showed significant variations among the evaluated treatments (Figure 5). Treatments N, P and NP resulted in the highest β-glucosidase activities (54.96; 48.73 and 55.33 µg p-nitrofenol g^{-1} solo h^{-1}, respectively), with no statistical difference between them ($p > 0.05$) (Figure 5). The control treatment showed intermediate activity, likely reflecting the presence of microbial communities naturally adapted to the soil's original conditions. On the other hand, CA treatment showed the lowest of this enzyme activity (42,56 µg p-nitrofenol g^{-1} solo h^{-1}), significantly differing from the others ($p < 0.05$) (Figure 6).

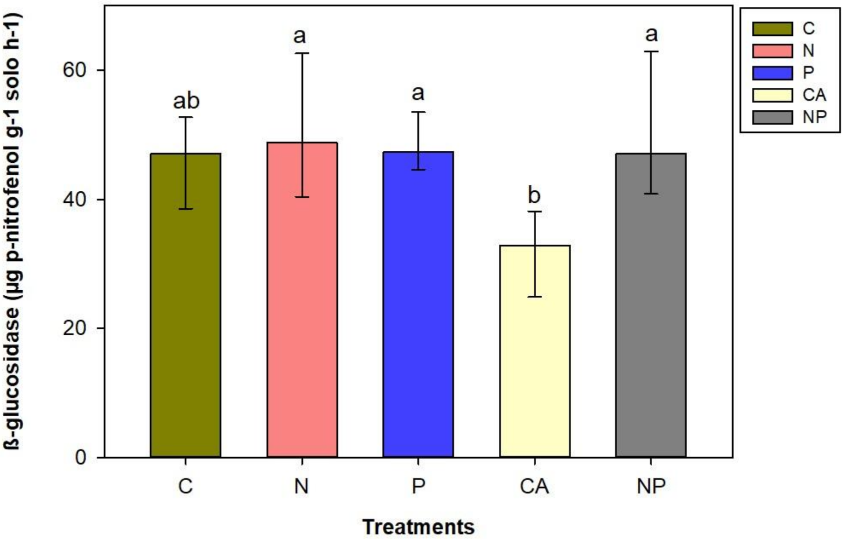

Figure 6. Values of β-glucosidase. Means followed by the same letter in the column graphics are not different from each other at the 5% probability level by the test of Tukey. C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment.

Phosphatase

The enzyme phosphatase plays a crucial role in phosphorus cycling in the soil, facilitating the release of inorganic forms available to plants (Campdelacreu Rocabruna et al., 2024). The results showed significant variations in phosphatase activity among the treatments. C treatment showed the highest phosphatase activities (699.91 µg p-nitrofenol g^{-1} solo h^{-1}), followed by N treatment (438,14 µg p-nitrofenol g^{-1} solo h^{-1}) with statistical difference between them ($p < 0.05$) (Figure 6). P treatment and NP showed the lowest phosphatase activity, significantly differing from the control treatment (389,59 and 381,834 µg p-nitrofenol g^{-1} solo h^{-1}, respectively) ($p < 0.05$). CA treatment exhibited the lowest values for the phosphatase enzyme with 361.12 µg p-nitrofenol g^{-1} solo h^{-1} ($p < 0.05$) (Figure 7).

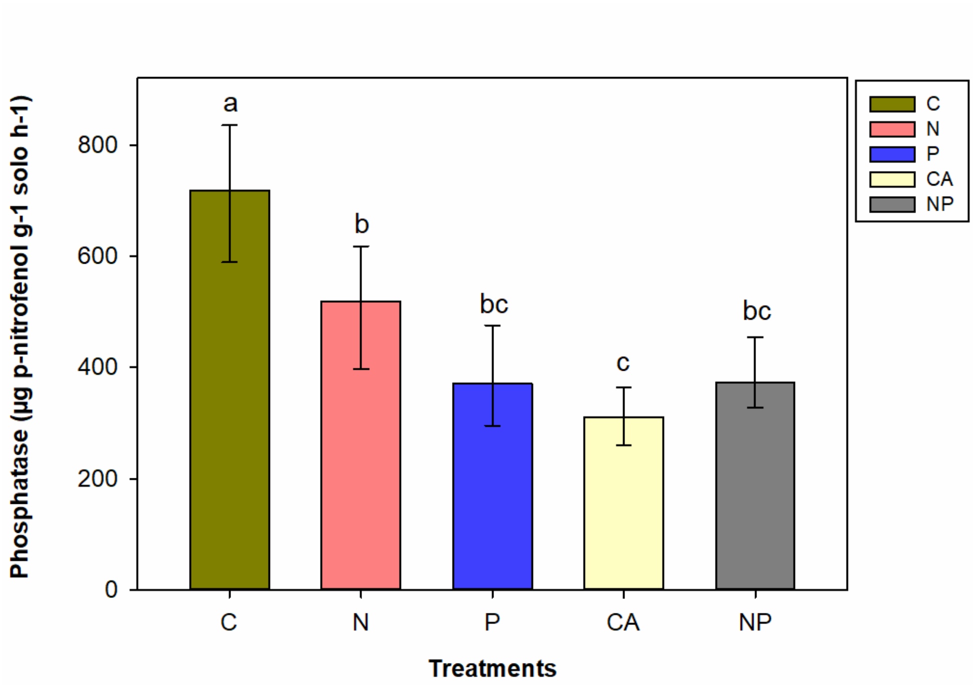

Figure 7. Values of phosphatase. Means followed by the same letter in the collum graphics are not different from each other at the 5% probability level by the test of Tukey. C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment.

Arylsulfatase

The activity of the enzyme arylsulfatase varied significantly among the soil treatments. Liming presented the highest enzymatic activity (45,61 µg p-nitrofenol g^{-1} solo h^{-1}) with statistical difference among other treatments ($p < 0.05$) (Figure 8). N and NP treatments exhibited similar patterns of the lowest values for arysulfatase, with no statistical difference between them (19.53 and 24,22 µg p-nitrofenol g^{-1} solo h^{-1}, respectively) ($p > 0.05$). The Control and the Phosphorus treatment showed intermediary values (32,37 and 29.96 µg p-nitrofenol g^{-1} solo h^{-1}, respectively) ($p > 0.05$).

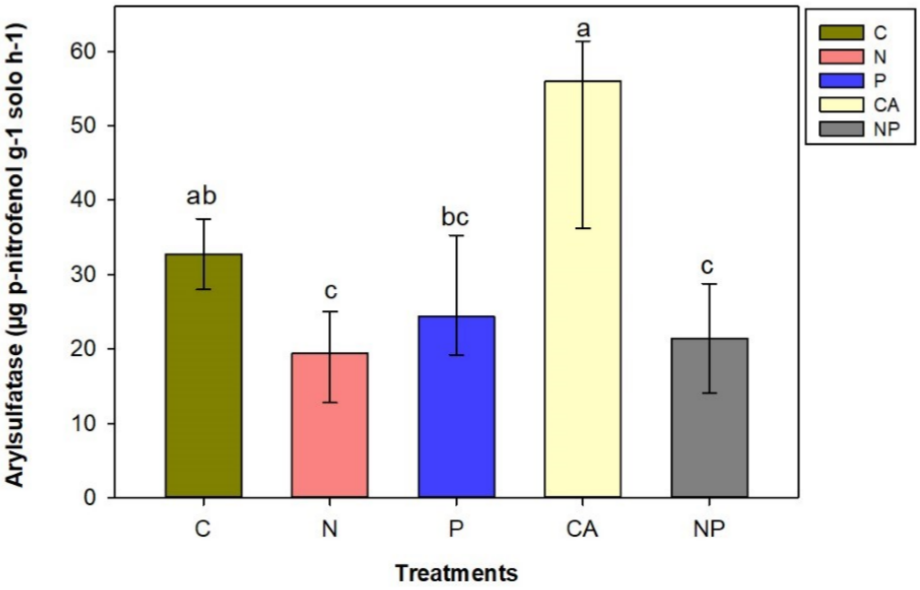

Figure 8 Values of arylsulfatase. Means followed by the same letter in the collum graphics are not different from each other at the 5% probability level by the test of Tukey. C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment.

Integrated Assessment of Soil Quality Based on the Soil Quality Index (SQI)

Evaluation of the IQS results from the soil fertility attributes (Table 4) like pH, Al, Ca, K, P, Mg, OM and the levels of β-glucosidase and arylsulfatase enzymes (Figure 6 and 8 respectively), resulted in the FERTBIO IQS values. The evaluation of the IQS in this long-term experiment reveals important insights into the trajectory of soil health under different management regimes.

We found that this index value for this soil is mostly average or inadequate. The control treatment, representing unamended soil, exhibited the lowest values across most indices, including Biological ISQ (0.41) and Nutrient Cycling (0.41) (Table 4). The application of lime significantly modified soil quality according to this index, yielding the highest values in Biological IQS (0.55), Chemical IQS (0.59), Nutrient Storage (0.66), and Nutrient Supply (0.52). The NP combined fertilization strategy led to moderate values across all indices. Biological IQS was 0.43, and Nutrient Storage reached 0.63 (Table 5).

Nitrogen addition resulted in the highest Nutrient Storage Index (0.82), indicative of strong microbial immobilization or accumulation of organic matter. However, the Nutrient Supply Index was low (0.23), suggesting potential imbalances or nutrient lock-up, possibly due to acidification or altered microbial community dynamics. Phosphorus treatment yielded intermediate Biological IQS (0.42) and the lowest Nutrient Cycling Index (0.36) (Table 4). Interestingly, the nutrient supply index was the only one with statistical difference among the indexes and the only index that all treatments are in a bad quality. The liming treatment had a value of 0,52 and the C and the N treatment had 0,23. The other treatments (CA and NP) had intermediate values (0.52 and 0.31 respectively).

Table 4. Average activity values of soil quality index (FERTIBIO IQS), biological quality index (biological IQS), chemical quality index (chemical IQS), cycling, storage, and nutrient supply functions of areas under different nutrient addition treatments

Treatment	FERTIBIO IQS	IQS Biological	IQS Chemical	Nutrient cycling	Nutrient storage	Nutrient supply
C	0,32^{a}	0,41^{a}	0,28^{a}	0,41^{a}	0,32^{a}	0,23
CA	0,58^{a}	0,55^{a}	0,59^{a}	0,55^{a}	0,66^{a}	0,52
NP	0,46^{a}	0,43^{a}	0,47^{a}	0,43^{a}	0,66^{a}	0,31
N	0,05^{a}	0,45^{a}	0,52^{a}	0,45^{a}	0,82^{a}	0,23
P	0,46^{a}	0,42^{a}	0,48^{a}	0,42^{a}	0,58^{a}	0,37

Means followed by the same letter in the column are not different from each other at the 5% probability level by the test of Tukey. * BioAS report generated by the Soil Quality Interpretation Module MIQS platform. In the BioAS report, indices are also represented in a chromatic pattern, where dark green or light green mean adequate values (very high and high, respectively), yellow, intermediate values, and orange or red, inadequate values. Treatments: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphorus treatment), and; P (phosphorus treatment).

Soil microbiota composition

Alpha and Beta diversity for bacteria

The alpha diversity indices (Figure 9, Table S3) obtained through 16S rRNA gene sequencing showed no statistically significant differences ($p > 0.05$) among the treatments for any of the parameters evaluated (Table S3). The mean values for richness indices—Observed, Chao1, and ACE—remained similar among treatments, ranging from 3,497.33 (NP) to 4,187.67 (P) for Observed, 3,499.59 (NP) to 4,195.90 (P) for Chao1, and 3,502.01 (NP) to 4,204.04 (P) for ACE. The high variability in the data, especially under the P treatment, may have contributed to the lack of statistically significant differences between groups.

The Shannon diversity index, which accounts for both species richness and evenness, showed average values ranging from 7.80 (C and NP) to 7.93 (P), also with no significant differences. Similar results were observed for the Simpson and Inverse Simpson indices, indicating high community evenness across all treatments, with Simpson values close to 1.00 and Inverse Simpson values ranging from 1,802.25 (C) to 2,197.92 (P) (Figure 9, Table S3).

To investigate the beta diversity of soil bacterial communities under different fertilization treatments, a Principal Coordinates Analysis (PCoA) was performed. The first two axes (PCo1 and PCo2) each explained 7.1% of the variation, reflecting differences in community composition across treatments (Figure 10).

The CA treatment (blue ellipse) formed a clearly separated cluster from the other treatments, indicating a distinct bacterial community structure. This separation suggests that pH correction and Al^{3+} neutralization associated with liming significantly influenced the microbial assemblage, potentially favoring taxa adapted to more neutral pH and improved nutrient availability. In contrast, the C (red ellipse), N (green ellipse), P (orange ellipse), and combined NP (purple ellipse) treatments showed greater overlap and clustering near the origin, indicating more similar microbial communities among these groups.

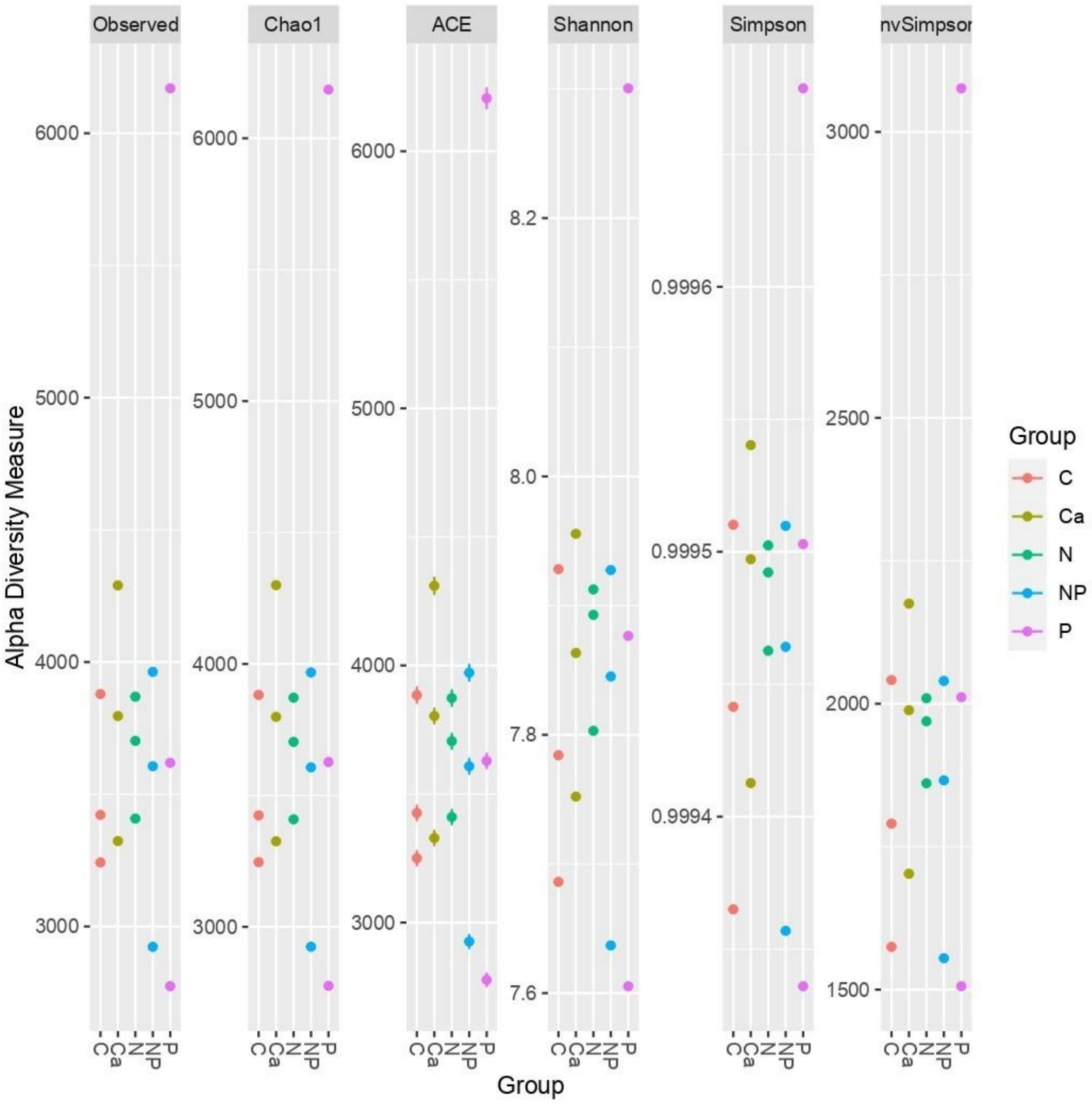

Figure 9. Graph containing the values of Alpha diversity in soil of a Cerrado native area with different treatments. Alpha diversity results (observed, chao1, ACE, shannon, simpson, invSimpson) obtained for all soil treatments by analysis based on the 16S rRNA amplicon sequences. Treatments: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphorus treatment), and; P (phosphorus treatment).

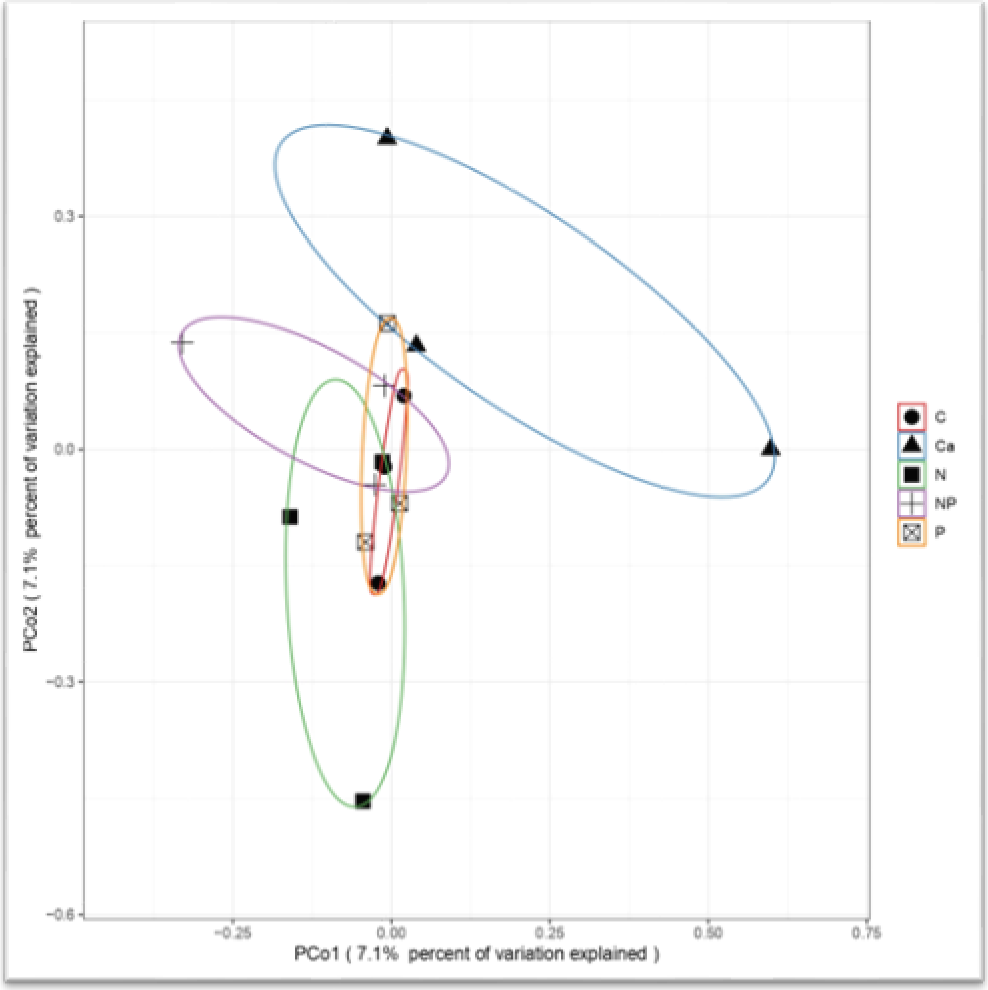

Figure 10. Graph containing the values of betadiversity in soil of a Cerrado native area with different treatments. Beta diversity of bacteria. PCo1 and PCo2 represent the X and Y axes, respectively. Treatments: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphorus treatment), and; P (phosphorus treatment).

Alpha Diversity of Fungi

The alpha diversity analysis based on ITS sequencing (Figure 11 and Table S4) revealed no statistically significant differences ($p > 0.05$) in most diversity indices across treatments. The Observed, Chao1, and ACE richness estimators showed similar patterns, with the highest values in the control (C; 934), followed by the nitrogen (N; 912.33) and NP (881.67) treatments. The lowest richness values were found in the C (652.33) and P (809.33) treatments.

The standard error of ACE (se.ACE) displayed a notable difference, with significantly lower values under P addition (9.43), suggesting reduced richness variability within that treatment ($p < 0.05$). Conversely, other treatments showed higher se.ACE values, particularly under N (13.27) and NP (13.37).

In terms of diversity indices, Shannon and Simpson indices were generally high among treatments, with nitrogen application yielding the highest Shannon diversity (5.28), followed by NP (4.95), while CA showed the lowest value (3.53). Nevertheless, these variations were not statistically significant. The Simpson index remained consistently high across all treatments (>0.82), indicating overall high community evenness.

The Inverse Simpson index, however, revealed greater variation. N treatment exhibited a significantly higher diversity (78.99) compared to CA; (9.47), with NP (47.10), C (35.23), and P (29.35) presenting intermediate values. This suggests a potential enhancing effect of nitrogen on fungal diversity, while liming may have suppressed it.

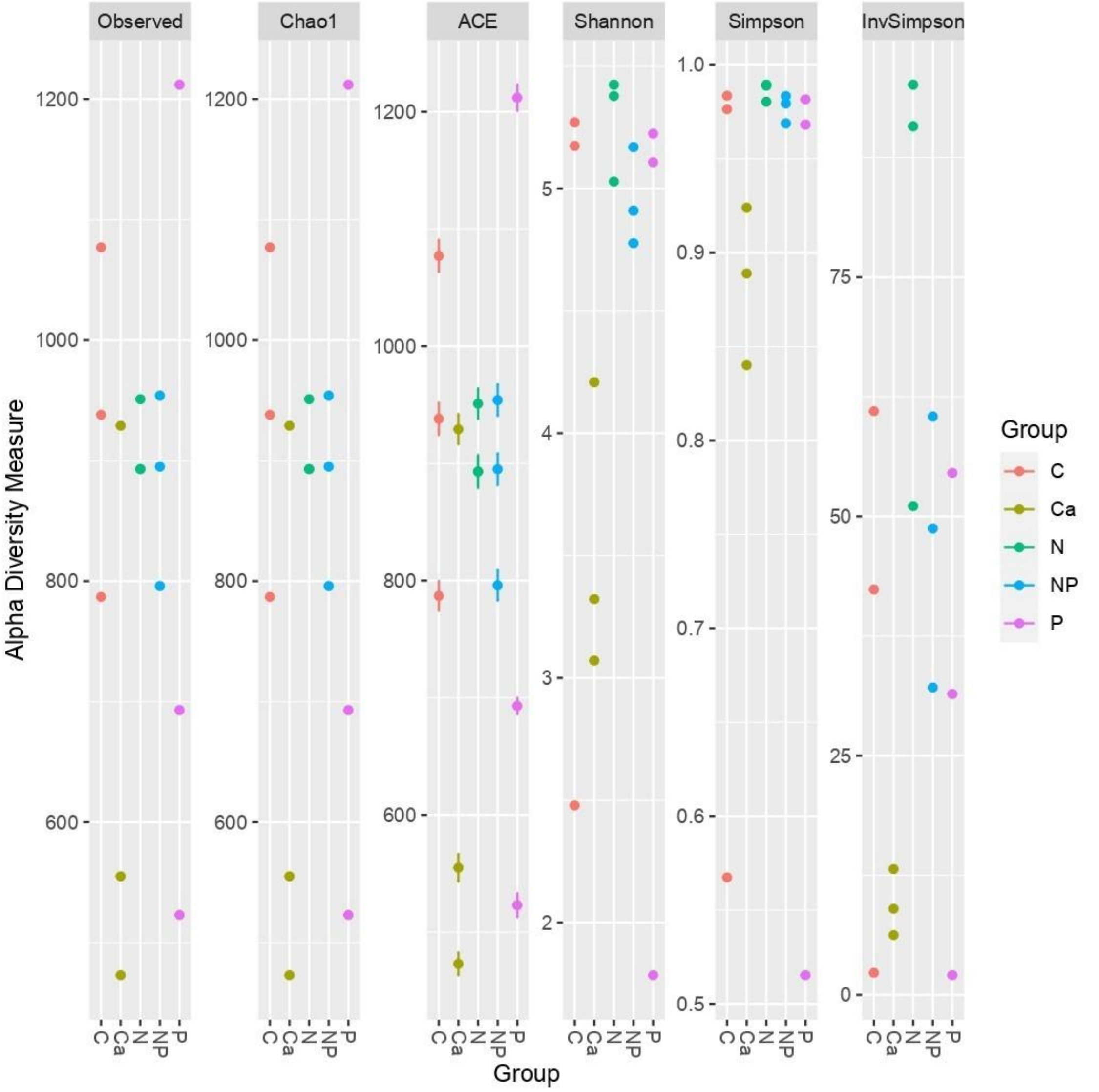

Figure 11. Alpha diversity results (observed, chao1, ACE, shannon, simpson, invSimpson) obtained for all soil treatments by analysis based on the ITS amplicon sequences. Treatments: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphorus treatment), and; P (phosphorus treatment).

Relative Abundance of Bacteria

The soil bacterial community in the Cerrado *sensu stricto* was dominated by a few major phyla across all treatments, particularly Acidobacteriota, Proteobacteria, Chloroflexi, and Actinobacteriota. Acidobacteriota, was especially prevalent in the control and nitrogen treatments, suggesting its adaptation to low-input environments. In contrast, Proteobacteria, showed higher relative abundance in the control and nitrogen treatments compared to phosphorus and NP additions (Figure 12 and Table S5).

Chloroflexi showed increased abundance under combined nitrogen and phosphorus (NP) input, while Actinobacteriota was more prominent in fertilized plots, particularly with liming and NP. Gemmatimonadota was moderately more abundant in the liming and phosphorus treatments. Other phyla, such as Verrucomicrobiota, Myxococcota, Bacteroidota, WPS-2, and Crenarchaeota, remained below 5% in relative abundance, with minimal variation among treatments (Figure 12 and Table S7).

At a finer taxonomic resolution, some differences emerged among bacterial orders in response to fertilization and liming (Figure 13 and Table S7). Unknown taxonomic groups were abundant across treatments, especially in CA (16.46%) and NP (18.18%) treatments, underscoring the presence of unclassified or poorly studied microbial taxa in the Cerrado soils.

Among copiotrophic orders, Gammaproteobacteria showed a significant reduction in the CA (3.82%) and P (3.21%) treatments compared to the control (10.41%) and nitrogen addition (N: 9.80%). Similarly, Myxococcales, known for their predatory lifestyle, were significantly less abundant in NP (0.24%), N (0.32%), and P (0.52%) treatments compared to control (1.20) (Figure 13 and Table S7).

Frankiales and Solibacterales, both associated with Actinobacteriota and Acidobacteriota respectively, showed treatment-specific responses. Frankiales increased significantly under NP (4.25%) compared to CA (1.29%), while Solibacterales were significantly lower in CA (1.26%) than NP (3.99%) (Figure 12 and Table S7), reflecting a possible sensitivity to liming. Likewise, Bryobacterales exhibited higher abundance in NP (3.42%) than in CA (0.68%).

In contrast, Polyangiales and Micropepsales showed clear sensitivity to treatment. Polyangiales were significantly more abundant in CA (1.59%) than in C (0.79%) and nitrogen (0.70%), while Micropepsales increased in NP (1.02%) and N (1.13%) treatments, with significantly lower levels under CA (0.33%) and P (0.32%). Elsterales were significantly more abundant in N treatment (2.47%) compared to C (1.06%) and other treatments (Figure 12 and Table S7).

At the genus level (Table S9), several bacterial taxa showed significant variation in their relative abundances in response to the different fertilization treatments. *Acidothermus* was significantly more abundant in the NP treatment (3.76%) compared to CA (0.66%). Similarly, Candidatus *Solibacter* and *Conexibacter* also presented higher abundances in NP than in CA, with values of 3.99% and 3.20% under NP, respectively.

The relative abundance of *Gammaproteobacteria Incertae Sedis* was significantly reduced under CA and P treatments (3.82% and 3.21%, respectively) compared to C (10.41%) and N (9.80%). Subgroup 1 and *Bryobacter* also showed higher values in the NP treatment (2.76% and 3.42%, respectively) when compared to CA and P. Proteobacteria *Sutterellaceae* exhibited reduced abundance under CA, NP, and P relative to the control. *Vicinamibacterales Unknown* had significantly higher abundance under CA (2.35%) compared to N (0.54%). *Elsterales Unknown* and *Micropepsaceae* genera were significantly more abundant in the N treatment (2.07% and 1.13%, respectively) compared to CA and P.

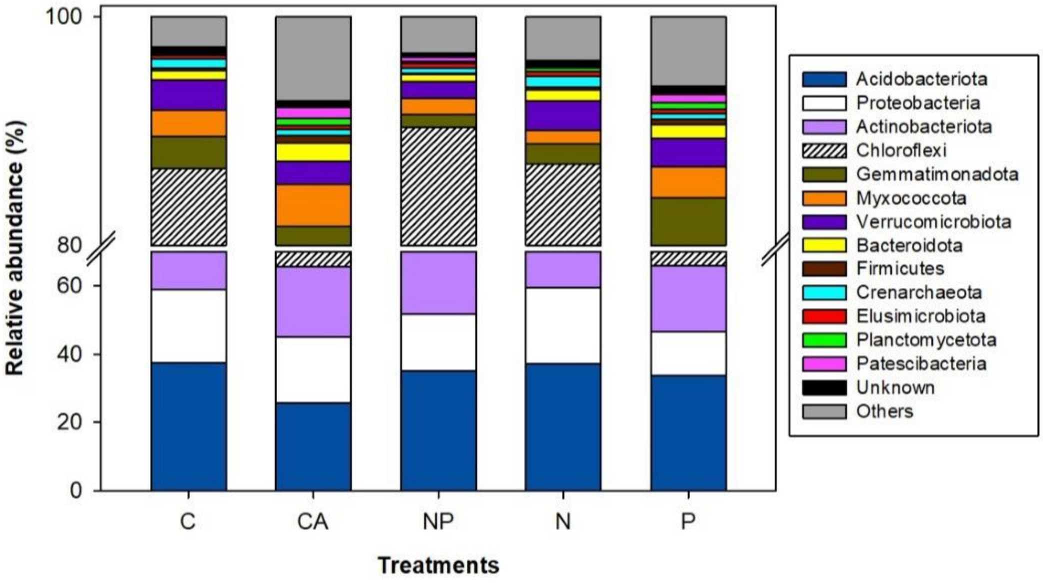

Figure 12. Relative abundance of soil bacteria community at the phyllum taxonomic level in a Cerrado sensu stricto area under long-term nutrient addition treatments. Treatments: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphate treatment), and P (phosphate treatment).

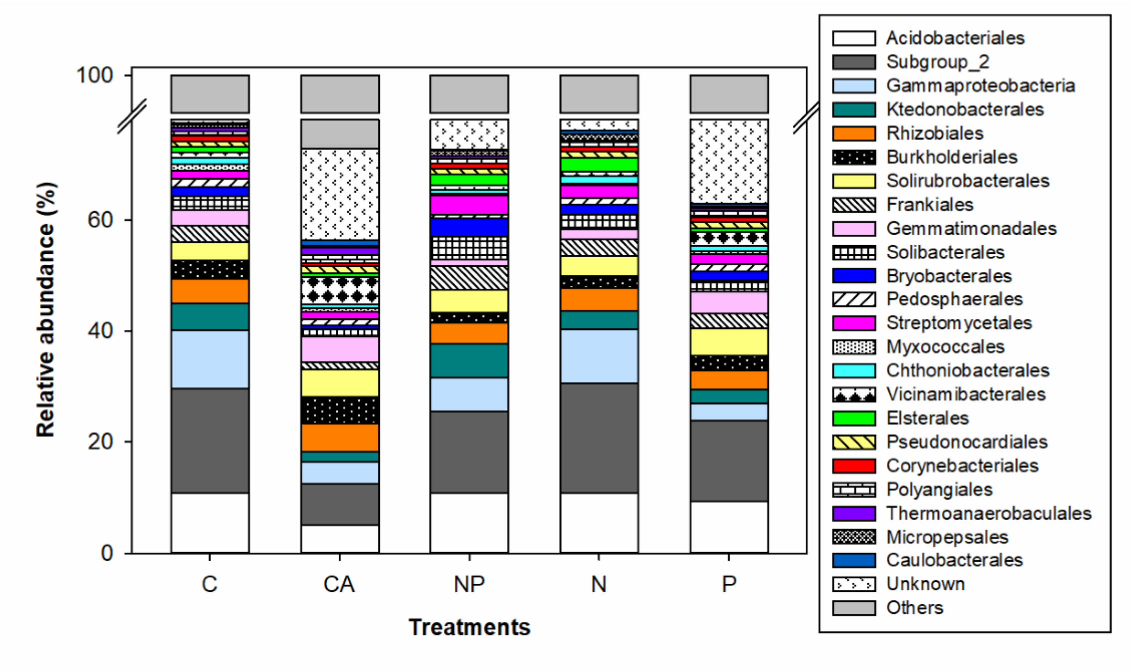

Figura 13. Relative abundance of soil bacteria community at the order taxonomic level in a Cerrado sensu stricto area under long-term nutrient addition treatments. Treatments: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphate treatment), and P (phosphate treatment).

Relative Abundance of Fungi

Seven fungal phyla were identified across the treatments (Figure 14 and Table S10). I was the dominant phylum under all treatments, with relative abundance ranging from 53.69% (P) to 91.36% (N). Although no significant differences were detected among treatments ($p > 0.05$), a notable numerical increase in Ascomycota was observed in N treatment.

Basidiomycota abundance was significantly higher under CA (37.94%) compared to all other treatments ($p < 0.05$), where values ranged from 4.04% (C) to 10.13% (P). At the order level (Figure 15 and Table S12), significant differences were observed in the abundance of fungal groups across treatments. Ascomycota Incertae sedis exhibited a notably higher relative abundance in N (14.43%) when compared to the C, CA, and P treatments ($p < 0.05$). Pleosporales were more abundant under C and N conditions relative to CA and P ($p < 0.05$). In contrast, Chaetothyriales was significantly stimulated by the combined NP treatment, showing the highest abundance (20.35%) and significantly exceeding values recorded under C and N alone ($p < 0.05$).

A strong effect of liming was also evident for Agaricales, which reached a significantly higher relative abundance under CA (36.92%) compared to all other treatments ($p < 0.05$) (Figure 14 and Table S12). Lastly, Onygenales exhibited a sharp increase in NP-treated soils (9.14%), with statistically higher values than in any other treatment ($p < 0.05$).

At the genus level (Table S14), several groups demonstrated significant treatment responses. Ascomycota *Incertae sedis* was significantly more abundant in the N treatment (14.43%) than in CA and P treatments ($p < 0.05$). The genus *Pseudorobillarda* was markedly more prevalent in the control treatment (11.50%) compared to CA and P ($p < 0.05$), suggesting a negative effect of fertilization and liming on this taxon. A particularly strong enrichment was seen for Herpotrichiellaceae *Incertae sedis* under the NP treatment (13.16%), where its abundance was significantly higher than in all other treatments ($p < 0.05$).

Leohumicola responded positively to nitrogen addition, with a significantly greater abundance in the N treatment (3.39%) compared to CA, NP, and P ($p < 0.05$). Similarly, *Glutinomyces* was significantly more abundant under N treatment relative to CA and P ($p < 0.05$). Additionally, *Onygenales Incertae sedis* followed a pattern similar to its corresponding order, showing the highest relative abundance under NP treatment (8.90%) compared to all others ($p < 0.05$) (Table S14).

Liming had a strong and specific effect on *Hygrocybe*, a genus within Basidiomycota, which reached 28.81% under CA. This was significantly higher than in C, N, and NP ($p < 0.05$).

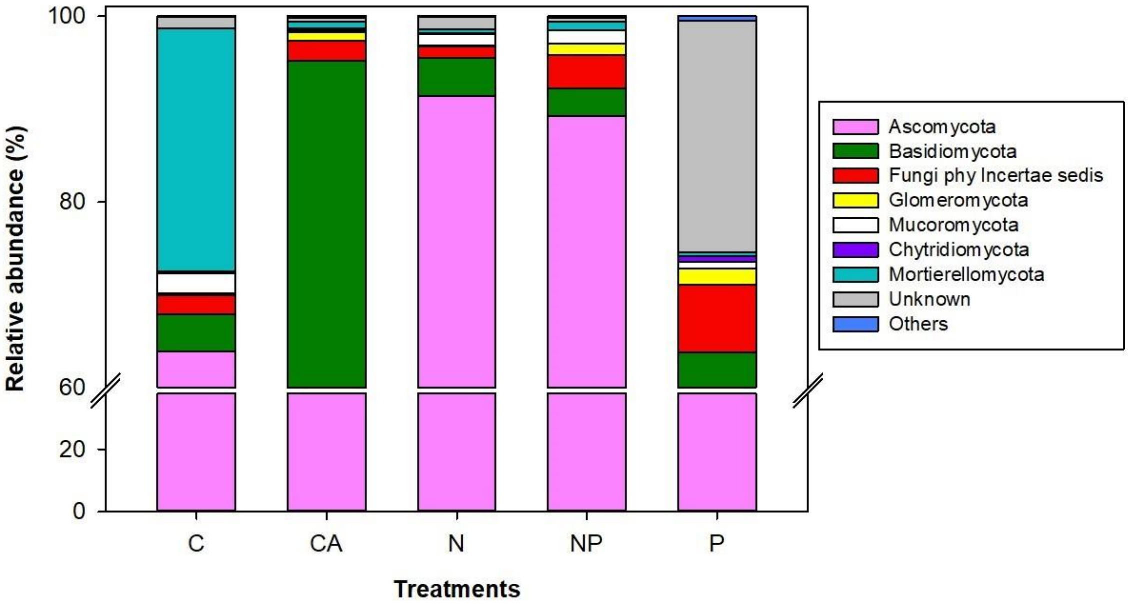

Figure 14. Relative abundance of soil fungi community at the phylum taxonomic level in a Cerrado sensu stricto area under long-term nutrient addition treatments. Treatments are: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphate treatment), and P (phosphate treatment).

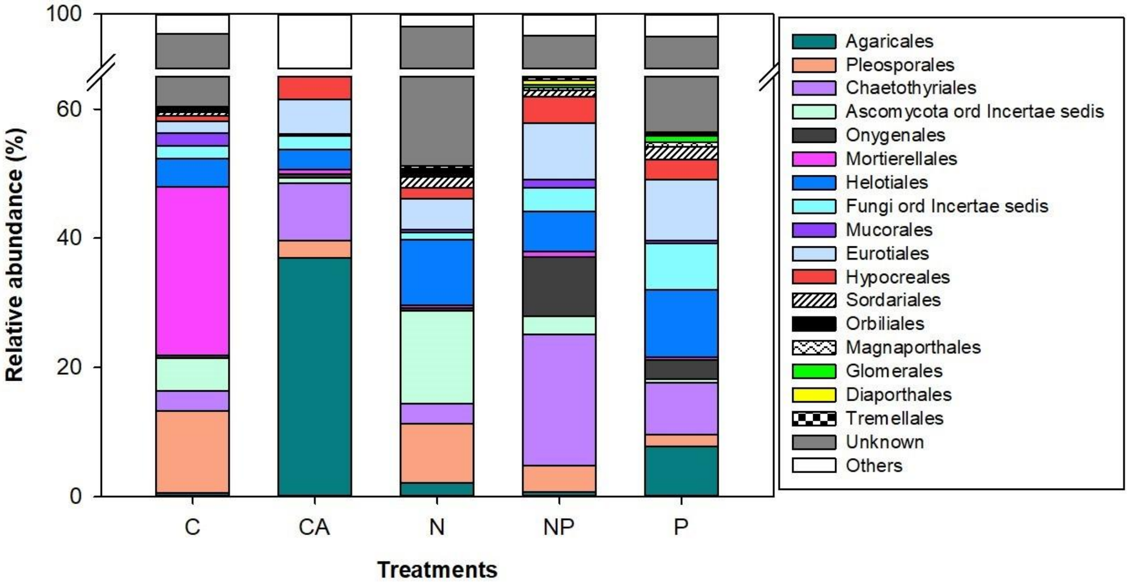

Figure 15. Relative abundance of soil fungi community at the order taxonomic level in a Cerrado sensu stricto area under long-term nutrient addition treatments. Treatments are: C: (control), CA (liming treatment), N (nitrogen treatment), NP (nitrogen + phosphate treatment), and P (phosphate treatment).

8.7.DISCUSSION

Soil has its own characteristics depending on climate, mineral formation and the weathering process the soil has been subjected to (Junior et al., 2020). The Cerrado soil is naturally acidic due to many factors like the removal of basic elements, especially Ca^{2+} from the colloidal part of soil. In the studied area the effects of the addition of nutrients have been analyzed along the years (Bustamante et al., 2012; De Mello et al., 2014; Silveira et al., 2021b) and there are many studies with long-term addition of nutrients around the world (Wang et al., 2024b), such as the classic nitrogen addition experiment at Cedar Creek, USA (Tilman, 1987), multi-site European field trials examining atmospheric nitrogen deposition (Phoenix et al., 2012), and nutrient enrichment experiments in tropical forests of Central America (Cusack et al., 2011).

The analysis we made in the soil follows the same direction that the addition of nutrients throughout the years caused the levels of P, S, Ca, Mg, Al, B, Cu, Fe, Mn, OM, SB and pH to change, differentiating the CA treatment from the C and the other treatments. Even five years after the last nutrient addition, these differences remain evident. The addition of calcium and gypsum can increase the pH, Ca^{2+}, Mg^{2+} and base saturation causing the reduction of Al^{3+} by utilizing the bicarbonate released from the lime dissolution which can react with the H^{+} from the soil neutralizing it (Fontoura, 2019). The CA treatment differs from the others in many aspects due to this nutrient addition. Other differences can be seen in Table S1 like the acidic reserve in soil (H+Al), the content of Cu, the total amount of cations (T), the Effective Cation Exchange Capacity (t), the balance between divalent (Ca^{2+} and Mg^{2+}) and monovalent (K^{+}) cations in the soil's exchange complex (Ca + Mg / K), the amount of clay and sand, and other components.

In a study, researchers found that the liming treatment has a continuous effect. After three years of the last lime addition, the soil still shows its effects. Not only in the surface, but also 1 meter below the surface of the soil (Bossolani et al., 2021). This corroborates with the present study because the last lime addition in the area occurred five years ago.

The differences caused by the addition of phosphorus and nitrogen in soil occurred in the contents of sulfur in those treatments (Table S1) and the treatments that most increased this nutrient were the addition of phosphorus and nitrogen, together or separated. The combination of those two nutrients gave the highest level of sulfur (48,94). This may have occurred because the nutrients added to the soil were combined with sulfur (Ammonium sulfate + Single superphosphate 20%).

Costa (2019), in her study conducted in the same experimental area, reported that plants in limed plots had leaves with five times less manganese (Mn) and twice as much magnesium (Mg) compared to control plots. This contrasts with our findings in the soil, where Mn content was five times higher and Mg was sixty-five times higher in the limed plots relative to the control. These contrasting patterns can be explained by the effects of liming on nutrient availability and plant uptake. Liming raises soil pH, which decreases the solubility and bioavailability of Mn, causing it to precipitate as insoluble oxides, even if total Mn content in the soil increases (Fageria, 2001). As a result, plants absorb less Mn despite its higher concentration in the soil. In contrast, the increase in Mg in the soil likely reflects both a release of exchangeable Mg and reduced leaching under higher pH conditions (Caires et al., 2015). While Mg uptake by plants also depends on various factors such as root physiology and cation competition—particularly with Ca^{2+} added through liming—plant absorption tends to increase when Mg is more available in the exchange complex (Marschner et al., 2012). Therefore, the discrepancy between soil and plant nutrient levels can be attributed to the complex interactions between soil chemistry, nutrient speciation, and plant uptake mechanisms influenced by liming.

Over the years, were added 1100 kg of Ammonium sulfate (N treatment), 1100kg of Single superphosphate 20% (P treatment), 2,200kg of Ammonium sulfate + Single superphosphate 20% (NP treatment) and 4,4000kg of Dolomitic limestone 60% + Agricultural gypsum 40% (CA treatment) in the area. The long-term nutrient addition experiment in the Cerrado has revealed important trends in soil chemical properties, further enriched by our 2023 data (Table S2). According to datasets compiled from studies by Jacobson (2009), Costa (2019), Mello (2019), Sousa (2020), and our own measurements, pH values significantly increased in the liming (CA) treatment—from approximately 4.36 in 2007 to 6.01 in 2023—demonstrating the enduring effect of liming on soil acidity correction. This consistent pH elevation led to a sustained reduction of aluminum (Al^{3+}) toxicity, with values dropping to 0.00 in 2023. In contrast, the pH in NP, N, and P treatments remained below 4.5, maintaining levels of exchangeable Al. Phosphorus levels, especially in the P and NP treatments, showed a clear accumulation over time, with values peaking in 2023 (P = 7.77 mg/dm^3, NP = 6.00 mg/dm^3), confirming the legacy effect of continued P input and reduced P fixation over time, as also highlighted in Mello (2019) and Costa (2019). Meanwhile, potassium (K) levels declined in CA—from ~42.75 in 2007 to ~11.33 mg/dm^3 in 2020—and remained low in 2023, indicating the need for replenishment.

Calcium (Ca) and magnesium (Mg), as expected, increased substantially in limed soils over the years (Ca = 5.83, Mg = 3.21 in 2023), while remaining low under acidic treatments. Organic matter (OM), which strongly contributes to cation exchange capacity (CEC), remained variable across treatments. In 2023, OM was highest in the control (2.44%), with somewhat elevated values in N (3.45%) and P (2.88%), possibly due to differences in plant litter or root dynamics, aligning with previous findings by Sousa (2020) and the studies of Pompermaier et al. (2022) that affirms that the combined addition of N and P can alter the dynamics of OM in the Cerrado, intensifying litter decomposition through the action of mesofauna, which may impact nutrient cycling and soil carbon stocks. The total iron (Fe) content, which is related to the adsorption of phosphorus, decreased in NP and N treatments in 2023, potentially reflecting shifts in mineral stabilization or plant uptake. These results reinforce the idea that while liming continues to correct acidity and improve base saturation, sustained nutrient additions such as P and N influence long-term nutrient accumulation, biological activity, and potentially biodiversity. Importantly, our 2023 data contribute new insights to this long-term experiment, showing how legacy effects of treatments persist or evolve under Cerrado conditions.

Studies by Minato, et al. (2023) revealed that the application of 10 t ha^{-1} of lime significantly increased soil pH, reduced aluminum (Al^{3+}) toxicity, and enhanced base saturation (V%), improving the availability of essential nutrients such as calcium (Ca^{2+}) and magnesium (Mg^{2+}). they found that these changes create a more favorable root environment, boosting the uptake of macronutrients (N, P, K) and stimulating microbial activity and nutrient mineralization. However, higher lime doses may lead to nutrient imbalances, especially by reducing the availability of phosphorus (P), potassium (K), and some micronutrients due to excessive pH elevation. In this study, liming was applied annually for several consecutive years without any vegetation being planted in the area, different from a cultivated area, which may have exacerbated the effects of liming over time and led to the invasion of non native grasses (Lannes et al., 2016).

Soil respiration analysis

The absence of statistically significant differences in soil respiration among the nutrient addition treatments suggests that long-term fertilization and liming did not substantially alter microbial respiratory activity in this Cerrado *sensu stricto* system. Although CA and P treatments presented numerically higher respiration rates, this variation was not sufficient to differentiate them statistically from the control. One possible explanation is the strong influence of abiotic conditions at the time of sampling. The measurements were conducted on the hottest day of the year, and elevated temperatures are known to stimulate microbial metabolism and root respiration, increasing overall CO_2 fluxes independently of nutrient status (Davidson et al., 2006). Under such thermal stress, microbial communities may exhibit enhanced metabolic activity across all treatments, leading to a homogenization of respiration rates. Additionally, the high variability observed within treatments, particularly in C and N, may reflect microsite-level differences in soil moisture or substrate availability, which become more pronounced under extreme temperature (Moyano et al., 2012; Qu et al., 2024)

While previous studies have shown that soil respiration can be useful for detecting differences in microbial activity, this tool is less effective when assessing long-term effects of treatments, as in our case where five years have passed since the last application. In studies like ours, other tools, such as metagenomics or metabarcoding, are necessary to detect more subtle differences that persist even after such a prolonged period without nutrient addition. These results highlight the importance of considering climatic conditions during sampling when interpreting soil respiration data and suggest that single-point measurements under extreme weather may obscure treatment effects. Thus, while soil respiration is a useful proxy for biological activity, its reliability as an indicator of soil health should be evaluated in the context of environmental variability and temporal replication.

Soil enzyme analysis

β-glucosidase

β-glucosidase activity varied among treatments, with the lowest values observed in the liming treatment (CA), which was significantly lower than the others (letter “b”, $p < 0.05$). Treatments receiving nitrogen (N), phosphorus (P), and the combination of N+P

(NP) presented higher mean values, although with no significant differences among them (letter “a”). These results support the understanding that nutrient addition—particularly N and P—stimulates the microbial communities responsible for producing β-glucosidase (Daughtridge et al., 2024; Liu et al., 2021), while liming alone may negatively impact enzyme activity, especially when accompanied by reductions in organic matter or labile carbon availability (Mockeviciene et al., 2022).

β-glucosidase plays a key role in the degradation of organic residues and reflects the quality and quantity of carbon substrates available in the soil. The low enzymatic activity observed in the CA treatment (approximately 34 µg pNP g^{-1} soil h^{-1}) may indicate organic matter loss over time or rapid mineralization following pH increase due to liming. This aligns with studies showing decreased enzyme activity in limed soils where organic inputs were not replenished (Pospíšilová et al., 2023). In contrast, the N and P treatments maintained activity levels above 45 µg pNP g^{-1} soil h^{-1} suggesting that even five years after nutrient inputs ceased, their residual effects on microbial function persist, although not enough to raise activity above the “moderate” threshold (91 mg PNP kg^{-1} soil h^{-1}) proposed by Barbosa et al. (2023).

Despite numerical variation, all treatments exhibited β-glucosidase activity below 91 mg pNP kg^{-1} soil h^{-1}, indicating low biological activity across the system, based on the classification by Barbosa et al. (2023). This outcome likely reflects the long-term absence of nutrient additions, the naturally low fertility of Cerrado soils, and extreme abiotic conditions at the time of sampling—performed on the hottest day of the year—which may have suppressed microbial activity and enzyme production (Wang et al., 2023b). These findings underscore that although β-glucosidase is a useful indicator of soil biological quality, its interpretation must account for environmental variability and long-term management history.

Soils under cultivation typically show significantly lower β-glucosidase activity compared to adjacent native forests or grasslands—a reflection of degraded organic matter quality and reduced microbial functioning. For example, (Saviozzi et al., 2001) found that after 45 years of continuous corn production, adjacent native grasslands exhibited higher β-glucosidase activity and water-soluble organic carbon than cultivated fields, indicating greater carbon turnover and soil quality in undisturbed systems (Saviozzi et al., 2001). Similarly, De Almeida et al., (2015)reviewed multiple land-use studies and concluded that β-glucosidase activity is generally elevated in preserved soils or those under organic management, and tends to decrease in intensively managed croplands due to lower labile carbon inputs.

Phosphatase

Acid phosphatase activity varied significantly among treatments, with the highest values observed in the control (C), followed by N, and the lowest in the CA (liming) treatment. The control group exhibited significantly greater activity compared to all nutrient addition treatments ($p < 0.05$). This result aligns with the established understanding that acid phosphatase is up regulated in phosphorus-deficient conditions, as it facilitates the mineralization of organic phosphorus (Conte et al., 2002; Margalef et al., 2017). The high enzymatic activity in the control indicates a strong microbial and plant demand for phosphorus in nutrient-limited conditions, serving as a biological indicator of P limitation. In this study, values ranged from approximately 330 to 730 µg p-nitrophenol g^{-1} soil h^{-1}, which are considered intermediate when compared to general literature values, where average phosphatase activity in acidic soils typically falls around 678 µg p-nitrophenol g^{-1} soil h^{-1} (Barbosa et al., 2023).

Nitrogen addition (N treatment) resulted in intermediate phosphatase activity, significantly lower than the control but higher than the P, NP, and CA treatments. This pattern is consistent with the dual influence of nitrogen on enzyme dynamics. Nitrogen is essential for enzyme synthesis and microbial growth, but depending on its form and application rate, it can also influence soil pH and alter phosphorus availability, which may reduce phosphatase activity (Arenberg et al., 2021; Liu et al., 2022). In treatments where phosphorus was added (P and NP), enzyme activity declined notably, supporting the theory that phosphatase production is down regulated when P is no longer limiting (Marklein et al., 2012). The values, although numerically lower than the control, still remained well above typical averages reported in the literature, suggesting robust biological activity across all treatments despite relative differences.

The CA treatment showed the lowest phosphatase activity, significantly different from the control and N treatment. This result reflects the sensitivity of acid phosphatases to pH changes, as these enzymes are generally more active in acidic environments. Liming raises soil pH, potentially leading to conformational changes in enzyme structure or shifts in microbial community composition, ultimately reducing enzymatic expression or stability (Liu et al., 2022). Nonetheless, the activity observed in CA (~330 µg pNP g^{-1} soil h^{-1}) remains an average value commonly reported in tropical soils. These findings reinforce the relevance of acid phosphatase as a sensitive bioindicator for evaluating both nutrient limitation and chemical shifts induced by soil management practices.

Arylsulfatase

Arylsulfatase activity varied significantly among treatments, with the highest values observed in the CA (liming) and C (control) treatments, and the lowest in the NP and N treatments ($p < 0.05$). These results indicate that nutrient additions, particularly nitrogen and phosphorus, suppressed microbial activity related to the sulfur cycle because the nutrients added contained sulfur in the formula ($(NH_4)_2SO_4$ and $Ca(H_2PO_4)_2$ + $CaSO_4.2H_2O$). Arylsulfatase plays a key role in the mineralization of organic sulfur, and its production is often enhanced under sulfur-limiting conditions. The low sulfur availability reported in C and CA treatments (Table S1) may have stimulated microorganisms to increase arylsulfatase production, compensating for the lack of readily available S. This pattern is supported by Kunito et al (2022), who reported higher arylsulfatase activity in soils with reduced soluble and adsorbed sulfur. Thus, arylsulfatase appears to be a sensitive biological indicator of sulfur availability and microbial adaptation to nutrient limitations (Wyszkowska et al., 2016).

Despite lower organic matter (OM) in the CA treatment, which exhibited the highest arylsulfatase activity, the N treatment—where OM was higher—showed the lowest activity, suggesting that OM alone does not fully explain the observed patterns. This finding contrasts with Longo et al., (2011), who described a direct relationship between OM and arylsulfatase due to the role of organic matter as a source of sulfate esters. However, enzyme activity is regulated by multiple soil properties, including pH, texture, microbial community structure, and nutrient dynamics. For instance, Chen et al., (2019) found that soil pH and texture were more influential than organic carbon in controlling arylsulfatase activity, especially in acidic soils. In this study, the increase in pH from liming in CA may have created a favorable microenvironment for enzyme stabilization or microbial expression, outweighing the effect of reduced OM.

According to the classification proposed by Barbosa et al. (2023) arylsulfatase activity is around 106 µg p-nitrophenol g^{-1} soil h^{-1}. In the present study, the treatments all registered low activity, below 30 µg pNP g^{-1} soil h^{-1}. These results suggest that long-term nutrient enrichment may have reduced microbial demand for sulfur mineralization or altered community composition in a way that suppresses arylsulfatase expression. Consequently, arylsulfatase emerges as a useful indicator of soil biological quality and nutrient cycling status, particularly sensitive to sulfur dynamics and affected by both management history and physicochemical soil conditions

Integrated Assessment of Soil Quality Based on the Soil Quality Index (IQS)

It is noteworthy to mention that the IQS (Soil Quality Index) was designed to evaluate soils under agricultural use and cropping systems, where fertility and productivity are primary concerns. Importantly, this index is generally measured after the crop is harvested, reflecting soil conditions resulting from cultivation and management practices. This timing may limit its applicability in natural ecosystems, as it does not account for the continuous, undisturbed nutrient cycling and biological interactions present throughout the year. In contrast, the Brazilian Cerrado is a naturally adapted ecosystem that evolved under nutrient-poor, acidic soil conditions, and its rich biodiversity is well adapted to low fertility without the need for external fertilization or pH correction. Native Cerrado vegetation maintains ecological equilibrium through specialized root systems, symbiotic associations (e.g., mycorrhizae and nitrogen-fixing bacteria), and efficient nutrient recycling mechanisms (Ratter, 1997). Therefore, while fertilization and liming may enhance soil quality metrics from an agricultural perspective, they are not necessary for the persistence or ecological functioning of the native Cerrado biome.

The IQS indexes are a complex analysis that needs from the sample data from the biome, the cultivated plant, the local where the sample was collected, the quantity of silt sand and clay from the ambient. The values for the arylsulfatase and β-glucosidase, the organic matter in soil, total cation exchange capacity (CTC T), amount of exchangeable hydrogen (H^+) and aluminum (Al^{3+}) ions that are adsorbed on soil colloids (H+Al). The values for K, Al, Ca^{+2} and Mg. The values of pH, the P in Mehlich and in resin, and other components. Even though these indexes are primarily used for cultivated soils, the principle can also be applied to native soils since the objective of this long-term experiment is to observe the effects of nutrient addition on native soils simulating the nutrient addition that agriculture may do in the native soil.

The observation that the CA treatment has the highest values for biological IQS, Chemical IQS, Nutrient storage and nutrient supply align with Cardoso et al., (2013) emphasis on pH correction as a crucial step in enhancing microbial biomass and enzymatic activity. The increased biological activity is likely due to improved pH, Ca^{+2} availability, and better conditions for microbial proliferation. Caires et al. (2008) found that in comparison to the non-limed area, surface liming increased the concentrations of Ca and Mg and improved the overall nutrient uptake by soybean plants, demonstrating the positive effect of lime on soil fertility and plant nutrition under no-till conditions.

While NP fertilization can stimulate microbial activity—often raising enzymes such as β-glucosidase—it does not necessarily yield balanced communities over time unless organic matter is maintained; in Cerrado Oxisols, microbial indicators closely track soil organic carbon, underscoring the role of OM inputs in sustaining biological function (Lopes et al., 2013; Dong et al., 2015; Zhao et al., 2016). The distinctive vegetation of the Cerrado biome is largely shaped by its highly weathered, acidic, and nutrient-poor soils—including Oxisols and deep sandy Entisols—and native plant communities have evolved specific morphological and physiological adaptations to thrive under high aluminum levels and low nutrient availability in these edaphic conditions (Haridasan, 2008).

In the experiments of Costa, (2019), in the same area as this study, invasive species such as *Melinis minutiflora* were predominantly observed in the N and NP treatments, while they were absent in the control plots, whereas in the N treatment there was an increase in the dominance of the native grass *Echinolaena inflexa* (De Mello et al., 2014) indicating that nutrient enrichment facilitated the establishment of exotic grasses in the native Cerrado area, demonstrating that nutrient addition in the Cerrado may have a negative effect above ground. The addition of nutrients, particularly nitrogen and phosphorus, can significantly enhance the invasion success of fast-growing exotic plant species. By alleviating resource limitations typical of native ecosystems—such as those found in nutrient-poor tropical soils—nutrient enrichment shifts the competitive balance in favor of invasive species. These species, which often exhibit rapid growth rates and high nutrient demands, can outcompete native flora adapted to oligotrophic conditions, ultimately altering plant community composition and ecosystem functioning (Liu et al., 2016; Uddin et al., 2020).

Regarding nutrient storage, there was no statistical difference between the treatments. The cation exchange capacity (CEC) of the soil is significantly influenced by the content of organic matter, which, upon decomposition, releases negatively charged functional groups (such as carboxyl and phenolic groups). These groups contribute to the soil's ability to retain and exchange essential nutrient cations like calcium, magnesium, and potassium. As a result, increasing soil organic matter typically enhances CEC (Table S1), thereby improving the nutrient retention and overall fertility of the soil (EMBRAPA, 2018; Brady et al., 2016; Sparks, 2003). Even with no statistical difference, the IQS analysis demonstrated that N treatment had a good quality for nutrient storage followed by CA and NP treatments. CA had the highest value for CEC but the lowest value for organic matter, and the N treatment was the opposite. This may be because organic matter has more influence in the result of the index than the cation exchange.

As for the nutrient supply function, the only index with statistical difference between treatments, C and N were statistically different than CA and intermediate values in NP and P. The nutrient supply considers exchangeable bases (SB) (Table S1) and P content; thus, every treatment scored low for this index, the difference is in the CA treatment is due to its higher SB. These results indicate that the CA treatment is supplying more nutrients due to the high pH, and this is modifying the microorganisms present in that area by elevating the abundance of copiotrophic organisms adapted to this condition.

Importantly, the samples in this study were collected on November 14, 2023—an abnormally hot day in Brasília marked by extreme temperatures and thermal sensation. This day fell within Brazil's eighth major heat wave of the year and was characterized by intense solar radiation, low relative humidity, and temperatures exceeding 32 °C (INMET, 2023). Such extreme weather conditions may have significantly impacted biological indicators, including enzyme activities and microbial responses, which are known to be sensitive to temperature and moisture fluctuations (Hammerl et al., 2019; Steinweg et al., 2013). Elevated temperatures can accelerate metabolic rates and microbial turnover but may also induce thermal stress, particularly when coupled with low moisture (Cruz-Paredes et al., 2021). Therefore, some of the results—especially those related to enzyme activity and microbial biomass—should be interpreted in light of these environmental constraints. This highlights the importance of contextualizing soil biological data with concurrent climatic conditions, particularly in field-based assessments of soil quality.

Soil microbiota composition

Alpha and beta diversity for bacteria

Alpha diversity metrics provide insights into the richness and evenness of microbial communities within a given sample. In this study, commonly used indices—including Observed OTUs, Chao1, ACE, Shannon, Simpson, and Inverse Simpson—were employed to evaluate how soil bacterial communities respond to nutrient inputs in a native Cerrado system. Despite visible trends among treatments, no statistically significant differences were detected among treatments for any of the alpha diversity indices (as shown by shared letters in Tukey's or t-tests at $p > 0.05$).

Growing evidence suggests that soil bacterial alpha diversity frequently remains unchanged following nutrient additions, even under long-term N or P fertilization regimes—highlighting microbial resilience in many ecosystems. For instance, in semi-arid winter wheat systems, irrigation significantly altered bacterial alpha diversity, but nitrogen fertilization had no statistically significant effect on alpha diversity indices (Li et al., 2021). Similarly, a 2022 study reported that short-term nitrogen addition did not affect bacterial or fungal alpha diversity in forest soils, suggesting that nutrient enrichment alone may not overcome community-level inertia or resistance (Chen et al., 2022). In a six-year nitrogen addition experiment conducted in a subtropical *Castanopsis carlesii* natural forest, researchers found no significant changes in bacterial or fungal alpha-diversity (e.g., Chao1 and Shannon indices) or beta-diversity across treatments differing in nitrogen input levels (He et al., 2023). This research showed that soil bacterial phyla (e.g., Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes) and major fungal phyla remained compositionally stable despite nutrient enrichment, suggesting functional redundancy and resilience of microbial communities under moderate N deposition.

Although P showed numerically higher values for Observed OTUs (4187.67) and ACE (4204.04), these differences were not statistically significant compared to C, which had 3514.3 observed OTUs and 3520.42 ACE. These overlapping ranges suggest high intra-treatment variability and potentially the influence of site heterogeneity or legacy effects. This aligns with findings from Fierer et al., (2012) and Carey et al., (2015), who emphasized that microbial community structure can shift taxonomically or functionally without necessarily affecting alpha diversity metrics.

The N and NP treatments showed only a subtle separation in bacterial beta diversity (Figure 10), which can be attributed to the persistence of similar environmental filters, such as high acidity (pH 4.0–4.4) and elevated exchangeable aluminum, both exerting strong selective pressure on the Cerrado microbial community. However, some chemical differences account for the partial divergence between these treatments. Both have sulfur levels much higher than the control (45–49 mg dm^{-3}), a condition that may favor microbial groups associated with the sulfur cycle. The N treatment exhibits a higher organic matter content (3.45 dag kg^{-1}), favoring copiotrophic taxa such as Proteobacteria (Fierer et al., 2007; Stone et al., 2023), while NP stands out due to its greater phosphorus availability (6.00 mg dm^{-3}), which may relieve P limitation and support the growth of groups with higher phosphorus demands. Thus, variations in S, OM, and P, combined with the dominant edaphic filters, explain the slight separation between N and NP in bacterial community structure.

Moreover, the stability of diversity indices like Shannon (range 7.80–7.93) and Simpson (all ~1.00) across treatments further supports the idea that nutrient addition did not disrupt community evenness. This may be due to the dominance of generalist taxa capable of maintaining equilibrium under varying nutrient scenarios, a trait common in soils with fluctuating nutrient inputs, especially in tropical biomes (Tedersoo et al., 2014).

Overall, while nutrient additions (particularly P) appear to influence bacterial abundance and possibly community structure, they do not significantly affect within-sample diversity (alpha diversity) in this Cerrado soil context. This reinforces the notion that alpha diversity may be functionally conservative, and shifts in microbial roles may not be captured by richness-based indices alone (Shade, 2017). Future work incorporating longer treatment durations or metatranscriptomic data may reveal more subtle or delayed responses in microbial functioning.

Alpha diversity for fungi

The analysis of fungal alpha diversity in response to soil nutrient additions revealed nuanced patterns across all measured indices, suggesting a complex interaction between nutrient availability and fungal community richness and evenness. Based on the observed, Chao1, and ACE estimators, which emphasize richness, P addition showed a tendency to maintain or increase the number of taxa compared to the C and other treatments. However, the standard deviations were large, and statistical comparisons (Tukey's or t-test) indicated no significant differences between treatments across most diversity metrics. For instance, the Observed, Chao1, and ACE values for P ranged around 809–934 taxa, not statistically distinct from the values of the NP and N treatments. This suggests that, although nutrient inputs might influence fungal community richness, they do not induce substantial compositional shifts under the conditions of this study.

Diversity indices that incorporate species evenness, such as the Shannon and Inverse Simpson indices, presented more variable responses to nutrient amendments. The Shannon index, which balances richness and evenness, was highest in the N treatment (5.28), followed by NP (4.95), suggesting that nitrogen, either alone or in combination with phosphorus, can support a more even distribution of fungal taxa. This could be attributed to the promotion of competitive balance among fungal guilds under moderate nutrient enrichment. Nevertheless, the large overlaps in standard deviations and lack of significant differences indicate that none of the treatments consistently disrupted or promoted community evenness to a significant extent.

Zheng et al., (2022) reported that soil fungal diversity, as measured by the Shannon index, is strongly influenced by phosphorus availability, suggesting that treatments alleviating P limitation promote more diverse communities, particularly those with higher nutrient requirements. Additionally, studies on nitrogen enrichment have shown that increased N availability can enhance fungal diversity and significantly alter community structure (Li et al., 2023b). Liming mostly reduces abundance, biomass and activity of most fungi which tend to favor acidic soils (Wenyika et al., 2025). As was also observed by Bååth et al., (2003) that reported that increasing soil pH, such as through liming, reduces the fungal-to-bacterial ratio, indicating the preference of fungi for more acidic environments.

The Simpson and Inverse Simpson indices further confirmed this interpretation. While the Simpson index values were relatively high (>0.8) across all treatments—indicating a generally even distribution of dominant taxa—no statistically significant differences were observed, as denoted by identical superscripts in the table. Interestingly, the Inverse Simpson index showed a wider range, with the N treatment (78.99) reaching the highest value, but again lacking statistical differentiation from the other treatments. This points to a general resilience in fungal alpha diversity in native Cerrado soils, where even significant nutrient enrichment does not drastically alter fungal community structure at the alpha level. These findings align with previous research that indicates fungal communities often exhibit higher resistance to nutrient perturbations than bacterial communities due to their functional plasticity and physiological tolerance (Fu et al., 2024; Zheng et al., 2021).

Relative Abundance of Bacteria

The analysis of bacterial taxonomic composition revealed significant changes in some microbial groups in response to nutrient addition and liming. These alterations likely reflect shifts in microbial niches and functional capacities, modulated by resource availability and soil chemical changes. Proteobacteria phylum (Figure 14 and Table S4) are among the dominant bacterial groups in Cerrado soils, especially in native vegetation and agricultural systems (De Castro et al., 2016; Silveira et al., 2021b; Souza et al., 2016), reflecting their ecological importance in nutrient cycling and soil health. They often increase in relative abundance in soils under agricultural management such as no-till and conventional tillage systems compared to native soils (Souza et al., 2016). Proteobacteria generally prefer acidic soil (Wang et al., 2019).

Members of the phylum Gemmatimonadota are consistently detected in soils across diverse environments and frequently represent a notable portion of the microbial community—often among the top nine most abundant bacterial phyla in terrestrial ecosystems (up to ~1 % relative abundance) (Mujakić; Piwosz; Koblížek, 2022). While typically oligotrophic and adapted to dry soils with low moisture, these bacteria are also positively correlated with soil nutrients such as organic carbon, nitrogen, and phosphorus, suggesting a role in nutrient cycling and soil organic matter turnover (Du et al., 2024). Moreover, genome-resolved and environmental studies indicate that many members encode polyphosphate accumulation mechanisms, anoxygenic photosynthesis, nitrogen-cycling genes (e.g., nosZ), and enzymes for degrading complex organic compounds—underscoring their metabolic versatility and potential functional significance in C, N, and P cycling within soils (Venkatachalam et al., 2024).

Members of the phylum Chloroflexi are consistently present in soils and play crucial roles in the degradation of microbial biomass and complex organic substrates. Genome-resolved analyses indicate that Chloroflexi species possess metabolic pathways for decomposing lysed bacterial cell debris, transporting and metabolizing N-acetylglucosamine (a component of cell walls), and engaging in fermentation and beta-oxidation of fatty acids—processing key components of soil nutrient turnover and organic matter transformation (Bovio-Winkler et al., 2023). Additionally, in engineered systems like activated sludge and constructed wetlands, Chloroflexi are known to form filamentous networks that support floc structure, contribute to carbon fermentation, and assist in nutrient removal—including nitrogen and phosphorus cycling (Speirs et al., 2019).

The order Frankiales showed significantly higher relative abundance in the NP compared to CA. Members of this order are recognized for their capacity in nitrogen fixation and their role in the breakdown of complex organic matter, particularly under nutrient-rich conditions (Sellstedt et al., 2013). Their increase under NP suggests a favorable environment for actinobacterial groups involved in N cycling and soil organic matter (SOM) transformation (Bao et al., 2021; Zhang et al., 2025a).

Solibacterales also increased significantly in the NP treatment compared to CA. This group belongs to the phylum Acidobacteriota and is known for its ability to degrade recalcitrant carbon compounds under acidic and nutrient-limited conditions, although it can also proliferate under moderate nutrient enrichment (De Chaves et al., 2019). The observed increase under NP implies that the combination of macronutrients may enhance the abundance of stress-tolerant, carbon-degrading taxa, especially those adapted to fluctuations in nutrient stoichiometry.

In contrast, the order Gammaproteobacteria was significantly less abundant in the CA and P treatments when compared to the C and N. Gammaproteobacteria are generally considered copiotrophic, responding positively to labile carbon and nitrogen availability (Fierer et al., 2007). Their decline under liming may be due to pH shifts, which can alter the competitive balance among bacterial taxa (Wang, 2024). The suppressive effect of P-only addition may reflect either P-induced microbial stress or limitations in complementary resources such as C or N.

The order Bryobacterales, which also belongs to Acidobacteriota, was significantly enriched in the NP treatment compared to CA. This order is typically associated with oligotrophic environments but has been shown to increase in abundance when SOM degradation is stimulated (Liu et al., 2022). The enrichment in NP-treated soils reinforces the idea that balanced nutrient inputs (especially N and P) support microbial taxa involved in mineralization and organic matter cycling.

Predatory bacteria of the order Myxococcales are ubiquitous in soils—often comprising up to 75 % of all bacterial micropredators in mineral soils—and act as key regulators of microbial community structure and nutrient cycling through their ability to lyse diverse prey taxa (bacteria, fungi, protozoa) via swarming behavior and secretion of lytic enzymes and secondary metabolites (Petters et al., 2021). Their predatory activity promotes nutrient turnover and organic matter decomposition, influencing carbon sequestration and N cycling by releasing biomolecules from prey cells into the soil matrix (Phillips et al., 2022; Ye et al., 2020).

Interestingly, the order Elsterales increased significantly in the N treatment compared to CA and P. Although this order is still poorly characterized, it is generally found in low-nutrient or disturbed environments (Kelleher et al., 2025). Members of the bacterial order Elsterales have been identified as key contributors to soil microbial communities, ranking among the main bacterial groups in plantation soils, as observed across different management stages in Chinese fir (*Pinus*) monocultures In China (Liao et al., 2024b). Analyses revealed that Elsterales is among the five most abundant bacterial groups, along with Subgroup2, Xanthobacteraceae, and others, and its predominance has been associated with carbon, nitrogen, and phosphorus cycling, acting in conjunction with soil variables such as pH, total N, and available phosphorus (Liao et al., 2024b). These findings indicate that Elsterales may play a central role in organic matter processing, nutrient turnover, and the functional structure of the soil microbial community, particularly in forest management systems.

Polyangiales, another oligotrophic order, was significantly more abundant under CA than under C and N treatments. Their response suggests that pH neutralization through liming may favor slow-growing, pH-sensitive bacteria, as previously reported in acid soils subjected to long-term liming (Sridhar et al., 2022). In a long-term paddy cropping system under legume rotation, Polyangiales—a predatory lineage within Myxococcota—

correlated positively with soil phosphorus availability and rice yield (Xia et al., 2023). Network analyses suggest that Polyangiales thrives in high-nutrient environments, likely due to increased prey availability resulting from nitrogen fixation and phosphorus mobilization by leguminous crops (Wang et al., 2023a). This points to a broader ecological role for Polyangiales in enhancing nutrient turnover (particularly P cycling) through predation-driven microbial interactions, indirectly promoting crop productivity.

Micropepsales showed a distinct response, with significantly higher abundance in NP and N treatments compared to CA and P. Although little is known about the functional roles of this order, its consistent enrichment in nitrogen-amended treatments suggests a degree of sensitivity to nitrogen availability, possibly linked to carbon processing or competitive microbial interactions (Harbison et al., 2017). Members of the order Micropepsales, including the genus *Micropepsis*, are chemoheterotrophic Alphaproteobacteria widespread in soils, often inhabiting mildly acidic, oligotrophic environments where they ferment diverse sugars such as cellobiose, glucose, and maltose (Harbison et al., 2017). These bacteria contribute to the decomposition of microbial and plant-derived organic matter, especially under low-pH conditions, and are adapted to survive in nutrient-poor matrices by utilizing complex carbon substrates (Harbison et al., 2017). Their metabolic versatility and common presence in poorly buffered soils underscore a subtle yet important role in soil carbon turnover and energy flow, particularly in acidic and nutrient-limited ecosystems.

The genus *Bryobacter*, a member of the family Bryobacteraceae within the Acidobacteriota phylum, is typically classified as an oligotrophic taxon associated with acidic and organic-rich environments (Kulichevskaya et al., 2010). It was significantly more abundant in the NP treatment (3.42%) compared to C, CA, or P. Although traditionally associated with nutrient-poor soils, *Bryobacter* possesses genes for the degradation of plant-derived polysaccharides and the utilization of low-molecular-weight organic compounds, suggesting it can adapt to moderate nutrient increases when microhabitat conditions—such as moisture, pH, and organic matter heterogeneity—become favorable (Berlemont et al., 2015). The increase of *Bryobacter* under NP may therefore reflect a balance between oligotrophic persistence and localized nutrient enrichment that allows niche expansion.

Unclassified Gammaproteobacteria (Incertae Sedis) were significantly more abundant in the control (10.41%) and N (9.80%) treatments compared to CA (3.82%) and P (3.21%). Gammaproteobacteria, including groups such as Enterobacteriaceae and Pseudomonadaceae, are known for their copiotrophic lifestyle, rapid growth, and strong responses to labile carbon and nitrogen additions (Kim et al., 2021; Kurm et al., 2017). Their high abundance in unfertilized and N-only plots suggests stimulation by nitrogen-driven increases in root exudation or microbial turnover, while liming and phosphorus addition may reduce competitiveness by altering soil pH or resource stoichiometry. (Liao et al., 2024a; Mastný et al., 2020). The high abundance of copiotrophic Gammaproteobacteria accelerates the mineralization of organic nitrogen, increasing NH_4^+ and NO_3^- availability and stimulating nitrification–denitrification processes that may enhance N_2O emissions (Fierer; Bradford; Jackson, 2007; Zhou et al., 2016). Their rapid metabolism of labile carbon sources promotes elevated microbial respiration and CO_2 release while limiting the formation of stable soil organic matter (Pascault et al., 2013). In addition, fast microbial growth can cause temporary P immobilization, while Pseudomonadaceae within Gammaproteobacteria produce organic acids and phosphatases that solubilize P and alter soil C:N:P stoichiometry (Richardson; Simpson, 2011).

In contrast, Sutterellaceae, another family within Gammaproteobacteria, showed significantly higher abundance in the control (2.02%) than in NP, CA, or P treatments. Although primarily studied in gut microbiomes, some members of this group have been detected in soil and are thought to be sensitive to environmental disturbances. Their decline under fertilization may reflect displacement by faster-growing competitors or sensitivity to shifts in nutrient balance and soil chemistry, a phenomenon commonly observed among native or stress-adapted microbial taxa (Baldrian, 2017; Luo et al., 2018)

Subgroup 1 of Acidobacteriota, which includes poorly classified but functionally relevant soil bacteria (family incertae sedis), was significantly more abundant in NP (2.76%) compared to CA and P. Members of this subgroup are typically oligotrophs involved in the decomposition of recalcitrant organic matter and the cycling of carbon under nutrient-limited conditions (Kielak et al., 2016; Männistö et al., 2013). Their increased abundance under NP suggests that nutrient enrichment may not exclusively favor copiotrophs but can also support the coexistence of oligotrophic taxa, likely due to increased resource heterogeneity and spatially distinct microhabitats.

The family Micropepsaceae, within Alphaproteobacteria, was significantly more abundant in NP and N treatments (1.02–1.13%) than in C, CA, and P. Although ecological information on this family is limited, Alphaproteobacteria are frequently associated with nitrogen transformations such as ammonification, nitrification, and nitrogen fixation (Liu et al., 2023). Their positive response to nitrogen addition supports the view that nitrogen availability is a key determinant of community structure and function in microbial assemblages.

A similar pattern was observed for Elsterales, an order within Alphaproteobacteria, which was more abundant in N and NP treatments compared to CA and P. While their functional capabilities are not fully understood, recent metagenomic evidence suggests Elsterales may be involved in organic nitrogen metabolism and respond positively to nitrogen-driven shifts in root exudation profiles. Their enrichment under N and NP indicates a role in nitrogen turnover, especially under conditions of increased nutrient input.

On the other hand, Vicinamibacterales, an order within the Acidobacteriota phylum (family Vicinamibacteraceae), were more abundant in CA (2.35%) than in N (0.54%). These taxa are typically associated with low-fertility, acidic soils and exhibit traits consistent with oligotrophic life strategies, such as slow growth and high-affinity nutrient transport systems (Eichorst et al., 2018; Navarrete et al., 2013). The increase in limed plots may reflect favorable pH conditions and improved cation exchange that reduce competitive exclusion by copiotrophic taxa, thereby supporting more pH-sensitive and functionally specialized lineages.

The study by Sousa, (2020) in the same area of this study, explored the effects of long-term nutrient addition and liming on nitrogen-fixing bacteria, focusing on the relative abundance of the *nif*H gene, a molecular marker for biological nitrogen fixation. The authors identified *Bradyrhizobium* as the dominant genus associated with *nif*H sequences, emphasizing its versatility as both symbiotic and free-living diazotrophs. Interestingly, the study found that the abundance of the *nif*H gene was significantly reduced in nutrient-treated plots—especially those receiving lime and phosphorus—compared to the untreated control, suggesting that nutrient enrichment may suppress nitrogen fixation activity in free-living communities. This reduction was most pronounced in the liming treatment, likely due to increases in soil pH and calcium levels, which may disrupt conditions optimal for nitrogenase enzyme functionality.

These findings suggest that, although nitrogen-fixing bacteria are present in Cerrado soils, their abundance and activity are not significantly enhanced by fertilization. In particular, liming consistently reduced the relative abundance of the *nif*H gene compared to control, N, and NP treatments—indicating a suppression of nitrogenase enzyme potential likely driven by increased soil pH and calcium interfering with optimal conditions for nitrogen fixation (Silveira et al., 2021). Additionally, long-term lime application in semi-natural grassland soils was shown to buffer but not increase free-living diazotroph abundance or *nif*H expression, suggesting that elevated pH and base saturation may constrain the ecological advantage of nitrogen-fixing microbes under acid removal management (Mitsuta et al., 2025).

In our current study, metagenomic profiling revealed a similar trend regarding nitrogen-fixing bacteria. Genera such as *Bradyrhizobium* and *Burkholderia-Caballeronia-Paraburkholderia*, which are recognized for their diazotrophic capabilities, were present across treatments but did not exhibit significant increases in abundance following nitrogen or phosphorus fertilization. Notably, *Bradyrhizobium* remained relatively stable, while *Burkholderia-Caballeronia-Paraburkholderia* showed only minor, statistically insignificant variations. These findings suggest that while nitrogen-fixing bacteria are present in the Cerrado soil, their relative abundance is not strongly stimulated by fertilization, potentially due to shifts in community structure or suppression of *nif*H gene expression under nutrient-rich conditions.

Nitrogenase, the enzyme complex responsible for biological nitrogen fixation (BNF), is highly sensitive to external nutrient conditions—particularly nitrogen (N) and phosphorus (P). Under enhanced nutrient treatments, nitrogenase activity typically decreases, especially when reactive nitrogen (e.g., NH_4^+, NO_3^-) is abundant. This occurs because diazotrophic (nitrogen-fixing) microorganisms downregulate *nif* gene expression and nitrogenase activity when external N sources are readily available, making BNF energetically unnecessary and inefficient (Reed et al, 2011).

Relative Abundance of fungi

Fungal community composition in the Brazilian Cerrado is shaped by a range of environmental and nutrient factors. Previous studies have identified Ascomycota and Basidiomycota as the most abundant fungal phyla in these ecosystems (Ferreira De Araujo et al., 2017; Maia et al., 2015). In the current study, Ascomycota was the dominant phylum across all treatments, particularly under nitrogen addition, where it reached 91.36% relative abundance (Table S9). Interestingly, although Basidiomycota typically ranks second in Cerrado soils, it was only the third most abundant in the control group (4.04%), with Mortierellomycota surpassing it (26.14%). Basidiomycota was the second most abundant only under the CA treatment, possibly due to improved growth conditions such as increased pH, carbon availability, or reduced microbial competition.

Nutrient enrichment led to shifts in the abundance of specific fungal orders, reflecting their ecological functions and adaptability. Among these, Chaetothyriales responded most strongly to phosphorus input, exhibiting a relative abundance of 8.07% under P treatment. This group of black yeasts (Teixeira et al., 2017; Torres-Garcia et al., 2023), often found in soil and plant-associated environments (Tedersoo et al., 2009), includes species capable of decomposing organic matter in challenging habitats, including polluted soils (Badali et al., 2011). The increase in Chaetothyriales under P fertilization may be attributed to the availability of nutrients favoring stress-tolerant saprotrophs.

Members of the fungal order Pleosporales, which belong to the class Dothideomycetes, are recognized as ecological generalists and saprotrophic decomposers—capable of breaking down cellulose and lignin-rich plant material (Moreira et al., 2021). In our study, Pleosporales were particularly prevalent in the control (12.66%) and N treatments (9.17%), yet their relative abundance declined dramatically under phosphorus addition (1.89%), suggesting a negative response to elevated P availability. This pattern aligns with evidence that increased phosphorus inputs can shift fungal community composition by reducing saprotrophic taxa in favor of P-adapted or mutualistic groups (Ma et al., 2022). Changes in substrate quality and competition dynamics under P enrichment may further disadvantage generalist decomposers like those in Pleosporales (Geng et al., 2023).

Patterns of abundance for Onygenales indicate that environmental pH and nutrient status influence this group's distribution. The highest mean abundance was observed in the control (0.55%), with a slight reduction under CA (0.39%). Onygenales, including keratin-degrading and potentially pathogenic fungi (Brasch et al., 2024; Chaturvedi et al., 2020), may be better suited to more acidic conditions (Coleine et al., 2022), which could explain their reduced presence under liming.

Nitrogen availability emerged as a key driver of change for fungi involved in carbon and nitrogen turnover. The order Helotiales peaked under nitrogen addition (10.2%), supporting findings that associate this group with nitrogen-enriched systems (Cao et al., 2022; Gómez-Brandón et al., 2020). These fungi, which include both saprotrophs and ericoid mycorrhizal species, thrive in acidic, nutrient-poor soils and contribute to the decomposition of lignified plant matter.

Responses to combined nitrogen and phosphorus inputs were particularly notable among some decomposer taxa. Onygenales, when considered separately from their Incertae sedis members, increased markedly under NP treatment (9.14%). This shift suggests opportunistic growth in response to nutrient-rich substrates and elevated nitrogen availability, consistent with their role in decomposing nitrogenous organic material (Liu et al., 2020)

Soils amended with lime (CA) displayed marked increases in fungal groups associated with lignocellulose degradation and symbiosis. Agaricales, a diverse order within Agaricomycetes, were highly enriched under CA treatment (36.92%). Their known capacity for decomposing lignocellulose and forming ectomycorrhizal associations (Tedersoo et al., 2014) suggests that pH amelioration improves habitat suitability for both saprotrophic and symbiotic fungi.

Unclassified lineages within Ascomycota also responded to nitrogen addition. Ascomycota Incertae sedis showed a significantly higher abundance under N (14.43%) than in C, CA, or P treatments. These groups likely include saprotrophic fungi involved in rapid organic matter turnover under nutrient-rich conditions (Tedersoo et al., 2014), highlighting the role of nitrogen in stimulating decomposer activity.

Pseudorobillarda, a saprotrophic genus within Ascomycota (possibly Dothideomycetes), was significantly more abundant in the control (11.50%) and declined under CA and P treatments. This genus contributes to lignocellulosic decomposition in nutrient-poor environments and may be competitively excluded under fertilization (Longepierre et al., 2021)

Combined nutrient treatments also influenced the abundance of extremotolerant fungi. Herpotrichiellaceae Incertae sedis peaked under NP treatment (13.16%). These melanized fungi, including *Exophiala*, are known for their resistance to stress and their ability to degrade recalcitrant carbon compounds such as aromatic hydrocarbons (Teixeira et al., 2017), suggesting a niche for oxidative decomposers under nutrient-enriched conditions.

Nitrogen addition alone favored several fungi associated with acidic, low-nutrient environments. *Leohumicola*, a genus within Leotiomycetes, was more abundant under N (3.39%) than in other treatments. Found in oligotrophic forest soils, this group is associated with saprotrophic activity and thermotolerance, and may serve as an indicator of acidic, low-P conditions (Nguyen et al., 2016).

Some less common genera also responded to nutrient addition, despite their low abundance. *Glutinomyces*, a little-studied genus within Ascomycota, was more prevalent under N (1.26%) than CA and P treatments. Its association with decaying organic matter and saprotrophic activity in nitrogen-enriched environments suggests substrate specialization or niche expansion (Nakamura et al., 2018).

Taxonomically unresolved Onygenales groups also responded to combined nutrient enrichment. Onygenales Incertae sedis increased substantially under NP treatment (8.90%), likely due to their ability to exploit protein-rich, nitrogenous substrates such as keratin and chitin (Kandemir et al., 2022). Their success under NP indicates a role in processing complex organic nitrogen sources. The response of Onygenales across treatments varied depending on taxonomic resolution. While total Onygenales abundance remained low in most treatments, their increase in NP and slight reduction under CA highlight the interplay between pH sensitivity and nutrient preference. This group's keratinolytic capabilities and occasional pathogenicity suggest functional roles in nitrogen recycling and organic residue decomposition (Chaturvedi et al., 2020).

Liming not only altered dominant fungal groups but also favored rare and sensitive taxa. *Hygrocybe*, a genus of waxcap fungi typically associated with nutrient-poor grasslands, was most abundant under CA (28.81%). Known for forming potential symbiotic associations with plant roots (Halbwachs et al., 2018), their increased presence may reflect improved root colonization or competitive release due to pH amelioration.

The promotion of *Hygrocybe* and *Trichoderma* under higher calcium levels and increased soil pH is likely to impact N, P, and C cycling markedly. *Trichoderma* species are known to enhance phosphorus availability via secretion of phosphatases and organic acids while suppressing N_2O emissions by stimulating denitrifying bacteria or inhibiting nitrification (*Trichoderma asperellum* reduces N_2O emissions by up to ~47 %) (Wu et al., 2022). Additionally, some *Hygrocybe* species, as basidiomycetous macrofungi, are potent decomposers that contribute to breakdown of recalcitrant soil organic carbon and mobilization of nitrogen and phosphorus (as seen in peatland and forest ecosystems) (Halbwachs et al., 2018). By enhancing decomposition and enzyme activity (e.g. phosphatases, laccases, urease), these fungi can increase nutrient turnover rates, modulate plant nutrient supply, and influence microbial competition and food web dynamics. Meanwhile, liming-induced pH shifts can alter the composition of other fungal groups (e.g. suppressing acidophilic species), thereby adjusting fungal community interactions, niche occupation, and the overall functional structure of soil microbiomes .

Changes in fungal abundance in response to nutrient treatments support the notion that fungal communities in the Cerrado are structured by a complex interaction of pH, nutrient availability, and substrate composition. These patterns reveal functional shifts in decomposer and symbiotic guilds, emphasizing the importance of fungal diversity in mediating soil processes under changing environmental conditions.

8.8.CONCLUSION

The long-term application of nutrients and lime brought about notable improvements in several aspects of soil quality in the Cerrado. Treatments significantly altered soil chemistry—raising pH, increasing base saturation, and boosting phosphorus availability. These factors are typically limiting in native Cerrado soils, and their improvement helped to reduce aluminum toxicity and ease nutrient stress. The FertBio Soil Quality Index (IQS), which integrates biological, chemical, and physical indicators, confirmed that the NP and CA treatments led to the best soil conditions. These results emphasize the importance of correcting soil acidity when aiming to restore functionality in highly weathered tropical soils.

Soil enzyme activities—particularly β-glucosidase, acid phosphatase, and arylsulfatase—offered valuable insight into how microbial functions responded to the treatments. β-glucosidase activity peaked in the P and NP treatments, suggesting increased decomposition of organic matter and more active carbon cycling under higher nutrient availability. Acid phosphatase, on the other hand, showed a clear decrease when phosphorus was added, likely due to feedback inhibition as phosphorus became more readily available. Arylsulfatase activity, while more variable, tended to increase with fertilization, hinting at greater microbial involvement in sulfur cycling. Together, these biochemical indicators highlight how sensitive soil enzymes are to changes in nutrient availability and microbial dynamics.

Interestingly, microbial alpha diversity—measured through richness and evenness—remained statistically stable across all treatments for both bacteria and fungi. This suggests a certain resilience within the native Cerrado microbial communities, even after prolonged nutrient addition. However, the lack of change in alpha diversity doesn't mean that these communities were unaffected. Rather, it points to the limitations of using richness alone as a metric. Functional and compositional shifts can occur even when diversity indices stay constant, especially in complex ecosystems like the Cerrado.

Indeed, while alpha diversity remained unchanged, metagenomic analyses revealed substantial shifts in the structure of microbial communities. Copiotrophic bacterial groups such as Proteobacteria, Firmicutes, and Gammaproteobacteria thrived under NP and N treatments, benefiting from the availability of labile nutrients. In contrast, oligotrophic taxa like Acidobacteria and Actinobacteria declined—particularly in plots that received lime or phosphorus—likely outcompeted by faster-growing organisms. Fungal communities were more nuanced in their responses: phosphorus addition encouraged some saprotrophic and mycorrhizal fungi, but generalist decomposers like Pleosporales declined, possibly due to changing substrate composition or microbial competition.

These compositional changes carry important functional consequences. The rise of fast-growing, copiotrophic microbes could enhance nutrient cycling and organic matter breakdown. However, this might also reduce the long-term stability of soil organic matter. Notably, nitrogen-fixing bacteria like *Bradyrhizobium* and *Burkholderia* remained present in all treatments but didn't increase in abundance with fertilization. This may reflect a reduced need for biological nitrogen fixation when nutrients are readily available.

Such shifts suggest that while fertilization can boost short-term fertility, it may alter the ecological roles of key microbial groups and disrupt natural nutrient cycling processes.

In conclusion, this study offers strong evidence that nutrient addition and liming reshape both the chemistry and biology of Cerrado soils in significant and lasting ways. By combining enzyme assays, chemical analyses, and metagenomic profiling, and interpreting them through the FertBio IQS, we gained a comprehensive understanding of soil health under different management practices. Still, applying agricultural indices to native ecosystems calls for caution and refinement. Ongoing monitoring, especially under a changing climate, is crucial to balance productivity with ecological integrity. Ultimately, these findings underline the need to include microbial assessments in soil health evaluations, particularly in sensitive tropical biomes like the Cerrado, where conservation and sustainable use must go hand in hand.

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8.9.SUPLEMENTARY MATERIAL

Table S 1 Soil nutrient content in each treatment in an area of Cerrado sensu stricto.

Treatment	pH H2O	pH CaCl2	P meh-^{1} mg dm-^{3}	S mg dm-^{3}	Ca cmolc dm-^{3}	Mg cmolc dm-^{3}	Al cmolc dm-^{3}	MO dag kg-^{1}	C.O dag kg-^{1}	H+Al cmolc dm-^{3}
C	4.66±0.24^{b}	4.36±0.38^{b}	1.27±0.8^{a}	3.96±0.15^{c}	0.06±0.02^{b}	0.05±0.05^{b}	0.79±0.06^{b}	2.44±1.17^{a}	1.41±0.68^{a}	6.78±0.71^{a}
CA	6.01±0.13^{a}	5.86±0.11^{a}	1.11±0.38^{a}	5.21±1.05^{c}	5.83±1.63^{a}	3.21±0.72^{a}	0.00±0.00^{c}	1.81±1.32^{a}	1.05±0.77^{a}	2.01±0.20^{b}
NP	4.42±0.31^{b}	4.15±0.25^{b}	6.00±1.65^{a}	48.94±0.35^{a}	0.27±0.27^{b}	0.06±0.06^{b}	1.29±0.10^{a}	2.25±0.83^{a}	1.31±0.48^{a}	9.16±0.72^{a}
N	4.33±0.18^{b}	4.06±0.16^{b}	1.93±1.19^{a}	45.39±2.57^{a}	0.11±0.06^{b}	0.06±0.04^{b}	1.37±0.14^{a}	3.45±0.72^{a}	2.00±0.42^{a}	9.02±1.25^{a}
P	4.45±0.10^{b}	4.18±0.08^{b}	7.77±5.6^{a}	30.03±2.32^{b}	0.12±0.08^{b}	0.04±0.01^{b}	1.15±0.26^{ab}	2.88±1.04^{a}	1.67±0.60^{a}	8.64±1.56^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$), according to Tukey's test or t-test.

Table S1 (Continuation) Soil nutrient content in each treatment in an area of Cerrado sensu stricto.

Treatment	B mg dm-^{3}	Cu mg dm-^{3}	Fe mg dm-^{3}	Mn mg dm-^{3}	Zn mg dm-^{3}	SB cmolc dm-^{3}	T cmolc dm-^{3}	t cmolc dm-^{3}	V %
C	0.42±0.07^{a}	0.48±0.24^{a}	73.07±22.27^{ab}	3.76±0.52^{a}	0.00±0.00^{a}	0.11±0.07^{b}	6.89±0.74^{b}	0.9±0.07^{b}	0.02±0.01^{b}
CA	0.40±0.10^{a}	0.07±0.07^{b}	53.53±9.43^{ab}	16.67±11.98^{a}	0.11±0.18^{a}	9.04±2.33^{a}	11.05±2.29^{a}	9.04±2.33^{a}	0.81±0.04^{a}
NP	0.47±0.13^{a}	0.17±0.14^{ab}	32.95±21.82^{b}	2.94±0.68^{a}	0.1±0.17^{a}	0.33±0.33^{b}	9.49±0.67^{ab}	1.62±0.25^{b}	0.03±0.03^{b}
N	0.37±0.04^{a}	0.21±0.05^{ab}	72.23±5.23^{ab}	2.86±0.31^{a}	0.04±0.08^{a}	0.18±0.08^{b}	9.20±1.25^{ab}	1.55±0.2^{b}	0.02±0.01^{b}

P	0.35 ± 0.03^{a}	0.29 ± 0.15^{ab}	87.44 ± 13.8^{a}	3.12 ± 0.58^{a}	0.11 ± 0.14^{a}	0.16 ± 0.09^{b}	8.80 ± 1.50^{ab}	1.32 ± 0.22^{b}	0.02 ± 0.01^{b}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$), according to Tukey's test or t-test

Table S1 (Continuation) Soil nutrient content in each treatment in an area of Cerrado sensu stricto.

Treatment	m %	Ca/Mg	Ca/K	Mg/K	Ca+Mg/K	Ca/T %	Mg/T %	Na/T %
C	0.88 ± 0.07^{a}	1.84 ± 0.96^{b}	10.92 ± 3.43^{b}	9.23 ± 9.50^{b}	20.15 ± 12.89^{b}	0.01 ± 0.00^{b}	0.01 ± 0.01^{b}	10.00 ± 3.00^{a}
CA	0.00 ± 0.00^{b}	1.81 ± 0.16^{b}	2273.7 ± 636.42^{a}	1251.36 ± 282.51^{a}	3525.06 ± 910.55^{a}	0.52 ± 0.04^{a}	0.29 ± 0.01^{a}	8.33 ± 5.86^{a}
NP	0.81 ± 0.16^{a}	4.54 ± 0.71^{a}	52 ± 52.61^{b}	11.72 ± 11.70^{b}	63.72 ± 64.29^{b}	0.03 ± 0.03^{b}	0.01 ± 0.01^{b}	8.00 ± 6.08^{a}
N	0.89 ± 0.05^{a}	2.05 ± 1.13^{b}	21.71 ± 12.09^{b}	12.27 ± 8.14^{b}	33.98 ± 16.49^{b}	0.01 ± 0.01^{b}	0.01 ± 0.00^{b}	5.67 ± 3.06^{a}
P	0.87 ± 0.09^{a}	2.96 ± 1.18^{ab}	23.4 ± 15.6^{b}	7.27 ± 2.63^{b}	30.67 ± 18.22^{b}	0.01 ± 0.01^{b}	0.00 ± 0.00^{b}	8.00 ± 6.00^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-B: different lowercase letters substituted in the same line denote differences ($p < 0.05$), according to Tukey's test or t-test.

Table S1 (Continuation) Soil nutrient content in each treatment in an area of Cerrado sensu stricto.

Treatment	H+Al/T %	H+Al/T %	Ca+Mg/T %	Ca+Mg+Na+K/T %	Clay g/kg	Silt g/kg	Sand g/kg
C	0.98 ± 0.01^{a}	0.98 ± 0.01^{a}	0.01 ± 0.01^{b}	0.02 ± 0.01^{b}	570.83 ± 14.43^{a}	128.33 ± 14.43^{a}	300.83 ± 14.43^{b}
CA	0.19 ± 0.04^{b}	0.19 ± 0.04^{b}	0.81 ± 0.04^{a}	0.81 ± 0.04^{a}	375.00 ± 37.50^{b}	128.33 ± 38.19^{a}	496.67 ± 68.84^{a}
NP	0.97 ± 0.03^{a}	0.97 ± 0.03^{a}	0.03 ± 0.03^{b}	0.03 ± 0.03^{b}	466.67 ± 57.74^{ab}	99.17 ± 19.09^{a}	434.17 ± 40.18^{ab}
N	0.98 ± 0.01^{a}	0.98 ± 0.01^{a}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	450.00 ± 123.11^{a}	107.50 ± 12.50^{a}	442.50 ± 114.56^{a}

					^{b}		^{b}
P	0.98±0.01^{a}	0.98±0.01^{a}	0.02±0.01^{b}	0.02±0.01^{b}	516.67±50.52^{ab}	103.33±14.43^{a}	380.00±37.50^{ab}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-B: different lowercase letters substituted in the same line denote differences ($p < 0.05$), according to Tukey's test or t-test.

Table S 2 Comparison of the nutrients in soil throughout the years in an area of Cerrado sensu stricto

	pH water	pH CaCl2	OC	N	P	K	Ca	Mg	Al	Fe	OM
2007											
C	4.36± 0.18	3.37± 0.06		0.07± 0.03	1.60± 0.39	42.75± 7.41	0.08± 0.06	0.07± 0,01	0.92± 0.23	117.88± 32.35	7.61± 0.92
CA	5.34± 0.36	5.42± 0.40		0.07± 0.02	1.80± 1.22	28.25± 1.50	4.19± 1.93	2.95± 0.39	0.03± 0.05	102.45± 56.99	6.67± 6.68
NP	3.82± 0.13	3.53± 0.10		0.08± 0.04	40.45± 49.63	41.75± 12.76	0.26± 0.20	0.06± 0.02	1.57± 0.19	108.65± 14.95	108.65± 14.95
N	3.86± 0.12	3.56± 0.06		0.10± 0.04	2.10± 0.34	47.25± 10.72	0.11± 0.08	0.07± 0.02	1.64± 0.17	109.78± 9.85	8.55± 0.83
P	4.15± 0.23	3.81± 0.11		0.07± 0.02	36.90± 25.57	48.75± 18.98	102± 0.63	0.11± 0.05	0.63± 0.39	103.60± 22.19	7.72± 0.82
2009											
C	0.43± 0.18	3.73± 0.06	44.10± 5.30	0.68± 0.25	1.60± 0.39	42.75± 7.40	16.00± 12.80	78.90± 1.60	82.25± 20.50	117.90± 32.30	

CA	5.34±0.45	5.42±0.40	38.70±4.00	0.73±0.22	1.80±1.22	28.25±1.50	839.60±386.90	358.70±47.60	2.24±4.50	102.40±57.00	
NP	3.82±0.13	3.53±0.10	45.70±9.50	0.80±0.38	40.45±49.60	41.75±12.70	52.60±50.00	7.59±2.00	140.69±17.50	108.60±15.00	
N	3.86±0.12	3.56±0.06	49.60±4.80	0.95±0.41	2.10±0.34	47.20±10.70	22.00±16.80	8.50±2.90	147.20±15.50	109.80±9.80	
P	4.15±0.23	3.81±0.11	44.80±4.80	0.70±0.22	36.90±25.50	48.75±18.90	203.97±297.00	13.06±6.20	56.41±34.80	103.60±22.10	
2015											
C	4.94±0.16	4.02±0.16		0.21±0.06	0.78±0.17	28.25±7.32	0.11±0.04	0.07±0.01	1.03±0.36	78.13±17.47	6.72±1.48
CA	6.56±0.26	5.81±0.34		0.27±0.02	1.93±0.91	12.75±1.71	5.01±1.00	3.22±0.77	0.00±0.00	29.13±2.61	6.48±1.60
NP	4.59±0.06	3.98±0.04		0.22±0.06	11.05±0.97	22.75±7.41	0.12±0.01	0.05±0.01	1.48±0.15	75.98±6.98	6.75±0.52
N	4.61±0.10	3.93±0.03		0.19±0.06	0.83±0.17	22.25±4.57	0.13±0.04	0.07±0.01	1.53±0.26	76.38±11.12	6.57±0.73
P	5.00±0.09	4.03±0.03		0.19±0.03	13.75±6.77	28.50±3.00	0.24±0.07	0.09±0.02	1.28±0.25	81.78±9.91	7.44±0.39
2019											
C	4.72±0.13			0.19±0.02	1.70±0.31	28.75±5.36	0.19±0.04	0.11±0.03	72.00±5.25		
CA	6.43±0.05			0.18±0.02	0.65±0.29	10.75±1.64	4.69±0.18	2.73±0.13	0.00±0.00		
NP	4.11±0.15			0.20±0.01	6.98±2.48	28.50±2.87	0.40±0.15	0.13±0.07	71.25±7.48		
N	4.09±0.06			0.19±0.01	71.25±7.48	22.75±2.95	0.15±0.02	0.08±0.01	82.78±0.65		
P	4.30±0.03			0.17±0.01	6.60±2.32	26.75±2.17	0.30±0.05	0.10±0.02	69.05±5.19		

2019											
C	4,71± 0,14			0,20± 0,02	1,66± 0,33	27,00± 6,64	0,18± 0,05	0,10± 0,04	0,93± 0,15		
CA											
NP	4,16± 0,19			0,19± 0,02	6,40± 2,80	28,40± 2,89	0,44± 0,17	0,15± 0,09	1,44± 0,13		
N	4,09± 0,06			0,18± 0,03	1,28± 0,11	23,40± 3,29	0,15± 0,03	0,08± 0,02	1,40± 0,13		
P	4,31± 0,05			0,20± 0,02	6,84± 3,23	25,4 0± 1,35	0,32± 0,06	0,11± 0,03	0,99± 0,16		
2020											
C	4.55± 0.06				0.53± 0.06	30.00± 6.56	0.12± 0.03	0.08± 0,01	1.20± 0.17		7.08± 0.51
CA	5.92± 0.19				1.30± 0.40	11.33± 3.21	5.67± 0.53	3.33± 0.37	0.00± 0.00		5.82± 0.56
NP	3.75± 0.13				10.40± 2.43	20.00± 4.36	0.21± 0.03	0.07± 0.01	1.53± 0.21		7.61± 0.60
N	3.93± 0.04				0.40± 0.17	20.00± 2.00	0.13± 0.03	0.06± 0.01	1.47± 0.15		7.03± 0.51
P	3.99± 0.05				11.50± 3.84	49.33± 45.62	0.19± 0.04	0.06± 0.01	1.43± 0.21		8.01± 1.02
2023											
C	4.66± 0.24	4.36± 0.38	1.41± 0.68		1.27± 0.80		0.06± 0.02	0.05± 0.05	0.79± 0.06	73.07± 22.27	2.44± 1.17
CA	6.01± 0.13	5.86± 0.11	1.05± 0.77		1.11± 0.38		5.83± 1.63	3.21± 0.72	0.00± 0.00	53.53± 9.43	1.81± 1.32
NP	4.42± 0.31	4.15± 0.25	1.31± 0.48		6.00± 1.65		0.27± 0.27	0.06± 0.06	1.29± 0.1	32.95± 21.82	2.25± 0.83
N	4.33±	4.06±	2.00±		1.93±		0.11±	0.06±	1.37±	72.23±	3.45±

	0.18	0.16	0.42		1.19		0.06	0.04	0.14	5.23	0.72
	4.45±	4.18±	1.67±		7.77±		0.12±	0.04±	1.15±	87.44±	2.88±
P	0.10	0.08	0.60		5.60		0.08	0.01	0.26	13.8	1.04

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation;

Table S 3 Alfa diversity 16s of microorganisms in soil in an area of Cerrado sensu stricto

Alpha diversity 16S	C	CA	N	NP	P
Observed	3514.33±328.38^{a}	3803±483.54^{a}	3659.67±233.4^{a}	3497.33±528.45^{a}	4187.67±1767.98^{a}
Chao1	3517.55±328.24^{a}	3808.04±487.29^{a}	3661.43±234.37^{a}	3499.59±529.97^{a}	4195.9±1774.62^{a}
ACE	3520.42±327.49^{a}	3814.05±490.63^{a}	3663.12±234.25^{a}	3502.01±530.84^{a}	4204.04±1785.34^{a}
se.ACE	28.91±1.00^{a}	29.57±1.98^{a}	29.13±1.21^{a}	28.45±2.80^{a}	30.66±7.21^{a}
Shannon	7.80±0.12^{a}	7.86±0.10^{a}	7.87±0.06^{a}	7.80±0.15^{a}	7.93±0.35^{a}
Simpson	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
InvSimpson	1802.25±233.57^{a}	1955.50±237.94^{a}	1946.66±76.92^{a}	1820.31±245.68^{a}	2197.92±801.55^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same alfa diversity on 16S for different treatments, according to Tukey's test or t-test.

Table S 4 Alfa diversity ITS of microorganisms in soil in an area of Cerrado sensu stricto.

Alpha diversity ITS	C	CA	N	NP	P
Observed	934±145.04 ^{a}	652.33±243.08 ^{a}	912.33±33.49 ^{a}	881.67±79.84 ^{a}	809.33±358.93 ^{a}
Chao1	934±145.04 ^{a}	652.33±243.08 ^{a}	912.33±33.49 ^{a}	881.67±79.84 ^{a}	809.33±358.93 ^{a}
ACE	934±145.04 ^{a}	652.39±243.18 ^{a}	912.33±33.49^{a}	881.67±79.84 ^{a}	809.33±358.93 ^{a}
se.ACE	13.43±0.62 ^{a}	11.41±1.6 ^{ab}	13.27±0.62 ^{a}	13.37±0.34 ^{a}	9.43±2.2 ^{ab}
Shannon	4.31±1.58 ^{a}	3.53±0.60 ^{a}	5.28±0.22 ^{a}	4.95±0.20 ^{a}	4.04±1.95 ^{a}
Simpson	0.84±0.24 ^{a}	0.88±0.04 ^{a}	0.99±0.01 ^{a}	0.98±0.01 ^{a}	0.82±0.27 ^{a}
InvSimpson	35.23±29.99 ^{ab}	9.47±3.47 ^{b}	78.99±24.26 ^{a}	47.1±14.23 ^{ab}	29.35±26.31 ^{ab}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-B: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same alpha diversity on ITS for different treatments, according to Tukey's test or t-test.

Table S 5 Content of Phylum of bacteria in soil in an area of Cerrado sensu stricto.

Phylum	C	CA	NP	N	P
Acidobacteriota	37.48±6.73^{a}	25.78±15.98 ^{a}	35.05±7.68 ^{a}	37.29±5.57 ^{a}	33.79±16.57 ^{a}
Proteobacteria	21.38±0.95 ^{a}	19.33±4.58 ^{a}	16.65±1.17 ^{a}	22.04±2.00 ^{a}	12.89±6.05 ^{a}
Gemmatimonadota	2.77±0.19 ^{a}	5.00±2.15 ^{a}	1.19±0.54 ^{a}	1.77±0.46 ^{a}	4.24±3.41 ^{a}
Verrucomicrobiota	2.62±0.87 ^{a}	2.04±0.65 ^{a}	1.45±0.33 ^{a}	2.56±2.56 ^{a}	2.47±0.50 ^{a}
Myxococcota	2.36±0.50 ^{a}	3.67±1.51 ^{a}	1.43±0.34 ^{a}	1.20±0.28 ^{a}	2.72±1.29 ^{a}
Chloroflexi	14.75±8.90 ^{a}	11.13±4.43 ^{a}	19.60±0.50 ^{a}	13.02±0.93 ^{a}	13.98±5.50 ^{a}
Actinobacteriota	13.14±3.50 ^{a}	20.42±7.22 ^{a}	18.98±7.03 ^{a}	14.75±2.09 ^{a}	19.28±5.85 ^{a}
WPS-2	0.98±0.95 ^{a}	0.93±1.61 ^{a}	2.28±1.14 ^{a}	1.69±1.47 ^{a}	1.64±1.33 ^{a}
Crenarchaeota	0.89±0.48 ^{a}	0.55±0.36 ^{a}	0.48±0.21 ^{a}	0.94±1.10 ^{a}	0.46±0.14 ^{a}
Bacteroidota	0.74±0.15 ^{a}	1.63±1.16 ^{a}	0.59±0.32 ^{a}	1.00±0.84 ^{a}	1.20±0.77 ^{a}
GAL15	0.46±0.15 ^{a}	0.10±0.17 ^{a}	0.27±0.11 ^{a}	0.97±1.02 ^{a}	0.33±0.40 ^{a}
Unknown	0.42±0.22 ^{a}	0.56±0.18 ^{a}	0.36±0.11 ^{a}	0.46±0.11 ^{a}	0.73±0.26 ^{a}

Elusimicrobiota	0.25±0.04 ^{a}	0.35±0.34 ^{a}	0.41±0.02 ^{a}	0.40±0.08 ^{a}	0.43±0.31 ^{a}
Firmicutes	0.24±0.17 ^{a}	0.58±0.59 ^{a}	0.12±0.02 ^{a}	0.20±0.27 ^{a}	0.49±0.42 ^{a}
Planctomycetota	0.23±0.08 ^{a}	0.63±0.47 ^{a}	0.17±0.19 ^{a}	0.32±0.28 ^{a}	0.51±0.57 ^{a}
RCP2-54	0.13±0.13 ^{a}	0.44±0.13 ^{a}	0.25±0.09 ^{a}	0.53±0.66 ^{a}	0.48±0.56 ^{a}
Patescibacteria	0.09±0.08 ^{a}	0.98±1.00 ^{a}	0.33±0.20 ^{a}	0.19±0.20 ^{a}	0.73±0.96 ^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same phylum for different treatments, according to Tukey's test or t-test.

Table S 6 Content of Class of bacteria in soil in an area of Cerrado sensu stricto.

Class	C	CA	NP	N	P
Actinobacteria	8.23±3.25 ^{a}	6.65±1.48 ^{a}	12.58±6.45 ^{a}	9.42±2.30 ^{a}	8.73±2.78 ^{a}
AD3	6.97±4.77 ^{a}	2.80±3.96 ^{a}	10.14±1.94 ^{a}	5.48±1.33 ^{a}	6.95±5.73 ^{a}
Alphaproteobacteria	6.87±0.98^{a}	9.18±2.42^{a}	7.95±0.88^{a}	9.09±1.64^{a}	6.01±3.73^{a}
Ktedonobacteria	5.22±4.00 ^{a}	2.25±3.01 ^{a}	6.67±2.31 ^{a}	3.97±2.36 ^{a}	2.79±1.50 ^{a}
Acidobacteriae	34.23±6.55 ^{a}	14.93±22.05 ^{a}	33.26±7.49 ^{a}	35.46±6.18 ^{a}	27.98±21.52 ^{a}
Thermoleophilia	3.34±0.27 ^{a}	7.98±4.69 ^{a}	4.23±1.09 ^{a}	3.69±0.26 ^{a}	6.55±4.38 ^{a}
Gemmatimonadetes	2.77±0.19 ^{a}	4.63±1.85 ^{a}	1.19±0.54 ^{a}	1.77±0.46 ^{a}	3.95±2.92 ^{a}
Verrucomicrobiae	2.61±0.88 ^{a}	2.01±0.66 ^{a}	1.43±0.32 ^{a}	2.52±2.54 ^{a}	2.45±0.51 ^{a}
Unknown	2.39±1.34 ^{a}	5.02±1.05 ^{a}	3.69±1.47 ^{a}	3.96±1.24 ^{a}	4.58±0.86 ^{a}
TK10	2.10±0.08 ^{a}	1.39±0.17 ^{a}	2.06±0.64 ^{a}	2.95±2.30 ^{a}	1.89±1.02 ^{a}
Gammaproteobacteria	14.5±0.36 ^{a}	10.14±2.97^{abc}	8.70±0.52 ^{bc}	12.92±1.76 ^{ab}	6.87±2.69 ^{c}
Vicinamibacteria	1.68±0.32 ^{a}	5.58±3.82 ^{a}	0.71±0.18 ^{a}	0.79±0.40 ^{a}	2.87±2.38 ^{a}

Acidimicrobiia	1.55±0.20^{a}	4.94±3.04^{a}	2.16±0.82^{a}	1.64±0.53^{a}	3.38±2.81^{a}
Myxococcia	1.20±0.33^{a}	0.61±0.18^{ab}	0.24±0.10^{b}	0.32±0.12^{b}	0.52±0.34^{b}
Polyangia	1.16±0.31^{ab}	2.93±1.21^{a}	1.19±0.25^{ab}	0.88±0.23^{b}	2.13±1.04^{ab}
Nitrososphaeria	0.86±0.49^{a}	0.55±0.36^{a}	0.47±0.22^{a}	0.93±1.08^{a}	0.45±0.14^{a}
Thermoanaerobaculia	0.74±0.37^{a}	1.32±1.36^{a}	0.36±0.21^{a}	0.45±0.16^{a}	0.44±0.17^{a}
Bacteroidia	0.73±0.12^{a}	1.52±1.06^{a}	0.57±0.31^{a}	1.00±0.84^{a}	1.04±0.57^{a}
Subgroup_5	0.39±0.06^{a}	0.51±0.16^{a}	0.67±0.13^{a}	0.49±0.17^{a}	0.72±0.32^{a}
Lineage	0.21±0.04^{a}	0.27±0.36^{a}	0.38±0.02^{a}	0.37±0.08^{a}	0.37±0.37^{a}
Saccharimonadia	0.08±0.07^{a}	0.76±0.77^{a}	0.31±0.20^{a}	0.15±0.15^{a}	0.48±0.55^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same class for different treatments, according to Tukey's test or t-test.

Table S 7 Content of Order of bacteria in soil in an area of Cerrado sensu stricto.

Order	C	CA	NP	N	P
Ktedonobacterales	4.81±3.88^{a}	1.90±2.99^{a}	6.09±2.67^{a}	3.30±2.64^{a}	2.38±1.57^{a}
Rhizobiales	4.43±0.88^{a}	5.16±1.26^{a}	3.87±0.50^{a}	4.01±0.35^{a}	3.47±2.18^{a}
Burkholderiales	3.44±0.36^{a}	4.77±1.81^{a}	1.80±0.38^{a}	2.16±1.61^{a}	2.73±1.83^{a}
Solirubrobacterales	3.26±0.29^{a}	4.91±2.31^{a}	4.14±1.08^{a}	3.63±0.26^{a}	4.92±1.84^{a}
Frankiales	2.92±1.15^{ab}	1.29±0.93^{b}	4.25±0.87^{a}	3.03±0.90^{ab}	2.58±1.32^{ab}
Gemmatimonadales	2.77±0.19^{a}	4.63±1.85^{a}	1.19±0.54^{a}	1.77±0.46^{a}	3.95±2.92^{a}
Solibacterales	2.56±0.82^{ab}	1.26±1.49^{b}	3.99±0.49^{a}	2.57±0.52^{a}	2.08±1.30^{a}
Subgroup_2	18.84±4.42^{a}	7.48±12.41^{a}	14.61±2.31^{a}	19.86±5.19^{ab}	14.45±12.29^{ab}
Unknown	13.03±5.82^{a}	16.46±1.93^{a}	18.18±2.48^{a}	14.32±2.49^{a}	18.37±4.85^{a}
Acidobacteriales	10.83±1.48^{a}	5.04±6.87^{a}	10.82±4.51^{a}	10.74±1.53^{a}	9.32±6.94^{a}
Gammaproteobacteria	10.41±0.64^{a}	3.82±2.41^{b}	6.14±0.69^{ab}	9.80±1.56^{a}	3.21±2.67^{b}
Bryobacterales	1.52±0.42^{ab}	0.68±0.93^{b}	3.42±0.70^{a}	1.79±0.49^{ab}	1.55±0.97^{ab}

Pedosphaerales	1.49±0.60^{a}	1.22±0.62^{a}	0.64±0.10^{a}	1.20±1.00^{a}	1.36±0.28^{a}
Streptomycetales	1.47±1.07^{a}	1.32±0.72^{a}	3.46±3.32^{a}	2.41±0.40^{a}	1.85±1.02^{a}
Myxococcales	1.20±0.33^{a}	0.61±0.18^{ab}	0.24±0.10^{b}	0.32±0.12^{b}	0.52±0.34^{b}
Chthoniobacterales	1.09±0.27^{a}	0.71±0.23^{a}	0.77±0.22^{a}	1.28±1.50^{a}	0.91±0.21^{a}
Vicinamibacterales	1.07±0.23^{a}	4.94±3.49^{a}	0.71±0.18^{a}	0.73±0.36^{a}	2.52±2.03^{a}
Elsterales	1.06±0.35^{b}	0.67±0.49^{b}	1.98±0.29^{ab}	2.47±0.76^{a}	0.66±0.44^{b}
IMCC26256	0.99±0.14^{a}	2.11±1.01^{a}	1.17±0.35^{a}	1.02±0.40^{a}	1.59±1.00^{a}
Pseudonocardiales	0.97±0.31^{a}	1.20±0.03^{a}	0.99±0.27^{a}	1.22±0.43^{a}	1.09±0.07^{a}
Corynebacteriales	0.93±0.31^{a}	0.51±0.47^{a}	0.99±0.31^{a}	0.89±0.27^{a}	0.79±0.53^{a}
Polyangiales	0.79±0.20^{b}	1.59±0.34^{a}	0.93±0.18^{ab}	0.70±0.21^{b}	1.27±0.38^{ab}
Catenulisporales	0.77±0.25^{a}	0.28±0.48^{a}	0.83±0.35^{a}	0.94±0.26^{a}	0.66±0.57^{a}
Thermoanaerobaculales	0.74±0.37^{a}	1.32±1.36^{a}	0.36±0.21^{a}	0.45±0.16^{a}	0.44±0.17^{a}
Chitinophagales	0.60±0.07^{a}	0.71±0.40^{a}	0.45±0.28^{a}	0.83±0.70^{a}	0.49±0.20^{a}
Micropepsales	0.42±0.13^{b}	0.33±0.32^{c}	1.02±0.10^{ab}	1.13±0.38^{a}	0.32±0.16^{c}
B12-WMSP1	0.38±0.15^{a}	0.11±0.18^{a}	0.53±0.49^{a}	0.60±0.31^{a}	0.24±0.23^{a}
Subgroup_13	0.35±0.06^{a}	0.17±0.29^{a}	0.18±0.16^{a}	0.24±0.11^{a}	0.33±0.29^{a}
Caulobacterales	0.31±0.18^{a}	0.90±0.57^{a}	0.17±0.15^{a}	0.57±0.13^{a}	0.48±0.52^{a}
Haliangiales	0.28±0.11^{a}	1.09±0.71^{a}	0.24±0.09^{a}	0.16±0.03^{a}	0.70±0.85^{a}
Micrococcales	0.24±0.11^{a}	0.45±0.23^{a}	0.99±1.27^{a}	0.14±0.05^{a}	0.40±0.15^{a}
Saccharimonadales	0.08±0.07^{a}	0.76±0.77^{a}	0.31±0.20^{a}	0.15±0.15^{a}	0.48±0.55^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same order for different treatments, according to Tukey's test or t-test.

Table S 8 Content of Family of bacteria in soil in an area of Cerrado sensu stricto.

Family	C	CA	NP	N	P
Unknown	47.38±1.25 ^{a}	43.38±8.92 ^{a}	46.77±5.56 ^{a}	49.4±6.13 ^{a}	50.19±12.17 ^{a}
Ktedonobacteraceae	4.34±3.47 ^{a}	1.72±2.80 ^{a}	5.43±2.55 ^{a}	2.89±2.36 ^{a}	1.94±1.35 ^{a}
Xanthobacteraceae	3.73±0.83 ^{a}	2.20±0.64 ^{ab}	2.79±0.37 ^{ab}	2.98±0.21 ^{ab}	2.02±0.81 ^{b}
Solirubrobacteraceae	3.16±0.27 ^{ab}	2.42±0.05 ^{b}	4.04±1.02 ^{b}	3.54±0.23 ^{ab}	3.44±0.52 ^{ab}
Gemmatimonadaceae	2.77±0.19 ^{a}	4.63±1.85 ^{a}	1.19±0.54 ^{a}	1.77±0.46 ^{a}	3.95±2.92 ^{a}
Acidothermaceae	2.63±1.01 ^{ab}	0.66±1.14 ^{b}	3.76±0.68 ^{a}	2.71±0.80 ^{ab}	2.01±1.64 ^{ab}
Solibacteraceae	2.56±0.82 ^{ab}	1.26±1.49 ^{b}	3.99±0.49 ^{a}	2.57±0.52 ^{ab}	2.08±1.30 ^{ab}
Sutterellaceae	2.02±0.44 ^{a}	0.37±0.64 ^{b}	0.48±0.18 ^{b}	0.95±0.60 ^{ab}	0.56±0.48 ^{b}
Proteobacteria Gammaproteobacteria Incertae	10.41±0.65 ^{a}	3.82±2.41 ^{b}	6.14±0.69 ^{ab}	9.80±1.56 ^{a}	3.21±2.67 ^{b}
Bryobacteraceae	1.52±0.42 ^{ab}	0.68±0.93 ^{b}	3.42±0.70 ^{a}	1.79±0.49 ^{ab}	1.55±0.97 ^{ab}
Pedosphaeraceae	1.49±0.60 ^{a}	1.22±0.62 ^{a}	0.64±0.10 ^{a}	1.20±1.00 ^{a}	1.36±0.28 ^{a}
Streptomycetaceae	1.47±1.07 ^{a}	1.32±0.72 ^{a}	3.46±3.32 ^{a}	2.41±0.40 ^{a}	1.85±1.02 ^{a}
Acidobacteriaceae	1.00±0.16 ^{ab}	0.53±0.74 ^{b}	2.76±1.22 ^{a}	1.94±0.17 ^{ab}	1.14±1.03 ^{ab}

Myxococcaceae	0.99±0.26^{a}	0.51±0.16^{ab}	0.23±0.09^{b}	0.28±0.13^{b}	0.46±0.31^{ab}
Pseudonocardiaceae	0.97±0.31^{a}	1.20±0.03^{a}	0.99±0.27^{a}	1.22±0.43^{a}	1.09±0.07^{a}
Burkholderiaceae	0.85±0.17^{a}	0.72±0.71^{a}	1.23±0.39^{a}	1.13±0.97^{a}	0.59±0.22^{a}
Polyangiaceae	0.78±0.21^{a}	0.86±0.37^{a}	0.90±0.16^{a}	0.69±0.2^{a}	1.01±0.50^{a}
Mycobacteriaceae	0.78±0.19^{a}	0.41±0.31^{a}	0.77±0.25^{a}	0.71±0.26^{a}	0.64±0.43^{a}
1.1c	0.77±0.49^{a}	0.21±0.37^{a}	0.26±0.23^{a}	0.76±0.85^{a}	0.19±0.17^{a}
Thermoanaerobaculaceae	0.74±0.37^{a}	1.32±1.36^{a}	0.36±0.21^{a}	0.45±0.16^{a}	0.44±0.17^{a}
Chthoniobacteraceae	0.65±0.19^{a}	0.49±0.02^{a}	0.12±0.13^{a}	0.47±0.71^{a}	0.47±0.24^{a}
Chitinophagaceae	0.60±0.07^{a}	0.54±0.23^{a}	0.45±0.28^{a}	0.83±0.70^{a}	0.46±0.17^{a}
JG30-KF-AS9	0.44±0.45^{a}	0.13±0.23^{a}	0.62±0.14^{a}	0.36±0.24^{a}	0.43±0.38^{a}
Xiphinematobacteraceae	0.44±0.07^{a}	0.20±0.23^{a}	0.61±0.18^{a}	0.79±0.81^{a}	0.44±0.22^{a}
Micropepsaceae	0.42±0.13^{bc}	0.33±0.32^{c}	1.02±0.10^{ab}	1.13±0.38^{a}	0.32±0.16^{c}
Catenulisporaceae	0.42±0.08^{a}	0.16±0.27^{a}	0.48±0.22^{a}	0.51±0.22^{a}	0.37±0.32^{a}
Actinospicaceae	0.35±0.16^{a}	0.12±0.21^{a}	0.35±0.14^{a}	0.44±0.12^{a}	0.29±0.25^{a}
Caulobacteraceae	0.31±0.18^{a}	0.16±0.17^{a}	0.17±0.15^{a}	0.57±0.13^{a}	0.18±0.21^{a}
Haliangiaceae	0.28±0.11^{a}	1.09±0.71^{a}	0.24±0.09^{a}	0.16±0.03^{a}	0.70±0.85^{a}
Micromonosporaceae	0.27±0.10^{a}	0.61±0.37^{a}	0.26±0.04^{a}	0.21±0.05^{a}	0.42±0.32^{a}
Thermomonosporaceae	0.27±0.03^{a}	0.04±0.07^{a}	0.25±0.22^{a}	0.29±0.18^{a}	0.16±0.15^{a}
Koribacteraceae	0.24±0.05^{a}	0.30±0.10^{a}	0.24±0.07^{a}	0.20±0.05^{a}	0.33±0.03^{a}
Beijerinckiaceae	0.23±0.03^{a}	0.29±0.07^{a}	0.41±0.10^{a}	0.44±0.10^{a}	0.26±0.11^{a}
Micrococcaceae	0.22±0.10^{a}	0.40±0.21^{a}	0.93±1.21^{a}	0.10±0.10^{a}	0.35±0.19^{a}
Rhizobiales	0.19±0.05^{a}	0.45±0.23^{a}	0.20±0.04^{a}	0.19±0.05^{a}	0.21±0.26^{a}

Vicinamibacteraceae	0.16±0.05 ^{a}	2.59±2.20 ^{a}	0.00±0.00 ^{a}	0.17±0.19 ^{a}	1.15±1.59 ^{a}
Nocardiaceae	0.13±0.14 ^{a}	0.09±0.15 ^{a}	0.22±0.10 ^{a}	0.17±0.03 ^{a}	0.12±0.11 ^{a}
Frankiaceae	0.11±0.10 ^{a}	0.16±0.19 ^{a}	0.27±0.18 ^{a}	0.18±0.15 ^{a}	0.26±0.11 ^{a}
Sporichthyaceae	0.11±0.09 ^{a}	0.27±0.08 ^{a}	0.17±0.02 ^{a}	0.04±0.07 ^{a}	0.20±0.13 ^{a}
67-14	0.04±0.08 ^{a}	2.40±2.16 ^{a}	0.06±0.11 ^{a}	0.05±0.08 ^{a}	1.46±2.22 ^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same family for different treatments, according to Tukey's test or t-test.

Table S 9 Content of Genus of bacteria in soil in an area of Cerrado sensu stricto.

Genus	C	CA	NP	N	P
Acidobacteriota Acidobacteriae Acidobacteriales Unknown	9.59±1.38 ^{a}	4.20±6.04 ^{a}	7.82±3.45 ^{a}	8.61±1.50 ^{a}	7.83±5.99 ^{a}
Chloroflexi AD3 Unknown	6.97±4.77 ^{a}	2.80±3.96 ^{a}	10.14±1.94 ^{a}	5.48±1.33 ^{a}	6.95±5.73 ^{a}
Unknown	20.32±4.49 ^{a}	9.80±11.70 ^{a}	15.53±2.58 ^{a}	20.78±4.95 ^{a}	16.30±11.82 ^{a}
Proteobacteria Alphaproteobacteria Rhizobiales Xanthobacteraceae	2.86±0.78 ^{a}	1.69±0.71 ^{a}	2.40±0.35 ^{a}	2.47±0.24 ^{a}	1.40±0.61 ^{a}
Acidothermus	2.63±1.01 ^{ab}	0.66±1.14 ^{b}	3.76±0.68 ^{a}	2.59±0.95 ^{ab}	2.01±1.64 ^{ab}
Candidatus Solibacter	2.56±0.82 ^{ab}	1.19±1.54 ^{b}	3.99±0.49 ^{a}	2.57±0.52 ^{ab}	2.08±1.30 ^{ab}
Conexibacter	2.38±0.26 ^{ab}	1.01±0.85 ^{b}	3.20±0.68 ^{a}	2.72±0.22 ^{ab}	2.20±1.26 ^{ab}
Gemmatimonadota Gemmatimonadetes Gemmatimonadales Gemmatimonadaceae	2.26±0.06 ^{a}	4.00±2.01 ^{a}	0.89±0.53 ^{a}	1.39±0.45 ^{a}	3.32±2.69 ^{a}
Chloroflexi Ktedono Ktedonobacterales Ktedonobacteraceae	2.15±1.71 ^{a}	0.50±0.75 ^{a}	1.34±0.71 ^{a}	1.26±1.07 ^{a}	0.64±0.39 ^{a}
Chloroflexi TK10 Unknown	2.10±0.08 ^{a}	1.31±0.17 ^{a}	2.06±0.64 ^{a}	2.95±2.30 ^{a}	1.89±1.02 ^{a}
Proteobacteria Gammaproteo Burkholderiales Sutterellaceae	2.02±0.44 ^{a}	0.37±0.64 ^{b}	0.48±0.18 ^{b}	0.95±0.60 ^{ab}	0.56±0.48 ^{b}
Proteobacteria Gammaproteo Gammaproteo Incertae Sedis	10.41±0.65^{a}	3.82±2.41 ^{b}	6.14±0.69 ^{ab}	9.80±1.56 ^{a}	3.21±2.67 ^{b}

Subgroup 1	1.00±0.16 ^{ab}	0.53±0.74 ^{b}	2.76±1.22 ^{a}	1.94±0.17 ^{ab}	1.14±1.03 ^{ab}
Bryobacter	1.52±0.42 ^{b}	0.57±0.74 ^{b}	3.42±0.70 ^{a}	1.79±0.49 ^{ab}	1.55±0.97 ^{b}
1921-2	1.09±0.98 ^{a}	0.57±0.99 ^{a}	2.07±1.23 ^{a}	0.86±0.62 ^{a}	0.49±0.47 ^{a}
Actinobacteriota Acidimicrobiia IMCC26256 Unknown	0.99±0.14 ^{a}	2.11±1.01 ^{a}	1.17±0.35 ^{a}	1.02±0.40 ^{a}	1.59±1.00 ^{a}
WPS-2 Unknown	0.98±0.95 ^{a}	0.93±1.61 ^{a}	2.28±1.14 ^{a}	1.69±1.47 ^{a}	1.64±1.33 ^{a}
Acidobacteriota Vicinami Vicinamibacterales Unknown	0.91±0.18 ^{ab}	2.35±1.36 ^{a}	0.65±0.19 ^{ab}	0.54±0.19 ^{b}	1.37±0.50 ^{ab}
Streptacidiphilus	0.81±0.71 ^{a}	0.28±0.48 ^{a}	2.77±3.12 ^{a}	1.65±0.76 ^{a}	1.14±1.01 ^{a}
Mycobacterium	0.78±0.19 ^{a}	0.41±0.31 ^{a}	0.77±0.25 ^{a}	0.71±0.26 ^{a}	0.64±0.43 ^{a}
Actinobacteriota Thermoleophilia Solirubrobacterales Solirubrobacteraceae	0.78±0.08 ^{a}	0.51±0.18 ^{a}	0.83±0.40 ^{a}	0.82±0.15 ^{a}	0.74±0.20 ^{a}
Archaea Crenarchaeota Nitrososphaeria Group 1.1c	0.77±0.49 ^{a}	0.21±0.37 ^{a}	0.26±0.23 ^{a}	0.76±0.85 ^{a}	0.19±0.17 ^{a}
Pajaroellobacter	0.76±0.22 ^{a}	0.57±0.59 ^{a}	0.90±0.16 ^{a}	0.69±0.20 ^{a}	0.89±0.61 ^{a}
Subgroup 10	0.74±0.37 ^{a}	1.32±1.36 ^{a}	0.36±0.21 ^{a}	0.45±0.16 ^{a}	0.44±0.17 ^{a}
ADurb.Bin063-1	0.72±0.30 ^{a}	0.35±0.24 ^{a}	0.20±0.12 ^{a}	0.38±0.25 ^{a}	0.44±0.17 ^{a}
Proteobacteria Alphaproteobacteria Elsterales Unknown	0.68±0.30 ^{bc}	0.49±0.34 ^{c}	1.79±0.38 ^{ab}	2.07±0.63 ^{a}	0.52±0.39 ^{c}
Verrucomicrobiota Verrucomicrobiae Pedosphaerales Pedosphaeraceae	0.68±0.25 ^{a}	0.71±0.39 ^{a}	0.41±0.10 ^{a}	0.64±0.60 ^{a}	0.75±0.11 ^{a}
Burkholderia-Caballeronia-Paraburkholderia	0.67±0.12 ^{a}	0.49±0.69 ^{a}	1.13±0.35 ^{a}	1.00±0.94 ^{a}	0.50±0.26 ^{a}
Rhodoplanes	0.64±0.10 ^{a}	0.36±0.10 ^{a}	0.20±0.18 ^{a}	0.30±0.09 ^{a}	0.34±0.30 ^{a}
Candidatus Udaeobacter	0.56±0.19 ^{a}	0.37±0.12 ^{a}	0.05±0.08 ^{a}	0.44±0.66 ^{a}	0.40±0.20 ^{a}
Gemmatimonas	0.51±0.16 ^{a}	0.47±0.22 ^{a}	0.31±0.14 ^{a}	0.38±0.10 ^{a}	0.57±0.28 ^{a}

Actinobacteriota Acidimicrobiia Unknown	0.50±0.13 ^{a}	1.22±0.75 ^{a}	0.88±0.40 ^{a}	0.55±0.13 ^{a}	0.86±0.50 ^{a}
Puia	0.49±0.01 ^{a}	0.08±0.14 ^{a}	0.37±0.24 ^{a}	0.69±0.56 ^{a}	0.21±0.21 ^{a}
GAL15 Unknown	0.46±0.15 ^{a}	0.10±0.17 ^{a}	0.27±0.11 ^{a}	0.97±1.02 ^{a}	0.33±0.40 ^{a}
Chloroflexi Ktedono Ktedonobacterales JG30-KF-AS9	0.44±0.45 ^{a}	0.13±0.23 ^{a}	0.62±0.14 ^{a}	0.36±0.24 ^{a}	0.43±0.38 ^{a}
Candidatus Xiphinematobacter	0.44±0.07 ^{a}	0.20±0.23 ^{a}	0.61±0.18 ^{a}	0.79±0.81 ^{a}	0.44±0.22 ^{a}
Unknown	0.42±0.22 ^{a}	0.55±0.18 ^{a}	0.36±0.11 ^{a}	0.46±0.11 ^{a}	0.73±0.26 ^{a}
Proteobacteria Alphaproteobacteria Micropepsales Micropepsaceae	0.42±0.13 ^{bc}	0.33±0.32 ^{c}	1.02±0.10 ^{ab}	1.13±0.38 ^{a}	0.31±0.16 ^{c}
Catenulispora	0.42±0.08 ^{a}	0.16±0.27 ^{a}	0.48±0.22 ^{a}	0.51±0.22 ^{a}	0.37±0.32 ^{a}
Chloroflexi KtedonoB12-WMSP1 Unknown	0.38±0.15 ^{a}	0.11±0.18 ^{a}	0.53±0.49 ^{a}	0.60±0.31 ^{a}	0.24±0.23 ^{a}
Actinospica	0.35±0.16 ^{a}	0.12±0.21 ^{a}	0.35±0.14 ^{a}	0.44±0.12 ^{a}	0.29±0.25 ^{a}
Streptomyces	0.32±0.19 ^{a}	0.97±0.94 ^{a}	0.46±0.23 ^{a}	0.16±0.16 ^{a}	0.43±0.28 ^{a}
Haliangium	0.28±0.11 ^{a}	1.09±0.71 ^{a}	0.24±0.09 ^{a}	0.16±0.03 ^{a}	0.70±0.85 ^{a}
Actinobacteriota Actinobacteria Pseudonocardiales Pseudonocardiaceae	0.24±0.21 ^{a}	0.24±0.10 ^{a}	0.31±0.18 ^{a}	0.70±0.33 ^{a}	0.32±0.11 ^{a}
Candidatus Koribacter	0.24±0.05 ^{a}	0.30±0.10 ^{a}	0.24±0.07 ^{a}	0.20±0.05 ^{a}	0.33±0.03 ^{a}
P3OB-42	0.23±0.02 ^{a}	0.22±0.10 ^{a}	0.08±0.14 ^{a}	0.15±0.16 ^{a}	0.27±0.22 ^{a}
Elusimicrobiota Lineage IIa Unknown	0.20±0.03 ^{a}	0.22±0.37 ^{a}	0.36±0.02 ^{a}	0.36±0.08 ^{a}	0.35±0.35 ^{a}
RCP2-54 Unknown	0.13±0.13 ^{a}	0.44±0.13 ^{a}	0.25±0.09 ^{a}	0.53±0.66 ^{a}	0.48±0.56 ^{a}
Jatrophihabitans	0.10±0.10 ^{a}	0.09±0.08 ^{a}	0.27±0.18 ^{a}	0.18±0.15 ^{a}	0.17±0.05 ^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same genus for different treatments, according to Tukey's test or t-test.

Table S 10 Content of Phylum of fungi in soil in an area of Cerrado sensu stricto.

Phylum	C	CA	N	NP	P
Ascomycota	63.91±30.54 ^{a}	57.23±18.76 ^{a}	91.36±3.17 ^{a}	69.25±6.38 ^{a}	53.69±38.88
Basidiomycota	4.04±4.75 ^{b}	37.94±20.47 ^{a}	4.16±1.90 ^{b}	6.81±1.46 ^{b}	10.13±7.27 ^{b}
Mortierellomycota	26.14±35.34 ^{a}	0.75±1.09 ^{a}	0.41±0.37 ^{a}	0.70±0.84 ^{a}	0.41±0.71 ^{a}
Mucoromycota	2.16±1.82 ^{a}	0.23±0.40 ^{a}	1.21±0.78 ^{a}	1.02±0.53 ^{a}	0.77±1.02 ^{a}
Fungi Incertae sedis	2.01±2.81 ^{a}	2.17±1.21 ^{a}	1.18±0.54 ^{a}	2.92±3.15 ^{a}	7.30±9.10 ^{a}
Unknown	1.27±0.95 ^{a}	0.40±0.15 ^{a}	1.32±1.36 ^{a}	17.99±0.16 ^{a}	24.84±39.89 ^{a}
Glomeromycota	0.23±0.21 ^{a}	0.92±0.19 ^{a}	0.15±0.03 ^{a}	1.05±1.08 ^{a}	1.71±1.46 ^{a}
Chytridiomycota	0.12±0.11 ^{a}	0.14±0.03 ^{a}	0.10±0.09 ^{a}	0.04±0.09 ^{a}	0.61±0.84 ^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same phylum for different treatments, according to Tukey's test or t-test.

Table S 11 Content of Class of fungi in soil in an area of Cerrado sensu stricto.

Class	C	CA	N	NP	P
Eurotiomycetes	6.20±3.65b	15.10±15.16^{ab}	8.39±4.46^{b}	38.67±5.74^{a}	20.55±17.25 ^{ab}
Ascomycota Incertae sedis	5.06±3.97 ^{ab}	0.83±0.72 ^{b}	14.43±90 ^{a}	2.84±2.38 ^{ab}	0.55±0.46 ^{b}
Leotiomycetes	4.73±2.60^{a}	3.04±4.99^{a}	10.73±1.93^{a}	6.31±3.68^{a}	10.58±13.72 ^{a}
Unknown	31.77±15.96^{a}	4.39±4.52^{a}	43.74±8.98^{a}	28.43±12^{a}	35.28±32.17 ^{a}
Agaricomycetes	3.84±4.66^{b}	37.69±20.6^{a}	3.49±2.23^{b}	2.24±1.35^{b}	9.56±7.43^{b}
Mortierellomycetes	26.15±35.33^{a}	0.77±1.07^{a}	0.43±0.34^{a}	0.95±0.81 ^{a}	0.44±0.68 ^{a}
Sordariomycetes	2.64±1.32^{a}	16.98±16.8^{a}	4.89±1.11^{a}	8.02±3.47 ^{a}	7.55±5.64 ^{a}
Fungi Incertae sedis	2.01±2.81^{a}	2.17±1.21^{a}	1.18±0.54^{a}	3.59±3.15 ^{a}	7.30±9.10 ^{a}
Dothideomycetes	14.23±8.47^{a}	3.37±1.18^{ab}	9.73±2.46^{ab}	4.79±1.10^{ab}	3.03±1.24^{b}
Orbiliomycetes	0.43±0.28^{a}	0.44±0.50^{a}	0.68±0.36^{a}	0.30±0.26^{a}	0.32±0.29 ^{a}
Glomeromycetes	0.26±0.16^{a}	0.69±0.38^{a}	0.13±0.03^{a}	0.47±0.32^{a}	1.05±0.67 ^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same class for different treatments, according to Tukey's test or t-test

Table S 12 Content of Order of fungi in soil in an area of Cerrado sensu stricto.

Order	C	CA	N	NP	P
Ascomycota Incertae sedis	5.06±3.97^{ab}	0.83±0.72^{b}	14.43±9.00^{a}	2.84±2.38^{ab}	0.55±0.46^{b}
Helotiales	4.30±2.25 ^{a}	3.00±4.98^{a}	10.2±1.57^{a}	6.19±3.74^{a}	10.41±13.65^{a}
Unknown	34.18±16.76 a	6.22±3.52^{a}	45.32±8.71^{a}	29.3±11.79^{a}	37.42±31.3^{a}
Chaetothyriales	3.18±1.57^{b}	8.93±5.99^{ab}	3.09±1.31^{b}	20.35±5.01^{a}	8.07±6.49^{ab}
Mortierellales	26.14±35.34^{a}	0.75±1.09^{a}	0.41±0.37^{a}	0.94±0.84^{a}	0.41±0.71^{a}
Fungi Incertae sedis	2.01±2.81^{a}	2.17±1.21^{a}	1.18±0.54^{a}	3.59±3.15^{a}	7.30±9.10^{a}
Pleosporales	12.66±7.42^{a}	2.71±1.24^{b}	9.17±2.60^{ab}	4.03±1.44^{ab}	1.89±0.82^{b}
Mucorales	1.93±1.75^{a}	0.22±0.38^{a}	0.43±0.42^{a}	1.23±0.52^{a}	0.35±0.37^{a}
Eurotiales	1.80±1.29^{a}	5.48±8.99^{a}	4.77±2.85^{a}	8.73±5.44^{a}	9.48±9.06^{a}
Hypocreales	0.88±0.09^{a}	11.73±15.59^{a}	1.73±0.16^{a}	4.09±3.23^{a}	3.01±2.83^{a}
Sordariales	0.54±0.69^{a}	1.90±1.25^{a}	1.59±1.42^{a}	1.02±0.40^{a}	1.99±1.67^{a}
Agaricales	0.54±0.37^{b}	36.92±20.85^{a}	2.15±1.74^{b}	0.74±0.07^{b}	7.73±8.74^{b}
Onygenales	0.41±0.30^{b}	0.55±0.96^{b}	0.30±0.40^{b}	9.14±2.97^{a}	2.94±2.65^{b}
Orbiliales	0.32±0.25^{a}	0.39±0.55^{a}	0.61±0.34^{a}	0.00±0.00a	0.00±0.00^{a}
Magnaporthales	0.22±0.38^{a}	0.05±0.08^{a}	0.34±0.34^{a}	0.37±0.15^{a}	0.75±0.65^{a}
Glomerales	0.22±0.19^{a}	0.65±0.35^{a}	0.09±0.08^{a}	0.45±0.31^{a}	1.04±0.67^{a}
Diaporthales	0.18±0.16^{a}	0.17±0.30^{a}	0.27±0.10^{a}	0.68±0.62^{a}	0.17±0.30^{a}

Tremellales	0.00±0.00^{a}	0.08±0.14^{a}	0.40±0.37^{a}	0.43±0.07^{a}	0.32±0.02^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same order for different treatments, according to Tukey's test or t-test.

Table S 13 Content of Family of fungi in soil in an area of Cerrado sensu stricto.

Family	C	CA	NP	N	P
Ascomycota Incertae sedis	5.06±3.97^{ab}	0.83±0.72^{b}	14.43±90^{ab}	2.84±2.38^{a}	1.07±0.46^{b}
Unknown	36.9±18.91^{a}	11.99±4.49^{a}	48.04±7.56^{a}	34.12±12.21^{a}	18.92±28.79^{a}
Herpotrichiellaceae	2.11±0.85^{b}	6.57±4.02^{ab}	1.35±0.28^{a}	14.98±3.24^{b}	3.93±4.99^{b}
Fungi Incertae sedis	2.01±2.81^{a}	2.17±1.21^{a}	1.18±0.54^{a}	3.59±3.15^{a}	17.72±9.10^{a}
Pleosporales Incertae sedis	11.54±6.56^{a}	0.35±0.50^{b}	8.59±3.39^{ab}	3.14±2.05^{ab}	1.30±0.56^{b}
Myxotrichaceae	1.82±1.16^{a}	2.01±3.49^{a}	3.45±1.22^{a}	3.92±4.09^{a}	0.53±1.40^{a}
Aspergillaceae	1.5±1.28^{a}	5.39±8.89^{a}	4.02±2.53^{a}	8.30±5.73^{a}	9.82±8.90^{a}
Helotiales Incertae sedis	1.32±0.72^{b}	0.17±0.21^{b}	3.93±0.64^{b}	0.45±0.29^{a}	0.57±0.26^{b}
Hyaloscyphaceae	0.68±0.40^{a}	0.19±0.34^{a}	1.27±0.39^{a}	0.57±0.27^{a}	2.11±1.08^{a}
Trichomeriaceae	0.57±0.37^{a}	0.61±1.05^{a}	0.57±0.27^{a}	0.92±0.45^{a}	0.34±0.74^{a}
Clavicipitaceae	0.51±0.28^{a}	0.95±0.62^{a}	0.65±0.26^{a}	2.74±2.66^{a}	0.21±0.52^{a}
Onygenales Incertae sedis	0.38±0.26^{b}	0.40±0.70^{b}	0.26±0.33^{a}	8.90±2.63^{b}	5.00±2.53^{b}
Chaetomiaceae	0.35±0.51^{a}	1.25±1.67^{a}	1.34±1.21^{a}	0.71±0.35^{a}	1.15±1.56^{a}
Magnaporthaceae	0.22±0.38^{a}	0.05±0.08^{a}	0.34±0.34^{a}	0.28±0.06^{a}	1.05±0.57^{a}
Glomeraceae	0.15±0.15^{a}	0.63±0.37^{a}	0.04±0.07^{a}	0.37±0.26^{a}	1.05±0.59^{a}
Nectriaceae	0.10±0.10^{a}	4.62±7.50^{a}	0.47±0.26^{a}	0.24±0.22^{a}	0.59±0.21^{a}
Hygrophoraceae	0.00±0.00^{b}	29.2±20.87^{a}	1.63±1.96^{b}	0.11±0.20^{b}	3.06±6.35^{ab}
Ophiocordycipitaceae	0.00±0.00^{b}	1.02±0.70^{a}	0.13±0.12^{ab}	0.33±0.29^{ab}	0.17±0.18^{ab}

Cordycipitaceae	0.00 ± 0.00^a	0.41 ± 0.44^a	0.21 ± 0.09^a	0.38 ± 0.38^a	0.21 ± 0.52^a
Trimorphomycetaceae	0.00 ± 0.00^a	0.08 ± 0.14^a	0.39 ± 0.36^a	0.42 ± 0.08^a	0.19 ± 0.07^a

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same family for different treatments, according to Tukey's test or t-test.

Table S 14 Content of Genus of fungi in soil in an area of Cerrado sensu stricto

Genus	C	CA	N	NP	P
Ascomycota Incertae sedis	5.06±3.97^{ab}	0.83±0.72^{b}	14.43±90^{a}	2.84±2.38^{ab}	0.55±0.46^{b}
Unknown	43.68±20.92^{a}	19.68±6.99^{a}	51.41±7.16^{a}	38.15±11.64^{a}	47.80±26.17^{a}
Fungi Incertae sedis	2.01±2.81^{a}	2.17±1.21^{a}	1.18±0.54^{a}	3.59±3.15^{a}	7.30±9.10^{a}
Pseudorobillarda	11.50±6.54^{a}	0.24±0.42^{b}	8.56±3.42^{ab}	3.12±2.07^{ab}	0.87±0.54^{b}
Herpotrichiellaceae Incertae sedis	1.53±0.45^{b}	2.47±4.29^{b}	0.72±0.18^{b}	13.16±2.2^{a}	2.55±2.37^{b}
Oidiodendron	1.16±1.00^{a}	1.94±3.36^{a}	2.68±1.31^{a}	3.58±3.84^{a}	0.73±1.14^{a}
Leohumicola	1.09±0.61^{b}	0.04±0.06^{bc}	3.39±0.64^{a}	0.00±0.00^{c}	0.20±0.13^{bc}
Penicillium	1.07±0.74^{a}	4.50±7.37^{a}	3.11±1.67^{a}	3.82±0.43^{a}	4.16±4.08^{a}
Glutinomyces	0.65±0.38^{ab}	0.18±0.31^{b}	1.26±0.40^{a}	0.46±0.20^{ab}	0.23±0.30^{b}
Knufia	0.56±0.38^{a}	0.59±1.02^{a}	0.52±0.23^{a}	0.80±0.47^{a}	0.58±0.74^{a}
Onygenales Incertae sedis	0.38±0.26^{b}	0.40±0.70^{b}	0.26±0.33^{b}	8.90±2.63^{a}	2.72±2.53^{b}
Talaromyces	0.34±0.50^{a}	0.34±0.59^{a}	0.37±0.32^{a}	3.42±4.61^{a}	5.05±4.78^{a}
Arcopilus	0.25±0.43^{a}	0.12±0.20^{a}	0.88±0.88^{a}	0.32±0.07^{a}	1.12±1.49^{a}
Pseudophialophora	0.21±0.36^{a}	0.05±0.08^{a}	0.33±0.34^{a}	0.28±0.06^{a}	0.65±0.57^{a}
Keithomyces	0.18±0.22^{a}	0.33±0.35^{a}	0.35±0.07^{a}	0.69±0.64^{a}	0.00±0.00a
Melanconiella	0.17±0.16^{a}	0.15±0.26^{a}	0.24±0.08^{a}	0.58±0.55^{a}	0.11±0.20^{a}
Xylogone	0.16±0.18^{a}	0.10±0.18^{a}	0.25±0.02^{a}	0.41±0.31^{a}	0.06±0.11^{a}
Trichoderma	0.08±0.14^{a}	4.25±7.27^{a}	0.13±0.12^{a}	0.15±0.13^{a}	1.02±1.77^{a}

Hygrocybe	0.00±0.00^{b}	28.81±20.34^{a}	1.63±1.96^{b}	0.11±0.20^{b}	5.00±6.07^{ab}
Penicillifer	0.00±0.00^{a}	3.81±6.48^{a}	0.12±0.02^{a}	0.06±0.10^{a}	0.07±0.13^{a}
Humicola	0.00±0.00^{a}	0.12±0.12^{a}	0.18±0.20^{a}	0.18±0.06^{a}	0.13±0.13^{a}

C: control treatment; CA: liming treatment; N: Nitrogen treatment; NP: nitrogen with phosphorus treatment; P: Phosphorus treatment; Values are expressed as mean ± standard deviation; A-C: different lowercase letters substituted in the same line denote differences ($p < 0.05$) between the same genus for different treatments, according to Tukey's test or t-test.