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**DANIELA TOMAZELLI**

# SOIL MICROBIAL COMMUNITY DINAMICS ACROSS PASTURE SYSTEMS

LAGES, SC
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Tese apresentada ao Curso de Pós-graduação em Ciência do Solo do Centro de Ciências Agroveterinárias, da Universidade do Estado de Santa Catarina, como requisito parcial para obtenção do grau de Doutora em Ciência do Solo.
Orientador: Osmar Klauberg Filho

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**SOIL MICROBIAL COMMUNITY DINAMICS ACROSS PASTURE SYSTEMS**


ABSTRACT

Soils are habitats of microorganisms, that play important roles in the maintenance of life on the planet. The microbiota inhabiting pasture soils is vital for these production systems. However, little is known about the effect of land-use intensity on microbial structure and functionality and how microbial communities respond to the conversion of natural grasslands to cultivated pastures. In this study, we evaluated the effect of soil and vegetation management on the taxonomic profile and the functional potential of microbial communities in grassland systems. The assembly patterns and habitat specialization of microbial communities were investigated along a gradient of pasture management intensification (Chapter III). Was also evaluated the patterns of microbial selection in rhizosphere and bulk soils of natural grasslands and cultivated pastures (Chapter IV). The study areas followed a gradient of soil disturbance intensity: natural pasture (NG), improved natural pasture (IG), perennial cultivated pasture (PP) and annually cultivated pasture (AP). The results have shown taxonomic and functional differences among natural and improved grasslands (NG and IG) and cultivated pastures (PP and AP). This effect is mainly due to the decrease of available aluminum in the managed soils (Chapter II). The conversion of natural grassland to cultivated pastures has increased the abundance of habitat specialists (Chapter III). Finally, plant diversity and root morphology have influenced specific groups of soil Bacteria and Fungi (Chapter IV). Together, these results can help in the decision-making of grassland management strategies and biodiversity conservation priorities in natural grasslands from southern Brazil.



**Keywords:** Atlantic Forest; Forage systems; Microbial ecology; Plant diversity; Soil Microbiome.

DINÂMICA DA COMUNIDADE MICROBIANA EM SISTEMAS DE PASTAGEM


## RESUMO

Solos são habitats de micro-organismos, que desempenham papéis importantes na manutenção da vida no planeta. A microbiota que habita solos de pastagem é vital para a produção desses sistemas. No entanto, pouco se sabe sobre o efeito da intensidade do uso da terra na estrutura e funcionalidade microbiana e como as comunidades microbianas respondem à conversão de pastagens naturais em pastagens cultivadas. Neste estudo, foram avaliados o efeito do manejo do solo e da vegetação no perfil taxonômico e o potencial funcional das comunidades microbianas nos sistemas de pastagem (Capítulo II). Foi investigado os padrões de montagem e especialização de habitat das comunidades microbianas, ao longo de um gradiente de intensificação do manejo de pastagens (Capítulo III). Também foi avaliado os padrões de seleção microbiana na rizosfera e solos a granel de pastagens naturais e pastagens cultivadas (Capítulo IV). As áreas de estudo seguiram um gradiente da intensidade de perturbação do solo: pastagem natural (NG), pastagem natural melhorada (IG), pastagem cultivada perene (PP) e pastagem cultivada anualmente (AP). Os resultados mostraram diferenças taxonômicas e funcionais entre pastagens naturais e melhoradas (NG e IG) e pastagens cultivadas (PP e AP). Esse efeito deve-se principalmente à diminuição do alumínio disponível nos solos gerenciados (Capítulo II). A conversão de pastagens naturais em pastagens cultivadas aumentou a abundância de especialistas em habitat (Capítulo III). Finalmente descobrimos que a diversidade vegetal e a morfologia radicular influenciaram grupos específicos de bactérias e fungos do solo (Capítulo IV). Juntos, esses resultados podem ajudar na tomada de decisão das estratégias de manejo de pastagens e nas prioridades de conservação da biodiversidade em pastagens naturais do sul do Brasil.



**Palavras-chave:** Diversidade vegetal; ecologia microbiana; mata atlântica; microbioma do solo; sistemas forrageiros.

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**SUMMARY**

# 1 INTRODUCTION

Grasslands are present in various landscapes, formed by natives or exotic forage species, has great importance in world food production (MURRAY; CROTTY; EEKERREN, 2012). The highest plant richness is often found in native grasslands, such as those found in the Pampa and Atlantic Forest biomes (OVERBECK et al., 2007), in Brazil. The native grasslands, along with the other landscapes of the Atlantic Forest biome, are recognized worldwide as "*biodiversity hotspots*" by their high diversity of animals and plants, coupled with high levels of endemism (MYERS et al., 2000; SLOAN et al., 2014).

The aboveground diversity of plants is known to influence soil microbial communities (EO et al., 2021; GUO et al., 2021). High diversity of forage species is often related to increased microbial activity, carbon storage (LANGE et al., 2015) and plant biomass productivity (PELKOFER et al., 2016). However, the need for more productive systems has induced the conversion of native grasslands to cultivated pastures (METZGER et al., 2019; ZANELLA et al., 2021). As a result, both conversion and soil management intensification lead to shifts in soil physical and chemical characteristics, with consequences for the microbial diversity and ecosystem functions provided by soil microbial communities (MENDES et al., 2015; GOSS-SOUZA et al., 2020).

The structure and assembly of soil microbial is governed by biotic and abiotic factors, with a relative influence of random processes. Two models can be used to explain microbial community assembly: (i) Niche-based theory: deterministic factors, such as species characteristics and interspecific interactions (e.g., competition, predation, mutualism, and taxa trade-offs), and environmental conditions (e.g., pH, temperature, salinity, and moisture) govern the community assembly, as defined by deterministic processes (ZHOU; NING et al 2017); and (ii) Neutral theory: the microbial community assembly is governed by stochastic processes of birth, death, dispersal, extinction, and speciation (VELLEND, 2010). However, according to the model developed by Dini-Andreote et al. (2015), deterministic and stochastic processes are complementary and act simultaneously to shape the assembly of soil microbial communities.

The microbial assembly in grassland soils often present high complexity and stochastic behavior (GÓSS-SOUZA et al., 2017), which may be related to the continuous and homogeneous root system distribution, favoring the homogenization of microbial communities (NEAL et al., 2020). On the other hand, the processes that affect the composition and dispersal

of microbial communities are multiple and respond to specific environmental conditions for each geographic region or biome (DINI-ANDREOTE et al., 2015; GOSS-SOUZA et al., 2022). The processes governing soil microbial community assembly in native grasslands of the pampa biome were studied by Lupatini et al. (2019), which found microbial communities to be neutral and affected by deterministic process related to soil characteristics.

Changes in vegetation and soil management can alter the microbial composition, and therefore the microbial recruitment at the plant rhizosphere, the millimeter interface between soil and roots (MENDES; GARBEVA; RAAIJMAKERS, 2013). The microbiota that colonizes and inhabits the rhizosphere plays a key role in plant nutrition (WU et al., 2021), pathogen resistance (MENDES et al., 2014) and plant species co-occurrence (CAVALIERI et al., 2020).

The conversion from natural grasslands to cultivated pastures brings consequences to the taxonomic and functional structure of microbial communities, as verified in natural grasslands of the pampa biome (LUPATINI et al., 2013). However, to our knowledge, there is no report on the microbial responses to this transition in grasslands from the Atlantic Forest biome. Understanding the ecological responses of soil microbes and their functions is pivotal for the improvement of soil and forage management strategies, aiming the productivity maintenance and the provision of ecosystem services in forage systems.

## 1. 1 HYPOTHESES

### 1.1.1 Taxonomy and microbial functionality (Chapter II)

Changes in soil chemical and physical characteristics in management intensification affect microbial composition, diversity, and potential function. And the aboveground forage diversity is positively correlated with belowground microbial diversity and functional potential.

### 1.1.2 Ecological processes (Chapter III)

The management intensification of grassland system increases the weight of ecological deterministic process and habitat specialists on microbial assembly.

### 1.1.3 Rhizosphere (Chapter IV)

Plant diversity and root morphology drive changes on bacterial and fungal diversity and composition in rhizosphere of natural and cultivated pastures. Bacteria and fungi respond differently to changes in plant diversity and root morphology.

## 1. 2 OBJECTIVES

### 1.2.1 Taxonomy and microbial functionality (Chapter II)

Define a soil and pasture vegetation management that maintains soil microbiological quality and can be good alternatives for cattle ranchers.

### 1.2.2 Ecological processes (Chapter III)

Understand the ecological processes affected by pastureland soil and vegetation management.

### 1.2.3 Rhizosphere (Chapter IV)

Predicting about changes in the rhizosphere when conversion from natural to cultivated pastures occurs

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# 2 CHAPTER I: BIBLIOMETRIC REVIEW: STUDIES OF MICROBIAL ECOLOGY IN BRAZIL

ABSTRACT

The advent of large-scale DNA sequencing has led to advances in soil microbial ecology studies. In the last 10 years, several studies have sought to understand the complexity of soil microbial communities, their relationship with soil and environmental characteristics. Studies have focused on consequences of management intensification and the replacement of natural vegetation with cultivated areas (e.g., agricultural and pastures areas) for the microbial patterns of assembly and ecosystem functions. Here, we applied a structured systematics search on scientific publication databases and compiled the information regarding the progress of microbial ecology studies in Brazil in the last 10 years. The aim of this study was to understand the scope of the research in Soil Microbial Ecology in Brazilian soils and the consequences of the intensification of land use in microbial communities (Prokaryotes). The study has revealed 57 scientific articles, available in two databases. Most studies have investigated soils from the southeast and the north Brazil, with no record for microbial composition in 11 Brazilian states. Most studies surveyed soils from the Atlantic Forest and Amazon biomes, including natural environments, mainly forest. From 23 studies that evaluated the replacement of natural to cultivated vegetation, most authors concluded that soil management (mainly changes in pH), and vegetation cover were the main drivers for shifts microbial community patterns. Brazil, as a continental country, still has a gap in the knowledge about the microscopic life in soils. Understanding the ecology of microorganisms that inhabit Brazilian soils in agroecosystems is fundamental for establishing more efficient soil management strategies and developing safer technologies for agriculture.

**Keywords:** Brazilian biomes; Ecology; Land-use; Microbial communities; Vegetation cover.

# REVISÃO BIBLIOMÉTRICA: ESTUDOS DE ECOLOGIA DE MICROBIANA NO BRASIL


## RESUMO

O advento do sequenciamento de DNA em larga escala levou a avanços nos estudos de ecologia microbiana do solo. Nos últimos 10 anos, diversos estudos têm buscado compreender a complexidade das comunidades microbianas do solo, sua relação com o solo e características ambientais. Os estudos têm focado nas consequências da intensificação do manejo e na substituição da vegetação natural por áreas cultivadas (por exemplo, áreas agrícolas e pastagens) para os padrões microbianos de montagem e funções ecossistêmicas. Aqui, aplicamos uma pesquisa sistemática estruturada em bases de dados de publicação científica e compilamos as informações sobre o andamento dos estudos de ecologia microbiana no Brasil nos últimos 10 anos. O objetivo deste estudo foi compreender o escopo da pesquisa em Ecologia Microbiana de Solos em solos brasileiros e as consequências da intensificação do uso da terra em comunidades microbianas (Procariontes). O estudo revelou 57 artigos científicos, disponíveis em duas bases de dados. A maioria dos estudos tem investigado solos do sudeste e do norte do Brasil, sem registro de composição microbiana em 11 estados brasileiros. A maioria dos estudos pesquisou solos dos biomas Mata Atlântica e Amazônia, incluindo ambientes naturais, principalmente florestas. De 23 estudos que avaliaram a substituição da vegetação natural para cultivada, a maioria dos autores concluiu que o manejo do solo (principalmente mudanças no pH), e a cobertura vegetal foram os principais impulsionadores para os deslocamentos dos padrões microbianos da comunidade. O Brasil, como país continental, ainda tem uma lacuna no conhecimento sobre a vida microscópica nos solos. Compreender a ecologia de microrganismos que habitam solos brasileiros em agroecossistemas é fundamental para estabelecer estratégias mais eficientes de manejo do solo e desenvolver tecnologias mais seguras para a agricultura.


**Palavras-chave:** Biomas Brasileiros; cobertura vegetal; comunidades microbianas; Ecologia; Uso da terra.

## 2.1 INTRODUCTION

Soil is the habitat of several organisms, which form a network of complex interactions among microbiota, plants, and animals (FENG et al., 2019). Microorganisms play important roles in the maintenance of life on the planet and the benefits that the soil supplies to humans are called ecosystem services, these being the provisioning (food, fibers, fuel), regulating (carbon storage, clean air, temperatures control), supporting (habit, biodiversity conservation, soil formation), and cultural service (Education, recreation, stewardship) (MURRAY; CROTTY; EEKERREN, 2012). Many ecosystem services are supplied through microbial mediated processes, which perform important functions such as organic matter decomposition, nutrient cycling, soil structure maintenance and climate regulation (BRUSSAARD, 2012).

The growing demand for food production has generated the need to expand agricultural production and thereby the suppression of native vegetation, converting these environments into annual or perennial crop systems (OVERBECK et al., 2022). Land-use change affects soil physical and chemical characteristics and modifies the patterns of diversity and functionality of microbial communities (MENDES et al., 2015; GÓSS-SOUZA et al., 2019).

Grasslands are widespread all over the globe in the most contrasting landscapes (NEYRET et al., 2021). In Brazilian biomes, they can be found as natural grasslands or cultivated pastures (PINTO et al., 2017). The highest richness of plant species is often found in natural grassland, in these environments' diversity can be directly related to productivity (DELORY et al., 2019), due to the diversity of compounds exuded by the rhizosphere (PELKOFER et al., 2016). Plant diversity is directly related to microbial activity, decomposition of matter and consequently carbon storage in soil (LANGE et al., 2015). Moreover, the presence of livestock can modify the connection between soil microorganisms and plant rhizosphere in grasslands (YANG et al., 2019).

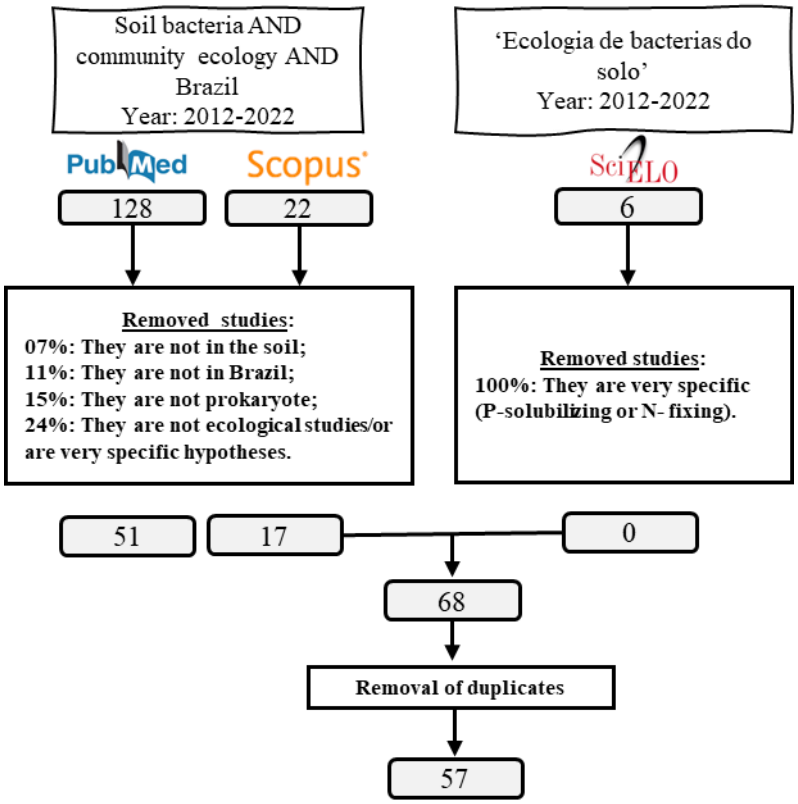
Beyond this, despite advances in studies in Brazilian soils (ARAUJO et al., 2021; GOSS-SOUZA et al., 2017; LUPATINI et al., 2014; NAVARRETE et al., 2015a, 2015b; PEDRINHO et al., 2020), there is still a knowledge gap about the diversity and ecology of microbial communities in Brazilian soils under natural vegetation, mainly natural grassland, and the consequences of substitution by agricultural crops. The aim of this study was to search a literature search using three databases to investigate the progress of microbial ecology studies in Brazil in the last 10 years and to search information on microbial ecology studies in natural vegetation, seeking to understand the progress in the study of Brazilian native pastures.

## 2.2 MATERIAL AND METHODS

### 2.2.1 Search criteria in databases

Scientific articles were searched for key terms in English '*Soil Bacteria*' AND '*community ecology*' AND '*Brazil*' in PubMed and SCOPUS databases. To cover the publications in Portuguese, the terms 'Ecologia de bactérias do solo' were used in the Scielo database. In both databases, the dating filter was used for the last 10 years (Figure 1). After searching for key terms, the studies were filtered one by one and, the following studies were eliminated: the research focus was not soil, studies in foreign soils, studies of fungi/nematodes/invertebrate's ecology, studies in a greenhouse with very specific hypotheses or focused on isolation of microorganisms (Figure 1). In a second stage, the article was a new screening, where only studies that evaluated the transition from natural to cultivated vegetation were selected.

Figure 2- Screening scheme and selection of scientific articles in the databases: PubMed, Scopus and Scielo in the years 2012 to 2022.




### 2.2.2 Extraction of evidence

The evidence extraction criterion was based on the questions listed, where the articles were analyzed to identify, step one: i. What is the progression of microbial ecology studies in Brazil in the last 10 years; ii. Which Brazilian regions and biomes were collected soil; iii. What types of land uses investigated (Natural, Agriculture); iv. What are the main methods of assessing microbial communities (Sequencing, fingerprint), and how the bioinformatic analysis of the sequences was performed (OTU: Operational taxonomic unit, AVS: Amplicon variant sequence).

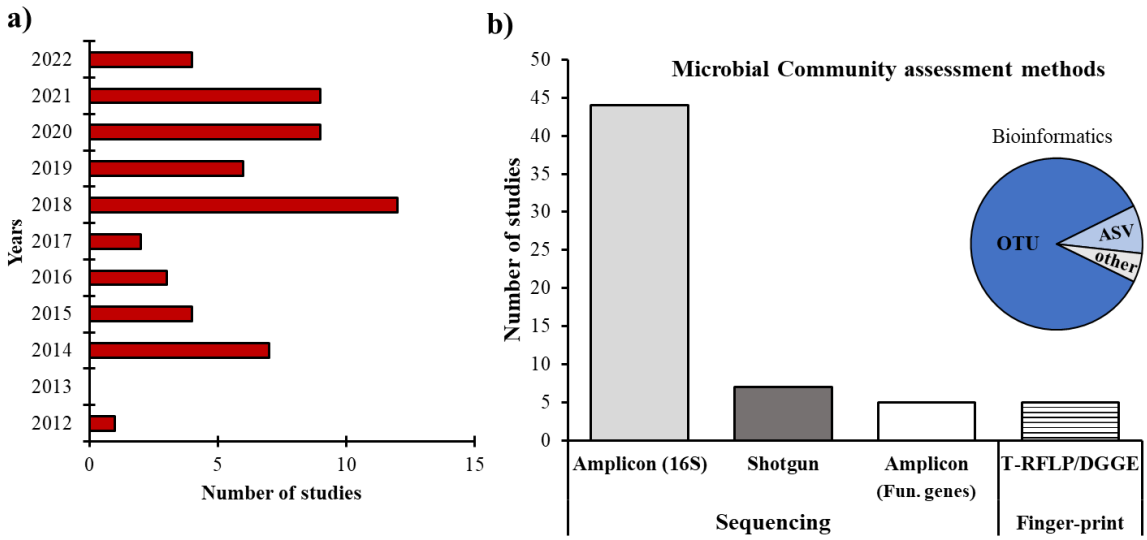
And, to understand the responses of the microbial community to the natural vegetation replacement for cultivated and the intensification of land-use, was conduced the step two, the selected studies were filters again, maintaining only fieldwork, which compared soils of natural vegetation with agricultural crops, from this was elaborated the following questions: v. What is the effect of natural vegetation substitution on soil microorganism alfa diversity and richness; vi. Which soil or vegetation attributes have the higher influence on the composition of microbial communities and which statistical methods explain these changes.

## 2.3 RESULTS

### 2.3.1 Advances in soil microbial ecology studies

Of the 57 articles selected, most of them were published in 2018, the main method of community evaluation is by sequencing, mainly, 16S amplification sequencing, following for metagenomic shot gun sequencing, and lastly functional genes sequencing and fingerprint techniques. Most of the studies used use OTU for the merge of the found sequences (Figure 2).

Figure 3- Evolution of the publications in microbial ecology in Brazil, from 2012 to 2020 (a) and (b) methods for evaluation of microbial communities in ecological studies.

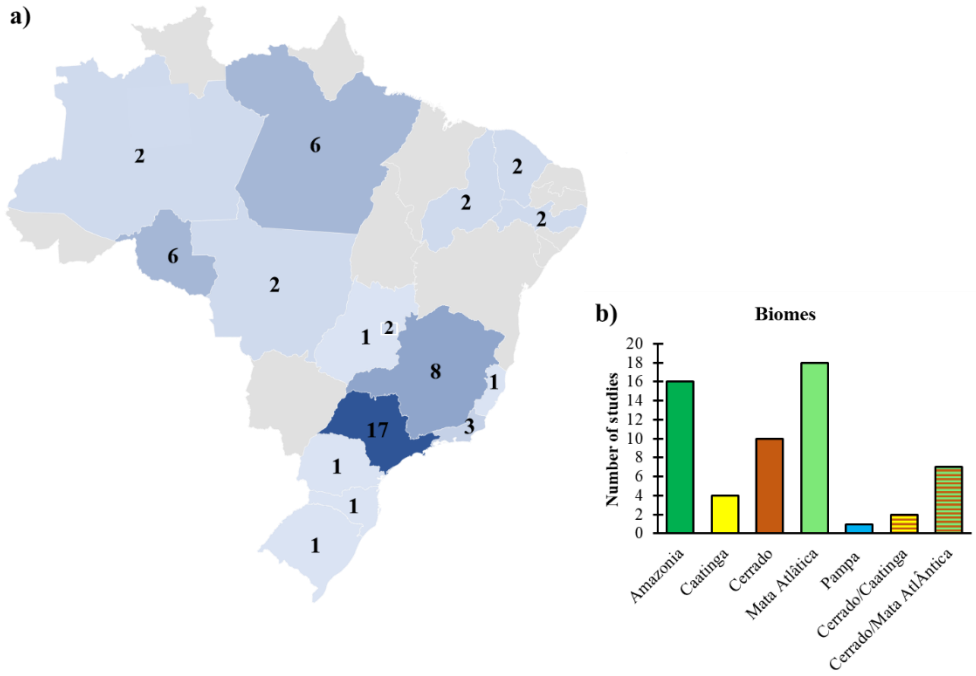



2.3.2 Brazilian regions and biomes of the studies

Most studies of microbial ecology in Brazil were concentrated in the state of Sao Paulo, with 17 published studies, followed by Minas Gerais with eight studies, Pará and Rondônia had six studies, at last Rio de Janeiro had three studies. The states, Amazônia, Ceará, Brasília (Federal District), Pernambuco and Piauí had two studies, and one study for Espírito Santo, Goiás, Paraná, Santa Catarina and Rio Grande do Sul (Figure 3a).

The Atlantic Forest is the most studied Brazilian biome, followed by the Amazon, Cerrado, and Caatinga biomes and studies that were done in intermediate regions between two biomes (Cerrado/Amazon). Only one study of microbial ecology was found for the Pampa biome (Figure 3b).

Figure 3- Numbers of studies of ecology of microorganisms in (a) Brazilian states and (b) biomes studied, in the last 10 years.

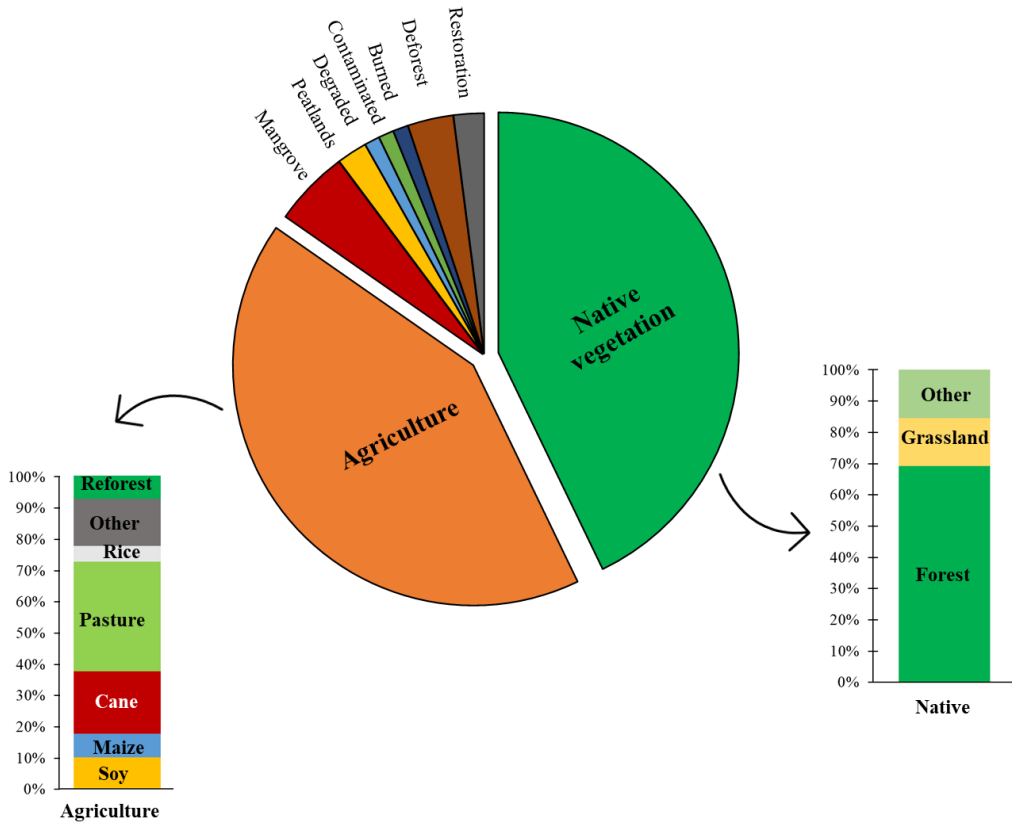



2.3.3 Land uses studied

Most investigated soils are under natural vegetation, representing 42% of the studies cataloged, followed by agricultural soils with 40%. Other studies are focused on mangrove soils (5%), deforestation of native forests (3%), peatland soils (2%), degraded soils (1%), contaminated soils (1%), and areas that burned naturally or induced (1%) (Figure 4).

From soils under natural vegetation, studies are concentrated in forest soils, with more than 65% of the studies, followed by soils of natural or naturalized pastures, and with less representativeness in other environments, such as island vegetation (Anthurium and bromeliads). While, in agricultural soils, pastures are the most studied land use, followed by sugarcane, soybean, maize, reforestation areas, rice, and other crops (Figure 4).

Figure 4- Proportion of microbial ecology studies in Brazilian soils, from 2012 to 2022.




### 2.3.4 Responses of microbial communities to land-use change and management intensification

The studies were filtered, with articles that did not evaluate the transition from natural to cultivated environments removed, so that we selected 23 studies, that evaluated the response of microbial communities to the conversion of natural vegetation into cultivated areas. Out of 23 studies, 17 evaluated forest replacement to agriculture cropping, three evaluated the land-use intensification of natural grasslands and two studied the effect of Cerrado vegetation intensification on the diversity and composition of microbial communities. In general, higher richness and microbial diversity were found in disturbed environments. And the main drivers of changes in microbial composition are pH, and vegetation cover (Table 1). The main statistical method used is RDA, followed by NMDS, Corr and with less representativeness PCA. Most of the studies were focused on the natural vegetation transition in soils of the Amazon biome, followed by Cerrado, Atlantic Forest and Caatinga (Table 1).

Table 1- Soil microbial richness, diversity, and structure responses to the intensification of soil land-use or substitution of natural vegetation by agricultural.

| Land-use | Diversity/Richness Response | Drivers | Statistics | Biome | Authors |
|---|---|---|---|---|---|
| Fo, Soy, CFo, Cane | Fo<CFo<Soy<Cane | pH | Corr | Cerrado | Bobul'ská et al. 2021 |
| Fo, Fo1dis, Fo2, Pa, Agri | Fo<Fo1dis<Fo2<Pa<Agri | pH | RDA | Amazon | Carvalho et al. 2016 |
| Fo, Pa, PaHP | Fo,Pa<PaHP | H+Al, P, EC, MBC, Na | RDA | Caatinga | Costa et al. 2022a |
| Fo, CFo | Fo<CFo | pH, Land-use | NMDS | Atlantic F. | Cuer et al. 2018 |
| Fo, Pa, Soy | Fo =Pa= Soy | P, Ca/Mg, $NO_3$ | RDA | Atlantic F. | Góss-Souza et al. 2017 |
| Fo1, Fo2, Pa | Fo1=Fo2<Pa | Land-use | NMDS | Amazon | Kroeger et al.2018; 2021 |
| Fo, noVeg, Re | noVeg<Fo<Re | pH,Al, Ca, OM, CEC, P, K | CCA | Atlantic F. | Lima et al. 2022 |
| Fo,Def, Agri, Pa | Flo<Def, Agri, Pa | pH, OC, Mn, K, N, $NO_3$ | RDA | Amazon | Mendes et al. 2015b |
| Fo1, Fo2, Pa | Fo2, Fo1<Pa | Land-use | PV | Amazon | Mirza et al. 2020 |
| Fo, CFo | Fo<CFo | Land-use | NMDS | Atlantic F. | Monteiro et al. 2020 |
| Fo, Def | Fo<Def | OM | Corr | Amazon | Navarrete et al. 2015a |
| Fo, Pa | Fo<Pa | pH,C:N,Ca, Mg e Al | RDA, Corr | Amazon | Navarrete et al. 2015b |
| Fo, Pa | Unregistered | Vegetation cover | LMM | Caatinga | Costa et al. 2022b |
| Fo1, Fo2, Pa | Fo1, Fo2<Pa | Al, FC | RDA | Amazon | Pedrinho et al. 2019; 2020 |
| Fo1, Fo2, Pa | Fo1, Fo2, Pa | Vegetation cover | NMDS | Amazon | Rajan et al. 2015 |
| Fo, Cane, Cane+Bu, Cane+Bu+Li | Fo<Cane<Cane+Bu<Cane+Bu+Li | OM,P,Mg e clay | RDA | Atlantic F. | Val-Moraes et al. 2016 |
| Fo2, NG, OG, GE | Unregistered | OC, N, Al e Na | RDA | Caatinga | Oliveira et al. 2021 |
| Ce, CeDg, Re | Ce>CeDg>Re | pH,SD, MBC, Ca, P, $qCO_2$ | RDA | Cerrado | Araujo et al. 2014 |
| Ce, Bu | Ce<Bu | Mn e Fe | PCA | Cerrado | Belmok et al. 2019 |
| Ce, CLIS | Ce<CLIS | Land-use | NMDS | Cerrado | Selari et al. 2021 |
| NG e Re | PN< Re | Vegetation cover | NMDS | Cerrado | Cardoso et al. 2020 |
| NG, Pa, GE | NG, Pa>GE | Na, Al, N, OC | RDA, Corr | Caatinga | Pereira et al. 2021 |
| NG,Ce, PaDg | Unregistered | pH | PCA | Cerrado | Sartori Silva et al. 2019 |

Label of Land-use: Forest (Fo), primary forest (Fo1), secondary forest (Flo2), Pasture (Pa), Agriculture (Agri), Natural Grassland (NG), Burnt areas (Bu), Liming (Li), Cerrado vegetation (Ce), Reforest (Re), Deforestation (Def), Cultivated Forest (CFo), Grazing exclusion (GE), Overgrazed (OG), Crop-livestock integrated systems (CLIS), area without vegetation (NoVeg), degraded (Dg), Disturbed area (Dis), High productivity (HP). Label of drivers: Microbial biomass carbon (MBC), Iron (Fe), metabolic coefficient ($qCO_2$), Electrical conductivity (EC), Soil organic matter (SOM), Organic carbon (OC), Manganese (Mn), Sodium (Na), Aluminum (Al), Phosphorus (P), Nitrogen (N), Nitrate ($NO_3$), Calcium (Ca), Potassium (K), Magnesium (Mg), potential acidity (H+Al) Cation exchange capability (CEC), Field capacity (FC), Soil density (SD). Label of statistic methods: Correlation (Corr), Partitioning variability (PV), Linear mixed Model (LMM), Redundancy analysis (RDA), non-metric multidimensional scaling (NMDS), Principal component analysis (PCA), canonical correspondence analysis (CCA).

## 2.4 DISCUSSION

The compiled data show the increase in the number of studies focused on ecology of microorganisms in Brazil in the last 10 years, with the largest number of public works in 2018, mainly due to the decrease in the cost of sequencing techniques and the emergence of service providers in this field.

Most of the studies evaluated identified the microbial community by sequencing the conserved region, with about 1500 base pairs, located in subunit 16S of the ribosomal gene (16S rRNA) (ARAUJO et al., 2021; PEREIRA et al., 2021; GONTIJO et al., 2021; LUPATINI et al., 2019; NAVARRETE et al., 2013). The universality of distribution of this gene, between prokaryotes and the large volume of information in databases, popularized the adoption 16S rRNA gene amplicon sequencing in microbial ecology, allowing to understand the spatial and temporal distribution of microorganisms and factors that shape their occurrence and evolution (KROEGER et al., 2021).

The wide Brazilian territorial extension has not been fully studied, and according to the searches in the databases, no studies regarding microbial ecology were found in 11 Brazilian states for the last 10 years (Acre, Roraima, Amapá, Maranhão, Sergipe, Alagoas, Rio Grande do Norte, Paraíba, and Bahia), and no there was record in the Pantanal biome. However, the fact that no studies have been found may be due to limited search in databases.

Most studies were focused on soils of the Atlantic Forest and Amazon biomes, mainly primary and secondary natural forest soils (BUSCARDO et al., 2018; CARVALHO et al., 2016; KROEGER et al., 2018, 2021). The Atlantic Forest in its large extension, has presented studies in island soils with native vegetation (ANDRADE et al., 2021; MENDES; TSAI, 2018; PYLRO et al., 2014). Other studies evaluated peatland soils in the Serra do Mar (ETTO et al., 2014), and microbial identification was also performed in mangroves of the Atlantic Forest biome (LINHARES et al., 2021; MENDES et al., 2014; SILVA et al., 2014), as shown in Table 1.

It is important to point out that the search did not list works on natural grasslands of the Atlantic Forest biome, nor works that demonstrated the effect of the transition from natural grasslands to cultivated pastures. Few studies addressed natural grasslands, with only one study in the Pampa biome (LUPATINI et al., 2019), and others in the typical fields of the Caatinga biome (COSTA et al., 2022a; PEREIRA et al., 2021; OLIVEIRA et al., 2021).

The listed studies have shown different drivers of changes in microbial diversity and composition in Brazilian soils. Soil pH was pointed out as an important factor, as some authors

have shown that certain bacterial groups, such as Acidobacteria have a negative correlation with soil pH, or preference for acidic soils (NAVARRETE et al., 2015). Other works exposed the effect of pH correction, with the increase in Ca and the decrease in Al contents, as drivers of microbial changes (LAMMEL et al., 2018). Agricultural practices have a prominent effect on the structure, composition, and assembly of microbial communities GOSS-SOUZA et al., 2017; MENDES et al., 2015b). Several researches have shown the correlation of alteration or substitution of vegetation cover with the decrease of microbial species diversity (RAJAN et al., 2015).

## 2.5 CONCLUSIONS

In the last 10 years, many advances have been made in the knowledge of microbial diversity and composition in Brazilian soils. Several studies have pointed out the effects of agricultural practices and the importance of vegetation cover on the microbial functions and ecological relationships among soil microbes. Despite these advances, the composition, richness, and functionality of the microbiome of Brazilian soils are still poorly known. Improving this knowledge shall bring advances in biotechnology and policy-making to improve both agricultural productivity and sustainability in Brazilian agroecosystems.

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# 3 CHAPTER II: PASTURE MANAGEMENT INTENSIFICATION SHIFTS THE SOIL MICROBIOME COMPOSITION AND ECOSYSTEM FUNCTIONS

ABSTRACT

Natural grasslands are important reservoirs of animal and plant biodiversity and provide several ecosystem services, through the action of soil microorganisms. The increased demand for food, energy, and cattle activity has led to the conversion of natural grasslands to cultivated systems. However, the consequences of this conversion for soil microbial diversity and ecosystem functioning are yet to be discovered. Here, we used the 16S rRNA amplicon sequencing and a large set of soil and environmental variables to understand the possible effects of natural grasslands to cultivated pasture conversion on the soil microbial structure, composition, diversity, and functions. The study areas followed a gradient of increasing soil disturbance intensity, as follows: Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP). Natural grassland conversion to managed and cultivated pastures decreased the abundances of Acidobacteria and Verrucomicrobia, while increased the α-, γ-, and δ-Proteobacteria classes, and Gemmatimonadetes, Bacteroidetes, Patescibacteria, Latescibacteria phyla. The predicted functional profiles have also changed, as functions like ‘cellulolytic and symbionts/parasites’ decreased after natural to cultivated pastures conversion, while ‘nitrogen respiration’, ‘sulfur respiration’, and ‘aromatic compound degradation’ functions increased. Aboveground plant diversity decrease influenced belowground microbial diversity. The main drivers of diversity, composition, and functional potential are associated with soil attributes affected by liming, like aluminum complexation. In conclusion, was found taxonomic and functional differences between natural and managed grasslands (NG and IG) and cultivated pastures (PP and AP), with consequences for management strategies and biodiversity conservation priorities.

**Keywords:** Community ecology; Faprotax functions; Microbial ecosystem functions; Natural grassland conversion; Plant-microbiome relationship; 16S rRNA sequencing

# INTENSIFICAÇÃO DO MANEJO DE PASTAGEM ALTERA A COMPOSIÇÃO DO MICROBIOMA DO SOLO E AS FUNÇÕES ECOSSISTÊMICAS

## RESUMO

As pastagens naturais são importantes reservatórios de biodiversidade animal e vegetal e prestam diversos serviços ecossistêmicos, através da ação de microrganismos do solo. O aumento da demanda por alimentos, energia e atividade pecuária levou à conversão de pastagens naturais em sistemas cultivados. No entanto, as consequências dessa conversão para a diversidade microbiana do solo e o funcionamento do ecossistema, ainda não foram descobertas. Aqui, foi utilizado o sequenciamento da amplificação do gene 16S rRNA e um grande conjunto de variáveis de solo, ambiente e vegetação para entender os possíveis efeitos da conversão de pastagens naturais em cultivadas, na estrutura, composição, diversidade e funções das comunidades microbianas do solo. As áreas de estudo seguiram um gradiente de intensidade de perturbação do solo: pastagem natural (NG), pastagem natural melhorada (IG), pastagem cultivada perene (PP) e pastagem cultivada anualmente (AP). A conversão de pastagens natural para melhoradas e cultivadas diminuiu as abundâncias dos filos, Acidobacteria e Verrucomicrobia, mas por outro lado, aumenta a abundância das classes α- γ-, e δ-Proteobacteria, e dos filos Gemmatimonadetes, Bacteroidetes, Patescibacteria e Latescibacteria. Os perfis funcionais previstos também mudaram, funções como ‘celulolíticos’ e ‘simbiontes/parasitas’ diminuíram após a conversão natural de pastagens, enquanto as funções de ‘respiração de nitrogênio’, ‘respiração de enxofre’ e ‘degradação de compostos aromáticos’ aumentaram nas pastagens cultivadas. A redução da diversidade de plantas acima do solo influenciou a diversidade microbiana abaixo do solo. Os principais fatores que afetaram a diversidade, composição e potencial funcional estão relacionados aos atributos do solo, modificados pela calagem, como a disponibilidade de alumínio em solos naturais. Em conclusão, foi encontrado diferenças taxonômicas e funcionais entre pastagens naturais (NG e IG) e pastagens cultivadas (PP e AP), esses resultados podem auxiliar na tomada de decisão sobre estratégias de manejo do solo e da vegetação e priorização em conservar a biodiversidade de pastagens naturais.

**Palavras-chave:** Conversão de pastagens naturais; Ecologia de comunidades; Funções do ecossistêmicas; Funções faprotax; Relação planta-microbioma; 16S rRNA sequenciamento.

## 3.1 INTRODUCTION

The Brazilian Atlantic Forest biome harbors one of the greatest global levels of biodiversity and endemism of both animals and plants (MYERS, et al., 2000) and has been identified as one of the 36 global hotspots of biodiversity (SLOAN et al., 2014). Despite being known for its "forest" component, a vast territory of this biome is composed of natural grasslands (ANDRADE et al., 2016; METZGER et al., 2019), located at the Highland Plateau of Santa Catarina and Rio Grande do Sul States, Southern Brazil (GIORGIA et al., 2014; OVERBECK et al., 2007). Those natural grasslands are important providers of ecosystem services (NABINGER et al., 2011) and reservoirs of biodiversity (ANDRADE et al., 2016; HAMAMOTO et al., 2018; MODERNEL et al., 2016; OVERBECK et al., 2007).

In the last century, the increased demand for wood, food, and fiber, among other primary products, has led to the vast conversion of natural grasslands to cultivated pastures (SÜHS et al., 2020). The transition from natural grasslands to cultivated pasture systems often leads to decreased diversity of forage species and consequently affects soil microbial diversity (DELORY et al., 2019; YIN et al., 2019). Natural and improved-natural grasslands are often overgrazed or excluded from grazing, both affecting the vegetation maintenance and soil attributes (GIUSTINA JUNIOR et al., 2019; RAUBER et al., 2021; SOARES et al., 2005). Together with management misconceptions, some studies have pointed out that grassland productivity and diversity might depend on climate (DU et al., 2022; GRACE et al., 2016).

The high thermal amplitude between the summer and winter season at the Highland Plateau mesoregion often leads to a huge decrease in pasture biomass production in the hibernal season, thus affecting cattle growth and meat production (PONTES et al., 2017; SBRISSIA et al., 2020). However, some studies have shown that the correct management of forage plants (ZANELLA et al., 2021), as well as the adoption of animal management strategies (RUGGIA et al., 2021), could ensure both the economic yield and the preservation of endemic fauna and flora in grassland areas, targeted as a global conservation goal (FAO, 2020).

Several studies have found a link between forage diversity and soil microbial activity, plant straw decomposition, and consequently, the storage of carbon in pasture soils (LANGE et al., 2015; OELMANN et al., 2021). The aboveground plant diversity can influence the belowground microbial diversity (GUO et al., 2021), by affecting the structure, diversity, and dispersal of soil microbial communities (GOSS-SOUZA et al., 2017; LEITE et al., 2021). However, the grassland systems amplify the magnitude of the interaction between animals, plants, and microorganisms within the heterogeneous soil matrix (NEAL et al., 2020), resulting

in a more complex ecological network when compared to forests (MENDES et al., 2015; PEDRINHO et al., 2020) and agricultural no-till cropping systems (GOSS-SOUZA et al., 2017).

The conversion of natural grasslands into agricultural systems often changes the relative abundance of certain microbial groups (LUPATINI et al., 2013). The decrease in the natural diversity of plant species is pointed out as a driver of changes in soil microbial activity (LANGE et al., 2015), with consequences to the multifunctionality of the soil microbiome (GUO et al., 2021; ZHANG et al., 2021). However, little is known about the effects of converting natural grasslands to cultivated pastures on the soil microbial community and potential ecosystem functions. We hypothesized that (i) the soil management intensification affects soil microbial composition, diversity, and functional potential; and (ii) the aboveground forage diversity is positively correlated with the belowground microbial diversity. By combining high throughput 16S rRNA gene sequencing with soil and vegetation analyses, was aimed (i) to determine the effect of soil and vegetation management on the taxonomic and functional potential profiles of microbial communities, and (ii) to evaluate the drivers of microbial community patterns in a gradient of soil and pasture management intensification.

## 3.2 MATERIAL AND METHODS

### 3.2.1 Site description and soil sampling

The chosen study areas belong to the Company of Agricultural Research and Rural Extension of Santa Catarina (Epagri/EEL), located in Lages, Santa Catarina State, Brazil (27°47'55" S and 50°19'25" W, altitude 922 m, annual rainfall, 1,668 mm) (APPENDIX A1). The Climate is humid mesothermal (Cfb) with harsh winters, mild summers, and rainfall well-distributed throughout the year, according to the Koppen-Geiger classification. The mean annual precipitation of the mesoregion in the last 85 years is 1543 mm (ALVARES et al., 2013), with 1117 mm in 2020 (EPAGRI/CIRAM, 2020) while the historic mean annual temperature is 14°C (APPENDICES A2 and A3).

The soil samples were collected in four grassland systems, representing a gradient of increasing soil management intensification, as follow: 1) Natural grassland (NG), with a predominance of *Andropogon lateralis* NESS; 2) Improved-natural grassland (IG), under the no-till system, where the native grassland was amended with overseeding of *Trifolium repens* L., *Festuca arundinacea* Schreb., *Lolium multiflorum* Lam., *Holcus lanatus* L., and managed

with nitrogen fertilization twice a year, NPK 9-32-12; 200 kg $ha^{-1}$, applied in the summer, and NPK 9-32-12; 300 kg $ha^{-1}$ in the winter, with the amendment of 400 kg $ha^{-1}$ of urea at grass tillering stage; 3) Perennial-cultivated pasture (PP), under the no-till system, with a intercropping of *Cynodon dactylus* and *Trifolium repens*, and overseeding of *L. multiflorum* in the winter. The nitrogen fertilization twice a year, NPK 9-32-12; 200 kg $ha^{-1}$, applied in the summer and NPK 9-32-12; 300 kg $ha^{-1}$ in the winter, and; 4) Annual-cultivated pasture (AP), in conventional land-use system, with harrowing before summer and winter sowing, where *Pennisetum glaucum* (L.) on the summer and *L. multiflorum* on the winter, were cultivated in succession, with nitrogen fertilization twice a year, NPK 9-32-12; 200 kg $ha^{-1}$, applied in the summer, and NPK 9-32-12; 300 kg $ha^{-1}$ in the winter (APPENDIX A4).

The IG, PP, and AP were implemented in 2015, when the soil pH was corrected, through liming, aiming to reach a bases saturation of 70% when forming the grazing system, using dolomitic limestone. One plowing, consisting in turnover of the topsoil layer and two harrowing practices were performed. The fertilization procedure was performed on the same day, for the three managed systems (IG, PP, and AP), in each season. Soil types were classified as Haplic Cambisols (IG, PP, and AP) and Humic Cambisol at the NG, using the World Reference Base for Soil Resources (ANJOS et al., 2015).

Each grassland system was composed of four replicates, as represented by grazing paddocks (25 × 35 m). Females of Rouge Flamand cattle breed, with an average weight of 600 ± 100 kg, were managed by the intermittent stocking method (ALLEN et al., 2011), entering each paddock when the vegetation had reached 20 cm height and leaving the paddock when vegetation had been grazed to 9 cm height. Plants height was monitored two times for month, with a sward stick (BARTHRAM, 1985), in a systematic walking path, with four lines of evaluation, totalizing 40 reading points per paddock. In NG, *A. laterallis* was the reference species for height measurement. Measurements were also performed in the lower stratum (between tussocks of *A. laterallis*).

To evaluate microbial diversity and functional categories (response variables), soil samples were collected in January and July 2020, comprising the summer and winter seasons of the southern hemisphere, respectively. The sampling campaigns were carried out 30 days after nitrogen fertilization (APPENDIX A4). Non-deformed soil samples from the 0-10 cm profile were collected with sterile PVC tubes (5 cm diameter × 10cm depth) in a geogrid scheme, equidistantly by 10 m from each other, with 5 m of the border in each paddock. The nine subsamples were homogenized to form a single composite sample per replicate (paddock). A total of 32 individual soil samples were collected (1 composite sample per paddock × 4

paddocks × 4 grassland systems × 2 sampling seasons). The samples were kept in a cooler box (~ 4°C) during sampling and transportation. Then, they were stored in an ultra-freezer at -80°C, until further processing for microbial analysis.

### 3.2.2. Soil and vegetation analyses

A total of 27 soil attributes were measured or analyzed. Soil chemical and physical parameters were determined for each of the 32 soil samples, based on 500 g of soil. For soil chemical characterization, the soil pH was measured in a pHmeter, in a 1:2.5 soil/water suspension. Soil exchangeable aluminum, calcium, and magnesium were extracted with 1 M KCl. Exchangeable $Ca^{2+}$ and $Mg^{2+}$ were determined by atomic absorption spectrometry, and $Al^{3+}$ by acid-base titration. Soil phosphorus and potassium were extracted by Mehlich-1, being exchangeable P determined by visible spectrophotometry and $K^{+}$ determined by flame atomic emission spectrometry. Potential acidity (H+Al) was estimated by an equation based on the pH determined Shoemaker-McLean-Pratt (SMP) buffer solution. We also calculated parameters such as exchangeable bases (EB), which is the sum of Ca, Mg, and K; cation exchange capacity (CEC), which is the sum of Ca, Mg, K, Al, and H; base saturation (V%), which is the percentage EB and CEC, and Al saturation (m%), which is the relation between exchangeable Al and CEC. Total nitrogen (TN) and total organic carbon (TOC) were extracted and determined by dry combustion catalytic oxidation at an elementary auto analyzer CNHS Vario EL Cube (Elementar, Langenselbold, Germany). Soil $N\text{-}NH_4^{+}$, $N\text{-}NO_3^{-}$ and $N\text{-}NO_2^{-}$ were extracted with 1 M KCl and titration with $H_2SO_4$. These parameters were analyzed at the Soil Analysis Laboratory, Santa Catarina State University, Lages, Brazil, following routine methodology (TEDESCO et al., 1995).

The physical soil attributes measured were soil bulk density, total porosity, macroporosity, microporosity, and biopores, through the ratio between the volume of water retained in the saturated soil, from samples collected at two points per paddock, using a volumetric ring (EMBRAPA, 2017). Soil granulometry (texture) was determined by pipette methodology, using NaOH as a dispersant, separating the sand fraction by sieve-washing, clay in suspension, and silt by difference (GEE; BAUDER, 1986). Microbial biomass carbon (MBC) was determined through the fumigation-extraction method (VANCE et al., 1987), was calculated by the difference between the carbon extracted from fumigated and non-fumigated soil samples. Microbial activity through soil microbial respiration (SMR) was evaluated by the determination of soil basal respiration ($CO_2$-C) in soil samples, incubated in the laboratory at

28°C for 10 days (ALEF; NANNIPIERI, 1995). Physical soil attributes and microbial activity analyses were performed at the Soil Ecology and Ecotoxicology Laboratory, Santa Catarina State University, Lages, Brazil.

The Plant diversity was also estimated, by the BOTANAL method (TOTHILL et al. 1992), which establishes a relationship of species composition and the participation of these species in the forage mass. For each sample, a "rank" was assigned, according to the participation of the most frequent species in the forage mass. Subsequently, the other species present inside the frames were identified, and a symbolic percentage (1%) was attributed to them, as they did not significantly contribute to the forage mass. The description of plant species found in each grassland system was exposed in APPENDIX A5.

Forage mass was obtained through the visual estimation, which was corrected by forage mass cuts (kg dry mass $ha^{-1}$, obtained in each cut). The cuts were performed close to the ground with shears and a shearing machine, allocating 0.25 $m^2$ (0.5 x 0.5 m) frames. Each collected forage sample was dried in an oven at 60°C for 72 hours and the weight was extrapolated to hectares (kg $ha^{-1}$), representing the production of dry mass (DM) on the sampling dates. The dry biomass of the plants was used for the determination of total nitrogen (TN) and total organic carbon (TOC), which were extracted and determined by dry combustion catalytic oxidation at an elementary auto analyzer CNHS Vario EL Cube (Elementar, Langenselbold, Germany), and the results used to calculate the C/N ratio.

### 3.2.3 Soil DNA extraction and 16S rRNA amplicon sequencing

The total DNA extraction from soil samples (250 mg) was performed for each of the 32 samples, using DNeasy PowerLyzer PowerSoil TM DNA Isolation Kit (Qiagen, Hilden, Germany), following the instructions of the manufacturer. DNA quality and concentration were evaluated using NanoDropTM 1000 spectrophotometer (Thermo Scientific, Wilmington, United States) and checked in gel electrophoresis with Tris-buffered saline with sodium boric acid, and 1.5% agarose (BRODY; KERN, 2004). The taxonomic characterization of microbial communities was performed through large-scale amplicon sequencing, on the Illumina MiSeq platform (Illumina, San Diego, United States). For this, the V3-V4 region of the 16S rRNA gene was amplified using the set of primers (F: 5' CCT ACG GGN GGC WGC AG 3' and R: 5' GAC TAC HVG GGT ATC TAA TCC 3') (KLINDWORTH et al., 2013). The PCR reaction was performed using 2 µL of DNA, 12.5 µL of 2x PCR Ultra Mix (PCR Biosystems, London, United Kingdom), 0.5 µL of each primer (10 mM), and ultrapure water to measure the final

volume of 25 µL. Amplification was carried out following the program: 3 minutes at 95ºC, 25 cycles of 30 seconds at 95ºC, 30 seconds at 55ºC, 30 seconds at 72ºC, and a final extension of 5 minutes at 72ºC. Amplicons were visualized on an agarose gel, resulting in 444 bp fragments. The amplicons were further purified using magnetic beads, AMPure XP beads (Beckman Coulter, Brea, United States). Purified amplicons were subjected to PCR reaction for ligation of Illumina adapters (Nextera XT Index Kit; Illumina, San Diego, United States). The PCR reaction for ligation of adapters was performed using 2.5 µL of purified PCR product, 12.5 µL of 2x PCR Ultra Mix (PCR Biosystems, London, United Kingdom), 2.5 µL of each adapter (Nextera XT Index 1 Primers, N7XX barcode, and; Nextera XT Index 2 Primers, S5XX), completing the volume with ultrapure water to 25 µL. PCR conditions were: 95°C for 3 minutes, 8 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with and final extension of 72°C for 5 minutes. All libraries were quantified and normalized to obtain an equimolar pool. The pool was quantified by qPCR, using the KAPA Biosystems kit (Roche, Basel, Switzerland) Then, the quantified pool was diluted to 2 mM and denatured with 0.1N NaOH and diluted to 20 M. Later, it was diluted to 7 pM, combined with 20% of denatured PhiX at 7 pM, for loading into the MiSeq Desktop Sequencer (Illumina). The sequencing consisted of paired end reads (2 × 250 bp), 500 cycles/39 hours run.

### 3.2.4 Bioinformatics analysis

The 16S rRNA gene paired-end reads were first merged using PEAR (ZHANG et al. 2014). Then, the merged sequences were analyzed using Quantitative Insights into Microbial Ecology (QIIME 2), version 2019.10. The sequences were demultiplexed and the quality control was carried out using DADA2 (CALLAHAN et al., 2016) with the consensus method to remove any remaining chimeric and low-quality sequences. The samples were then rarefied to 31,900 sequences, following the number of the lowest sample, and singletons and doubletons were removed. The taxonomic affiliation was performed at 97% similarity using the Silva Database, version 132 (QUAST et al., 2013), and the generated operational taxonomy units (OTUs) matrix was further used for statistical analyses. The bacterial functions were predicted using the Faprotax database, version 1.2 (LOUCA et al., 2016), which enabled us to map prokaryotic clades to metabolic and ecologically relevant functions (C, N, and S cycles). The relative abundances of the identified genes that belonged to functional groups were calculated as the cumulative abundance of OTUs assigned to each functional group. All sequencing data

in this study were submitted to the Metagenomics Rapid Annotation Server (MG-RAST), version 4.0.3. The amplicon data are available under project ID ‘PastureSC’ (APPENDIX A6).

### 3. 2.5 Statistical analysis

The statistical analyses were performed using the previously described experimental design: 4 grassland systems × 2 seasons × 4 replicates. Alpha diversity of OTUs, phylum level, functional microbial profiles, and forage plant diversity was calculated from the taxonomic relative abundance matrix, and estimated by Chao-1 and Shannon’s index, with the PAST software, version 4.0.3 (HAMMER et al., 2001). After checking the assumptions of homogeneity and normality, the means of alpha diversity indices and soil physical-chemical characteristics were compared through ANOVA with Tukey’s Honest Significant Difference test (Tukey’s HSD), with the function ‘tukeyHSD’, on R software, version 4.0.5 (R CORE TEAM, 2020). The ANOVA analysis model used nested repetitions in each grassland system, as the systems were in neighbor areas, at the same toposequence.

To test the possible effects of season and grassland management intensification on the taxonomic structure and functional clustering of microbial communities, we used permutational multivariate analysis of variance (PERMANOVA) (ANDERSON, 2001), from the resulting Bray-Curtis distance matrix through the ‘adonis’ function in ‘vegan’ R package, version 2.5-6 (OKSANEN et al., 2019). Aiming to identify the main environmental drivers of microbial taxonomic (OTU and phylum level) and functional potential profiles, we performed a distance-based redundancy analysis (db-RDA) of Bray–Curtis's dissimilarity matrices, with stepwise ‘forward selection’. Only significant ($P < 0.05$) and non-collinear environmental factors were considered in the model (BLANCHET; LEGENDRE; BORCARD, 2008; RAMETTE; TIEDJE, 2007). Then, we performed a variation partitioning of redundancy analysis (pRDA), generated by principal coordinates analysis of neighbor matrices (PCNM), with stepwise ‘forward selection’, to evaluate the possible single and joint effects of environmental variables, distance, and sampling season on the variation of taxonomic and functional potential profiles of microbial communities. Both analyses were performed using CANOCO software, version 5.2 (PETR SMILAUER; JAN LEPS, 2014).

Further, was explored the relationship of the selected environmental variables by db-RDA, with taxonomic diversity indices, through pairwise Spearman’s correlation analysis between alpha diversity Chao-1, and Shannon’s index with environmental and geographic variables. The analysis was performed and plotted with the ‘vegan’ R package. To correlate the

variability in pairwise OTU microbial community Bray–Curtis's similarities with geographical distance and environmental factors that could modulate diversity, we performed the ‘Mantel’ and ‘partial Mantel’ correlation tests (LEGENDRE; FORTIN, 1989), with 1000 permutations, using the functions ‘mantel’ and ‘partial.mantel’ (LEGENDRE; FORTIN, 1989), in R software, with ‘vegan’ R package. To understand the changes in microbial community composition between grassland systems, a Kruskal Wallis median test was performed for each relative abundance at the phylum level, with the function ‘kruskal.test’, with the ‘rstatix’ R package (KASSAMBARA, 2021). To explore the relationship between phylum composition and grassland management together with specific soil and vegetation characteristics, we performed and selected Spearman’s highly significant ($\rho > |0.6|$) correlations with Bonferroni correction and visualized the results with heatmap graphs.

Was performed co-occurrence network analyses to assess the complexity of interactions among microbial taxa in each grassland system. For this, non-random co-occurrence analysis was carried out using the Python module ‘SparCC’ (FRIEDMAN; ALM, 2012). For this, a table of frequency of OTUs was used. To decrease the number of correlations, was included only the OTUs with > 50 sequences, which represented an average of 80% of the total sequences. For each network, the SparCC correlations were calculated and only strong (SparCC > 0.8 or < -0.8) and highly significant ($P < 0.01$) were selected. The nodes in the reconstructed network represented the OTUs, whereas the edges represented significantly positive or negative correlations between nodes. The network graphs were based on a set of topological complementary measurements, including the number of nodes, number of edges, modularity, number of communities, average node connectivity, average path length, diameter, and cumulative degree distribution. The network visualization and topological measurements were performed with the Gephi interactive platform, version 0.8 (BASTIAN; HEYMANN; JACOMY, 2009).

To evaluate the functional potential, was used the Faprotax matrix, to estimate the alpha diversity indices Shannon, Chao-1. The differences among grassland systems were tested by Tukey’s HSD test. To verify the main drivers of the environment that influenced the functions db-RDA, and p-RDA were performed. The differences in the relative abundance of each function among grassland systems were evaluated by the Kruskal-Wallis's test. Finally, to verify the correlation between environmental variables and functional groups, Spearman correlations were performed and plotted as heatmaps. The parameters for statistical analyses of Faprotax functional groups followed the same setup as used for taxonomic profiles.

## 3.3. RESULTS

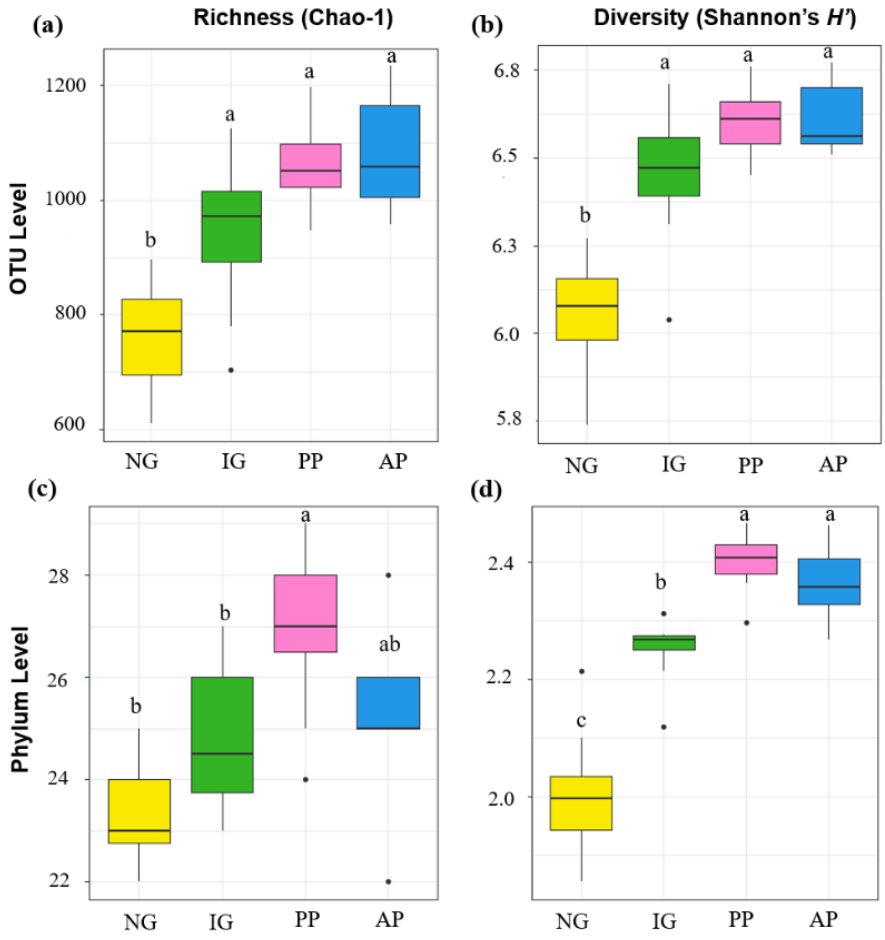
### 3.3.1. Soil and vegetation attributes

The Tukey's HSD test ($P < 0.05$) was performed for 26 soil physical-chemical parameters measured for the four grassland systems samples, collected in summer and winter seasons (APPENDIX A7). The potential acidity (H+Al), the $Al^{3+}$ concentration, and the biopores decreased after conversion of natural grassland (NG) to improved-natural grassland (IG) and perennial-cultivated pasture (PP), and annual-cultivated pasture (AP) while pH, N-$NH_4^+$ and $NO_3^-$, $Ca^{2+}$, $Mg^{2+}$, Ca:Mg ratio, bases saturation (BS), and cation exchange capacity (CEC) increased ($P < 0.001$). The soil density (SD) was higher in the PP ($P < 0.001$). No differences were observed for soil organic matter (SOM), total nitrogen (TN), microbial biomass carbon (MBC), soil microbial respiration (SMR), total porosity, microporosity (MiP), macroporosity (MaP), and soil texture. Forage systems presented different qualitative and quantitative characteristics (APPENDICES A8 e A9). The highest plant richness (Chao-1) and plant diversity (Shannon's *H'*) were observed in IG, followed by NG with the lowest values found in the cultivated pastures (PP and AP) (APPENDIX A8). The highest plant C:N ratio was found in NG, and the highest plant dry biomass production was found in NG and PP (APPENDIX A9).

### 3.3.2. Diversity and microbial community structure across grassland systems

From a total of 10,274 OTUs, was found 34 phyla (32 bacterial and 2 archaeal), 131 Classes, 349 orders, 618 families, and 1048 genera. Was found differences in taxonomic alpha diversities at the OTU and the phylum level (Tukey's HSD, $P < 0.05$) (Figure 5). At the OTU level, Chao-1 richness (Figure 5a) and Shannon's alpha diversity (Figure 5b) increased from the NG (Chao-1 = 761; *H'* = 6.05) to the IG (Chao-1 = 939; *H'* = 6.44) and cultivated pastures PP (Chao-1 = 1060; *H'* = 6.60), and AP (Chao-1 = 1084; *H'* = 6.62). Related results were found at the phylum level, Richness and Shannon diversity was lower in NG (Chao-1 = 23.25, *H'* = 2.00), and IG (Chao-1 = 24.75, *H'* = 2.25), with the highest values observed in PP (Chao-1 = 26.87, *H'* = 2.40), not differing from AP (Chao-1 = 25.28, *H'* = 2.36) (Figures 5c and d).

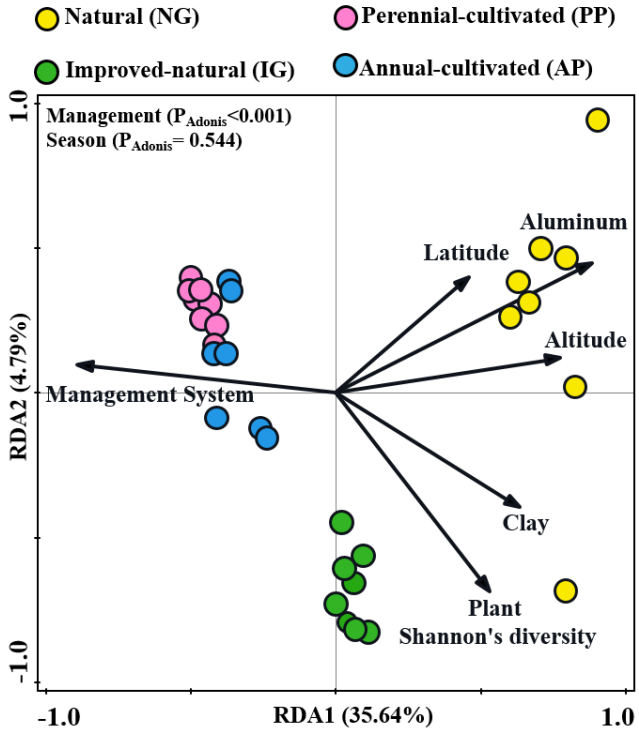
Figure 5- Soil microbial richness and alpha-diversity from Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated (PP) and Annual-cultivated pasture (AP). Comparisons based on the operational taxonomic units (OTU) level (a and b), and phylum level (c and d). Lower-case letters refer to significant differences among pasture systems, based on Tukey's HSD test ($P < 0.05$).




The PERMANOVA analysis showed differences in taxonomic clustering of microbial communities according to grassland systems at the OTU and the phylum level ($P_{Adonis} < 0.001$), but not within sampling seasons (OTU, $P_{Adonis} = 0.544$; Phylum, $P_{Adonis} = 0.137$), the reason we explored the further results only according to the management system, regardless season (Figure 6 and APPENDIX A11). Using distance-based RDA (db-RDA), the forward-selected variables explained together 37.4% of the total adjusted variation in the taxonomic Bray-Curtis beta diversity, at the OTU level (APPENDIX A10), with 40.47% of this variation explained in the first two axes of db-RDA. The variation partitioning of the redundancy analysis (pRDA) with 3 groups (Figure 6 and APPENDIX A12), showed that 0.2% of the variation in OTUs beta diversities was explained by biotic environmental factors, and 8.3% by abiotic environment variables, with also a low contribution of sampling location and season (1.6%), and the highest

contribution (27.9%) resulting from the overlap between the three sets of variables. The environmental variables that contributed most to the explanation of microbial structure community were management system, latitude, altitude, aluminum, clay, and plant Shannon's diversity.

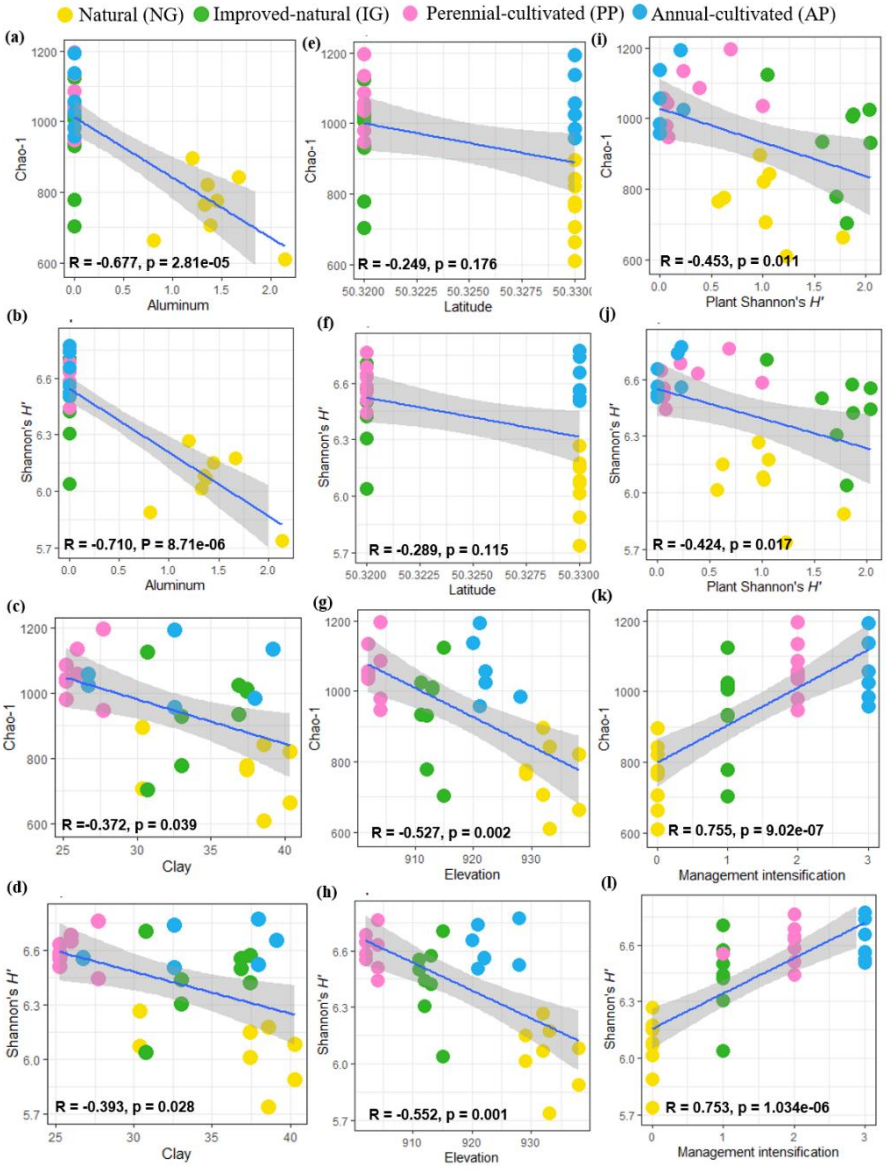
Figure 6- Partitioning redundancy analysis (pRDA) of soil microbial communities at the OTU level, in Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP). Vectors represent environmental variables, which were forward-selected with 1000 Monte-Carlo permutations and Bonferroni correction ($P_{adjusted}$ < 0.05).




The environmental variables that explained the taxonomic variation were selected, through pRDA, and we further explored the effect of these individual variables using Spearman's correlation. Was verified that the exchangeable aluminum concentration is negatively correlated with OTU richness (Chao-1) and alpha diversity (Shannon's), showing a correlation coefficient $\rho$ = -0.677 and $\rho$ = -0.710, respectively (Figs. 7a and 7b). In contrast, clay content showed a negative correlation with Chao-1 and Shannon ($\rho$ = -0.372 and $\rho$ = -0.393, respectively) (Figures 7c and 7d). The geographic variable, Latitude was not significantly correlated with Chao-1 and Shannon (Figures 7e and 7f). However, for the elevation, a negative correlation was verified with Chao-1 and Shannon ($\rho$ = -0.527 and $\rho$ = -0.552, respectively) (Figures 7g and 7h). Plant diversity (Shannon) was negatively correlated

with taxonomic richness and diversity ($\rho$ = -0.453 and $\rho$ = -0.424, respectively) (Figures 7 I and J). Finally, the management intensity is positively correlated with increased taxonomic richness and diversity ($\rho$ = 0.753 and $\rho$ = 0.755, respectively) (Figures 7k and 7l).

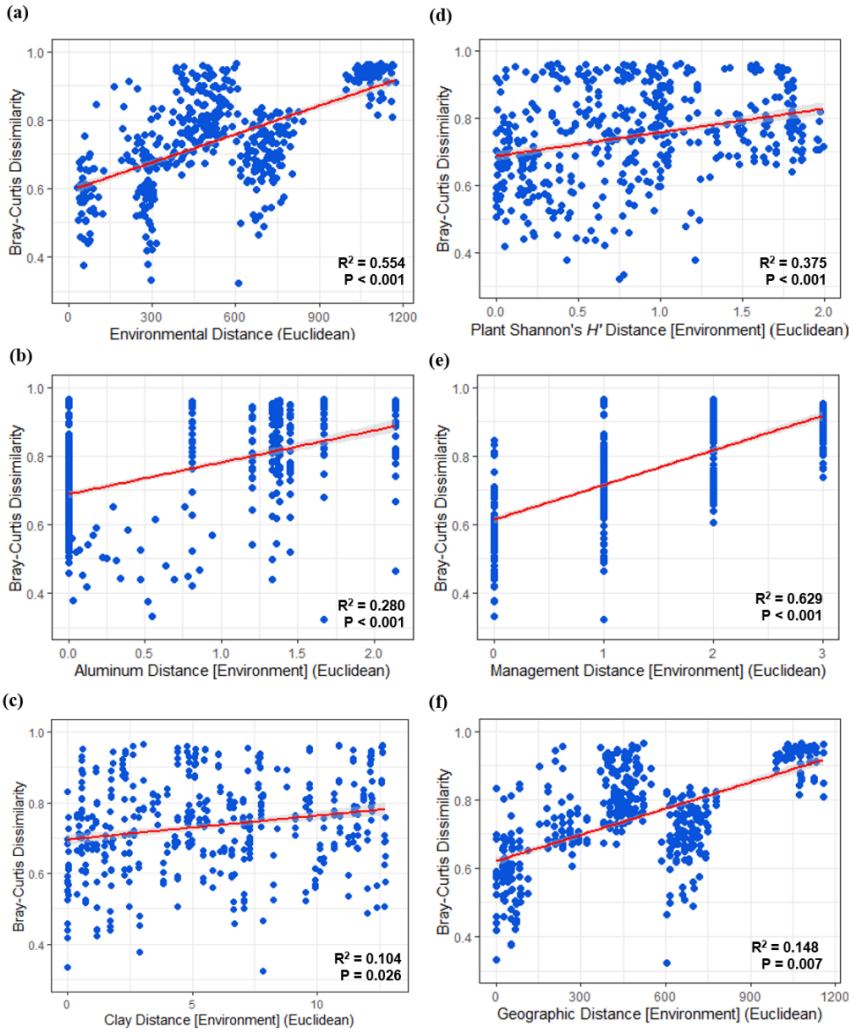
Figure 7- Spearman's rank correlation coefficients (R) and statistical significance between soil microbial OTU Chao-1 and Shannon's diversity with soil aluminum (a-b), soil clay (c-d), latitude (e-f) elevation (g-h), Plant Shannon's (i-j), and management intensification (k-l). Samples are colored as follows: Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP).




The mantel and partial mantel were performed using the variables selected by db-RDA, to verify the effect of the variables on the microbial communities at the OTU level. The Mantel showed that the variation in the similarities of microbial communities was correlated with a matrix of distance from the environmental variables (Mantel, $\rho$ = 0.554; P < .001) (Figure 8a).

Specific correlations were observed for aluminum (Partial Mantel, $\rho = 0.280$; $P < 0.001$) (Figure 8b), clay (Partial Mantel, $\rho = 0.104$; $P = 0.026$) (Figure 8c), and Shannon plant diversity (Partial Mantel, $\rho = 0.375$; $P < 0.001$) (Figure 8d). The significant effect of the intensification of management on the distance from the microbial community was also observed (Partial Mantel, $\rho = 0.629$; $P < 0.001$) (Figure 8e). The importance of geographic distance in the distribution of microbial OTU was emphasized by the partial mantel (Partial Mantel, $\rho = 0.148$; $P = 0.007$) (Figure 8f).

Figure 8 - Mantel correlograms of beta diversities among pairs of soil microbial communities, within neighborhoods from 0.0 to 1200 m, across grassland systems. (a) Mantel product-moment of Spearman's correlation between microbial pairwise Bray-Curtis's dissimilarities and all forward-selected environmental variables; (b) Partial Mantel for soil aluminum; (c) Partial Mantel for plant Shannon's H'; (d) Partial Mantel soil clay; (e) Partial Mantel for management intensification, and (f) Partial Mantel for geographic distance. The x-axis represents the distance between pairwise OTU level microbial communities and the y-axis represents the Bray-Curtis dissimilarity for each pair of microbial communities (blue circles). The red lines represent the fitted GLM model, with 1000 permutations.



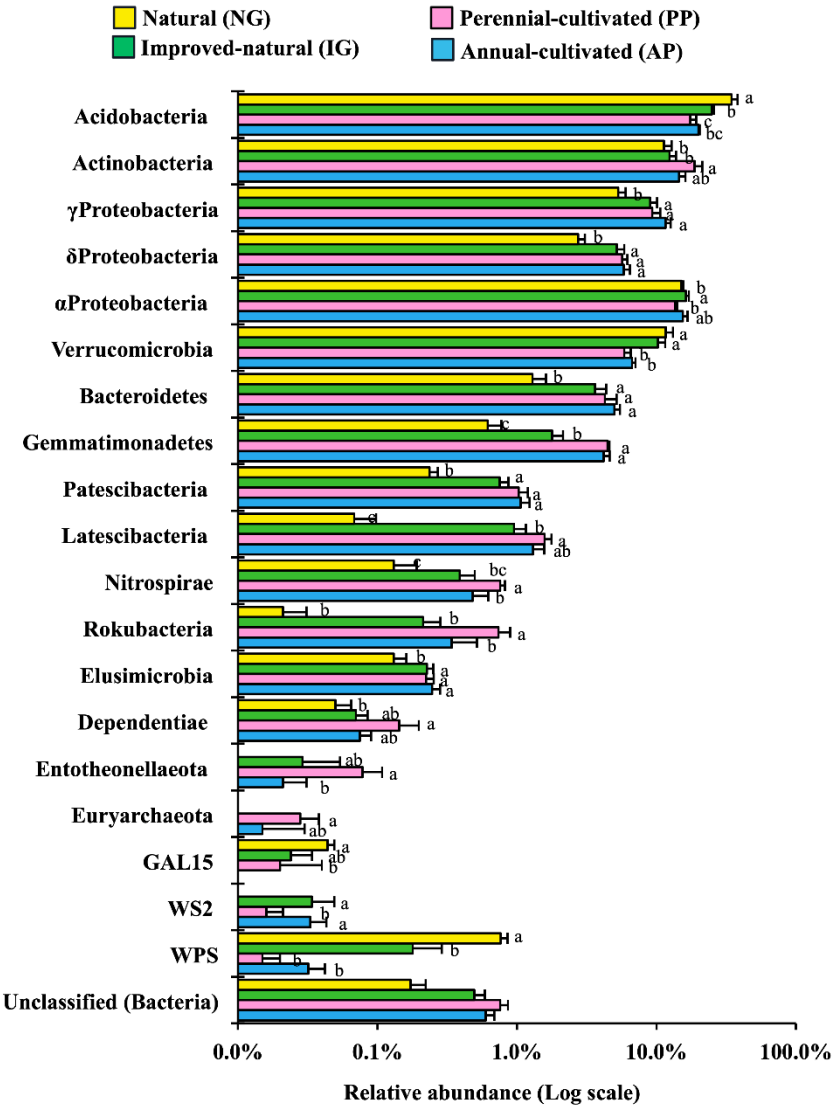
### 3.3.3. Microbial communities' composition

The sequences were affiliated to 34 phyla (32 bacterial and 2 archaeal). Overall, the most abundant phylum was Proteobacteria, with a mean of 28.6% of the assigned amplicon sequences. From this phylum, the most abundant class was α-Proteobacteria (15.1%), followed by γ-Proteobacteria (8.8%), and δ-Proteobacteria (4.9%). Following, Acidobacteria (24.2%), Actinobacteria (14.3%), Verrucomicrobia (8.6%). Sequences matching the phyla Chloroflexi, Planctomycetes, and Bacteroidetes ranged from 3.5 to 7.7%.

When comparing the relative abundance of microbial groups among grassland systems, the conversion of NG to IG and the cultivated pastures (PP and AP) led to significant changes in relative abundances for 16 bacterial phyla and one archaeal phylum. The changes in microbial phyla and classes of Proteobacteria across grassland systems were compared through the Kruskal-Wallis's test ($P < 0.05$) (Figure 9 and APPENDIX A13).

The relative abundance of the Acidobacteria phylum average decreased by 39%, from NG to IG, PP, and d AP. Similarly, Verrucomicrobia decreased from NG and IG to 57% for PP and AP. The GAL15 and WPS phyla also decreased by 50 and 90.19% their abundances with the conversion of natural to improved grassland and cultivated pastures. In addition, the presence of GAL15 in the AP was not observed. The relative abundance of α-Proteobacteria was 9.6% higher in IG compared to m NG, PP, and AP. The classes γ-Proteobacteria and δ-Proteobacteria were about 48% lower abundant in NG than in other systems. Actinobacteria was 32% more abundant in PP compared to other systems. The phylum Gemmatimonadetes was 58% more abundant in PP and AP than IG and 85.7% higher than NG. Similarly, Bacteroidetes increased 69% in IG, AP, and PP when compared to n NG. The Patescibacteria and Elusimicrobia were more abundant in the cultivated pastures (AP and PP) and Improved grassland (IG) compared to natural grassland (NG). Other low-abundant phyla (with relative abundances lower 1%), Latescibacteria, Nitrospirae, Entotheonellaeota, Dependentiae, Rokubacteria, and the archaeal phylum Euryarchaeota were more abundant in PP (Figure 9).

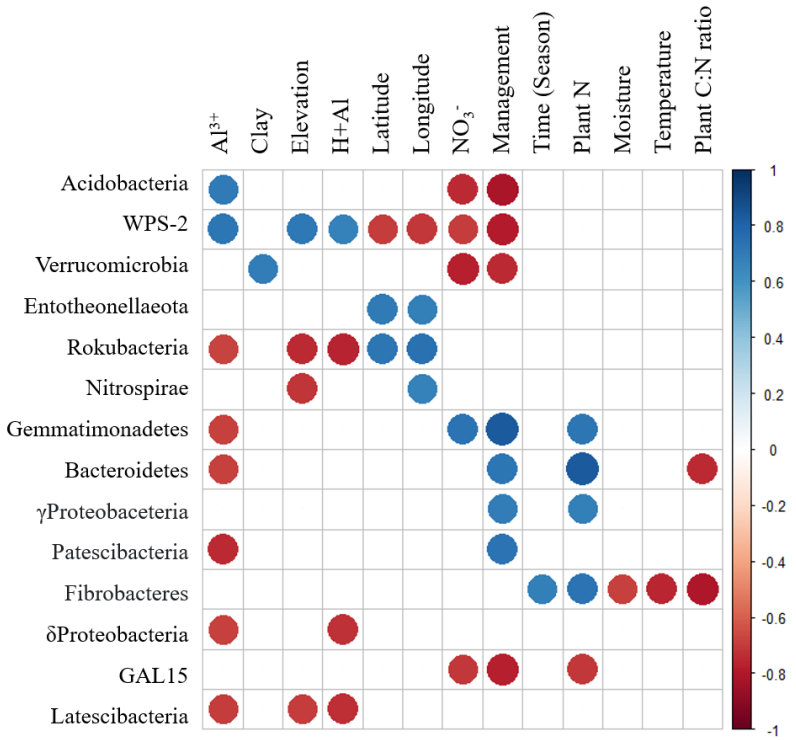
Figure 9- Relative abundance of soil bacterial and archaeal phyla and Proteobacteria classes in soils from Natural grassland (NG) - yellow bars, Improved-natural grassland (IG) - green bars, Perennial-cultivated (PP) - pink bars, and Annual-cultivated (AP) - light blue bars. Only significantly altered taxa are shown (Kruskal-Wallis H test; $P < 0.05$). Classification at the taxonomic phylum level (Silva Database). Error bars show the standard deviation and different lower-case letters refer to significant differences for the relative abundance across sites.




Was further depicted the correlations between phylum-level abundances and the environmental variables, through Spearman's correlations with Bonferroni correction ($P_{corrected} < 0.05$) (Figure 10). The $Al^{3+}$ concentration was positively correlated with the abundances of Acidobacteria and WPS-2 and negatively correlated with Rokubacteria, Gemmatimonadetes, Bacteroidetes, Patescibacteria, δ-Proteobacteria, and Latescibacteria abundances. The clay was only positively correlated with Verrucomicrobia. The potential acidity (H+Al) was positively correlated with WPS-2 abundance and negatively correlated with Rokubacteria, δ-

Proteobacteria, and Latescibacteria abundances. The elevation gradient was positively correlated with the abundance of WPS-2 and negatively correlated to the abundances of Rokubacteria, Nitrospirae, δ-Proteobacteria, and Latescibacteria. On the other hand, longitude and latitude showed negative correlations with WPS-2 and positive correlations with Entotheonellaeota and Rokubacteria, with longitude also positively correlated with Nitrospirae (Figure 10).

Figure 10- Heatmap of the main soil microbial phyla and Proteobacteria classes correlated with environmental variables, selected by Spearman's correlations and Bonferroni correction ($P < 0.05$) in soils from Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP).



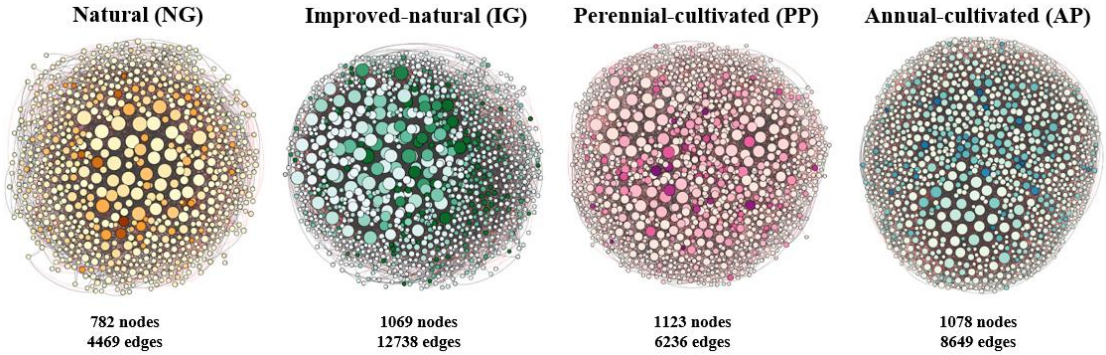

The concentration of $NO_3^-$ was negatively correlated with the abundances of Acidobacteria, WPS-2, Verrucomicrobia, and GAL15, while positively correlated with Gemmatimonadetes abundance. Soil moisture was negatively correlated with Fibrobacteres abundance, while clay proportion was positively correlated with the abundance of Verrucomicrobia. The Plant C:N ratio was negatively correlated with the abundance of Bacteroidetes and Fibrobacteres. Yet the plant N concentration was positively correlated with the abundances of Gemmatimonadetes, Bacteroidetes, γ-Proteobacteria, and Fibrobacteres.

Season and temperature were respectively positively and negatively correlated with the abundance of Fibrobacteres. Finally, the grassland system management was negatively correlated with the abundances of Acidobacteria, WPS-2, Verrucomicrobia, and GAL15. Meanwhile, it was positively correlated with the abundances of Gemmatimonadetes, Bacteroidetes, γ-Proteobacteria, and Patescibacteria (Figure 10).

### 3.3.4. Co-occurrence networks of microbial communities

Was used co-occurrence network analysis to assess the complexity of interactions among OTUs in microbial communities across the different grassland management systems (Figure 11). The largest number of nodes was found in PP (12,738), followed by AP (1,078), IG (1,069) and NG (782) with the lowest number of nodes. On the other hand, the highest number of edges was observed in IG (12,738), followed by AP (8,649), PP (6,236) and with fewer NG (4,469) correlations.

Figure 11- Network co-occurrence analysis of bacterial communities in the sampled soils Natural Grassland (NG), Improved-Grassland (IG), Perennial-cultivated (PP), Annual-cultivated (AP). A connection stands for SparCC correlation with magnitude of >0.8 or <0.8 and statistically significant (P <0.01). Each node represents different bacterial OTU, and the size of the node is proportional to the number of connections (degree).




The network topological properties indicate that the IG and the cultivated pastures (PP and AP) harbored more complex and connected communities when compared to the NG system. The IG exhibited the highest community complexity based on the high number of edges (number of connections). The degree means average number of connections per node, is a component that demonstrates the complexity of microbial co-existence, here we verified higher

average degree in IG (23.83), followed by AP (16.04), NG (11.43) and PP (11.10). As well as the average degree, modularity was also higher in IG (11.71), followed by PP (10.44), AP (9.23) and lower modularity NG (2.54) (Table 2).

Table 2 - SparCC correlations and topological properties of soil microbial community networks in Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP). Comparisons at the OTU level.

| **Network properties** | **NG** | **IG** | **PP** | **AP** |
|---|---|---|---|---|
| Number of nodes[a] | 782 | 1069 | 1123 | 1078 |
| Number of edges[b] | 4469 | 12738 | 6236 | 8649 |
| Positive edges[c] | 2615 | 6504 | 3220 | 4499 |
| Negative edges[d] | 1854 | 6234 | 3016 | 4150 |
| Modularity[e] | 2.538 | 11.71 | 10.44 | 9.231 |
| Number of communities[f] | 138 | 117 | 198 | 94 |
| Network diameter[g] | 15 | 18 | 22 | 14 |
| Average path length[h] | 5.303 | 5.690 | 6.754 | 5.707 |
| Average degree[i] | 11.43 | 23.83 | 11.10 | 16.04 |
| Av. clustering coefficient[j] | 0.657 | 0.650 | 0.717 | 0.698 |

[a] Number of features with at least one strong correlation (SparCC $\geq |0.8|$ and highly significant ($P < 0.01$).
[b] Number of connections obtained by pairwise correlations between nodes.
[c] Spearman's pairwise positive correlations (SparCC $\geq 0.8$) and highly significant ($P < 0.01$).
[d] Spearman's pairwise negative correlations (SparCC $\leq -0.8$) and highly significant ($P < 0.01$).
[e] The longest distance between nodes in the network, measured as the number of edges.
[f] The capability of the nodes to form a modular structure, that is, a structure with a high density of nodes and clustered topology. Values > 0.4 suggest that the network has a modular structure.
[g] Average network distance between all pairs of nodes or the average length of all edges in the network.
[h] How nodes are embedded in their neighborhood and the degree to which they tend to cluster together.
The average number of connections per node in the network, that is, the node connectivity.
[j] The average number of connections per node in the network, corrected according to the proportion of nodes and edges in the network.

All systems presented a higher number of total positive correlations compared with the negative ones. We identified the nodes with high betweenness centrality, which are considered keystone species (APPENDIX A14). Considering the top 10 OTUs with the highest betweenness centrality, we observe changes in some keystone groups. However, *Candidatus udaeobacter* showed up as a key species in three systems (NG, IG and PP). The OTUs with the highest betweenness centrality in each system were affiliated to Pyrinomonadaceae (NG system), Xanthobacteraceae (IG), *Agromyces* (PP), and *Acidothermus* (AP).

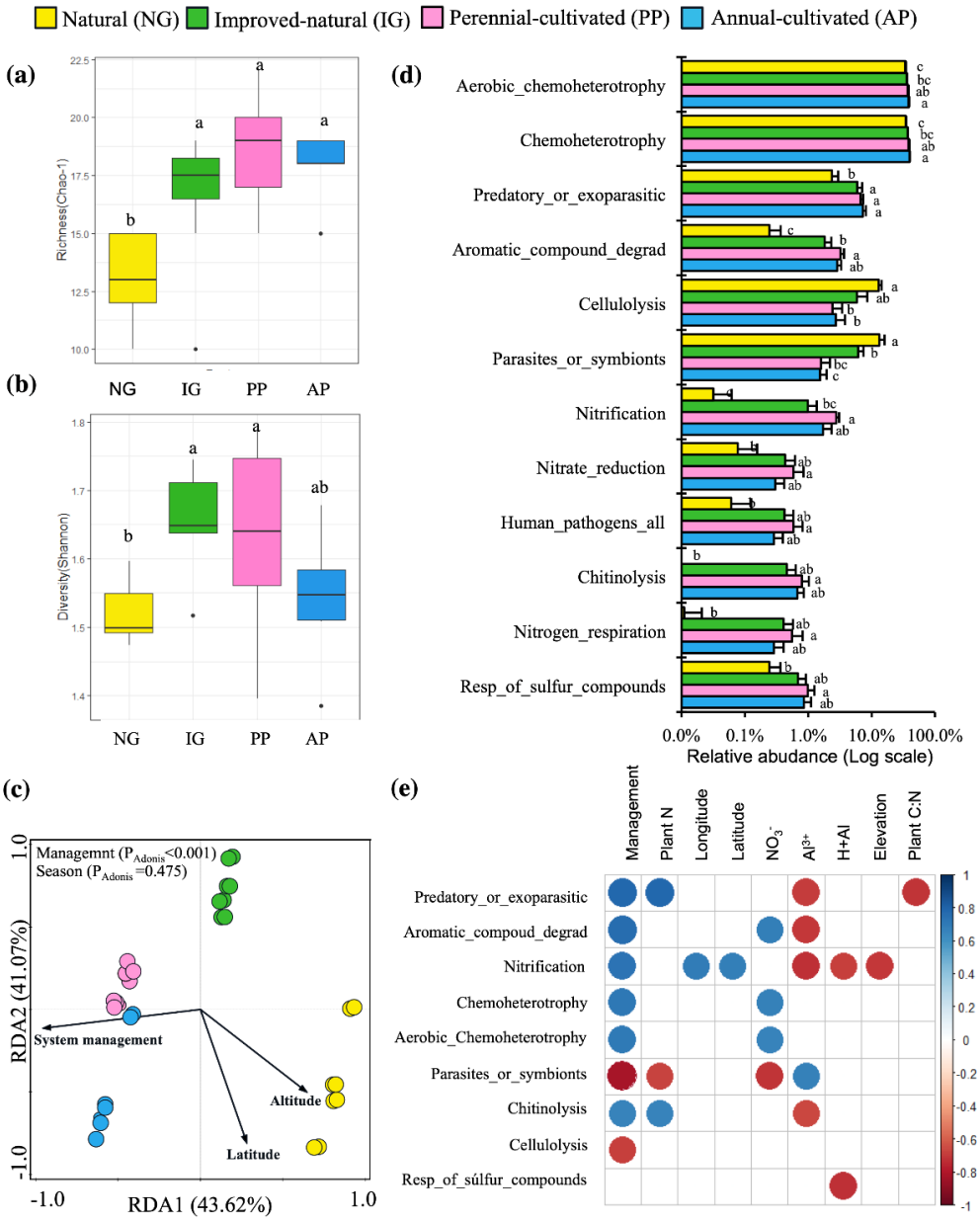
### 3.3.5. Functional prediction of microbial communities across grassland management systems

Was evaluated the alpha diversity patterns for a set of 24 functional categories, assigned through Faprotax. We found differences in Chao-1 richness and Shannon's *H'* alpha diversities (Tukey's HSD, $P < 0.05$) (Figure 12). The Chao-1 richness and Shannon diversity (Figures 12a and 12b) increased from the NG (Chao-1 = 13, *H'* = 1.52) to the IG (Chao-1 = 17, *H'* = 1.66), and the cultivated pastures PP (Chao-1 = 19, *H'* = 1.63), and AP (Chao-1 = 18, *H'* = 1.54).

The PERMANOVA for microbial functional categories showed the same patterns observed for OTUs, as was found differences in functional clustering of microbial communities, according to grassland systems ($P_{Adonis} < 0.001$), but not for seasons ($P_{Adonis} = 0.475$). The variation partitioning of the redundancy analysis (pRDA) corroborated the db-RDA results (APPENDIX A12), as 8.1% of the variation in functional categories was explained by environmental variables, with a lower contribution of sampling location and season (1.2%), and the highest contribution (33.6%) resulting from the overlap between the two sets of variables.

To measure the effects of environmental and spatial variables on the distance difference of functional categories, was performed the Mantel and partial mantel tests, selecting the variables separated by the partitioning of variances (p-RDA). The Mantel test showed that the variation in the similarities of functional categories was correlated with the matrix of distance from the environmental variables (Mantel, $\rho$=0.059; P =0.095) (APPENDIX A15). Specific correlations for functions were also observed for aluminum (Partial Mantel, $\rho$ =0.551; P <0.001) (APPENDIX A15), and plant diversity (Partial Mantel, $\rho$ = 0.145; P=0.02). Of all functional categories revealed by Faprotax, the most representative were 'chemoheterotrophy' and 'aerobic chemoheterotrophy', with 37.2 and 36.3% of the assigned reads, respectively. Of all the observed functional categories, 12 of them changed across systems (Figure 12d and APPENDIX A16).

Figure 12- Soil microbial functional patterns. (a and b) Alpha diversity and richness estimated by Shannon and Chao-1 Index, respectively; (c) partitioning of redundancy analysis (p-RDA) of soil microbial communities at functional categories; (d) Relative abundance of microbial functions, with only significantly altered functions shown (Kruskal-Wallis H test; $P < 0.05$) and (e) Heatmap of functions correlated with environmental variables, selected by Spearman's correlations and Bonferroni correction ($P < 0.05$) in soils from Natural Grassland (NG), Improved-Grassland (IG), Perennial -cultivated (PP), Annual cultivated (AP).




The relative abundances of 'cellulolysis' decreased 55.03% from NG to IG, and 80.2% compared NG with PP and AP. Likewise, 'parasites or symbionts', decreased 53.4% from NG conversion to IG and 88.2% to cultivated pastures. Meanwhile, we observed an increase in

‘chemoheterotrophy’, ‘aerobic chemoheterotrophy’ relative abundances about 14.7% from NG to AP. The same as found for ‘predatory or exoparasitic’ category, which was 200% more abundant in AP than NG. The ‘aromatic compound degradation’ increased 700% from NG to IG, and about 1000% to PP and AP. Other low-abundant functional categories (with relative abundances lower than 1%), such as ‘chitinolysis’, ‘respiration of sulfur compounds’ increased NG from to AP, and some functions like ‘nitrogen respiration’, ‘nitrate reduction’ and ‘human pathogens all’ were more abundant in PP.

Through Spearman’s correlations, was verified that the management intensity (grassland system) correlated with most of the measured functions, and with positive correlation for ‘predatory or exoparasitic’, ‘aromatic compound degradation’, ‘nitrification’, ‘chemoheterotrophy’, ‘chitinolysis’. In contrast, negative correlations were found between management and ‘parasites or symbionts’ and ‘cellulolysis’. The $Al^{3+}$ concentration was negatively correlated with ‘predatory or exoparasitic’, ‘aromatic compound degradation’, ‘nitrification’ and ‘Chitinolysis’ and positively correlated with ‘parasites or symbionts.’ The $NO_3^-$ concentration was negatively correlated with ‘parasites or symbionts’, and positively correlated with ‘aromatic compound degradation’, ‘chemoheterotrophy’, and ‘aerobic chemoheterotrophy’. The elevation was negatively correlated with ‘nitrification’. Yet ‘nitrification’ was positively correlated with longitude and latitude (Figure 12e).

## 3.4 DISCUSSION

In this study, was evaluated the effect of grassland management intensification on the soil microbiome composition and potential functions. First, our results have shown that soil parameters were altered with the conversion of natural grassland to cultivated soil pastures. The intensification of management has decreased the natural plant diversity, while the soil liming and fertilization have increased the pH and the concentrations of Ca, Mg, and $NO_3^-$, with the exchangeable $Al^{3+}$ being fully complexed in soil solution after liming practice. Second, the change in vegetation cover combined with the use of agricultural machinery has altered the soil density (LAI; KUMAR, 2020) and porosity ( ANDOGNINI et al., 2020).

Several studies have demonstrated the effects of land-use change and management intensification on the patterns of microbial communities' diversity in agricultural soils (NAVARRETE et al., 2015; PEDRINHO et al., 2020; GOSS-SOUZA et al., 2019; 2020; MENDES et al., 2015a). Here, was found that the change from natural grassland to cultivated pastures has increased taxonomic richness and alpha diversity. Unlikely, other authors have

found no differences in bacterial alpha diversity between natural grassland and cultivated vegetation in the pampa biome ( LUPATINI et al., 2013) or the conversion of native Atlantic Forest to pasture ( GOSS-SOUZA et al., 2017). On the other hand, collaborated with our results, many studies, have reported increases in taxonomic diversity in the conversion of natural forests to agricultural systems (MENDES et al., 2015a), and cultivated pastures when compared to primary and secondary forests (PEDRINHO et al., 2019). Comparable results have been also found diversity increased in deforested sites when compared to the pristine adjacent Amazon Forest (NAVARRETE et al., 2015). The richness and diversity of microbial communities can be altered by local dormancy or immigration from the species regional pool (LENNON; JONES, 2011). Also, the increased diversity in the fertilized areas has been suggested as a strategy to increase functional diversity and maintain essential ecosystem functions (MENDES et al., 2015b).

Many studies showed the difference between natural grassland and agricultural lands (LUPATINI et al., 2013) or the effects of land-use change and management intensification (MENDES et al., 2014; GOSS-SOUZA et al., 2019). These present results have demonstrated that vegetation change and the liming were the main determining factors in differential patterns of diversity, composition, and functional potential of microbial communities across grassland systems. Forage plant diversity has also affected the composition of the microbial communities in this study, through the relationship between plant species and rhizodeposits recruitment of microorganisms by plants (EISENHAUER et al., 2017; MENDES et al., 2018). The liming with dolomite ($CaMg(CO_3)_2$) leads to increased Ca and Mg contents in soil solution, with consequently increased base saturation, and $Al^{+3}$ complexation, through the formation of $Al(OH_3)$ precipitate in the solution (GREGOR et al., 1997; HAYNES, 1982). The effect of liming, together with the fertilization, has been found as pivotal for the changes in microbial composition (CASSMAN et al., 2016). The effect of pH on microbial communities has already been reported by several researchers (KURAMAE et al., 2012; MENDES et al., 2015a; PEDRINHO et al., 2020). Was observed an indirect pH effect, resulting from liming and $Al^{+3}$ complexation, as the main driver of the microbial diversity and ecosystem function changes in natural grassland conversion to cultivated pastures.

The intensification of management was also pointed out as a component that explains the taxonomic variability. All systems evaluated in this study belong to the same soil toposequence, but with differences in elevation, being pointed out as components of the variation of OTU diversity, through pRDA. The patterns of decreasing distance can be driven solely by differences in environmental conditions between space (MARTINY et al., 2011) as

differences in the mineral fraction of the soil and clay content. Dispersal limitation can also give rise to diversity, as it allows historical contingencies to conduct current biogeography standards, an effect observed by studies in the ecology of fungi (CEOLA et al., 2021), forage plants (DELORY et al., 2019), and microbial communities (HAWKES; KEITT, 2015).

Regarding microbial composition, was observed a decrease in Acidobacteria and Verrucomicrobia phyla abundances due to the shift from natural to managed (NG and IG) and for cultivated pastures (PP and AP). Many studies have described Acidobacteria as sensitive to changes in pH (LI et al., 2021; NAVARRETE et al., 2013). However, in the present study, was found a positive correlation between Acidobacteria and $Al^{3+}$ and negative with $NO_3^-$, like other studies (COSTA et al., 2022; MENDES et al., 2015; NAVARRETE et al., 2015b). The phylum Verrucomicrobia harbors microorganisms adapted to acidic soils and sensitive to land-use change ( COSTA et al., 2022; KANT et al., 2011). Was also verified a negative correlation of this phylum with management intensity and $NO_3^-$ soil concentration, suggesting that management practices such as nitrogen fertilization can decrease Verrucomicrobia abundance. Furthermore, Verrucomicrobia has been found associated with environments of high plant diversity (GUO et al., 2021), which could explain the decrease in the occurrence of this phylum in the gradient of both land-use intensification and plant diversity decrease from NG and IG for PP and AP, in the present study.

In contrast, the grassland management intensification and cultivation have increased the occurrence of several phyla such as Bacteroidetes, Gemmatimonadetes, Latescibacteria, Patescibacteria, Nitrospirae, Rokubacteria, and Proteobacteria classes in IG, PP, and AP. Bacteroidetes was found to be strongly negatively correlated with $Al^{+3}$, as it has increased in limed soils (IG, PP, and AP). Likewise, Gemmatimonadetes has shown a preference for slightly alkaline and low moisture pasture soils (DEBRUYN et al., 2011). Other studies have found a higher abundance of Gemmatimonadetes in pasture soils, compared to primary and secondary forests, during the dry season (PEDRINHO et al., 2019) or in deforested agricultural soils (MENDES et al., 2015a). Nitrogen fertilization can affect soil microbial groups differently in pastures (DING; WANG, 2021; LI et al., 2021). Markedly, Bacteroidetes, Firmicutes, and Proteobacteria were correlated positively with the levels of total N and N-ammonia, while Actinobacteria has shown a negative correlation, the same as found in another study (PAN et al. 2018). In addition, some authors have verified an increase in the abundance of the phylum Nitrospirae in pasture agricultural soils (CARBONETTO et al., 2014; GOSS-SOUZA et al., 2017), and negative correlation with Al, H+Al, and positive with Mg and pH (COSTA et al., 2022; PEDRINHO et al., 2019), corroborating the results we have found here. Nitrospirae

phylum is a member of the nitrite-oxidizing bacteria (NOB) group (VAN ELSAS et al., 2019), suggesting a large occurrence in pastures that have received nitrogen fertilization, as was found for IG, PP, and AP.

The increase in OTU richness and diversity with management intensification may be related to the decrease in available Al, the increase in cations by liming, and a fertilization effect, led by N, P, and K, through fertilizer application. In the present study, Al has presented a negative relationship with Rokubacteria, Gemmatimonadetes, Patescibacteria, δ-Proteobacteria, and Latescibacteria. Complementary, another study also showed a negative correlation of Al with Bacteroidetes, Cloroflexi, Actinobacteria, Nitrospirae, Firmicutes, and Cyanobacteria (MENDES et al., 2015a). In addition, Al showed a negative correlation with 25 of 32 phyla in a study with pasture and forest soils (PEDRINHO et al., 2019). However, in this study, these phyla are positively correlated with Ca and Mg, added by liming and mineral nitrogen fertilizer, explaining the influence of these soil management practices on soil microbial communities.

The Mantel and partial Mantel tests have confirmed the study's hypothesis that the conversion of natural grassland to cultivated pastures would affect the diversity, composition, and functional potential of microbial communities. Through network analysis, it was possible to confirm that the transition from natural grassland to cultivated systems has affected the interactions of the microbial groups and changed the network topology. Natural grassland (NG) has presented fewer interactions, which has indicated less complexity when compared to the cultivated pastures. Similar, network topological patterns have been found by other authors, with cultivated and fertilized pastures exhibiting more complex microbial networks, with a higher number of nodes when compared with natural systems ( COSTA et al., 2022; GOSS-SOUZA et al., 2017; PEDRINHO et al., 2020). A previous study has shown that microbial communities of soils from cultivated pastures of the Atlantic Forest biome had a higher number of nodes and stronger correlations when compared to forests and no-till crop soil communities, being more subjected to environmental selection (GOSS-SOUZA et al., 2017). Yet other authors, surveying in natural grassland of the pampa biome, found little structural change in the microbial community in the conversion to agricultural use (LUPATINI et al., 2013).

Was then identified the keystone groups within each network, which are presumed to have higher control over the entire network (HO et al., 2020). The main keystone phylum in NG is Acidobacteria, which presented a higher abundance, compared to the other evaluated systems. Members of this phylum have a high centrality in grasslands (BANERJEE et al., 2018) and are related to cellulose degradation (WARD et al., 2009), an important function for

vegetation with a high C:N ratio. In the IG, the system with the highest number of interactions, the family Xanthobacteraceae (Phylum Proteobacteria) was pointed as a key group within the network. Most microorganisms belonging to the Proteobacteria phylum are fast-growing (copiotrophic or r strategists), requiring high energy input for their development. These characteristics may explain the higher centrality of this group in the IG system that, together with fertilization, has a high diversity of root exudates from vegetation (ABIS et al., 2020). In the two cultivated pastures (PP and AP), two groups of the Actinobacteria phylum (*Agromyces* in PP and *Acidothermus* in AP) were appointed as the keystone in cultivated pastures, which may be related to the need for degradation of recalcitrant compounds (VAN ELSAS et al., 2019). The genus *Acidothermus* has been pointed as keystone species in soils and is related to nitrogen metabolism (BÁRTA et al., 2017). Interestingly, the Verrucomicrobia affiliated *Candidatus udaeobacter* was pointed as keystone species in all grassland systems, except AP, the more intensified pasture of this study. Together with Pyrinomonadaceae (keystone species in NG), the group *C. udaeobacter* was correlated with denitrification in agricultural soils (TATARIW et al., 2021). These results indicate that the most important groups within the networks have a role in nitrogen metabolism.

We have observed that the IG presented high microbial richness and alpha diversity, as well as the greatest diversity of plants in this system. A study showed that forage plant diversity was associated with the diversity and functionality of soil microbial communities (EO et al., 2021; GUO et al., 2021). Changing vegetation and adding practices such as liming and fertilization are the main drivers of changing the functional profile of microorganisms, as indicated by p-RDA. High forage richness leads to both increased microbial biomass and microbial functional diversity (LANGE et al., 2015; MORENO et al., 2021). Moreover, several works have concluded that soil multifunctionality or multiple ecosystem functions are governed by the aboveground diversity (i.e., plant diversity) (DING; WANG, 2021; GUO et al., 2021; ZHANG et al., 2021). The functional categories related to the metabolism of N, S, and degradation of aromatic compounds were increased in cultivated pastures. Some studies have also shown a decrease in microbial multifunctionality with the intensification of pasture management (EO et al., 2021; LEKBERG et al., 2021; YIN et al., 2019). According to present results, important groups from N metabolism, such as Bacteroidetes and Nitrospirae, have increased in fertilized pastures, the same as found previously (PAN et al. 2014). This result can also explain why the keystone species identified in our network analysis have a role in nitrogen metabolism. We have also observed a decrease in ‘cellulolytics’ and ‘symbiotic or parasites’ in the cultivated pastures. Other research has demonstrated higher activity of the beta-glucosidase

enzyme (cellulose metabolism) from less to more intensified systems, as AP with conventional land use (VRIES et al. 2015), corroborating our results, that have shown a negative correlation between the abundance of cellulolytic groups with the intensification of pasture management.

Finally, these results support the hypothesis that the management intensification has affected microbial community structure, diversity, dynamics, and consequently functional potential (ALI et al., 2021; GUO et al., 2021). Grasslands are agroecosystems of high socioeconomic importance and provide essential ecosystem services for the maintenance of human life, and for plant and animal biodiversity (NEYRET et al., 2021). Our study has demonstrated how soil and vegetation management impact the diversity, and composition, also affecting ecosystem functions performed by soil microbial communities. We argue that the conversion of natural grasslands to more intensified systems should be implemented with caution, by following the proposed in the "The Low Carbon Brazilian Program" (FAO, 2020). Moreover, as found by other authors, was demonstrated that improved natural grassland (IG) can be productive and contribute to maintaining ecosystem functionality, especially related to soil microbial activity (LÓPEZ ZIEHER et al., 2020; RUGGIA et al., 2021).

## 3.5 CONCLUSIONS

In this work, was demonstrated that the structure of soil microbial communities was affected by the diversity of forage plants due to the conversion of natural grasslands to cultivated pastures. Soil management practices such as nitrogen fertilization and liming have increased the soil microbial alpha diversity and altered potential ecosystem functions. Soil liming was the main driver of changes in microbial communities, by decreasing the levels of available Al in the soil, and favoring microbial groups related to N and S metabolism, such as 'nitrification', 'nitrate reduction', 'nitrogen respiration', and 'respiration of sulfur compounds', as represented by Bacteroidetes and Nitrospirae, with a decrease in potential functions associated with C cycling, such as 'cellulosys', as represented by Acidobacteria. We also have found spatial correlations in microbial diversity distributions, which will be better investigated in an upcoming ecological manuscript. Finally, we have found that the management of natural grasslands (IG) can be an alternative to improve the availability of food for cattle, maintaining the endemic diversity of forage plants and the soil biological quality, preserving important ecosystem functions performed by the soil microbes.

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# 4 CHAPTER III: NATURAL GRASSLAND CONVERSION TO CULTIVATED PASTURE INCREASED SOIL MICROBIAL HABITAT SPECIALIZATION AND ALTERED ECOLOGICAL PROCESSES

ABSTRACT

The substitution of natural grasslands with cultivated pastures alters soil habitat conditions, with consequences for habitat generalist and specialist niche occupation, and the assembly of microbial communities. Grasslands act as filters for homogenizing conditions, leading to stochastic assembly governed by the dispersal process. However, there is still a gap in knowledge about the possible effects of conversion from natural grasslands to cultivated pastures on microbial ecological processes and their relationship with microbial habitat specialization. We used 16S rRNA high-throughput sequencing to investigate the assembly patterns of microbial communities and evaluate the ecological models for generalist and specialist microbes, along with a sequence of management intensification in grassland systems. The results have shown that the conversion of natural grasslands to cultivated pastures increases the number of microbial habitat specialists. We have also found microbial communities from perennial pastures with homogeneous vegetation cover and lower plant diversity more influenced by stochastic processes.

**Keywords:** Community assembly; Microbial niche specialization; Neutral theory; Niche theory.

# CONVERSÃO DE PASTAGENS NATURAIS EM CULTIVADA AUMENTA A ESPECIALIZAÇÃO E ALTERA OS PROCESSOS ECOLÓGICOS MICROBIANOS DO SOLO

## RESUMO

A substituição de pastagens naturais por cultivadas, altera as condições de habitat do solo, e as proporções de micro-organismos especialistas e generalistas, assim como montagem de comunidades microbianas. As pastagens forçam condições homogêneas para a dispersão de micro-organismos, favorecendo processos estocásticos. No entanto, ainda não há clareza sobre o efeito da conversão de pastagens naturais em cultivadas e os efeitos nos processos ecológicos microbianos. Foi utilizado sequenciamento do gene 16S rRNA para investigar os padrões de montagem das comunidades microbianas e avaliar os modelos ecológicos para micro-organismos generalistas e especialistas, ao longo de uma sequência de intensificação de manejo dos solos de pastagens. Os resultados apontam que a conversão de pastagens naturais para pastagens cultivadas aumenta o número de micro-organismos especialistas. E descobriu-se, que micro-organismos de pastagens perenes com cobertura de vegetação homogênea e baixa diversidade vegetal são mais influenciados por processos estocásticos.

**Palavras-chave:** Montagem de comunidades; Nicho especialização de micro-organismos; Seleção; Teoria neutra; Teoria de nicho.

## 4.1 INTRODUCTION

Soil microorganisms perform the biogeochemical cycling of soil elements, and are pivotal to plant nutrition, also acting in the plants defense line (LANGE et al., 2015; MENDES et al., 2014; RISCH, 2019). Furthermore, the functioning of the ecosystem is dependent on the diversity of functions performed by microbial communities, which is directly correlated with their assembly (WANG et al., 2021; ZHOU et al., 2022).

Two models have been often used to explain microbial community assembly: (i) Neutral theory: microbial community assembly is governed by stochastic or random processes of birth, death, colonization, extinction and speciation; and (ii) Niche-based theory: deterministic factors such as species characteristics and interspecific interactions (competition, predation, mutualism and trade-offs) and environmental conditions (pH, temperature, salinity and moisture) govern community structure and assembly, defined as deterministic processes (ZHOU; NING, 2017; VELLEND, 2010). However, according to the model developed by Dini-Andreote et al. (2015), deterministic and stochastic processes often occur simultaneously in soil microbial community assembly and are complementary.

Microbial resilience to biotic and abiotic stressors may be influenced by habitat, where generalists adapt better to adverse conditions, while specialists are less tolerant to environmental disturbances (SRISWASDI; YANG; IWASAKI, 2017). Studies have shown that specialists are more influenced by deterministic processes and more susceptible to environmental filtering (LUO et al., 2019), while generalists are prone to be governed by stochastic processes (LIAO et al., 2016), because of higher genetic potential for resistance mechanisms, such as dormancy (XU et al., 2021).

The assembly of microbial communities is altered by land-use change (GOSS-SOUZA et al., 2020; WU et al., 2019). According to Goss-Souza et al. (2017) microbial communities associated with cultivated pasture soils in southern Brazil have presented stochastic behavior, probably related to the root system architecture, which provides high connectivity, favoring the homogenization of communities in the face of environmental changes. In natural grasslands of the pampa biome, it was found that stochastic and deterministic processes occur mutually, and that soil moisture was the main deterministic driver of the assembly patterns in soil microbial communities (LUPATINI et al., 2019). The increased demand for forage and the lack of information on the management of natural grasslands lead to their conversion to cultivated pastures (ZANELLA et al., 2021). The soil disturbance and consequent management intensification have been found to stimulate the deterministic (niche-based) assembly process

of soil microbial communities (WANG et al., 2021). However, little is known about the consequences of replacing natural grasslands with cultivated pastures over the habitat specialization and the resulting assembly of microbial communities.

In this study, was hypothesized that: (i) Natural grasslands conversion to cultivated pastures changes would increase the weight of ecological deterministic process on soil microbial assembly, and (ii) that the cultivated pastures would lead to higher soil microbial habitat specialization. The aim of this study was to investigate the assembly patterns of microbial communities and evaluate the ecological models for generalist and specialist microbes, along a sequence of management intensification in grassland systems.

## 4.2 MATERIAL AND METHODS

### 4.2.1 Grassland system descriptions

The experiment was carried out at the Company of Agricultural Research and Rural Extension of Santa Catarina (Epagri/EEL), located in Lages (Santa Catarina State, Brazil (27°47'55" S and 50°19'25" W); 922 m of elevation; and annual rainfall, 1,668 mm). The Climate is humid mesothermal (Cfb) according to the Koppen-Geiger classification. Soil types were classified as Haplic Cambisols (IG, PP, and AP) and Humic Cambisol at the NG, using the World Reference Base for Soil Resources (ANJOS et al., 2015).

The soil samples were collected in four grassland areas, representing a gradient of increasing soil management intensification, as follow: 1) Natural grassland (NG), with a predominance of *Andropogon lateralis* NESS; 2) Improved-natural grassland (IG), under the no-till system, where the native grassland was amended with overseeding of *Trifolium repens* L., *Festuca arundinacea* Schreb., *Lolium multiflorum* Lam., *Holcus lanatus* L., and managed with nitrogen fertilization twice a year, NPK 9-32-12; 200 kg $ha^{-1}$, applied in the summer and NPK 9-32-12; 300 kg $ha^{-1}$ in the winter, since 2015, with the amendment of 400 kg $ha^{-1}$ of urea at grass tillering stage; 3) Perennial-cultivated pasture (PP), under the no-till system, with a monoculture of *Cynodon dactylus*, with nitrogen fertilization twice a year, as described in IG, and; 4) Annual-cultivated pasture (AP), where *L. multiflorum* and *Pennisetum glaucum* (L.) R. Br., were cultivated in succession, with nitrogen fertilization twice a year, as described in IG. Except for NG, all the pastures had their soil pH corrected with dolomitic limestone in 2015, and nitrogen fertilization in summer and winter.

### 4.2.2 Sampling and soil and vegetation analysis

The soil samples were collected in January and July 2020, comprising the summer and winter seasons. Each soil sample was composed of nine subsamples collected in a georeferenced grid, equidistantly every 10 meters from each other. Non-deformed soil samples from the 0-10 cm profile were collected with sterile PVC tubes (5 cm diameter × 10cm depth) in a geogrid scheme, equidistantly by 10 m from each other, with 5 m of the border in each paddock. The nine subsamples were homogenized to form a single composite sample per replicate (paddock). A total of 32 individual soil samples were collected (1 composite sample per paddock × 4 paddocks × 4 pasture systems × 2 sampling seasons).

Soil chemical and physical parameters were determined for each of the 32 soil samples. For soil chemical characterization, was measured pH soil, and estimated aluminum (Al), calcium (Ca), and magnesium (Mg), phosphorus (P), potassium (K), exchangeable bases (EB), cation exchange capacity (CEC), Total nitrogen (TN), total organic carbon (TOC), and Soil N-$NH_4^+$ and N-$NO^-$. These parameters were analyzed at the Soil Analysis Laboratory, Santa Catarina State University, Lages, Brazil, following routine methodology (TEDESCO et al., 1995). The physical soil attributes measured were soil bulk density, total porosity, macroporosity, microporosity, and biopores, through undeformed sample, using a volumetric ring (EMBRAPA et al., 2017). Soil granulometry (texture) was determined by pipette methodology (GEE; BAUDER, 1986).

The Plant diversity was also estimated, by the BOTANAL method (TOTHILL et al., 1992), which establishes a relationship of species composition and the participation of these species in the forage mass. For each sample, a “rank” was assigned, according to the participation of the most frequent species in the forage mass. Forage mass was obtained through the visual estimation, which was corrected by forage mass cuts (kg dry mass $ha^{-1}$, obtained in the cuts) performed at each evaluation period in areas adjacent to those evaluated. The plant forage sample was dried in an oven at 60°C for 72 hours. The dry biomass of the plants was used for the determination of total nitrogen (TN) and total organic carbon (TOC) were extracted and determined by dry combustion catalytic oxidation at an elementary auto analyzer CNHS Vario EL Cube (Elementar, Langenselbold, Germany).

### 4.2.3 Soil microbial sequencing and bioinformatics analysis

For taxonomic characterization of microbial communities' total DNA extraction from soil samples and performed 16S rRNA (V3-V4 region) sequencing, on the Illumina miseq platform. The bioinformatics analysis was performed using QIIME 2, version 2019.10 and the sequences were demultiplexed and quality control was carried out using DADA2 (CALLAHAN et al., 2016). After, the taxonomic affiliation was performed at 97% similarity using the Silva Database, version 132 (QUAST et al., 2013), generated operational taxonomy units (OTUs) matrix was further used for statistical analyses.

### 4.2.4 Statistical Analysis

Permutational analysis of variance (PERMANOVA), as implemented by 'adonis' function in 'vegan' package (ANDERSON, 2001; OKSANEN et al., 2019), on R software (R CORE TEAM, 2020). Adonis-PERMANOVA allowed us to test whether distribution was separated by grassland system and season effect.

The Multinomial Species Classification Method (CLAM test) (CHAZDON et al., 2011) were performed to classify all the possible phylotypes (10,273 OTUs) according to their niche occupancies, as generalists, specialists, and rare taxa, using the 'clamtest' function, in 'vegan' R package, to the estimated species relative abundance. The test was applied using the supermajority rule ($K = 2/3$, $P < 0.005$).

From the separation of the matrices by CLAM, to investigate the species association patterns across grassland systems and seasons, the species rank abundance distributions (RADs) for each of the 32 samples were calculated and fitted to four different theoretical assembly models: the zero-sum multinomial (ZSM) and the broken stick (null model), which regard to neutral assembly. And the pre-emption, Zipf and log-normal, related to a niche-based assembly. Broken stick, pre-emption and log-normal models were calculated using the 'radfit' function from the 'vegan' R package. The ZSM model was calculated on TeTame software, version 2.16 (JABOT; ETIENNE; CHAVE, 2008). The models were compared based on the Akaike Information Criterion (AIC). The lowest AIC value, the best-fitted model for each sample (BOZDOGAN, 1987). The dispersal rates, related to the tendency to migrate from members of a certain community, were calculated for each sample, through Etienne's formula (ETIENNE; ALONSO, 2005), on TeTame Software.

From the Bray-Curtis dissimilarity matrices, the beta-diversity distributions were calculated for local and regional communities with the function ‘vegdist’ on the ‘vegan’ R package (OKSANEN et al., 2019). Then, was performed permutations resemblance of those Bray-Curtis dissimilarity distance distributions under the null model with the function ‘swap_count’ from the ‘vegan’ R package. Afterward, was generated the Z-scores for the set of microbial communities with the function ‘oecsimu’ (GOTELLI; ULRICH, 2010), also from the ‘vegan’ R Package.

The Z-score refers to the deviation of expected Bray-Curtis pairwise distributions under permutations to the observed value, indicating the distance of a certain set of pairwise beta diversities from the null expectation (KEIL, 2019). The co-occurrence patterns of microbial communities were considered non-random, resulting from deterministic, homogeneous (Z-score < -2) or variable selection (Z-score > +2) processes, while Z-scores within those values (-2 < Z-score < +2), indicated that communities co-occurred randomly, governed by drift and/or dispersal stochastic processes (DINI-ANDREOTE et al., 2015; GAO et al., 2019).

To investigate the importance of geographic coordinates as primary predictors of Euclidian dissimilarities across environmental variables and Bray-Curtis for microbial communities’ habitat specialization was first performed a Principal Coordinates Analysis of Neighbor Matrices (PCNM), with forward-selection, setting environmental variables as primary predictors and the resulting coordinates (PCNM axes). From the resulting PCNM non-collinear and significant variables (Bonferroni correction), was depicted the proportion of the variation in the microbial assembly of overall bacterial communities, generalists and, specialists explained by (1) environmental and (2) geography, via Mantel and partial Mantel tests, with Spearman correlations, according to pastureland, with the functions ‘mantel’ and ‘partial.mantel’ (LEGENDRE; FORTIN, 1989), in the ‘vegan’ R Package.

## 4.3. RESULTS

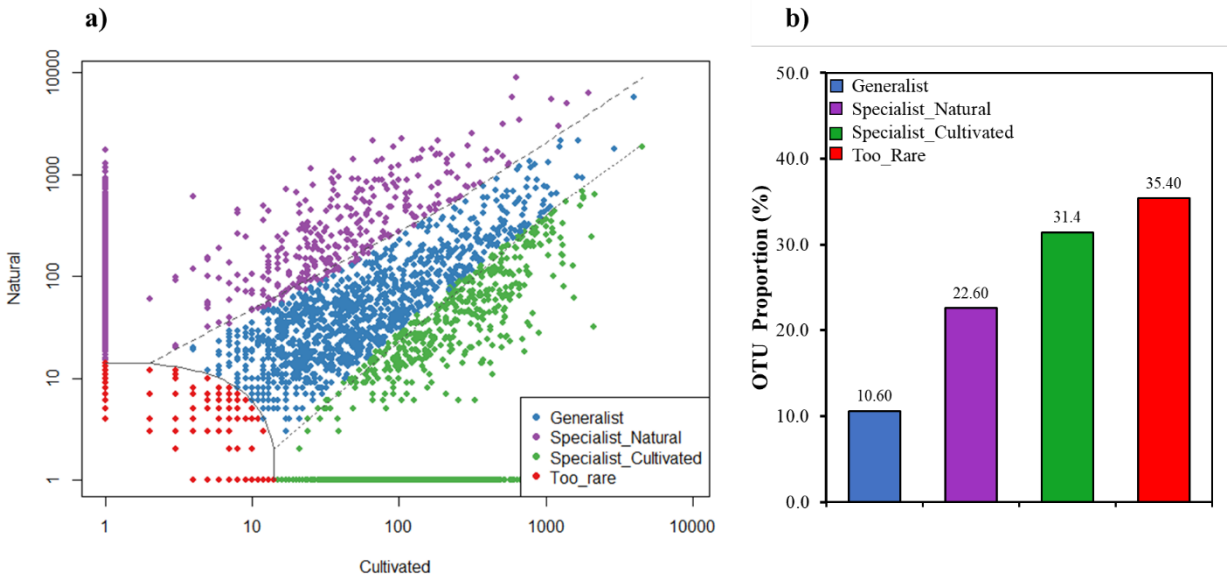
Through the PERMANOVA results, it was demonstrated that the microbial communities characterized as rare, generalists and specialists were correlated with the grassland systems ($p>0.001$), while no significant effect of sampling season (Table 3).

Table 3- Significance of Permutational multivariate analysis of variance (PERMANOVA) across Grassland system and season for Generalist, specialist of Natural grassland (Specialist N), specialist of cultivated pasture (Specialist C) and rare communities.

| Factor | Generalist | | Specialist N | | Specialist C | | Rare | |
|---|---|---|---|---|---|---|---|---|
| | F | Pr(>F) | F | Pr(>F) | F | Pr(>F) | F | Pr(>F) |
| Grassland | 4.4655 | 0.0009 | 6.9287 | 0.0009 | 6.1197 | 0.0009 | 1.065 | 0.0009 |
| Season | 1.1106 | 0.3007 | 0.764 | 0.6214 | 0.7689 | 0.6703 | 1.008 | 0.2148 |

The CLAM test revealed 10.6% of OTUs as generalist (1,088 OTUs), and 35.40% as rare (3,636 OTUs). The specialist in natural grasslands (NG and IG) represented 22.6% of the OTUs (2,321 OTUs), while the specialist in cultivated pastures (PP and AP) were 31.4% (3,225 OTUs) (Figure 13a and b).

Figure 13- Habitat microbial specialization between natural grassland and cultivated pasture. The x and y axes represent the OTUs abundance turnover between grassland system (a). The number and the percentage of generalists and specialists for each habitat comparison (b). The classification of generalists and specialists was performed through the CLAM test, according to the estimated species relative abundance.

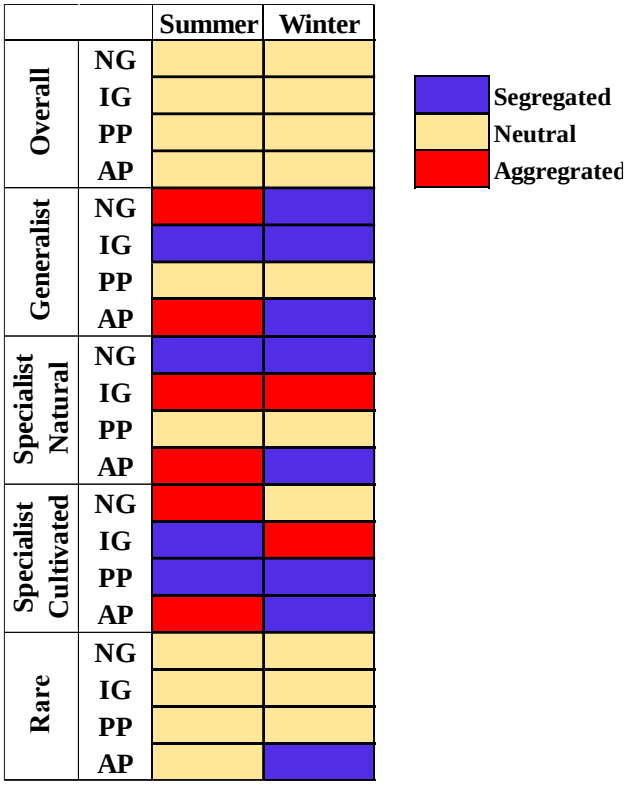



The Overall communities fitted predominantly as neutral. However, different patterns of ecological models have been verified for the assembly of generalist, specialist, and rare taxa. For the generalist occupancy, microbial communities were segregated in IG, neutral in PP, and aggregated for NG and AP for summer. NG and AP for winter were segregated.

The specialists of the Natural Grasslands were segregated in NG, aggregated for IG, neutral for PP, and aggregated for AP summer, while segregated for AP winter. On other hand, the specialists of cultivated pastures were segregated for PP, IG summer, and AP winter, and

aggregated for NG summer, IG winter, and AP summer, while neutral for NG winter (Figure 14 and APPENDIX B1). For the rare taxa, almost all samples were fitted as neutral, except for AP winter, which was segregated (Figure 14 and APPENDIX B1).

Figure 24- The Z-score at grassland system and summer and winter season. Across pasture system: Natural Grassland (NG); Improved Natural grassland (IG); Perennial Cultivated Pasture (PP) and Annual Cultivated Pasture (AP).




Through the Rank Abundance Distributions (RADs), microbial communities fitted predominantly to Lognormal niche-based model. The assembly of the generalist microbial communities was fully explained by Lognormal model in IG, PP, AP, and 63% of NG, while 37% of the NG samples adjusted to Zipf.

For the natural grassland specialists, it was found that 12% of the samples fitted Zipf, and all of them IG samples as Lognormal. For both cultivated pastures, 12% of the samples fitted Null, neutral model. In PP, it was found that 12% of the samples fitted as Preemption and Zipf, both explained by the niche model.

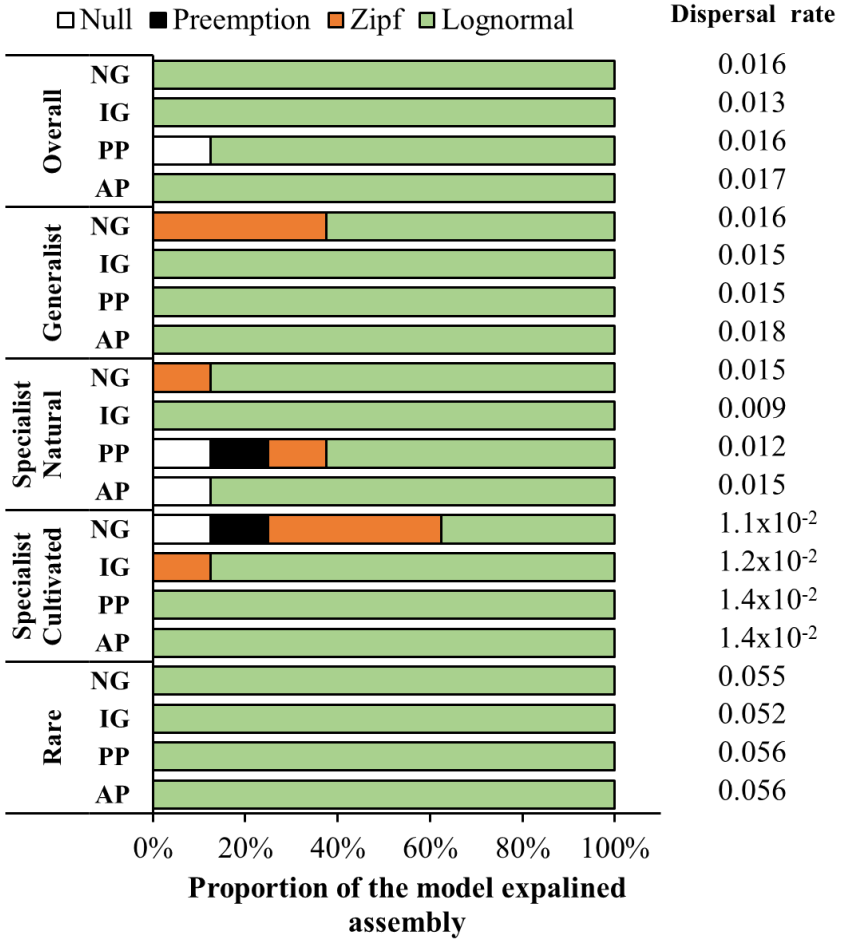
For the specialist in cultivated pastures, it was verified that 37% of NG samples fitted as Zip and lognormal, and 12% as Preemption, totaling 87.7% of the samples fitted to niche

theory and 12.5% fitted as Null (neutral theory). In IG, 12.5% of the samples fitted as Zipf, while in the cultivated pastures, 100% of the samples fitted lognormal (Figure 15 and APPENDIX B3).

For the rare taxa, all treatments fitted lognormal, based on niche theory, while for the generalist habitat 37% of the samples fit Zipf, niche-based theory. None of the samples were explained by the ZSM model (neutral theory).

Finally, the dispersal rate was numerically higher in AP in all niches occupancies, while IG had the lowest dispersal rate for overall, generalist, specialist of natural grasslands, and rare communities. The dispersal rate of NG and PP was found intermediate (Figure 15 and APPENDICES B1, B2 and B3).

Figure 15- Bacterial community assembly processes, based on Akaike information criterion (AIC) for rank abundance distributions of microbial OTUs. Across pasture system: Natural Grassland (NG); Improved Natural grassland (IG); Perennial Cultivated Pasture (PP) and Annual Cultivated Pasture (AP). The ZSM and Broken-stick are null models regarding to theoretical neutral assembly while Preemption, Lognormal and Zipf are niche-based models regarding to deterministic assembly. Dispersal rates were compared through grassland by Etienne's formula.



The Mantel product-moment correlation tests showed significant correlations for with all environmental variables and environmental variables selected for PCNM with overall communities and for generalist, specialist in cultivated pastures and rare taxa (Figure 6). Meanwhile, the specialists in natural grasslands were not correlated with overall environmental variables. The partial mantel tests showed effect of the grassland management and the plant diversity for all niche occupancies. The aluminum had no correlation for specialist in natural grasslands, while the clay was only correlated with overall and natural grasslands specialists. The elevation and the distance selected for PCNM, had no correlation on overall communities and niche occupancies (Table 4).

Table 4- Relative contribution environmental factors influencing bacterial communities with different niche occupancies. Was calculated Spearman product-moment correlations from the simple (Mantel test; 1000 permutations; $P < 0.05$) and the controlled effects (partial Mantel test; 1000 permutations; $P < 0.05$).

| ***Mantel*** | **Overall** | | **Generalist** | | **Specialist N** | | **Specialist C** | | **Rare** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *ρ* | *P* | *ρ* | *P* | *ρ* | *P* | *ρ* | *P* | *ρ* | *P* |
| Environmental overall | 0.193 | 0.008 | 0.196 | 0.003 | 0.085 | 0.058 | 0.185 | 0.008 | 0.175 | 0.004 |
| Environmental selected | 0.509 | 0.001 | 0.506 | 0.001 | 0.379 | 0.001 | 0.495 | 0.001 | 0.359 | 0.001 |
| ***Partial Mantel*** | *ρ* | *P* | *ρ* | *P* | *ρ* | *P* | *ρ* | *P* | *ρ* | *P* |
| Management | 0.612 | 0.001 | 0.227 | 0.002 | 0.484 | 0.001 | 0.547 | 0.001 | 0.312 | 0.001 |
| Aluminum | 0.323 | 0.001 | 0.377 | 0.001 | -0.044 | 0.681 | 0.565 | 0.001 | 0.130 | 0.013 |
| Plant diversity | 0.312 | 0.001 | 0.222 | 0.001 | 0.327 | 0.002 | 0.259 | 0.001 | 0.222 | 0.001 |
| Clay | 0.111 | 0.026 | -0.091 | 0.992 | 0.249 | 0.002 | 0.042 | 0.153 | 0.054 | 0.115 |
| Elevation | -0.301 | 1.000 | -0.170 | 0.999 | -0.109 | 0.997 | -0.380 | 1.000 | -0.121 | 0.997 |
| Distance | -0.387 | 1.000 | -0.238 | 1.000 | -0.351 | 1.00 | -0.335 | 1.000 | -0.159 | 1.000 |

## 4.4 DISCUSSION

Our results have not supported our first hypothesis, where it has been expected that the change from natural grasslands to cultivated pastures would increase the influence of deterministic processes. Meanwhile, we have verified the predominance of the lognormal model (deterministic) for all grassland systems. We have found a predominantly neutral assembly in PP, for all niche occupancies. This may be related to the root system of grasses from the genus *Cynodon*, in which the soil area is uniformly covered, favoring homogeneous conditions, and consequently higher stochasticity with lesser competition and variable selection (HORNER-DEVINE et al., 2004). Results from Richter-Heitmann et al. (2020) have demonstrated the effect of root architecture on microbial assembly processes. Authors have

found a predominance of homogenizing dispersal compared to variable selection in grasslands with dense and homogeneous roots, such as PP. The rare taxa have fitted most to the lognormal model and the neutral z-score. Some authors have shown that rare are shaped by deterministic processes, while in this work, the rare taxa were most driven by stochastic processes. Despite the high dispersal rate of the rare biosphere, dispersal can result in homogenizing dispersal (JIA; DINI-ANDREOTE; FALCÃO SALLES, 2018). In this study, the rare taxa were also found in higher proportion compared to the other niche occupancies, which may be due to high horizontal gene transfer and rapid development of new generations (JIA; DINI-ANDREOTE; FALCÃO SALLES, 2018).

The higher number of specialists in the cultivated pastures (PP and AP) may be due to the responses of generalists to habitat changes, which can induce genetic changes when generalist taxa lose genes if they specialize (SRISWASDI; YANG; IWASAKI, 2017), supporting our second hypothesis. Generalists, on the other hand, were found to be fewer in proportion, fitting to lognormal and, expected to be driven by niche-based model. They have also shown variations in Z-scores, demonstrating that deterministic processes have a great influence on the assembly of generalists, the same as found by Luo et al. (2019). The spatial variables in the present study have had no influence for any niche occupancies, just as found in a recent study (ZENG; AN, 2021), where the spatial variables contributed only with 1.2% of the explained variability of the rare community and less than 5% of the variability for the other niche occupancies.

## 4.5 CONCLUSIONS

In the present study, the stochasticity was related to the homogeneity of the vegetation cover. our results have shown that the conversion of natural grasslands to cultivated pastures have increased the number of microbial habitat specialists. We have also found microbial communities from perennial pastures with homogeneous vegetation cover and lower plant diversity more influenced by stochastic processes.

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# 5 CHAPTER IV: GRASSLAND MANAGEMENT AND ROOT MORPHOLOGY ALTER RHIZOSPHERE MICROBIAL COMPOSITION AND DIVERSITY

ABSTRACT

Soil microorganisms are essential for plant nutrition and protection. Recent studies show that the rhizosphere composition is passively or actively selected by plants, according to microbial functions. Natural grasslands have higher diversity of plant species, with root morphology and physiology resulting from long-term evolutionary processes, which lead to habitat adaptation and resilience to environmental disturbance events, while domestic grasses have morphological characteristics selected by breeding programs, which is expected to influence the ability of microbial recruitment and filtering capability by the plant root system. Here, we used bacterial 16S rRNA and fungal ITS region, to test the hypothesis that, plant diversity and root morphology influence the diversity and microbial composition on the rhizosphere of natural grasslands and cultivated pastures. We evaluated whether Bacteria and Fungi have different responses to changes in plant diversity and root morphology. Rhizosphere and bulk soil were collected in two subtropical highland grasslands: improved natural grassland (IG) and annual cultivated pasture (AP). Our results have indicated that the bacteria and fungi community structure differed between grassland systems and soil compartments—rhizosphere and bulk soil. No difference was found for bacterial richness and alpha diversity between grassland systems, while the fungal alpha diversity was higher in the AP rhizosphere. The diversity, composition, and root characteristics of grassland plants have influenced the selection of specific groups of soil bacteria and fungi.

**Keywords:** Plant microbial selection; rhizosphere bacterial community; rhizosphere fungi; root traits.

# O MANEJO DE PASTAGENS E A MORFOLOGIA RADICULAR ALTERAM A COMPOSIÇÃO MICROBIANA DA RIZOFERA


## RESUMO

Os micro-organismos do solo são fundamentais para a nutrição e proteção das plantas, estudos recentes mostram que a composição da rizosfera é selecionada pelas plantas, devido características funcionais dos micro-organismos. As diferenças entre plantas nativas e plantas selecionadas por programas de reprodução, resultam em diferentes necessidades de funções microbianas pelas plantas recrutadoras. As pastagens naturais possuem alta diversidade de espécies vegetais, e trazem características radiculares resultantes da evolução ao longo dos anos, adaptando-se às características de solo e ambiente, enquanto as gramíneas domésticas têm características morfológicas selecionadas pelo homem. Aqui, foi utilizado o sequenciamento do gene 16S rRNA e da região ITS rRNA, para testar a hipótese, de que a diversidade vegetal e a morfologia radicular influenciam a diversidade e composição microbiana da rizosfera de pastagens naturais e cultivadas, e verificar se bactérias e fungos respondem de forma diferente às mudanças na diversidade e morfologia radicular vegetal. A rizosfera e o solo a granel foram coletados em duas áreas de pastagens subtropicais de solos de altitude: pastagem natural melhorada (IG) e pastagem anual cultivada (AP). Os resultados indicam, que a estrutura da comunidade de bactérias e fungos é diferente entre sistemas de pastagem e no compartimento de solo (Rizosfera, solo a granel). No entanto, não foi encontrado diferença para a riqueza e alfa diversidade de bactérias entre os sistemas de pastagem, mas a diversidade de fungos é maior na rizosfera AP. A diversidade, a composição e as características radiculares das plantas de pastagens influenciam grupos específicos de bactérias e fungos do solo.


**Palavras-chave:** Característica de raízes; Comunidade microbiana; Fungos do solo; Riqueza das plantas.

## 5.1 INTRODUCTION

The rhizosphere is a narrow soil layer influenced by the plant root system (MENDES; GARBEVA; RAAIJMAKERS, 2013), in which plants establish complex interactions with several soil organisms, markedly bacteria, fungi, and protozoa (ROSSMANN et al., 2020). Rhizosphere microbes come from the bulk soil, which serves as a microbial reservoir (LING; WANG; KUZYAKOV, 2022). The recruitment of microorganisms by the plant is often related to nutrition and the seek for protection against pathogens (FAVELA; BOHN; KENT, 2021; MENDES et al., 2018). The composition and microbial assembly in the rhizosphere are altered by the vegetation cover and diversity, cropping system, and soil management (GOSS-SOUZA et al., 2020).

Plant domestication and breeding programs often aim the adaptation to field conditions, high yield, and resistance/tolerance to pathogens, which are intimately tied to the recruiting of microorganisms by the roots (MENDES et al., 2014). Modern cultivars have less genetic complexity in the rhizosphere when compared to the wild ones (ROSSMANN et al., 2020). A study by Albuquerque et al., (2022) has shown that breeding affects the dynamics of the rhizosphere microbial communities, decreasing the complexity of the interactions between microbes and plants.

Together with plant selection, increasingly intensified agricultural practices have led to the shifts in interaction patterns between plants and the root microbiome (FAVELA; BOHN; KENT, 2021). A recent study has identified a higher abundance of microbial chemotaxis genes in wild rice rhizosphere compared to that from commercial cultivars (SUN et al., 2021). The differential rhizosphere recruitment found in indigenous plants may be associated to long-term environmental and the co-evolutionary processes linking those wild plants with their microbiomes (FUKAMI; NAKAJIMA, 2011).

The interspecific coexistence and competition of plants alter the rhizosphere selection (CAVALIERI et al., 2020). A recent work has found that grasslands mixed of with cultivated grasses and legumes have a more stable and complex rhizosphere microbiome, and even higher forage production (YAN et al., 2022). Furthermore, aboveground plant diversity may reflect on the selection of microbes in the rhizosphere (ZVEREV et al., 2021). The aboveground diversity of plants affects the belowground microbial competition, and the differences in root morphology can give competitive advantages or disadvantages to plants to select their microbiome (PELLKOFER et al., 2016; WU et al., 2021).

Plant genotype is believed to be a major driver of the rhizosphere microbiome selection, as the release of specific exudates may be linked to the recruitment of certain groups of bacteria (FAVELA; BOHN; KENT, 2021; MENDES et al., 2014). Indeed, root architecture has the potential to affect the rhizosphere microbiome, especially by altering the plant-microbiome interaction interface (HERMS et al., 2022). According to Zeng et al., (2022), root phenotypic traits have selected rhizosphere bacterial communities. Authors also have found that Acidobacteria and Bacteroidetes might play important roles in modifying the root development.

In this study, we tested the hypothesis that (i) plant diversity and root morphology influence the bacterial and fungal diversity and composition in the rhizosphere of natural and cultivated pastures, and that (ii) Bacteria and Fungi respond differently to changes in plant diversity and root morphology. The aim of this study was to evaluate the differences in microbial diversity, structure and composition of rhizosphere and bulk soils of improved natural grasslands and cultivated annual pastures.

## 5.2 MATERIAL AND METHODS

### 5.2.1. Site description and soil sampling

The grassland areas are in Company of Agricultural Research and Rural Extension of Santa Catarina (Epagri), located in Lages (Santa Catarina State, Brazil (27°47'55" S and 50°19'25" W). The soil samples were collected in two grassland areas: 1) Improved-natural grassland (IG), under the no-till system, where the native grassland was amended with overseeding of *Trifolium repens* L., *Festuca arundinacea* Schreb., *Lolium multiflorum* Lam., *Holcus lanatus L*; 2) Annual-cultivated pasture (AP), *Pennisetum glaucum* (L.) R. Br. were cultivated in conventional system with soil harrowing. The grassland systems were composed of three replicates, as represented by grazing paddocks (25 × 35 m).

The IG and AP were implemented in 2015, when the soil pH was corrected, through liming, aiming to reach a bases saturation of 70% when forming the grazing system, using dolomitic limestone. The fertilization procedure was performed on the same day, twice a year. The study areas are in the same toposequence, both are classified as Haplic Cambisols (ANJOS et al., 2015). Soil analysis was evaluated hydrogen potential (pH), potential acidity(H+Al), Soil Organic Matter (SOM), Phosphor (P), Aluminum (Al), cation exchange capability (CEC), Total

organic carbon (TOC), Total nitrogen (TN), soil density (SD), and Clay (TEDESCO, 1995), the soil attributes can be seen in the Table 5.

Tabe 5- Soil analysis of grassland systems: Improved Native Grassland (IG) and Annual Cultivated Pasture (AP).

| Soil attributes | IG | AP |
|---|---|---|
| **pH** | 6.20±0.1 | 6.15±0.2 |
| **H+Al** | 2.71±0.2 | 3.15±0.4 |
| **SOM** | 4.22±0.2 | 4.42±0.7 |
| **CEC** | 16.18±0.9 | 14.08±0.7 |
| **P** | 18.07±2.0 | 15.61±2.5 |
| **Al** | 0.00±0.0 | 0.00±0.0 |
| **TOC** | 70.85±9.6 | 56.31±3.7 |
| **TN** | 3.52±0.2 | 3.60±0.2 |
| **Clay** | 34.5±1.0 | 34.09±2.0 |
| **Soil density** | 1.12±0.0 | 1.22±0.0 |

Units: pH -log [H+ mol $L^{-1}$]; potential acidity= H+Al; SOM= %; CEC= $cmol_c\ dm^{-3}$; P= $mg\ dm^{-3}$; Al= $cmol_c\ dm^{-3}$; TOC= $mg\ g^{-1}$; TN= $mg\ g^{-1}$; soil density= $g\ cm^{-3}$; Clay=%.

To evaluate microbial taxonomy and functional categories of bulk soil and rhizosphere, soil samples were collected in January 2020. Soil and vegetation were collected in monoliths (25 x 25 cm area, and 20 cm depth), with three replicates (paddocks) and two grassland systems (Figure 16). The monolith samples were kept in a cooler box (~ 4°C) during the sampling and transportation. The soil excess has been manually removed from the monoliths and considered as bulk soil, while the rhizosphere—attached to the roots— was collected using a sterile brush and stored in an ultra-freezer at -80°C, until further processing for microbial analysis.

### 5.2.2 Soil DNA extraction and sequencing

The total DNA extraction from soil samples (250 mg) was performed for each of the 12 samples (12 samples: 3x AP Rhizosphere; 3x AP bulk soil; 3x IG Rhizosphere; 3x IG bulk soil) using DNeasy PowerLyzer PowerSoil ™ DNA Isolation Kit (Qiagen, Hilden, Germany), following the instructions of the manufacturer. DNA quality and concentration were evaluated using NanoDrop™ 1000 spectrophotometer (Thermo Scientific, Wilmington, USA) and checked in gel electrophoresis with Tris-buffered saline with sodium boric acid, and 1.5% agarose (BRODY; KERN, 2004). The taxonomic characterization of microbial communities was performed through large-scale amplicon sequencing, on the Illumina MiSeq platform

(Illumina, San Diego, USA). For this, the V3-V4 region of the 16S rRNA gene was amplified using the set of primers (F: 5' CCT ACG GGN GGC WGC AG 3' and R: 5' GAC TAC HVG GGT ATC TAA TCC 3') (KLINDWORTH et al., 2013). The PCR reaction was performed using 2 µL of DNA, 12.5 µL of 2x PCR Ultra Mix (PCR Biosystems, London, UK), 0.5 µL of each primer (10 mM), and ultrapure water to measure the final volume of 25 µL. The amplification of ITS1/ITS2 was performed using the same polymerase as above.

### 5.2.3 Bioinformatics analysis

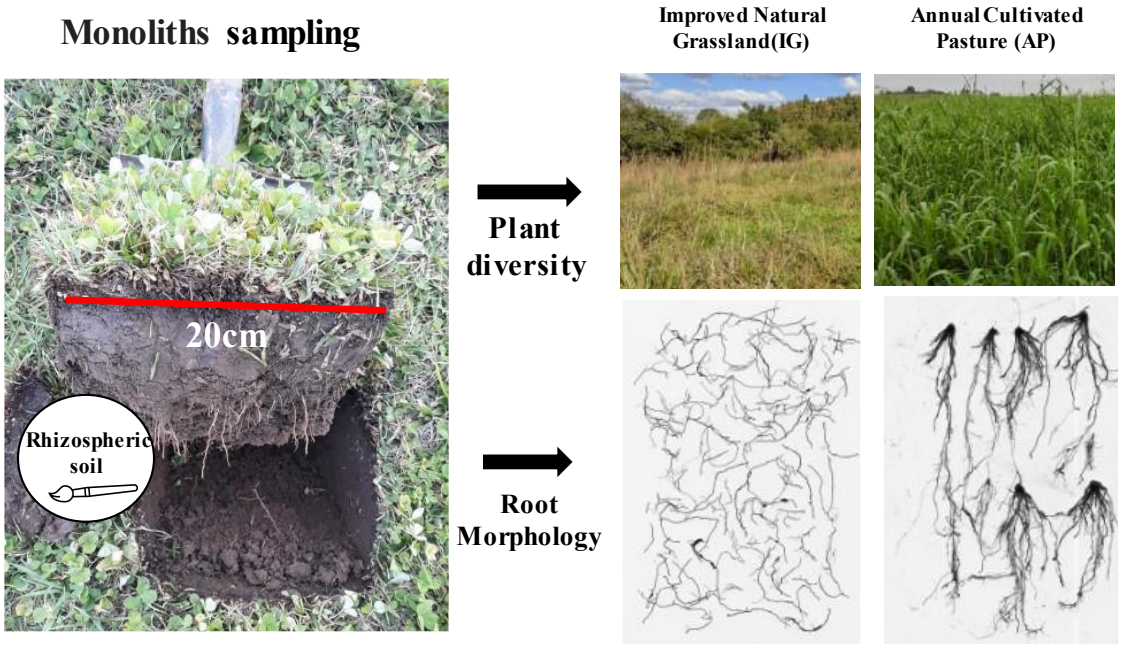
The 16S rRNA gene paired-end reads were first merged using PEAR (ZHANG et al., 2014). Then, the merged sequences were analyzed using Quantitative Insights into Microbial Ecology (QIIME 2), version 2019.10. The sequences were demultiplexed and the quality control was carried out using DADA2 (CALLAHAN et al., 2016) with the consensus method to remove any remaining chimeric and low-quality sequences. The samples were then rarefied to 20,065 sequences for 16S sequences and 27,952 sequences for ITS region, following the number of the lowest sample, and singletons and doubletons were removed. The taxonomic affiliation was performed at 97% similarity using the Silva Database, version 132 (QUAST et al., 2013), and the generated operational taxonomy units (OTUs) matrix was further used for statistical analyses. The bacterial functions were predicted using the Faprotax database, version 1.2 (LOUCA et al., 2016), which enabled us to map prokaryotic clades to metabolic and ecologically relevant functions (C, N, and S cycles). The relative abundances of the identified genes that belonged to functional groups were calculated as the cumulative abundance of OTUs assigned to each functional group. All sequencing data in this study were submitted to the Metagenomics Rapid Annotation Server (MG-RAST), version 4.0.3. The amplicon data are available under project ID ‘Rhizo_Soil_Grassland_SC’ (Appendix C1).

### 5.2.4 Vegetation analyses

The forage roots were collected in soil monoliths. After separating the rhizosphere soil, the roots were washed, stored in 50% ethanol, scanned (Epson Expression 10,000 XL scanner), and analyzed using the WinRhizo Pro (2009) software (Regent Instruments, Quebec, Canada). We determined root architecture and morphology parameters, such as total root length (cm), root volume ($cm^3$), root average diameter (mm), and number of tips. After scanning, the roots

were dried in an oven at 70°C for 48 hours and weighted for the determination of root density (mass/$m^2$). The monolith and root sampling and analysis is shown in Figure 16.

Figure 16- Root sampling by monoliths and architecture and morphology analysis of roots from Improved Natural Grassland (IG) e Annual cultivated pasture (AP).




The plant diversity was also estimated, by the BOTANAL method (TOTHILL et al., 1992), which establishes a "rank" for species composition and forage mass. Forage mass was obtained through the visual estimation and corrected through forage mass cuts (kg dry mass $ha^{-1}$, obtained for each cut), performed in adjacent areas to those evaluated.

### 5.2.5 Statistical analysis

The statistical analyses were performed using the previously described experimental design: 2 grassland systems × 3 replicates x 2 soil compartments. Alpha diversity of OTUs, phylum level, functional microbial profiles, and forage plant diversity were calculated from the taxonomic relative abundance matrix, and estimated by Chao-1 and Shannon's index, with the PAST software, version 4.0.3 (HAMMER; HARPER; RYAN, 2001). Alpha diversity index were compared through ANOVA with Tukey's Honest Significant Difference test (Tukey's HSD), with the function 'tukeyHSD', on R software, version 4.0.5 (R CORE TEAM, 2020). For the phyla relative abundance, the difference between grassland systems was measured

through the Kruskal Wallis median test. From the resulting Bray-Curtis distance matrix, was used permutational multivariate analysis of variance (PERMANOVA) (ANDERSON, 2001), through the 'adonis' function in 'vegan' R package, version 2.5-6 (OKSANEN et al., 2019), to test the effect of soil compartments (Rhizosphere and bulk soil) and grassland systems (IG and AP) on bacterial and fungal communities.

Principal Coordinates Analysis (PCoA) was performed, based on the similarity matrix of the Bray-Curtis index, to visualize the dissimilarity of the community of bacteria among grassland system, the plot was performed using Canoco software, version 5.0 (PETR SMILAUER; JAN LEPS, 2014).

To determine the differences in abundance of bacterial and archaeal groups among soil samples, the Statistical Analysis of Metagenomic Profiles (STAMP) software v.3.0 (PARKS; BEIKO, 2010) was used. The q-values were calculated using two-sided Welch's t-test (WELCH, 1947), with corrections performed using the Benjamin–Hochberg false discovery rate (BENJAMINI; HOCHBERG, 1995). The Spearman's correlation coefficients were calculated between the relative abundance bacterial and fungi and plant diversity, composition and root morphology using the "multtest" package (POLLARD et al., 2005) in R (R CORE TEAM, 2020). P-values were corrected filtered and only significant correlations ($P<0.05$) were demonstrated.

## 5.3 RESULTS

### 5.3.1 Plant diversity and root morphology

The richness and Shannon diversity of plants was higher in IG (H' = 1.98; Chao-1 = 14) compared to AP (H' = 0.0; Chao-1 = 1) (Table 6). For the root morphology, only the root mass was different between grassland systems, being higher in IG. The other variables (diameter, tips, length, and volume) did not differ (Table 6).

Table 6- Vegetation richness, alpha-diversity, and root morphology from four grassland management systems: Improved-natural grassland (IG) and Annual-cultivated pasture (AP). Mean ± standard deviation. Lines followed by different letters differ significantly (Tukey <0.05) among grassland systems.

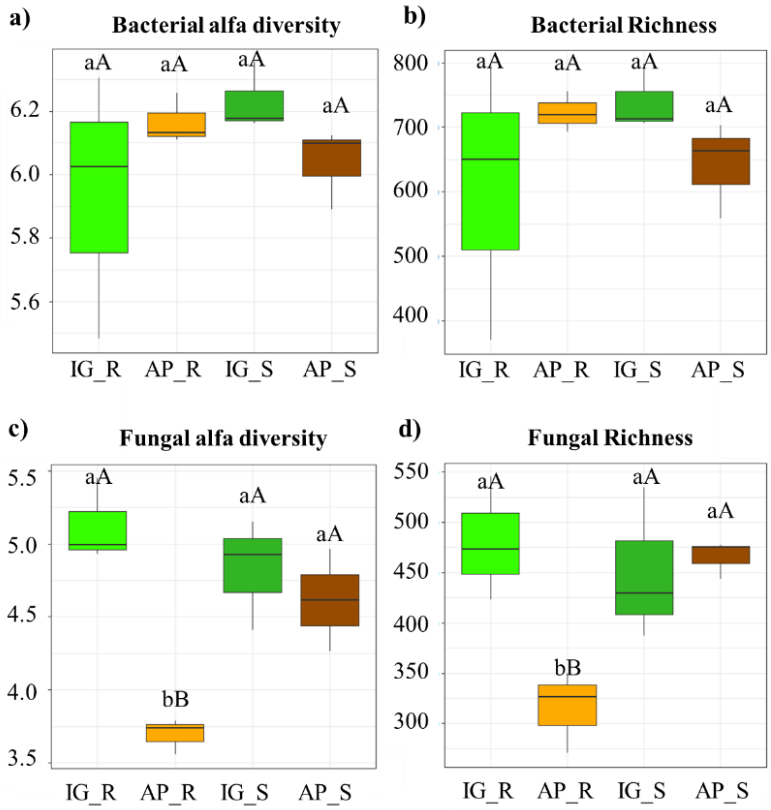
| | **Plant diversity** | | **Root Morphology** | | | | |
|---|---|---|---|---|---|---|---|
| **Grassland** | Diversity (Shannon *H'*) | Richness (Chao-1) | Mass ($g/m^2$) | Diam (mm) | Tips (number) | Length (cm) | Volume ($cm^3$) |
| **IG** | 1.98±0.10a | 14.00±2.0a | 48.09±14a | 0.47±0.04a | 83,614a | 340,370,102a | 61,174a |
| **AP** | 0.00±0.00b | 1.00±0.00b | 21.82±3b | 0.44±0.01a | 87,028a | 274,376,767a | 43,242a |
| *p-value* | <0.001 | <0.001 | 0.033 | 0.421 | 0.881 | 0.448 | 0.375 |

5.3.2 Diversity and composition of soil bacterial and fungal communities

For Bacteria (16S rRNA gene), we found a total of 4,828 OTUs, 30 phyla, 97 Classes, 258 orders, 452 families, and 798 genera. We did not found differences in bacterial taxonomic Shannon's alpha diversities and Richness at the OTU level between rhizosphere samples of IG and AP, and there was also no difference between bulk soil samples, according to grassland system (Tukey's HSD, $P < 0.05$) (Figure 17a and 17b).

For Fungi (ITS region), we had 2877 OTUs, 12 phyla, 39 classes, 82 orders, 13 families, and 260 genera identified. The fungal diversity was different between the rhizosphere of the two grassland systems, with the higher Shannon's diversity and richness found in the improved natural grassland (IG). However, we found no differences in the richness and diversity of Fungi in the bulk soil between IG and AP and verified higher Shannon's diversity and richness in AP bulk soil, compared to AP rhizosphere (Figure 17c e 17d).

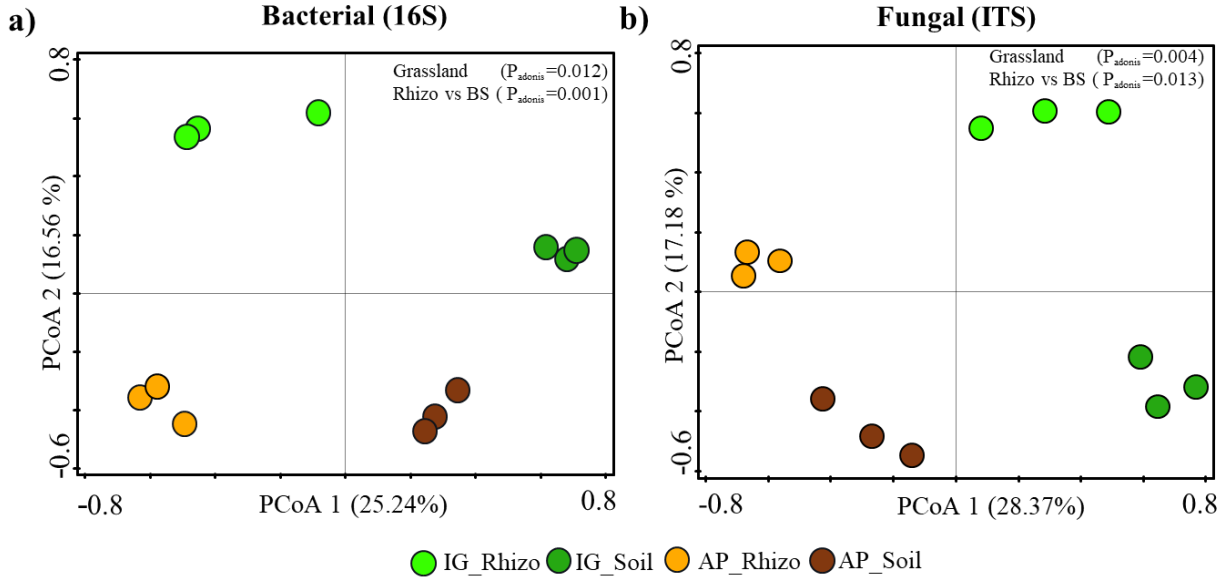
Figure 17 - Soil microbial Shannon's alpha-diversity (a) and Richness (b) from Rhizosphere (R) and bulk soil (S) of Improved-natural grassland (IG) and Annual-cultivated pasture (AP). Bacterial taxonomy for operational taxonomic units (OTU) level. Lowercase letters represent differences between grassland system (IG and AP) in the same soil compartment (Rhizosphere and bulk soil), and uppercase letters represent differences between soil compartments in the same grassland system.




In the PCoA, based on the Bray-Curtis similarity matrix, for the bacterial community, the axis of the main coordinate (PCO1) was responsible for 25.2% of the data variation explanation and the coordinate 2 (PCO2) explained16.6%. There was a clear separation for both the grassland systems and soil compartments (Figure 18a), corroborated by the PERMANOVA analysis (grassland systems; $p = 0.012$) (rhizosphere vs. bulk soil; $p = 0.001$) (Figure 18a).

For the fungal community, the axis of the main coordinate (PCO1) was responsible for 28.3% of the data variation explanation and the coordinate 2 (PCO2) explained 17.2%. Both the grassland systems and soil compartments were separated in the PCoA (Figure 18b). According to PERMANOVA analysis, the soil fungal community changed with grassland system ($p = 0.004$), as well between rhizosphere and bulk soil ($p = 0.013$) (Figure 18b).

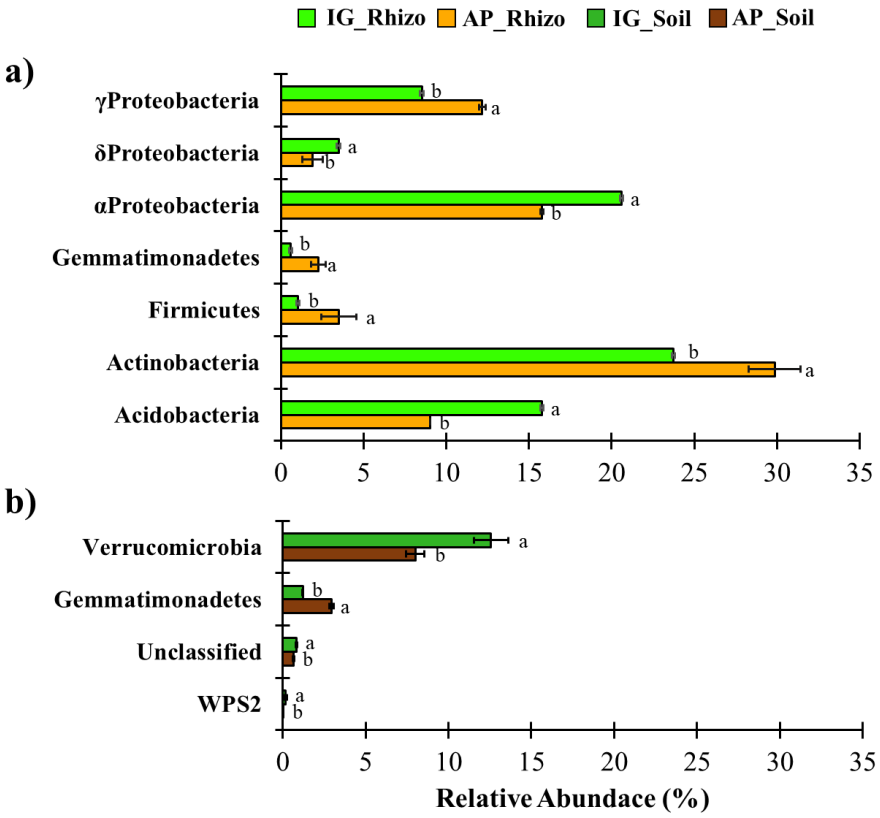
Figure 18 - Principal coordinate analysis (PCoA) using the Bray-Curtis distance, based on the abundance of bacterial (a) and fungal (b) communities, in rhizosphere (Rhizo) and bulk soil (soil) from Improved Natural Grassland (IG) and Annual Cultivated pasture (AP).




## 5.3.3 Bacterial composition changes

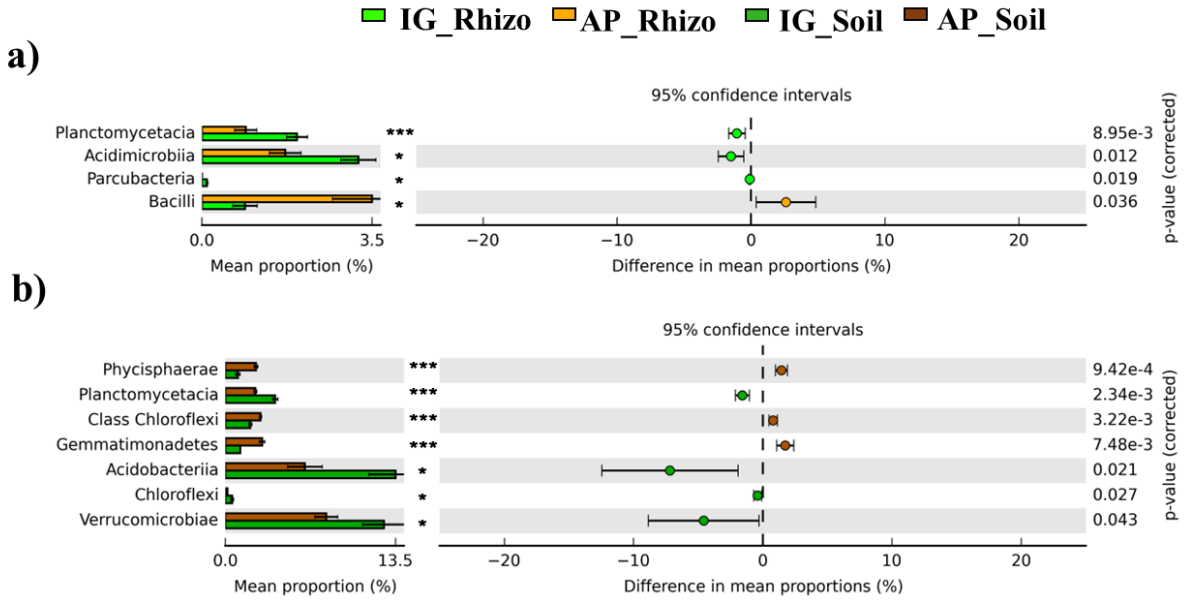
The main changes in bacterial community composition were observed in the rhizosphere. The class of αProteobacteria and the phyla Actinobacteria, Firmicutes, Gemmatimonadetes were higher in the *P. glaucum* rhizosphere (AP), while the δProteobacteria and γProteobacteria classes were more abundant in the rhizosphere of improved natural grassland (IG) (Figure 19a). In bulk soil, only four phyla were different between grasslands, with Verrucomicrobia more abundant in IG soils, while WPS and Gemmatimonedetes were more abundant in AP soils (Figure 19b and Appendix C2).

Figure 19- Relative abundance of soil bacterial phyla and Proteobacteria classes of Rhizosphere (a) and bulk soil (b) from soil of Improved-natural grassland (IG) – dark and light green bars, and Annual-cultivated (AP) – brown and orange bars. Only significantly altered taxa are shown (Kruskal-Wallis H test; $P < 0.05$). Classification at the taxonomic phylum level (Silva Database). Error bars show the standard deviation.




The bacterial class Acidimicrobia (Acidobacteria), Parcubacteria (Patescibacteria), Planctomycetacia (Planctomycetes) were more abundant in the IG rhizosphere, while Bacili (Firmicutes) was more abundant in the AP rhizosphere (Figure 20a). In bulk soil, the Verrucomicrobiae (Verrucomicrobia), Acidobacteriia (Acidobacteria) and unclassified (Cloroflexi) were more abundant in IG bulk soil. On the other hand, Planctomycetacia (Planctomycetes), unclassified class (Gemmatimonadetes), Phycisphaerae (Planctomycetes) and unclassified (Cloroflexi) were more abundant in AP bulk soil (Figure 20b).

Figure 20- Relative abundance of soil bacterial class of Rhizosphere (a) and bulk soil (b) from soil of Improved-natural grassland (IG) – dark and light green bars, and Annual-cultivated (AP) – brown and orange bars. Differences in mean proportions are shown for comparison between grassland. Welch's t-test with corrected q-values calculated using the Benjamini–Hochberg false discovery rate was performed for the significance levels: $*q \leq 0.05$, $**q \leq 0.01$, $***q \leq 0.001$.




The Spearman's correlation between phyla and vegetation parameters revealed that the phylum Entotheonellaeota established a positive correlation with the occurrence of IG plants, and with the root diameter and mass (Table 7). In AP, the richness of microbial phyla was positively correlated with root volume and length, and negatively with plant mass and diversity (Table 7).

In the overall correlations, Gemmatimonadetes showed negative correlations with plant diversity and with several plant species found in IG, while WPS-2 showed a positive correlation with the IG forage plants, and a negative correlation with *P. glaucum* in AP. The phylum Entotheonellaeota showed the same pattern of correlations established in IG. Finally, the phylum Firmicutes showed a negative correlation with the root mass and a positive with *P. glaucum* (AP) (Table 7).

Table 7- Spearman's correlation coefficients (R) statistical significance between soil bacterial at Phylum level and vegetation composition, diversity, and root morphology. Samples are follows: Improved-natural grassland (IG), and Annual-cultivated pasture (AP) and overall data. Only significant correlations are shown ($P \leq 0.05$).

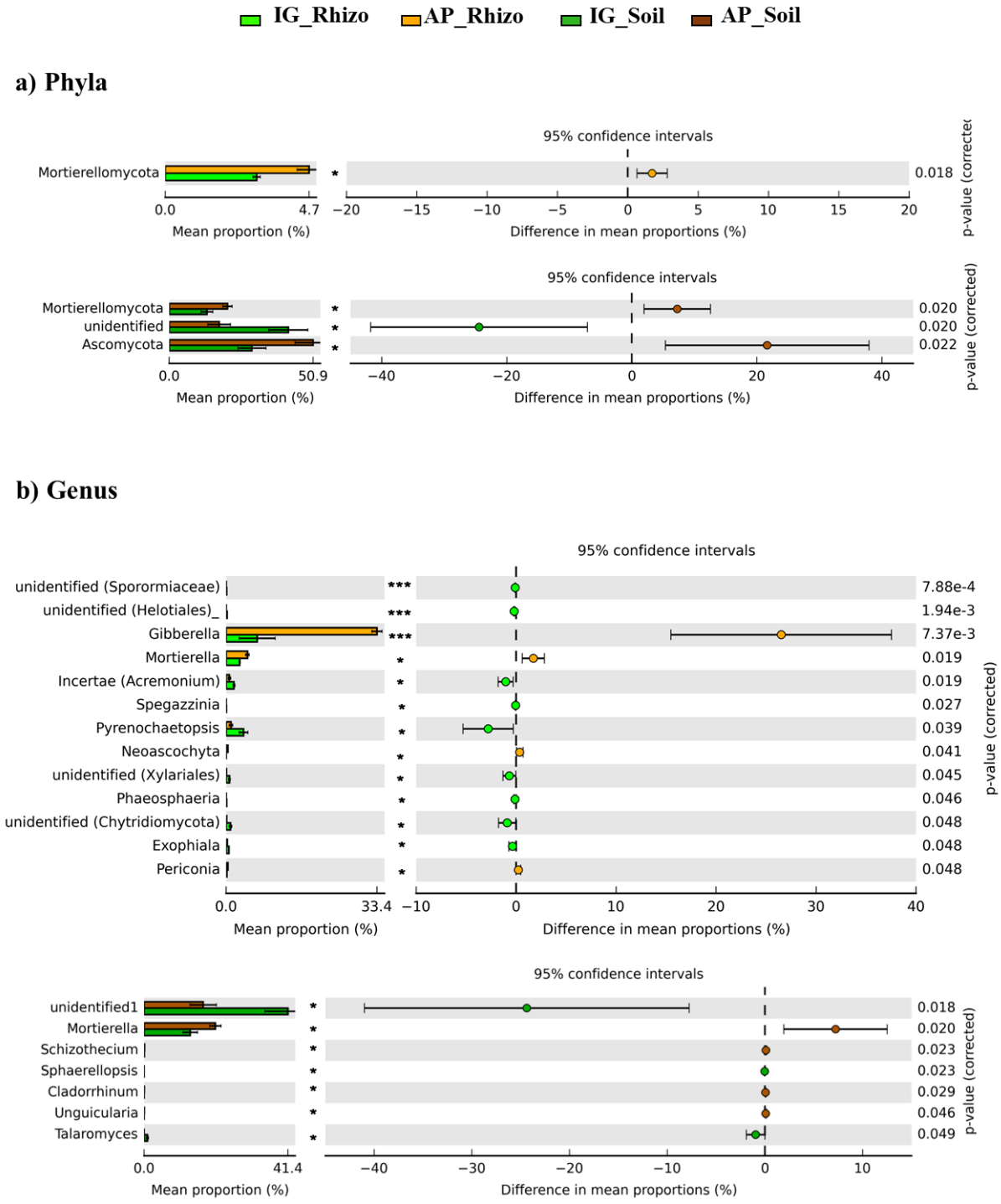
| **Vegetation** | **IG** | **AP** | **Overall** | | | |
|---|---|---|---|---|---|---|
| | Entotheonellaeota | Phyla Richness | Gemmatimonadetes | WPS-2 | Entotheonellaeota | Firmicutes |
| Vegetantion diversity | | **-0.84** | **-0.78** | | | |
| Root Mass | **0.85** | **-0.84** | **-0.78** | | | **-0.61** |
| Root Diameter | **0.85** | | | | | |
| Root Tips | **-0.85** | | | | **-0.59** | |
| Root Length | | **0.84** | | | | |
| Root volume | | **0.84** | | | | |
| *Andropogon lateralis* | **-0.85** | | **-0.83** | **0.62** | | |
| *Anthoxanthum* ssp. | **0.98** | | **-0.80** | | | |
| *Apium leptophyllum* | **0.85** | | | | **0.62** | |
| *Axonopus affins* | **0.98** | | **-0.80** | **0.59** | | |
| *Cyperus* spp. | **-0.85** | | **-0.83** | **0.62** | | |
| *Dichondra sericea* | **-0.85** | | **-0.83** | **0.62** | | |
| *Paspalum* spp. | **0.85** | | **-0.80** | **0.59** | | |
| *Paspalum notatum* | **0.98** | | | | **0.62** | |
| *Pennisetum glaucum* | | | **0.87** | **-0.65** | | **0.58** |
| *Plantago major* | **-0.85** | | **-0.84** | **0.70** | | |
| *Sida* spp. | **0.98** | | | | **0.62** | |
| *Trifolium repens* | **-0.85** | | **-0.84** | **0.70** | | |

### 5.3.4 Fungal composition changes

In order, the phylum Ascomycota was the most abundant, representing 57.1% of the assigned reads, followed by an unidentified phylum with 19.97%, Mortierellomycota with 10.41%, and Basidiomycota with 9.98% of the reads (Appendix C3). Mortierellomycota was more abundant in the AP Rhizosphere, and the most abundant phylum in AP bulk soil, the same as for Ascomycota. An unidentified phylum was more abundant in the IG bulk soil (Figure 21a).

The genus Mortierella (Phylum: Mortierellomycota) and the unidentified genus (Phylum: Chytridiomycota) were more abundant on AP. The genera Gibberella, Neoascochyta, Periconia, belonging to the phylum Ascomycota were more abundant in AP. The other genera of the phylum Ascomycota: Exophiala, unidentified (Family: Sporormiaceae), unidentified (Order: Helotiales), Incertae (Acremonium), Spegazzinia, Pyrenochaetopsis, unidentified (Order: Xylariales), Phaeosphaeria had higher abundances in IG (Figure 21b).

Figure 21- Relative abundance of soil fungal Phyla (a) and genus (b) of Rhizosphere and bulk soil from soil of Improved-natural grassland (IG) – dark and light green bars, and Annual-cultivated (AP) – brown and orange bars. Differences in mean proportions for comparison between grassland. Welch's t-test with corrected q-values calculated using the Benjamini–Hochberg false discovery rate for the significance levels: ∗q ≤ 0.05, ∗∗q ≤ 0.01, ∗∗∗q ≤ 0.001.




The correlations between the fungal phyla and the plant characteristics were performed between the IG, AP, and overall data. In IG, we found positive correlations between the phylum

Kickxellomycota with root tips, and the plants *Axonopus*, *Holcus lanatus*, *Plantago major*, *Trifolium* ssp., while for the phylum Mucoromycota, we found a negative correlation with root mass, root diameter, *Axonopus* ssp., *Paspalum* spp., *Sida* spp., *Trifolium* spp., *Cyperus* spp., and positive with *Andropogon lateralis* (Table 8). In AP, only the phylum Chytridiomycota presented positive correlations with plant diversity and root mass, while presenting negative correlations with root volume and length.

We found overall positive correlations between the phylum Kickxellomycota and *Axonopus* ssp., *Holcus lanatus*, *Paspalum* spp., *Plantago major*, *Trifolium* ssp. The phylum Mucoromycota established a positive correlation with vegetation diversity and with over 10 plant species. Otherwise, it had negative correlation with *Pennisetum glaucum* species cultivated in AP. Finally, the phylum Zoopagomycota was negatively correlated with the root mass, *Axonopus* ssp., *Paspalum* spp., and positive with *Pennisetum glaucum*.

Table 8- Spearman's correlation coefficients (R) statistical significance between soil fungal Phylum level and vegetation composition, diversity, and root morphology. Samples are follows: Improved-natural grassland (IG), and Annual-cultivated pasture (AP) and overall data. Only significant correlations are shown ($P \leq 0.05$).

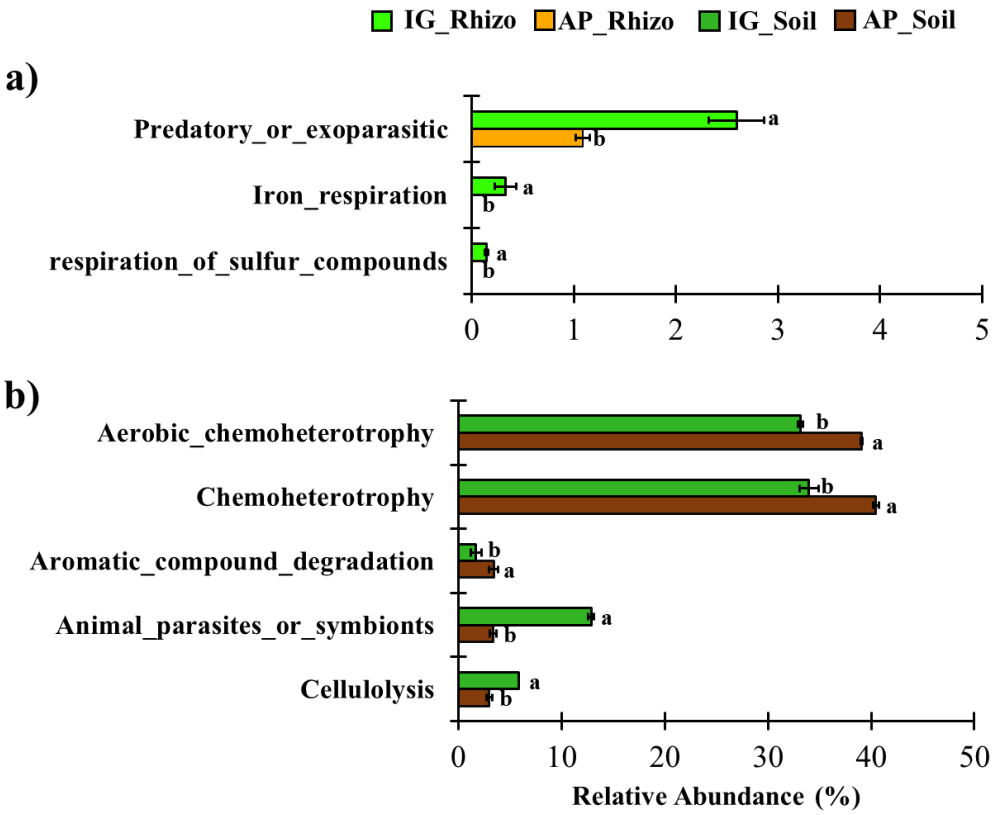
| **Vegetation** | **IG** | | **AP** | **Overall** | | |
|---|---|---|---|---|---|---|
| | Kickxellomycota | Mucoromycota | Chytridiomycota | Kickxellomycota | Mucoromycota | Zoopagomycota |
| Vegetantion diversity | | | **0.96** | | **0.64** | |
| Root Mass | | **-0.85** | **0.96** | | | **-0.6** |
| Root Diameter | | **-0.85** | | | | |
| Root Tips | **0.84** | | | | | |
| Root Length | | | **-0.96** | | | |
| Root volume | | | **-0.96** | | | |
| *Andropogon lateralis* | | **0.85** | | | **0.88** | |
| *Anthoxanthum* spp | | | | | **0.68** | |
| *Axonopus affins* | **0.83** | **-0.85** | | **0.65** | **0.65** | **-0.62** |
| *Cyperus* spp | | **0.85** | | | **0.88** | |
| *Dichondra sericea* | | | | | **0.88** | |
| *Holcus lanatus* | **0.84** | | | **0.62** | **0.84** | |
| *Paspalum* spp | | **-0.85** | | **0.58** | **0.61** | **-0.62** |
| *Pennisetum glaucum* | | | | | **-0.69** | **0.59** |
| *Piptochaetium* ssp | | | | | **0.69** | |
| *Plantago major* | **0.84** | | | **0.63** | **0.84** | |
| *Sida* spp | | **-0.85** | | | | |
| *Trifolium repens* | **0.84** | **-0.85** | | **0.63** | **0.84** | |

### 5.3.5 Changes in Bacterial soil functions

Only three functions were different between the IG and AP rhizospheres, with a higher abundance of 'Predador_or_Parasite', found in IG, and 'Iron_Respiration' and 'Respiration_of_sulfur_compounds', only observed in IG rhizosphere (Figure 22a). For bulk

soil, a higher abundance of 'Aerobic_Chemohetrophy', 'Chemoheterotrophy', 'Aromatic_ compound_degradation', was found in the bulk soil of AP, and higher abundances of 'Animal_Parasites_or_symbionts' and 'Cellulolysis' were found in IG (Figure 22b and Appendix C4).

Figure 22- Relative abundance of soil bacterial functions by Faprotax of rhizosphere (a) and bulk soil (b) from soil of Improved-natural grassland (IG) – dark and light green bars, and Annual-cultivated (AP) – brown and orange bars. Only significantly altered taxa (Kruskal-Wallis H test; $P < 0.05$). Error bars show the standard deviation.




## 5.4 DISCUSSION

Our study has demonstrated that the rhizosphere and bulk soils of natural improved grasslands, which higher plant diversity (IG) have had different compositions of fungal and bacterial communities, when compared to the monoculture cultivated pastures (AP). There was no difference in the richness and Shannon diversity of bacteria between IG and AP rhizospheres, and no differences between rhizosphere and bulk soil diversity and richness. Other studies have already found no differences in taxa diversity, with changes (decrease/increase) in the abundance of specific groups (SCHÖPS et al., 2018).

We have observed higher abundance of αProteobacteria and δProteobacteria in the IG rhizosphere when compared to AP. The high abundance of colonizers, characterized as fast growing or r-strategists, such as Proteobacteria has been reported by several studies (LING; WANG, KUZYAKOV, 2022; SHI et al., 2015). The study of Yan et al. (2022), demonstrated that grass rhizosphere bacterial stability and complexity under monoculture is lower than in grass-legume cropping, evidencing that the increased recruiting of these microbial groups may be related to plant genotypes or plant-mixing.

Another important point is the adaptation of wild forage grasses to soil conditions and climate along time, compared to commercial cultivars. The maize cultivars in the last 50 years, from a germplasm bank, the selection (via breeding) of the plant genotype across a changing agronomic environment drives changes in recruitment of the rhizosphere plant microbiome (FAVELA; BOHN and KENT). In grassland soils dominated by annual plants, as in AP, the rhizosphere is a transient microenvironment that lasts during the lifetime of a root. Unlikely, in perennial environments, as in IG, the rhizosphere persists across seasons (NUCCIO et al., 2016). In addition, bacterial taxonomic groups are preferentially associated with native plant species, potentially affecting plant dominance and the resulting plant-microbe interactions (TOJU; KUROKAWA; KENTA, 2019).

Our results have indicated the correlation of certain phyla of Bacteria and Fungi with root morphology and plant species diversity. The root morphology, mainly the proportion of fine roots is known to influence rhizosphere microbiome recruitment, and the root hairs and root tips have more complex microbial structure compared with other rhizodeposition zones (RÜGER et al., 2021). Furthermore, looking to the individual effect of the root, in environments with high density of plants, such as found in IG, it can potentialize the selection by the rhizosphere. Cavalieri et al. (2020) have found that increased plant density has changed the rhizosphere bacterial communities, with the plant recruiting more groups of ‘plant growth-promoting bacteria’. In addition, the decrease of vegetation diversity and density by the suppression of natural vegetation and the introduction of agricultural crops in AP have culminated with the loss of microbial diversity in both bulk soil and rhizosphere, as verified by Goss-Souza et al. (2020) in long-term forest-to-agriculture conversion.

The dynamics of rhizosphere colonization by fungi and bacteria follow different patterns. In our study, we have noticed that the diversity of Fungi is higher in the rhizosphere of IG, with no differences for Bacteria. Similar results were found by Sweeney et al. (2021) in grasslands, where the fungal rhizosphere was found to be influenced by root traits. Differently, a search

conducted by Kurokawa e Kenta (2019) has suggested that Fungi do not establish preference for native or exotic species of grasslands.

The fungal phyla Mortierellomycota and Ascomycota were more abundant in the AP rhizosphere. Similarly, we have found an increase in the abundance of Ascomycota in the AP bulk soil. The higher abundance of Mortierellomycota may be explained by the negative correlation that this group establishes with root area and length (SWEENEY et al., 2021), but may also be related to plant-fungus specificity and the stage of development (SHANG et al., 2021). Mortierella are known to generate antagonistic substances in plants, to suppress plant disease (ALI et al., 2021). Likewise, Ascomycota are groups found in abundance in pastures (NEIRA et al., 2021), that some genotypes produce toxins used in the defense against pathogens and organic acids capable of solubilizing phosphate (CHALLACOMBe et al. 2019).

Furthermore, to the known symbiotic relationship between plants and fungal communities (TEDERSOO; BAHRAM; ZOBEL, 2020), the present study shows that, despite the lower relative abundance of Kickxellomycota (less than 1% in all compartments in both grasslands) (Appendix C2), it is correlated with several plant species (IG) and the root tip number. This phylum has a positive correlation with carbon availability (TARIN et al., 2021), which is higher in IG (Table 5). The Mucoromycota has presented positive correlation with *Andropogon lateralis*, similar to the other found results, that have found high abundances of Mucoromycota in native savanna grasslands compared to the crop-livestock integrated system and low-input recovering areas (SELARI et al., 2021).

We have found higher abundance of 'predators_or_parasites', 'Iron_respiration', 'respiration_of_Sulfur_compounds' in IG rhizosphere, while in bulk soil there was an increase in both functions related to 'Animal parasite_or_symbionts' and 'Cellulosis'. Yet in AP bulk soil, we have found a higher abundance of 'Chemoheterotrophic' and 'Aromatic_compound_degradation'. The higher abundance of potential functions in the rhizosphere of native/wild plants was also verified by Sun et al. (2021). Authors concluded that wild and cultivated rice accessions can recruit specific core groups of chemotactic bacteria, some of which may play a critical role in the associated rhizosphere bacterial communities. The functional complexity of rhizosphere is often related to the need of plant genotypes. MENDES et al. (2014) found that soybean cultivar selects a specific microbial community inhabiting the rhizosphere based on functional traits. Furthermore, *Fusarium* resistant bean cultivars (breeding) have higher functional complexity in the rhizosphere than cultivars susceptible to the disease (MENDES et al., 2018).

## 5.5 CONCLUSION

The plant composition, diversity and root morphology have influenced the rhizosphere microbiome diversity and composition in perennial natural grasslands (IG) and cultivated annual pastures (AP). IG has presented a higher diversity of Fungi in the rhizosphere, and the Kickxellomycota fungal phylum has had a positive correlation with plant species in IG, while the Mucoromycota phylum has had a negative correlation with IG plant species. The diversity and richness of Bacteria has not been modified by the grassland system. However, the bacterial phylum Gemmatimonadetes have established negative correlations with plant diversity and several IG plants, meanwhile Entotheonellaeota has presented positive correlations with plants and root traits in IG. The differences in the rhizosphere of plants in monoculture compared to high plant diversity systems has led to differences in microbial functional potential, as functions related to 'Predador_or_Parasite', 'Iron_respiration', 'respiration_of_Sulfur_compounds' were higher in IG rhizosphere. Finally, the adoption of grassland systems composed of high plant diversity could contribute to maintaining rhizosphere microbial diversity and functionality, leading to more resilient and productive and resilient grassland systems.

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# 6 GENERAL CONCLUSION

In the last decade, advances in sequencing technologies and bioinformatics have led soil microbial ecology studies to another level. In Brazil, several studies have pointed out to the effects of agricultural practices and the importance of vegetation cover on soil microbial ecology and functionality.

In this work, we have shown that the assembly, diversity, and composition of soil microbial communities was affected by the diversity of forage plants due to the conversion of natural grasslands to cultivated pastures. Soil management practices, such as nitrogen fertilization and liming have increased the soil microbial alpha diversity and altered potential ecosystem functions. Soil liming was the main driver of changes in microbial communities, by decreasing the levels of available Al in the soil, and favoring microbial groups related to Nitrogen and Suffer metabolism.

Regarding the ecological processes that govern the assembly of microbial communities, the stochasticity was related to the homogenization of the vegetation cover, showing no pattern in relation to natural grasslands and cultivated pastures. The shifts in vegetation cover, coupled with soil management intensification, have culminated with changes in soil microbial community assembly, with consequences for niche occupancy, leading to an increased microbial specialization to keep functional resilience.

Finally, the selection of bacterial and fungal taxa in the rhizosphere was intimately linked with aboveground diversity and root morphology.

This knowledge can be used for improving soil management and biotechnology, aiming to improve agricultural productivity, and making grassland systems more sustainable. The management and improvement of natural grasslands (IG) and perennial pastures (PP) can be a suitable alternative to improve the availability of food for cattle, maintaining the endemic diversity of forage plants and the soil biological quality, while preserving important ecosystem functions performed by the soil microbes.

# APPENDICES

## APPENDIX A – CHAPTER II

A1 to A16

## APPENDIX B – CHAPTER III

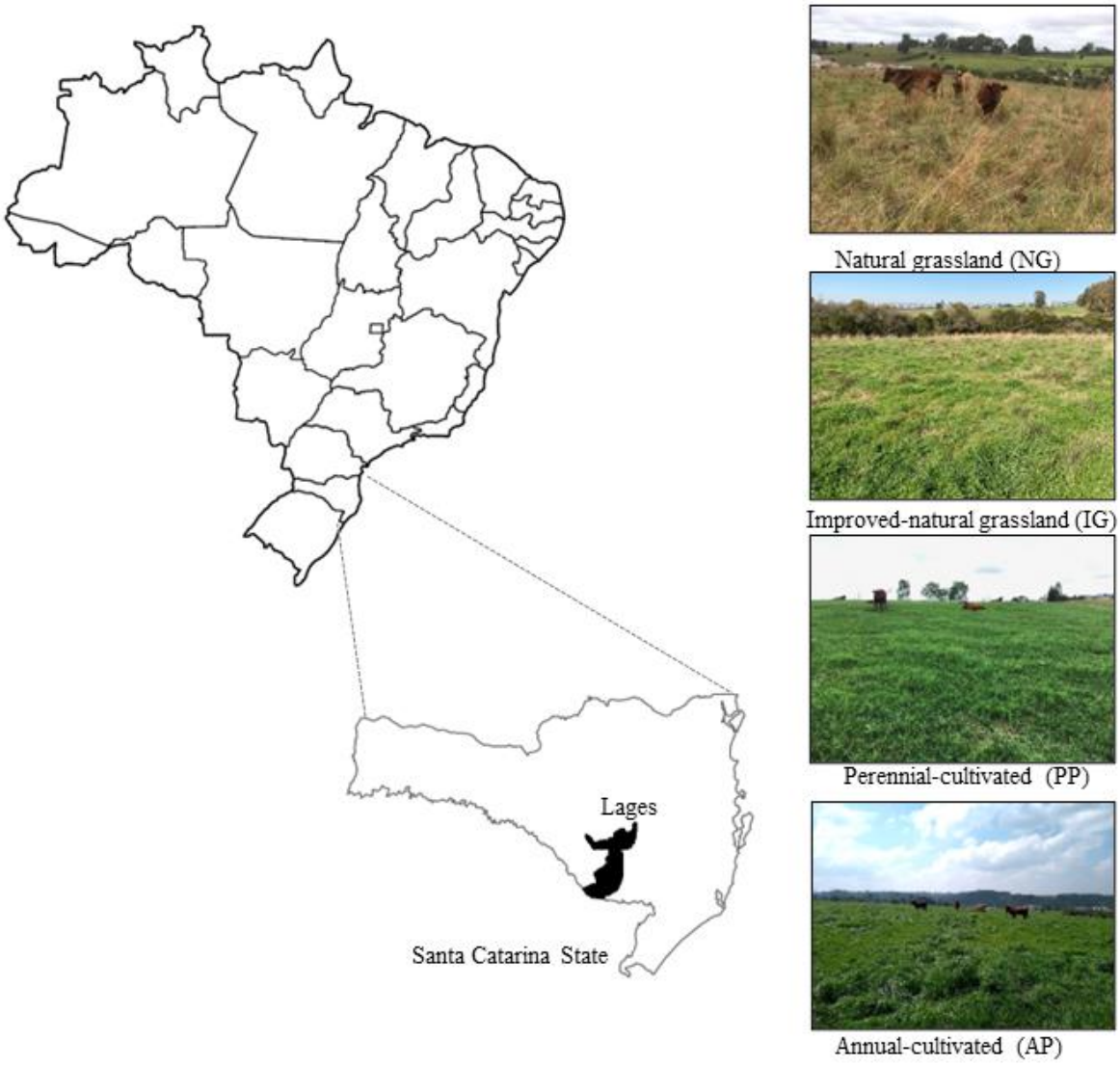
B1 to B4

## APPENDIX C – CHAPTER IV

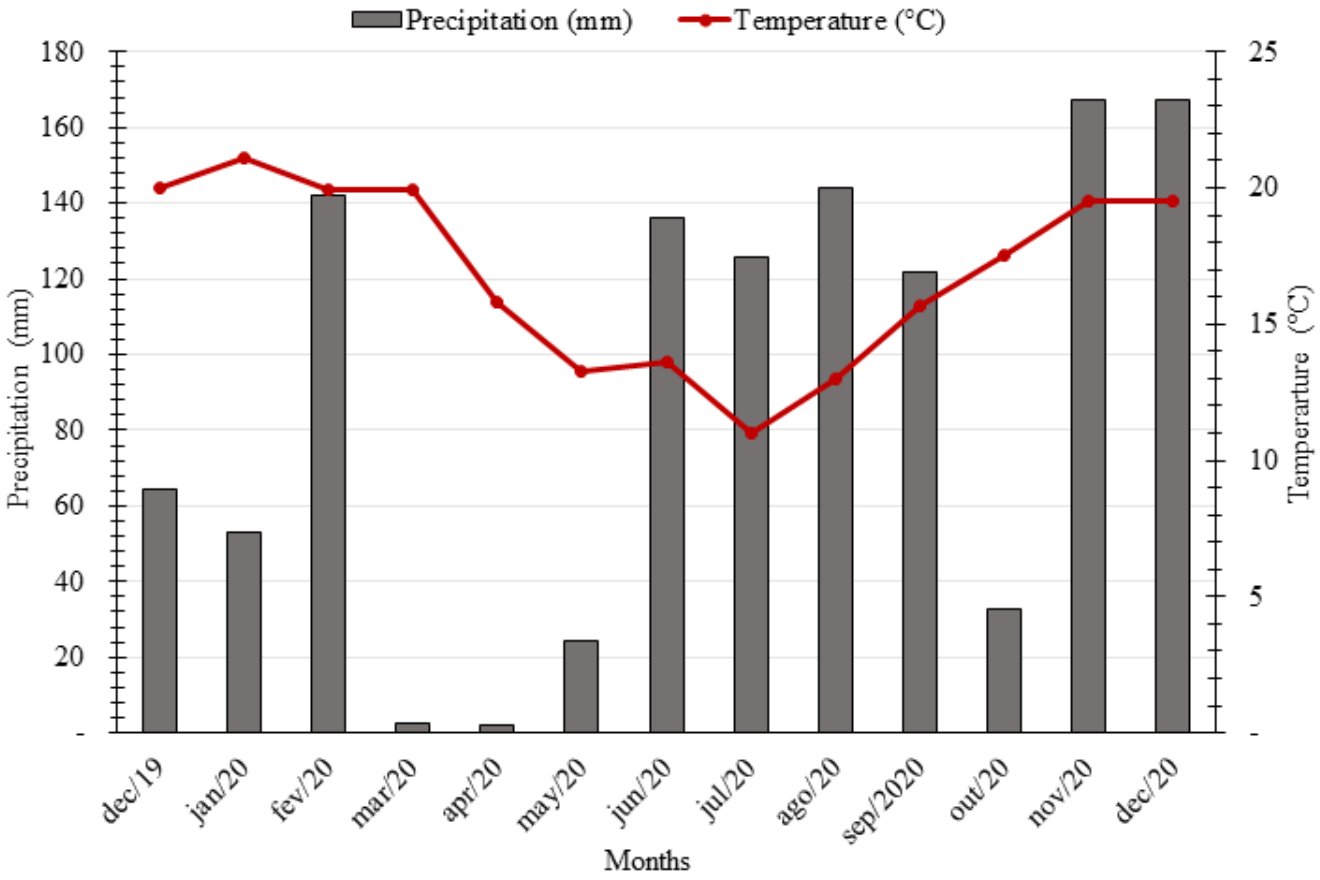
C1 to C4

## APPENDIX A – CHAPTER II

APPENDIX A1 - Sites located at Santa Catarina State, Brazil. Lages city is represented in black, and Grassland management system are shown: Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP).



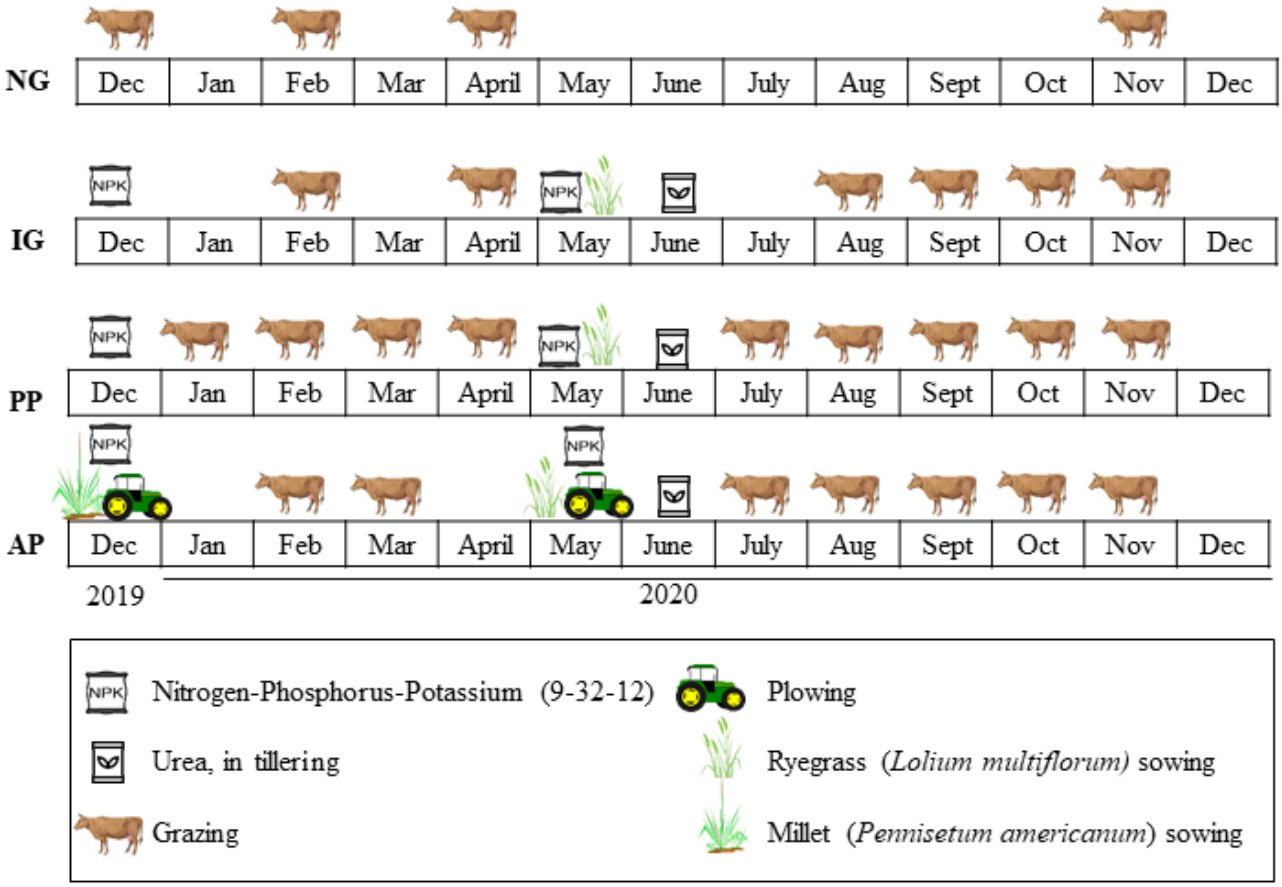
APPENDIX A2- Precipitation and temperature in Lages, SC, Brazil, from December 2019 to December 2020.



APPENDIX A3 - Studies site geographic coordinates. Samples were collected in four grassland management system: Natural grassland (NG), Improved-natural grassland (IG); Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP) in areas belonging to the Atlantic Rainforest Biome, Santa Catarina State, Brazil.

| Grassland | Paddocks | Latitude | Longitude | Elevation (m) |
|---|---|---|---|---|
| NG | 1 | 27º 48’ 13.2” S | 50º 19’ 55.3” W | 938 |
| | 2 | 27º 48’ 14.6” S | 50º 19’ 55.5” W | 932 |
| | 3 | 27º 48’ 12.3” S | 50º 19’ 53.7” W | 929 |
| | 4 | 27º 48’ 10.9” S | 50º 19’ 55.6” W | 933 |
| IG | 1 | 27º 47’ 52.4” S | 50º 19’ 41.7” W | 911 |
| | 2 | 27º 47’ 53.3” S | 50º 19’ 42.3” W | 912 |
| | 3 | 27º 47’ 53.9” S | 50º 19’ 43.0” W | 913 |
| | 4 | 27º 47’ 54.4” S | 50º 19’ 43.8” W | 915 |
| PP | 1 | 27º 47’ 49.4” S | 50º 19’ 28.2” W | 902 |
| | 2 | 27º 47’ 48.4” S | 50º 19’ 27.6” W | 902 |
| | 3 | 27º 47’ 47.8” S | 50º 19’ 27.1” W | 904 |
| | 4 | 27º 47’ 47.0” S | 50º 19’ 26.7” W | 904 |
| AP | 1 | 27º 48’ 00.3” S | 50º 19’ 49.3” W | 928 |
| | 2 | 27º 47’ 59.8” S | 50º 19’ 48.4” W | 921 |
| | 3 | 27º 47’ 59.3” S | 50º 19’ 47.7” W | 922 |
| | 4 | 27º 47’ 58.5” S | 50º 19’ 47.0” W | 920 |

APPENDIX A4- Management of grassland systems from December 2019 to December 2020: Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP).



APPENDIX A5 - List of frequency of forage plant species cataloged in grassland management system: Natural grassland (NG), Improved-natural grassland (IG); Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP) estimated by BOTANAL method.

| Forage plant species | NG | IG | PP | AP | NG | IG | PP | AP |
|---|---|---|---|---|---|---|---|---|
| | Summer | | | | Winter | | | |
| *Achyrocline satureioides* | 0.00 | 0.50 | 0.00 | 0.00 | 7.19 | 0.37 | 0.00 | 0.00 |
| *Andropogon lateralis* (Nees) | 59.29 | 14.70 | 0.00 | 0.00 | 49.18 | 11.81 | 0.00 | 0.00 |
| *Anthoxanthum odaoratum* (L.) | 0.81 | 0.52 | 0.00 | 0.00 | 3.91 | 1.12 | 0.00 | 0.00 |
| *Apium leptophyllum* (Pers.) | 0.00 | 0.55 | 0.00 | 0.00 | 0.00 | 0.46 | 0.00 | 0.00 |
| *Axonopus affins* (Chase) | 7.97 | 10.05 | 0.00 | 0.00 | 6.04 | 1.78 | 0.00 | 0.00 |
| *Axonopus compressus* (Sw.) | 0.99 | 0.00 | 0.00 | 0.00 | 3.38 | 7.14 | 0.00 | 0.00 |
| *Axonopus siccus* (Nees) | 0.30 | 0.00 | 0.00 | 0.00 | 5.07 | 0.00 | 0.00 | 0.00 |
| *Baccharis tridentata* (Vahl) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 20.89 | 0.00 | 10.28 |
| *Centella asiatica* (L.) | 1.45 | 0.49 | 0.00 | 0.00 | 0.73 | 0.41 | 0.00 | 0.00 |
| *Cerastium glomeratum* (Thuill.) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Chascolytrum subaristatum* (Lam.) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.46 | 0.00 | 0.00 |
| *Coelorachis selloana* (Hack.) | 0.49 | 0.20 | 0.00 | 0.00 | 0.42 | 0.42 | 0.00 | 0.00 |
| *Conyza bonariensis* (L.) | 0.00 | 0.00 | 0.66 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Cynodon dactylon* (L.) *cv Jiggs* | 0.00 | 0.00 | 88.75 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Cyperus rotundus* (L.) | 0.00 | 3.09 | 0.00 | 0.00 | 0.00 | 0.41 | 0.00 | 0.00 |
| *Cyperus* spp. | 0.83 | 1.08 | 0.00 | 0.00 | 2.10 | 1.21 | 0.00 | 0.00 |
| *Desmodium incanum* (Sw.) | 0.00 | 0.43 | 0.00 | 0.00 | 0.00 | 0.36 | 0.00 | 0.00 |
| *Dichanthelium sabulorum* (Lam.) | 0.30 | 0.00 | 0.00 | 0.00 | 0.25 | 0.00 | 0.00 | 0.00 |
| *Dichondra sericea* (Sw.) | 0.57 | 0.40 | 0.00 | 0.00 | 0.49 | 0.34 | 0.00 | 0.00 |
| *Diodia brasiliensis* (Spreng.) | 0.00 | 0.55 | 0.00 | 0.00 | 0.00 | 0.46 | 0.00 | 0.00 |
| *Elephantopus mollis* (Kunth) | 0.00 | 0.68 | 0.93 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Erianthus angustifolius* (Nees) | 0.00 | 4.41 | 0.00 | 0.00 | 0.00 | 0.43 | 0.00 | 0.00 |
| *Festuca arundinacea* (Schreb.) | 0.00 | 0.47 | 0.00 | 0.00 | 2.96 | 0.39 | 0.47 | 0.00 |
| *Galium humile* (Cham. & Schltdl.) | 0.69 | 0.40 | 0.00 | 0.00 | 0.59 | 0.34 | 0.00 | 0.00 |
| *Gamochaeta americana* (Mill.) | 0.49 | 0.68 | 0.00 | 0.00 | 0.42 | 0.57 | 0.00 | 0.00 |
| *Holcus lanatus* (L.) | 1.01 | 5.68 | 0.00 | 0.00 | 0.87 | 7.34 | 8.63 | 0.00 |
| *Hypochaeris catharinensis* (Cabrera) | 0.00 | 0.00 | 0.00 | 0.00 | 2.11 | 0.57 | 0.00 | 7.71 |
| *Hypoxis decumbens* (L.) | 0.42 | 0.55 | 0.00 | 0.00 | 0.36 | 0.46 | 0.00 | 0.00 |
| *Lolium multiflorum* (Lam.) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 18.92 | 47.30 | 74.84 |
| *Lotus uliginosus* (Schkuhr) | 0.00 | 1.13 | 0.00 | 0.00 | 0.00 | 0.31 | 0.00 | 0.00 |
| *Oxalis* spp. | 0.00 | 0.00 | 0.87 | 0.00 | 0.00 | 0.57 | 0.65 | 0.00 |
| *Paspalum lepton* (Schult) | 0.78 | 11.73 | 0.00 | 0.00 | 3.45 | 0.40 | 0.00 | 0.00 |
| *Paspalum nicorae* (Parodi) | 0.42 | 8.59 | 0.00 | 0.00 | 0.36 | 0.00 | 0.00 | 0.00 |
| *Paspalum notatum* (Flüggé) | 14.19 | 7.17 | 0.00 | 0.00 | 3.44 | 0.47 | 0.00 | 0.00 |
| *Paspalum umbrosum* (Trin) | 0.00 | 7.39 | 1.48 | 0.00 | 0.00 | 0.45 | 1.04 | 0.00 |
| *Pennisetum glaucum* | 0.00 | 0.00 | 0.00 | 99.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Pfaffia tuberosa* (Spreng.) | 0.00 | 0.68 | 0.00 | 0.00 | 0.00 | 0.57 | 0.00 | 0.00 |
| *Piptochaetium montevidense* (Spreng.) | 5.15 | 0.60 | 0.00 | 0.00 | 2.30 | 0.50 | 0.00 | 0.00 |
| *Plantago major* (L.) | 1.01 | 0.40 | 0.94 | 0.00 | 0.87 | 0.43 | 0.66 | 0.00 |
| *Pluchea sagittalis* (Lam.) | 0.69 | 1.55 | 0.00 | 0.00 | 0.59 | 0.46 | 0.00 | 0.00 |
| *Poaceae* spp. | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.46 | 0.00 | 0.00 |
| *Rumex* spp. | 0.00 | 0.55 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Schizachyrium tenerum* (Nees) | 0.30 | 0.00 | 0.00 | 0.00 | 0.25 | 0.00 | 0.00 | 0.00 |
| *Setaria parviflora* (Poir.) | 0.00 | 0.89 | 0.66 | 0.95 | 0.00 | 0.18 | 0.47 | 0.75 |
| *Sida spp.* | 0.00 | 0.45 | 0.77 | 0.00 | 0.00 | 6.69 | 0.54 | 0.00 |
| *Sorgastrum setosum* | 0.00 | 0.00 | 0.00 | 0.00 | 1.08 | 0.00 | 0.00 | 0.00 |
| *Stachytarpheta cayennensis* (Rich.) | 0.00 | 7.29 | 1.15 | 0.00 | 0.00 | 3.95 | 0.81 | 0.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Steinchisma hians* (Elliott) | 0.00 | 0.51 | 0.00 | 0.00 | 0.00 | 0.43 | 0.00 | 0.00 |
| *Trifolium pratense* (L.) | 0.00 | 2.27 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Trifolium repens* (L.) | 0.99 | 3.36 | 3.79 | 0.00 | 0.85 | 5.98 | 14.78 | 6.42 |
| *Trifolium riograndense Burkart* | 0.86 | 0.00 | 0.00 | 0.00 | 0.74 | 0.00 | 0.00 | 0.00 |
| *Vicia sativa L.* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.32 | 24.64 | 0.00 |

APPENDIX A6 - Samples sequencing through 16S RNA gene. Sample name, Identification (ID), Number of sequencing reads, sequence length, and percentages after quality control on *Metagenomic Rapid Annotations using Subsystems Technology (*MG-RAST) pipeline. Samples: Natural grassland (NG), Improved-natural grassland (IG); Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP), for summer and winter season.

| Season | Grassland | Sample | ID MG-RAST | Number of sequences reads | Mean sequence length |
|---|---|---|---|---|---|
| Summer | NG | PN-P1-C1 | 7ea55cff3a6d676d343931363638392e33 | 39,329 | 450 ± 19 bp |
| | | PN-P2-C1 | d44fd78ede6d676d343931363638372e33 | 45,305 | 450 ± 19 bp |
| | | PN-P3-C1 | 45ea82166e6d676d343931363639302e33 | 40,108 | 446 ± 29 bp |
| | | PN-P4-C1 | ad963354d76d676d343931363639372e33 | 48,330 | 448 ± 22 bp |
| | IG | PM-P1-C1 | 3c56cca0276d676d343931363734372e33 | 40,439 | 454 ± 14 bp |
| | | PM-P2-C1 | e80de211156d676d343931363639382e33 | 37,051 | 453 ± 16 bp |
| | | PM-P3-C1 | 953f6b7e1a6d676d343931363639332e33 | 39,807 | 455 ± 12 bp |
| | | PM-P4-C1 | 7b3470b11f6d676d343931363639362e33 | 37,942 | 449 ± 18 bp |
| | PP | PP-P1-C1 | e6c4d379b96d676d343931363639352e33 | 42,917 | 451 ± 22 bp |
| | | PP-P2-C1 | 9cc1519e706d676d343931363639322e33 | 41,917 | 453 ± 20 bp |
| | | PP-P3-C1 | 90cdda37606d676d343931363639312e33 | 41,524 | 452 ± 21 bp |
| | | PP-P4-C1 | 78759e99566d676d343931363638332e33 | 39,429 | 452 ± 18 bp |
| | AP | PC-P1-C1 | 9b2350c0536d676d343931363639342e33 | 37,445 | 453 ± 18 bp |
| | | PC-P2-C1 | 00fafe987a6d676d343931363638362e33 | 36,101 | 453 ± 21 bp |
| | | PC-P3-C1 | 30abbe38236d676d343931363638382e33 | 43,009 | 452 ± 19 bp |
| | | PC-P4-C1 | 83ed0554e16d676d343931363638352e33 | 43,567 | 453 ± 17 bp |
| Winter | NG | PN-P1-C2 | 8acf84ff046d676d343931363735302e33 | 47,365 | 451 ± 20 bp |
| | | PN-P2-C2 | cce5d468d36d676d343931363734362e33 | 32,687 | 448 ± 23 bp |
| | | PN-P3-C2 | | | |
| | | PN-P4-C2 | 63decb89ec6d676d343931363734322e33 | 39,147 | 450 ± 21 bp |
| | IG | PM-P1-C2 | fa28599c546d676d343931363733382e33 | 40,439 | 454 ± 14 bp |
| | | PM-P2-C2 | 007206df936d676d343931363735312e33 | 31,717 | 454 ± 12 bp |
| | | PM-P3-C2 | a1a388c4d86d676d343931363734312e33 | 39,807 | 455 ± 12 bp |
| | | PM-P4-C2 | 0ca7e415466d676d343931363734352e33 | 43,154 | 453 ± 20 bp |
| | PP | PP-P1-C2 | 105886194c6d676d343931363733392e33 | 41,618 | 452 ± 18 bp |
| | | PP-P2-C2 | 06fcb80c9d6d676d343931363734302e33 | 39,856 | 453 ± 18 bp |
| | | PP-P3-C2 | 95ce5b5bdf6d676d343931363734332e33 | 39,213 | 453 ± 18 bp |
| | | PP-P4-C2 | 1e5c13631d6d676d343931363735322e33 | 45,546 | 454 ± 18 bp |
| | AP | PC-P1-C2 | fdb49e531f6d676d343931363734382e33 | 46,416 | 453 ± 17 bp |
| | | PC-P2-C2 | 2143a49ae06d676d343931363733372e33 | 42,643 | 454 ± 19 bp |
| | | PC-P3-C2 | f35520e51c6d676d343931363734392e33 | 38,320 | 452 ± 19 bp |
| | | PC-P4-C2 | f91a157de56d676d343931363734342e33 | 22,052 | 453 ± 18 bp |

APPENDIX A7 - Soil physical-chemical characteristics from the 0-10 cm profile from four grassland management system: Natural grassland (NG), Improved-natural grassland (IG); Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP), for summer and winter season. Mean ± standard deviation. Rows followed by different letters differ significantly (Tukey's HSD test, $P < 0.05$) among pasture systems.

| Soil attributes | NG | IG | PP | AP | *P* |
|---|---|---|---|---|---|
| **pH** | 5.25±0.1b | 6.2±0.1a | 6.13±0.6a | 6.15±0.2a | <0.001 |
| **H+Al** | 11.38±0.7a | 2.71±0.2b | 2.58±0.1b | 3.15±0.4b | <0.001 |
| **BS** | 14.75±1.7b | 85.29±1.7a | 83.11±1.0a | 81.52±2.7a | <0.001 |
| **OC** | 2.84±0.4 | 2.45±0.1 | 1.92±0.2 | 3.47±0.7 | 0.080 |
| **Ca/Mg** | 0.67±0.2b | 1.33±0.1a | 1.66±0.1a | 1.48±0.0a | <0.001 |
| **OM** | 4.90±0.7 | 4.22±0.2 | 3.21±0.4 | 4.42±0.7 | 0.195 |
| **P** | 10.20±1.4b | 18.07±2.0a | 16.21±1.5ab | 15.61±2.5ab | 0.033 |
| **K** | 0.33±0.0 | 0.37±0.0 | 0.31±0.0 | 0.31±0.0 | 0.566 |
| **Ca** | 0.66±0.1b | 9.00±0.6a | 7.85±0.7a | 8.13±0.4a | <0.001 |
| **Mg** | 0.92±0.1c | 6.79±0.4a | 4.76±0.3b | 5.64±0.3b | <0.001 |
| **Al** | 1.41±0.1a | 0.00±0.0b | 0.00±0.0b | 0.00±0.0b | <0.001 |
| **CEC** | 3.33±0.2c | 16.18±0.9a | 12.93±0.7b | 14.08±0.7ab | <0.001 |
| **$NH_4^+$** | 6.39±0.8b | 9.61±1.3ab | 8.14±0.8ab | 10.72±1.3a | 0.036 |
| **$NO_2^- + NO_3^-$** | 4.07±0.6b | 5.31±1.0b | 12.58±0.5b | 36.09±9.4a | <0.001 |
| **TOC** | 58.24±6.0ab | 70.85±9.6a | 38.69±2.2b | 56.31±3.7ab | 0.007 |
| **TN** | 3.37±0.2 | 3.52±0.2 | 3.68±0.4 | 3.6±0.2 | 0.820 |
| **MBC** | 0.34±0.0 | 0.49±0.1 | 0.19±0.0 | 0.5±0.2 | 0.050 |
| **SMR** | 2.10±0.2 | 2.76±0.3 | 2.83±0.3 | 2.45±0.4 | 0.346 |
| **Porosity** | 0.64±0.1 | 0.68±0.1 | 0.57±0.1 | 0.64±0.1 | 0.536 |
| **MiP** | 0.51±0.0 | 0.39±0.0 | 0.45±0.1 | 0.39±0.1 | 0.199 |
| **MaP** | 0.14±0.0 | 0.27±0.1 | 0.12±0.0 | 0.25±0.1 | 0.099 |
| **BioP** | 0.07±0.0a | 0.06±0.0ab | 0.04±0.0b | 0.04±0.0b | <0.001 |
| **BD density** | 1.17±0.0b | 1.12±0.0b | 1.39±0.0a | 1.22±0.0b | <0.001 |
| **Sandy** | 34.96±0.8ab | 29.46±2.4b | 42.89±2.1a | 32.82±3.1b | 0.002 |
| **Clay** | 36.65±1.4a | 34.50±1.0a | 26.04±0.4b | 34.09±2.0a | <0.001 |
| **Silt** | 28.38±1.9 | 36.03±1.5 | 31.06±2.4 | 33.08±2.0 | 0.064 |

Units: pH= -log[H+ mol $L^{-1}$]; potential acidity = [H+Al] mEq 100 $g^{-1}$; Base Saturation= Mg+Ca+K; Organic Carbon= %; Calcium and Magnesium ratio= Ca/Mg; Organic Matter=%; Phosphorus= $mg.dm^{-3}$; Potassium = $mg.dm^{-3}$; Calcium = $cmol_c\ dm^{-3}$; Magnesium = $cmol_c\ dm^{-3}$; Aluminium= $cmol_c\ dm^{-3}$; Cation exchange capability= $cmol_c\ dm^{-3}$ ; Ammonium= $mg.dm^{-3}$ ; Nitrite+Nitrate = $mg.dm^{-3}$; Total organic carbon = $mg.g^{-1}$; Total nitrogen= mg $g^{-1}$; Microbial biomass Carbon= $mg.kg^{-1}$; Soil microbial respiration= mg $C\text{-}CO_2.kg^{-1}$ $soil.h^{-1}$; Total porosity= $cm^3\ cm^{-3}$; Microporosity= $cm^3\ cm^{-3}$ ; Macroporosity = $cm^3\ cm^{-3}$ ; Biopores =$cm^3\ cm^{-3}$; Bulk soil density= $g.cm^{-3}$; Sand =%; Clay= %; Silt =%.

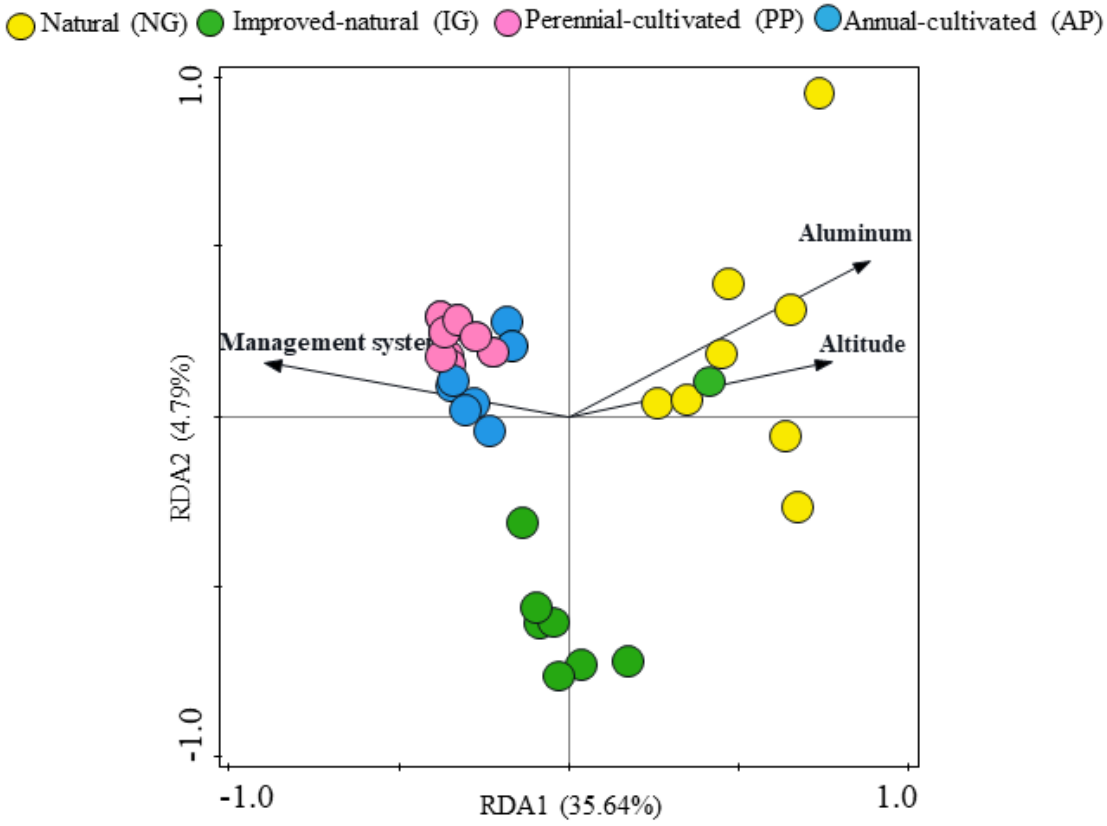
APPENDIX A8 - Vegetation characteristics from four grassland management systems: Natural grassland (NG), Improved-natural grassland (IG); Perennial-cultivated pasture (PP) and Annual-cultivated pasture (AP), for summer and winter season. Mean ± standard deviation. Lines followed by different letters differ significantly (Tukey <0.05) among forage systems.

| **Grassland** | **Diversity Index** | | | |
|---|---|---|---|---|
| | **Simpson** | **Pielou** | **Shannon** | **Chao-1** |
| | ***Summer*** | | | |
| **NG** | 0.47±0.17a | 0.37±0.08b | 0.96±0.27b | 13.50±3.69b |
| **IG** | 0.82±0.01b | 0.63±0.04a | 1.97±0.10a | 23.50±4.43a |
| **PP** | 0.89±0.08c | 0.11±0.07c | 0.20±0.14c | 5.50±2.08c |
| **AP** | 0.00±0.00c | 0.00±0.00c | 0.00±0.00c | 1.00±0.00c |
| ***P*** | <0.001 | <0.001 | <0.001 | <0.001 |
| | ***Winter*** | | | |
| **NG** | 0.42±0.10ab | 0.38±0.07 | 0.94±0.22ab | 12.00±3.26a |
| **IG** | 0.71±0.11a | 0.59±0.11 | 1.50±0.34a | 12.75±1.7a |
| **PP** | 0.26±0.28bc | 0.39±0.04 | 0.47±0.48bc | 3.25±0.50b |
| **AP** | 0.07±0.04c | 0.17±0.11 | 0.18±0.98c | 3.50±1.29b |
| ***P*** | <0.001 | 0.119 | <0.001 | <0.001 |

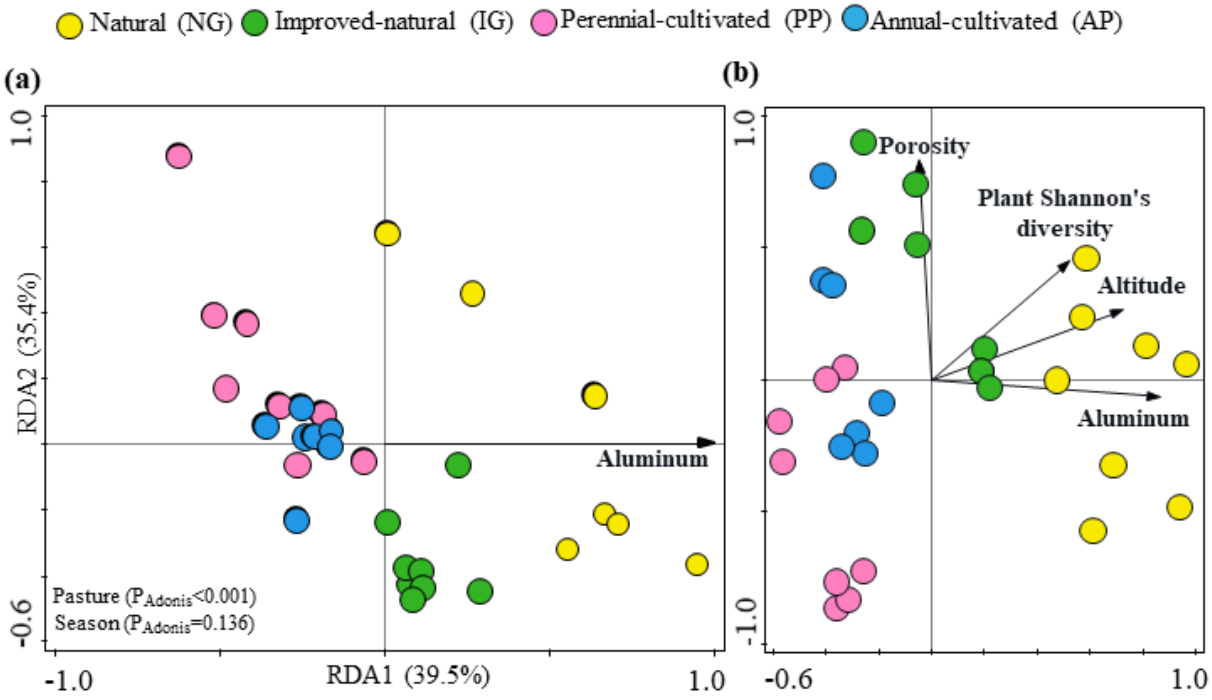
APPENDIX A9 - Production of dry mass (DM) and Carbon/Nitrogen ratio from four grassland system management: Natural grassland (NG), Improved-natural grassland (IG); Perennial-cultivated pasture (PP) and Annual-cultivated pasture (AP), for summer and winter season. Mean ± standard deviation. Lines followed by different letters differ significantly (Tukey <0.05) among forage systems.

| **Grassland** | **Production (kg.DM.ha$^{-1}$)** | | **Carbo/Nitrogen ratio** | |
|---|---|---|---|---|
| | ***Summer*** | ***Winter*** | ***Summer*** | ***Winter*** |
| **NG** | 4543±1841a | 2771±2112a | 98.71±7.24a | 30.63±3.30a |
| **IG** | 2784±670ab | 2183±259a | 69.50±6.78b | 12.13±0.85b |
| **PP** | 3580±830a | 1602±308a | 36.89±5.78c | 11.84±1.35b |
| **AP** | 1203±446b | 1596±192a | 35.89±17.05c | 9.07±1.91b |
| ***P*** | 0.006 | 0.394 | <0.001 | <0.001 |

APPENDIX A10- Distance-based redundancy analysis (db-RDA) of soil microbial communities at the OTU level. Plots were generated using Euclidean distance matrices with 1000 Monte-Carlo permutations. Vectors represent environmental variables, which were forward selected ($P_{adjusted} < 0.05$). Samples are colored as follows: Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated (PP), and Annual cultivated (AP).



APPENDIX A11- Soil microbial communities at the phylum level (a) distance-based redundancy analysis (db-RDA), and (b) p-RDA. Plots were generated using Euclidean distance matrices with 1000 Monte-Carlo permutations. Vectors represent environmental variables, which were forward selected ($P_{adjusted} < 0.05$). Samples are colored as follows: Natural grassland (NG), Improved-natural grassland (IG), Perennial cultivated (PP), and Annual-cultivated (AP).



APPENDIX A12 - Variation partitioning of redundancy analysis (pRDA) generated by principal coordinates of neighbor matrices (PCNM) with a forward selection of explanatory variables generated from Euclidean distance matrices, with1000 Monte-Carlo permutations and corrected by Benjamini Hochberg false discovery rate approach (FDR). Data show the adjusted coefficient of multiple determination (R2) from simple effects of environmental variables, geography +time, and their interactions. For OTU level partitioning of variances considered 3 groups (Biotic, abiotic, and geographic+time). For phylum level and Function (FAPROTAX) partitioning of variances considered 2 groups (Environment = Biotic+abiotic, and geographic+time).

| | **Environment** | | ***P*** | **Geographic+time** | ***P*** | **Overlap** | ***P*** | **Residual** |
|---|---|---|---|---|---|---|---|---|
| | **Biotic** | **Abiotic** | | | | | | |
| OTU | 0.20% | 8.30% | 0.001 | 1.60% | 0.001 | 27.90% | 0.001 | 62.00% |
| Phyla | 31.71% | | 0.001 | 2.37% | 0.001 | 23.43% | 0.001 | 42.56% |
| Functions | 8.10% | | 0.001 | 1.20% | 0.001 | 33.60% | 0.001 | 57.10% |

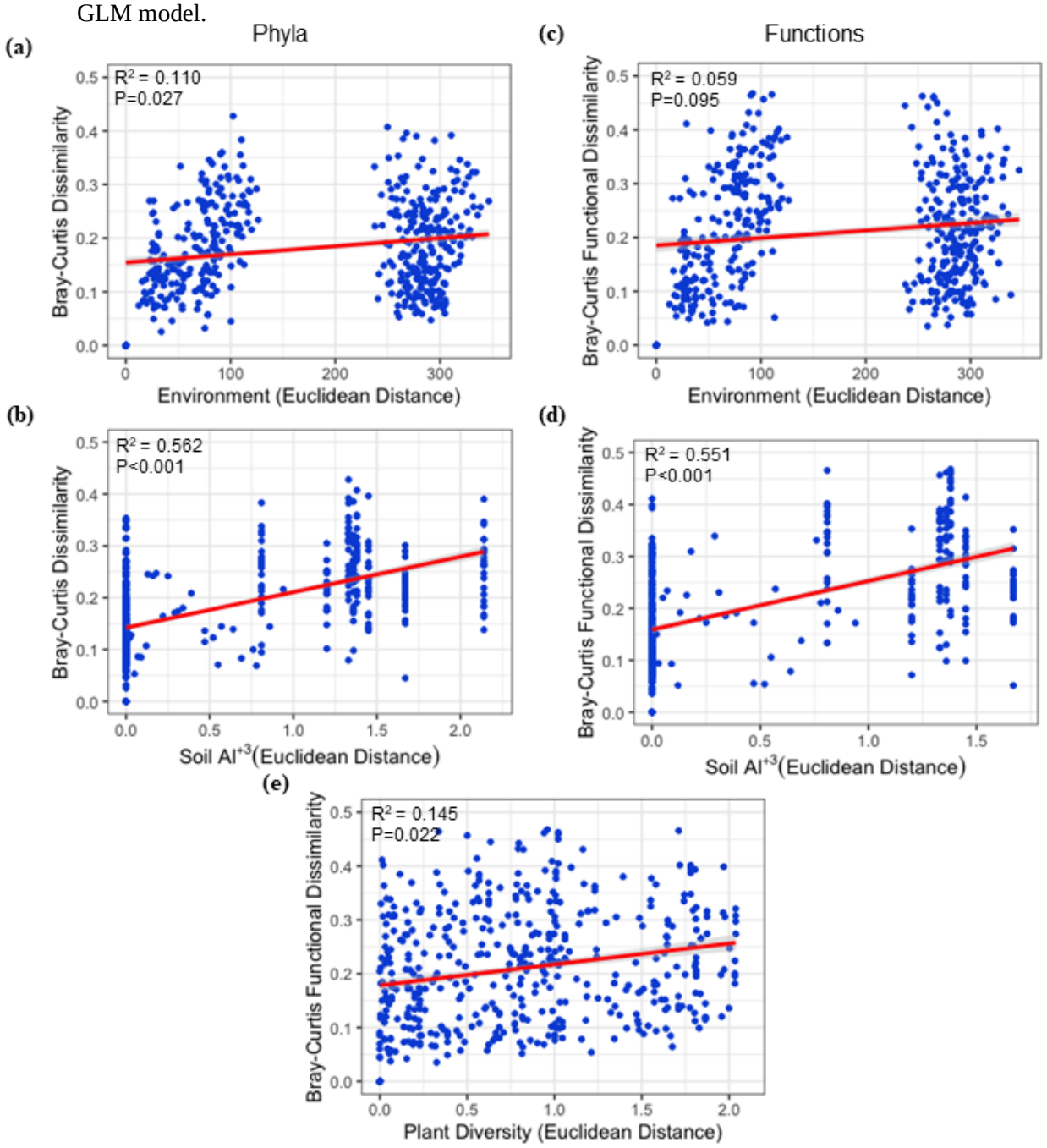
APPENDIX A13 - Relative abundance of de all phyla found by 16S RNA gene sequencing for the summer and winter season. Soil sample collected Natural grassland (NG), Improved-natural grassland (IG), Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP). The phyla in bold showed a significant difference for the Kruskal-Wallis median test ($P < 0.05$).

| **Phylum** | **Grassland management system** | | | | ***P*** |
|---|---|---|---|---|---|
| | **NG** | **IG** | **PP** | **AP** | |
| Crenarchaeota | 0.001±0.00 | 0.000±0.00 | 0.000±0.00 | 0.000±0.00 | 0.5846 |
| Euryarchaeota | 0.002±0.01b | 0.003±0.01b | 0.028±0.02a | 0.015±0.03ab | 0.0111 |
| Acidobacteria | 34.550±7.20a | 24.850±2.1b | 17.480±3.6c | 19.970±0.9bc | <0.0001 |
| Actinobacteria | 11.330±3.05b | 12.426±2.79b | 18.750±5.14a | 14.542±3.21ab | 0.0093 |
| Armatimonadetes | 0.130±0.04 | 0.131±0.10 | 0.117±0.05 | 0.124±0.06 | 0.6412 |
| BRC1 | 0.000±0.00 | 0.013±0.02 | 0.016±0.02 | 0.014±0.014 | 0.0919 |
| Bacteroidetes | 1.294±0.65b | 3.625±1.48a | 4.271±1.82a | 5.001±0.96a | 0.0007 |
| Chlamydiae | 0.094±0.06 | 0.135±0.07 | 0.087±0.03 | 0.078±0.04 | 0.2521 |
| Chloroflexi | 9.164±3.43 | 7.255±4.11 | 8.498±2.56 | 6.129±0.91 | 0.0181 |
| Cyanobacteria | 0.118±0.05 | 0.133±0.08 | 0.125±0.08 | 0.135±0.07 | 0.9403 |
| Deferribacteres | 0.000±0.00 | 0.003±0.01 | 0.000±0.00 | 0.003±0.01 | 0.5301 |
| Dependentiae | 0.050±0.03b | 0.070±0.03ab | 0.143±0.11a | 0.075±0.03ab | 0.0335 |
| Elusimicrobia | 0.131±0.06b | 0.226±0.05a | 0.223±0.06a | 0.246±0.07a | 0.0146 |
| Entotheonellaeota | 0.002±0.00b | 0.029±0.05ab | 0.078±0.06a | 0.021±0.02b | 0.0011 |
| FBP | 0.000±0.00 | 0.009±0.01 | 0.019±0.02 | 0.009±0.01 | 0.0989 |
| FCPU426 | 0.021±0.01 | 0.007±0.01 | 0.018±0.02 | 0.010±0.01 | 0.2023 |
| Fibrobacteres | 0.034±0.04 | 0.097±0.11 | 0.092±0.11 | 0.092±0.08 | 0.2948 |
| Firmicutes | 1.635±0.21 | 0.686±0.80 | 2.011±1.42 | 1.466±1.27 | 0.1294 |
| Fusobacteria | 0.009±0.01 | 0.012±0.01 | 0.007±0.01 | 0.008±0.01 | 0.8869 |
| GAL15 | 0.044±0.01a | 0.024±0.02ab | 0.020±0.04ab | 0.000±0.00b | 0.0004 |
| Gemmatimonadetes | 0.617±0.31c | 1.784±0.71b | 4.464±0.27a | 4.196±0.85a | <0.0001 |
| Hydrogenedentes | 0.000±0.00 | 0.000±0.00 | 0.001±0.00 | 0.001±0.00 | 0.5229 |
| Latescibacteria | 0.068±0.06c | 0.954±0.41b | 1.578±0.39a | 1.307±0.53ab | 0.0003 |
| Nitrospirae | 0.131±0.12c | 0.389±0.22bc | 0.759±0.12a | 0.483±0.28b | 0.0004 |
| Omnitrophicaeota | 0.000±0.00 | 0.000±0.00 | 0.004±0.01 | 0.001±0.00 | 0.0772 |
| Patescibacteria | 0.236±0.07b | 0.751±0.24a | 1.029±0.34a | 1.064±0.34a | 0.0003 |
| Planctomycetes | 4.589±0.89 | 4.635±0.51 | 4.174±0.58 | 4.419±0.36 | 0.6248 |
| αProteobacteria | 15.050±0.99ab | 16.313±1.59a | 13.597±1.06b | 15.461±2.46ab | 0.0114 |
| δProteobacteria | 2.748±0.64b | 5.198±1.38a | 5.686±1.03a | 5.815±1.29a | 0.0015 |
| γProteobacteria | 5.323±1.41b | 9.029±2.16a | 9.311±2.66a | 11.605±2.05a | 0.0010 |
| Otherproteobacteria | 0.000±0.00 | 0.001±0.00 | 0.005±0.01 | 0.001±0.01 | 0.2164 |
| Rokubacteria | 0.021±0.02b | 0.213±0.14b | 0.735±0.32a | 0.341±0.35b | 0.0002 |
| Spirochaetes | 0.000±0.00 | 0.002±0.01 | 0.004±0.00 | 0.005±0.01 | 0.1678 |
| Verrucomicrobia | 11.658±2.97a | 10.281±2.59a | 5.876±1.31b | 6.694±0.79b | <0.0001 |
| WPS | 0.765±0.18a | 0.179±0.22b | 0.015±0.01b | 0.032±0.02b | <0.0001 |
| WS2 | 0.000±0.00b | 0.034±0.03a | 0.016±0.01ab | 0.033±0.02a | 0.0039 |
| WS4 | 0.009±0.01 | 0.002±0.00 | 0.001±0.00 | 0.000±0.00 | 0.1869 |

APPENDIX A14 - The 10 OTUs with the highest betweenness centrality (BC) and number of correlations (that is, degree) for each factor.

| | OTU ID | Classification | BC | Degree |
|---|---|---|---|---|
| **Natural** | OTU_71 | Acidobacteria – Pyrinomonadaceae | 21410.2 | 37 |
| | OTU_152 | Chloroflexi – Ktedonobacterales | 17442.1 | 39 |
| | OTU_7 | Verrucomicrobia – *C. udaeobacter* | 16723.8 | 23 |
| | OTU_173 | Verrucomicrobia – *C. xiphinematobacter* | 15984.6 | 20 |
| | OTU_16 | Verrucomicrobia – *C. xiphinematobacter* | 15895.6 | 17 |
| | OTU_614 | Chloroflexi - unclassified | 15303.0 | 9 |
| | OTU_75 | Verrucomicrobia – *C. udaeobacter* | 15045.9 | 43 |
| | OTU_14 | Proteobacteria – Myxococcales | 13422.1 | 8 |
| | OTU_213 | Acidobacteria – Acidobacteriales | 11165.3 | 4 |
| | OTU_39 | Actinobacteria – Micromonosporaceae | 10723.4 | 9 |
| **Improved-natural** | OTU_5 | Proteobacteria – Xanthobacteraceae | 63758.3 | 56 |
| | OTU_123 | Acidobacteria – Subgroup 6 | 56074.4 | 13 |
| | OTU_24 | Verrucomicrobia – *C. udaeobacter* | 36701.9 | 22 |
| | OTU_61 | Acidobacteria – *Edaphobacter* | 36095.3 | 10 |
| | OTU_21 | Chloroflexi – Ktedonobacterales | 31986.8 | 12 |
| | OTU_511 | Acidobacteria – Solibacteraceae | 30598.3 | 35 |
| | OTU_16 | Verrucomicrobia – *C. xiphinematobacter* | 27411.8 | 58 |
| | OTU_3 | Proteobacteria – Xanthobacteraceae | 27378.4 | 27 |
| | OTU_4 | Acidobacteria – Subgroup 2 | 26040.2 | 118 |
| | OTU_10 | Acidobacteria – *Occallatibacter* | 22998.4 | 71 |
| **Perennial-cultivated** | OTU_450 | Actinobacteria – *Agromyces* | 37517.0 | 6 |
| | OTU_426 | Actinobacteria – unclassified | 37114.5 | 34 |
| | OTU_1800 | Verrucomicrobia – *C. udaeobacter* | 35556.5 | 4 |
| | OTU_28 | Gemmatimonadetes – Gemmatimonadaceae | 34921.6 | 15 |
| | OTU_497 | Acidobacteria – Blastocatellia | 33898.3 | 29 |
| | OTU_122 | Chloroflexi – unclassified | 32142.3 | 29 |
| | OTU_149 | Verrucomicrobia – Pedosphaeraceae | 30922.2 | 9 |
| | OTU_455 | Gemmatimonadetes – Gemmatimonadaceae | 30622.2 | 14 |
| | OTU_79 | Actinobacteria – Gaiellales | 28896.3 | 8 |
| | OTU_294 | Proteobacteria – Rhizobiales | 28330.7 | 39 |
| **Annual-cultivated** | OTU_13 | Actinobacteria – *Acidothermus* | 44704.5 | 11 |
| | OTU_95 | Proteobacteria – Gammaproteobacteria | 41740.6 | 23 |
| | OTU_65 | Acidobacteria – Acidobacteriales | 34842.8 | 6 |
| | OTU_193 | Actinobacteria – *Ilumatobacter* | 33468.9 | 16 |
| | OTU_252 | Proteobacteria – Micropepsaceae | 33241.7 | 30 |
| | OTU_262 | Acidobacteria – Subgroup 6 | 31909.5 | 34 |
| | OTU_344 | Latescibacteria – unclassified | 31455.8 | 16 |
| | OTU_701 | Planctomycetes – Tepidisphaerales | 28270.0 | 29 |
| | OTU_57 | Acidobacteria – Pyrinomonadaceae | 27971.6 | 13 |
| | OTU_1 | Proteobacteria - *Bradyrhizobium* | 27348.9 | 44 |

APPENDIX A15- Mantel patterns among pairs of microbial communities, within neighborhoods from 0.0 to 1200 m, across grassland management system, in the Brazilian Atlantic Biome, Southern Brazil. (a) Mantel patterns of all environmental attributes for Phylum level; (b) Partial Mantel of Aluminum for Phylum level and (c) Mantel patterns of all environmental attributes for functions; (d) Partial Mantel of Aluminum Partial Mantel of soil density for functions; (e) Partial Mantel of Plant Shannon's *H'* for functions. The x-axis represents the environmental distance (Euclidean) between pairwise microbial communities at the phylum level and FAPROTAX functions, while the y-axis represents the Bray-Curtis dissimilarity for each pair of microbial communities (blue circles). The red lines represent the fitted GLM model.



APPENDIX A16 - Relative abundance of de all functions found by FAPROTAX for the summer and winter season. Soil sample collected Natural grassland (NG), Improved-natural grassland (IG); Perennial-cultivated pasture (PP), and Annual-cultivated pasture (AP). The phyla in bold showed a significant difference for the Kruskal Wallis median test (P < 0.05).

| **Functions** | **Grassland management systems** | | | | ***P*** |
|---|---|---|---|---|---|
| | **NG** | **IG** | **PP** | **AP** | |
| Aerobic chemoheterotrophy | 33.977±1.93c | 35.634±1.34bc | 37.081±2.25ab | 38.380±1.58a | 0.0023 |
| Aromatic compound degrad. | 0.243±0.25c | 1.812±1.00b | 3.233±0.84a | 2.840±0.93ab | 0.0002 |
| Cellulolysis | 12.913±3.04a | 5.838±5.41ab | 2.427±1.98b | 2.736±2.13b | 0.0003 |
| Chemoheterotrophy | 34.309±2.06c | 36.711±1.69bc | 38.361±2.17ab | 39.488±1.51a | 0.0007 |
| Chitinolysis | 0.000±0.00b | 0.461±0.35ab | 0.796±0.46a | 0.676±0.35a | 0.0014 |
| Chlorate reducers | 0.000±0.00 | 0.000±0.00 | 0.026±0.07 | 0.061±0.10 | 0.2032 |
| Chloroplasts | 0.182±0.13 | 0.292±0.09 | 0.245±0.19 | 0.247±0.08 | 0.3635 |
| Fermentation | 0.463±0.43 | 0.512±0.16 | 0.215±0.16 | 0.236±0.23 | 0.0243 |
| Human pathogens (all) | 0.061±0.13b | 0.421±0.32ab | 0.580±0.46a | 0.289±0.22ab | 0.0296 |
| Hydrocarbon degradation | 0.000±0.00 | 0.108±0.21 | 0.265±0.20 | 0.202±0.26 | 0.0373 |
| Intracellular parasites | 0.566±0.18 | 0.905±0.42 | 0.643±0.31 | 0.563±0.25 | 0.4084 |
| Iron respiration | 1.009±0.69 | 2.324±1.15 | 2.449±1.84 | 1.803±1.20 | 0.0732 |
| Mammal gut | 0.000±0.00 | 0.028±0.08 | 0.000±0.00 | 0.000±0.00 | 0.4113 |
| Methylotrophs | 0.000±0.00 | 0.006±0.01 | 0.004±0.01 | 0.004±0.01 | 0.7702 |
| Nitrate reduction | 0.077±0.16b | 0.433±0.37ab | 0.585±0.51ab | 0.303±0.23ab | 0.0699 |
| Nitrification | 0.032±0.06c | 0.986±0.76bc | 2.749±0.65a | 1.716±1.25ab | <0.0001 |
| Nitrogen fixation | 0.014±0.03 | 0.000±0.00 | 0.000±0.00 | 0.000±0.00 | 0.1145 |
| Nitrogen respiration | 0.011±0.02b | 0.405±034ab | 0.557±0.52a | 0.289±0.24ab | 0.0180 |
| Parasites_or_symbionts | 13.143±5.79a | 6.109±2.63b | 1.593±1.21bc | 1.529±0.86c | <0.0001 |
| Photoautotrophs | 0.371±0.14 | 0.252±0.27 | 0.194±0.07 | 0.227±0.15 | 0.0526 |
| Predatory or exoparasitic | 2.367±1.21b | 5.910±2.35a | 6.686±1.36a | 7.230±1.81a | 0.0017 |
| Resp_of_sulfur_compounds | 0.244±0.27b | 0.692±0.45ab | 0.981±0.55a | 0.852±0.51ab | 0.0167 |
| Respiration of sulfur (S) | 0.000±0.00 | 0.000±0.00 | 0.000±0.00 | 0.008±0.02 | 0.3301 |
| Urease | 0.000±0.00 | 0.022±0.05 | 0.093±0.15 | 0.004±0.01 | 0.2284 |

## APPENDIX B – CHAPTER III

APPENDIX B1- Z-scores of distributions across Pasture system and seasons showing the Z-scores distributions (Z = -2 and +2, respectively; P < 0.05) for habitat Rare, generalist and specialist of natural and specialist of cultivated. Z-scores were generated under null model method 'swap_count' with 10000 simulations. Dispersal rates were calculated by Etienne's formula. Values of dispersal are between 0 and 1, where the higher the value the higher the tendency to migration of members of a local microbial community.

| Sample | **Generalist** | | **Specialist Natural** | | **Specialist Cultivated** | | **Rare** | |
|---|---|---|---|---|---|---|---|---|
| | Z-score | Dispersal rate | Z-score | Dispersal rate | Z-score | Dispersal rate | Z-score | Dispersal rate |
| NG-1-S | -24.85 | 0.011 | 18.36 | 0.014 | -8.35 | 5.6E-05 | 1.95 | 0.051 |
| NG-2-S | | 0.020 | | 0.015 | | 2.7E-02 | | 0.054 |
| NG-3-S | | 0.021 | | 0.019 | | 4.8E-05 | | 0.056 |
| NG-4-S | | 0.015 | | 0.017 | | 8.3E-03 | | 0.055 |
| NG-1-W | 3.44 | 0.021 | 3.06 | 0.018 | 0.2 | 1.8E-02 | 0.93 | 0.060 |
| NG-2-W | | 0.013 | | 0.010 | | 7.8E-03 | | 0.048 |
| NG-3-W | | 0.019 | | 0.016 | | 1.5E-02 | | 0.056 |
| NG-4-W | | 0.009 | | 0.010 | | 1.1E-02 | | 0.057 |
| IG-1-S | 87.64 | 0.015 | -6.51 | 0.011 | 39.07 | 1.3E-02 | 0.08 | 0.055 |
| IG-2-S | | 0.016 | | 0.010 | | 1.1E-02 | | 0.051 |
| IG-3-S | | 0.017 | | 0.012 | | 1.5E-02 | | 0.058 |
| IG-4-S | | 0.000 | | 0.008 | | 3.2E-05 | | 0.049 |
| IG-1-W | 3.48 | 0.014 | -22.16 | 0.010 | -18.48 | 1.2E-02 | -0.16 | 0.049 |
| IG-2-W | | 0.011 | | 0.000 | | 7.6E-03 | | 0.045 |
| IG-3-W | | 0.019 | | 0.011 | | 1.1E-02 | | 0.058 |
| IG-4-W | | 0.024 | | 0.009 | | 2.4E-02 | | 0.054 |
| PP-1-S | 1.97 | 0.015 | -0.93 | 0.013 | 11.92 | 1.2E-02 | -0.35 | 0.053 |
| PP-2-S | | 0.015 | | 0.012 | | 1.5E-02 | | 0.059 |
| PP-3-S | | 0.019 | | 0.009 | | 1.6E-02 | | 0.059 |
| PP-4-S | | 0.012 | | 0.024 | | 1.1E-02 | | 0.051 |
| PP-1-W | -1.17 | 0.017 | 1.24 | 0.012 | 4.29 | 1.3E-02 | 1.53 | 0.059 |
| PP-2-W | | 0.015 | | 0.014 | | 1.6E-02 | | 0.056 |
| PP-3-W | | 0.010 | | 0.008 | | 1.2E-02 | | 0.055 |
| PP-4-W | | 0.014 | | 0.006 | | 1.6E-02 | | 0.058 |
| AP-1-S | 5.55 | 0.016 | -3.83 | 0.013 | -9.54 | 1.2E-02 | 1.34 | 0.050 |
| AP-2-S | | 0.014 | | 0.010 | | 1.1E-02 | | 0.053 |
| AP-3-S | | 0.022 | | 0.024 | | 1.4E-02 | | 0.055 |
| AP-4-S | | 0.019 | | 0.014 | | 1.5E-02 | | 0.057 |
| AP-1-W | -2.04 | 0.015 | 5.93 | 0.009 | 7.27 | 1.6E-02 | 3.49 | 0.067 |
| AP-2-W | | 0.019 | | 0.012 | | 1.7E-02 | | 0.058 |
| AP-3-W | | 0.018 | | 0.026 | | 1.2E-02 | | 0.051 |

APPENDIX B2 - Samples fitting to theoretical ecological models, based on Akaike information criterion (AIC) for rank abundance distributions of OTUs of Overall communities. Across pasture system: Natural Grassland (NG); Improved Natural grassland (IG); Perennial Cultivated Pasture (PP) and Annual Cultivated Pasture (AP) in the summer (S) and winter (W) season. The Rank abundance models based on corrected AIC value from Poisson distributions using maximum likelihood estimation. The lowest AIC value for each sample represented the best-fitted model for general community's assembly. The ZSM (Zero Sum Multinomial) and Broken-stick are null models regarding to theoretical neutral assembly while Preemption (Preemp), Lognormal (LogN) and Zipf are niche-based models regarding to deterministic assembly.

| Sample | **Akaike information criterion (AIC)** | | | | |
|---|---|---|---|---|---|
| | **Overall** | | | | |
| | **Neutral** | | **Niche-based** | | |
| | Null | ZSM | Preemp | LogN | Zipf |
| NG-1-S | 7928.9 | 9889.3 | 11326.0 | 4270.2 | 5332.5 |
| NG-2-S | 7324.5 | 13302.5 | 10170.7 | 4809.5 | 6500.3 |
| NG-3-S | 8094.3 | 10832.1 | 11016.6 | 4309.9 | 6054.9 |
| NG-4-S | 7073.4 | 12409.3 | 9692.3 | 4409.3 | 6539.8 |
| NG-1-W | 5833.9 | 11789.3 | 6851.1 | 5140.8 | 8414.6 |
| NG-2-W | 5808.1 | 11137.5 | 7315.1 | 4817.5 | 7570.9 |
| NG-3-W | 6824.0 | 11358.1 | 9091.3 | 5031.0 | 7530.6 |
| NG-4-W | 5082.0 | 9322.3 | 6865.2 | 5031.0 | 6941.3 |
| IG-1-S | 8890.0 | 15936.7 | 12480.4 | 4920.0 | 6056.1 |
| IG-2-S | 5508.4 | 14339.7 | 7636.0 | 3890.2 | 6359.0 |
| IG-3-S | 6229.3 | 15222.5 | 8842.2 | 4370.5 | 6628.7 |
| IG-4-S | 8219.3 | 12603.5 | 11313.4 | 3750.9 | 5386.6 |
| IG-1-W | 5671.9 | 14511.9 | 6156.3 | 4789.7 | 7895.1 |
| IG-2-W | 4909.7 | 12439.6 | 5545.1 | 4159.8 | 7340.6 |
| IG-3-W | 5774.5 | 15366.2 | 6255.9 | 5135.5 | 8559.3 |
| IG-4-W | 6597.8 | 16845.2 | 6748.2 | 5584.4 | 8723.9 |
| PP-1-S | 6189.9 | 15939.8 | 7524.0 | 5237.3 | 8160.2 |
| PP-2-S | 6302.7 | 17072.7 | 6922.7 | 6922.7 | 8872.5 |
| PP-3-S | 6291.5 | 15878.0 | 7121.5 | 5379.0 | 8397.7 |
| PP-4-S | 5888.7 | 14767.3 | 7602.5 | 4797.4 | 7455.5 |
| PP-1-W | 6480.9 | 15550.7 | 7646.8 | 5111.6 | 7507.8 |
| PP-2-W | 5783.5 | 15627.9 | 6664.1 | 4948.4 | 8020.0 |
| PP-3-W | 6951.3 | 15054.4 | 8670.9 | 5562.1 | 7842.1 |
| PP-4-W | 6776.3 | 17844.3 | 7978.1 | 5707.2 | 8481.1 |
| AP-1-S | 6033.2 | 14869.9 | 6988.1 | 5194.9 | 8253.0 |
| AP-2-S | 6198.7 | 14649.9 | 6320.5 | 5373.0 | 8723.3 |
| AP-3-S | 5453.4 | 15370.7 | 6356.5 | 5033.9 | 8633.0 |
| AP-4-S | 6625.2 | 16674.1 | 6678.6 | 5948.2 | 9527.9 |
| AP-1-W | 6685.4 | 18721.3 | 7219.1 | 5986.1 | 9386.5 |
| AP-2-W | 6773.8 | 17427.6 | 7440.1 | 5788.9 | 8785.8 |
| AP-3-W | 6374.9 | 15410.1 | 7593.2 | 5203.2 | 7812.8 |

APPENDIX B3 - Samples fitting to theoretical ecological models, based on Akaike information criterion (AIC) for rank abundance distributions of OTUs of Generalist communities. Across pasture system: Natural Grassland (NG); Improved Natural grassland (IG); Perennial Cultivated Pasture (PP) and Annual Cultivated Pasture (AP) in the summer (S) and winter (W) season. The Rank abundance models based on corrected AIC value from Poisson distributions using maximum likelihood estimation. The lowest AIC value for each sample represented the best-fitted model for general community's assembly. The ZSM (Zero Sum Multinomial) and Broken-stick are null models regarding to theoretical neutral assembly while Preemption (Preemp), Lognormal (LogN) and Zipf are niche-based models regarding to deterministic assembly.

| Sample | **Akaike information criterion (AIC)** | | | | |
|---|---|---|---|---|---|
| | **Generalist** | | | | |
| | **Neutral** | | **Niche-based** | | |
| | ZSM | Null | Preemp | LogN | Zipf |
| NG-1-S | 1006.7 | 541.8 | 713.0 | 400.1 | 496.9 |
| NG-2-S | 2483.2 | 2729.2 | 3586.1 | 1419.0 | 1118.8 |
| NG-3-S | 1308.4 | 654.6 | 802.2 | 527.7 | 737.7 |
| NG-4-S | 1141.7 | 588.2 | 771.3 | 435.2 | 540.2 |
| NG-1-W | 1536.5 | 757.6 | 910.5 | 562.1 | 686.8 |
| NG-2-W | 1628.0 | 922.1 | 1249.0 | 649.0 | 890.5 |
| NG-3-W | 1674.3 | 1423.5 | 1868.6 | 939.5 | 923.0 |
| NG-4-W | 1038.2 | 1379.5 | 1585.9 | 618.2 | 574.4 |
| IG-1-S | 5762.6 | 2664.4 | 2946.1 | 2075.6 | 3007.5 |
| IG-2-S | 4759.1 | 2209.2 | 2239.0 | 1698.4 | 2524.6 |
| IG-3-S | 4846.1 | 2076.5 | 2323.7 | 1719.9 | 2525.2 |
| IG-4-S | 1729.4 | 553.7 | 729.7 | 366.2 | 417.3 |
| IG-1-W | 5077.6 | 2416.4 | 2104.5 | 1852.2 | 2945.5 |
| IG-2-W | 4657.0 | 2038.3 | 1986.5 | 1739.9 | 3041.6 |
| IG-3-W | 6009.6 | 3173.3 | 2569.6 | 2208.0 | 3292.5 |
| IG-4-W | 5893.6 | 3261.5 | 2615.3 | 2223.0 | 3121.3 |
| PP-1-S | 3247.2 | 1580.5 | 1649.0 | 1231.3 | 1704.8 |
| PP-2-S | 2924.5 | 1245.0 | 1142.7 | 987.8 | 1414.4 |
| PP-3-S | 3056.6 | 1641.4 | 1721.5 | 1193.1 | 1482.2 |
| PP-4-S | 3259.1 | 1562.5 | 2000.1 | 1203.0 | 1742.6 |
| PP-1-W | 3226.8 | 1752.2 | 1958.0 | 1256.7 | 1563.4 |
| PP-2-W | 2803.0 | 1259.3 | 1116.5 | 995.8 | 1497.9 |
| PP-3-W | 2388.8 | 1358.9 | 1568.3 | 991.2 | 1209.1 |
| PP-4-W | 2850.8 | 1210.4 | 1143.2 | 960.8 | 1419.4 |
| AP-1-S | 3795.4 | 1841.6 | 1679.5 | 1378.4 | 2040.6 |
| AP-2-S | 3660.1 | 1867.0 | 1516.6 | 1297.2 | 1907.5 |
| AP-3-S | 4259.5 | 2772.0 | 3538.7 | 2042.3 | 2273.7 |
| AP-4-S | 4003.9 | 1873.4 | 2096.2 | 1509.4 | 2085.5 |
| AP-1-W | 3622.8 | 1518.2 | 1452.5 | 1209.4 | 1729.4 |
| AP-2-W | 4131.9 | 1815.9 | 1699.6 | 1466.4 | 2147.0 |
| AP-3-W | 4406.5 | 2554.1 | 3061.9 | 1837.9 | 2245.7 |

APPENDIX B4 - Samples fitting to theoretical ecological models, based on Akaike information criterion (AIC) for rank abundance distributions of OTUs of Natural Specialist. Across pasture system: Natural Grassland (NG); Improved Natural grassland (IG); Perennial Cultivated Pasture (PP) and Annual Cultivated Pasture (AP) in the summer (S) and winter (W) season. The Rank abundance models based on corrected AIC value from Poisson distributions using maximum likelihood estimation. The lowest AIC value for each sample represented the best-fitted model for general community's assembly. The ZSM (Zero Sum Multinomial) and Broken-stick are null models regarding to theoretical neutral assembly while Preemption (Preemp), Lognormal (LogN) and Zipf are niche-based models regarding to deterministic assembly.

| Sample | **Akaike information criterion (AIC)** | | | | |
|---|---|---|---|---|---|
| | **Specialist Natural Grassland** | | | | |
| | **Neutral** | | **Niche-based** | | |
| | ZSM | Null | Preemp | LogN | Zipf |
| NG-1-S | 7769.9 | 7238.3 | 10227.6 | 3942.9 | 3961.5 |
| NG-2-S | 8219.7 | 4528.3 | 5529.6 | 3095.1 | 4012.2 |
| NG-3-S | 8137.3 | 6968.7 | 9610.2 | 3766.7 | 4288.4 |
| NG-4-S | 9301.7 | 6024.5 | 8137.5 | 3758.7 | 4616.8 |
| NG-1-W | 8356.4 | 7573.0 | 10798.1 | 4296.8 | 4032.9 |
| NG-2-W | 8042.0 | 4804.5 | 5987.6 | 3073.3 | 4047.1 |
| NG-3-W | 7989.9 | 4916.8 | 6550.7 | 3294.2 | 4351.0 |
| NG-4-W | 7169.3 | 6541.2 | 9399.9 | 3296.2 | 3890.7 |
| IG-1-S | 4941.8 | 2414.6 | 2188.9 | 1585.0 | 2045.4 |
| IG-2-S | 5882.4 | 1573.6 | 1955.5 | 339.9 | 686.5 |
| IG-3-S | 6553.6 | 3897.4 | 5098.6 | 2574.5 | 3068.5 |
| IG-4-S | 8662.5 | 4392.5 | 5644.0 | 3003.1 | 4756.6 |
| IG-1-W | 5296.1 | 2734.5 | 2640.1 | 1802.3 | 2329.5 |
| IG-2-W | 9662.8 | 2743.4 | 2699.4 | 1695.4 | 2151.8 |
| IG-3-W | 5092.5 | 2415.1 | 2428.1 | 1679.4 | 2299.3 |
| IG-4-W | 2875.7 | 1252.8 | 1172.5 | 963.4 | 1451.5 |
| PP-1-S | 474.3 | 192.9 | 177.0 | 162.3 | 196.1 |
| PP-2-S | 237.9 | 135.8 | 148.1 | 100.9 | 92.0 |
| PP-3-S | 175.7 | 67.1 | 72.7 | 82.5 | 111.3 |
| PP-4-S | 811.8 | 517.2 | 616.5 | 363.7 | 418.7 |
| PP-1-W | 342.5 | 167.0 | 174.0 | 146.4 | 190.8 |
| PP-2-W | 341.4 | 211.9 | 148.1 | 135.5 | 168.4 |
| PP-3-W | 140.3 | 83.1 | 65.4 | 66.7 | 85.0 |
| PP-4-W | 146.4 | 63.2 | 65.6 | 59.8 | 66.9 |
| AP-1-S | 1012.7 | 420.4 | 474.2 | 389.4 | 602.9 |
| AP-2-S | 725.2 | 293.7 | 316.9 | 256.7 | 353.0 |
| AP-3-S | 1321.8 | 709.0 | 777.9 | 539.3 | 694.4 |
| AP-4-S | 733.2 | 301.0 | 293.5 | 288.1 | 425.3 |
| AP-1-W | 374.2 | 140.1 | 148.7 | 135.7 | 167.2 |
| AP-2-W | 554.1 | 202.6 | 205.2 | 203.6 | 279.9 |
| AP-3-W | 1590.8 | 743.4 | 849.1 | 619.7 | 898.2 |

APPENDIX B5 - Samples fitting to theoretical ecological models, based on Akaike information criterion (AIC) for rank abundance distributions of OTUs Specialist Cultivated communities. Across pasture system: Natural Grassland (NG); Improved Natural grassland (IG); Perennial Cultivated Pasture (PP) and Annual Cultivated Pasture (AP) in the summer (S) and winter (W) season. The Rank abundance models based on corrected AIC value from Poisson distributions using maximum likelihood estimation. The lowest AIC value for each sample represented the best-fitted model for general community's assembly. The ZSM (Zero Sum Multinomial) and Broken-stick are null models regarding to theoretical neutral assembly while Preemption (Preemp), Lognormal (LogN) and Zipf are niche-based models regarding to deterministic assembly.

| Sample | **Akaike information criterion (AIC)** | | | | |
|---|---|---|---|---|---|
| | **Specialist Cultivated Pasture** | | | | |
| | **Neutral** | | **Niche-based** | | |
| | ZSM | Null | Preemp | LogN | Zipf |
| NG-1-S | 129.6 | 31.3 | 32.8 | 33.8 | 37.7 |
| NG-2-S | 452.7 | 208.2 | 198.0 | 179.7 | 213.3 |
| NG-3-S | 134.3 | 79.3 | 72.0 | 62.3 | 46.5 |
| NG-4-S | 168.8 | 350.5 | 192.5 | 101.0 | 81.7 |
| NG-1-W | 182.6 | 62.7 | 68.0 | 60.3 | 64.6 |
| NG-2-W | 129.2 | 48.6 | 44.0 | 45.2 | 49.4 |
| NG-3-W | 177.5 | 81.1 | 84.8 | 78.4 | 83.0 |
| NG-4-W | 134.8 | 65.3 | 63.5 | 55.9 | 53.6 |
| IG-1-S | 2838.6 | 1247.9 | 1216.7 | 1016.0 | 1588.5 |
| IG-2-S | 1757.3 | 684.0 | 663.3 | 603.6 | 917.8 |
| IG-3-S | 1757.4 | 713.5 | 682.1 | 573.3 | 747.5 |
| IG-4-S | 133.1 | 176.1 | 61.2 | 50.2 | 39.9 |
| IG-1-W | 2414.8 | 1197.2 | 1112.1 | 857.8 | 1194.6 |
| IG-2-W | 1621.0 | 930.7 | 671.4 | 565.6 | 811.4 |
| IG-3-W | 2039.7 | 1071.3 | 815.0 | 740.6 | 1094.0 |
| IG-4-W | 4791.2 | 3020.9 | 2316.9 | 1785.3 | 2227.4 |
| PP-1-S | 9972.8 | 4624.4 | 5174.3 | 3401.7 | 4777.3 |
| PP-2-S | 11138.9 | 5700.0 | 5048.9 | 3798.3 | 5288.0 |
| PP-3-S | 10401.2 | 5254.1 | 4871.0 | 3649.0 | 5170.7 |
| PP-4-S | 8659.9 | 4367.3 | 4226.7 | 3039.5 | 4152.6 |
| PP-1-W | 9910.5 | 4894.9 | 4462.5 | 3386.2 | 4854.4 |
| PP-2-W | 10447.9 | 5627.1 | 4639.1 | 3672.5 | 5241.8 |
| PP-3-W | 10060.9 | 4697.8 | 4240.9 | 3493.3 | 5576.2 |
| PP-4-W | 11330.1 | 6309.2 | 4708.4 | 3959.7 | 5546.7 |
| AP-1-S | 8341.5 | 4936.1 | 5072.1 | 3194.5 | 3672.8 |
| AP-2-S | 8270.1 | 4327.4 | 4385.8 | 2923.4 | 4008.3 |
| AP-3-S | 7862.8 | 3944.0 | 3920.7 | 2818.0 | 3805.0 |
| AP-4-S | 9513.9 | 5056.1 | 5076.5 | 3470.2 | 4383.4 |
| AP-1-W | 11728.7 | 5652.3 | 5028.5 | 3926.6 | 5420.0 |
| AP-2-W | 9993.6 | 5263.0 | 4937.7 | 3513.5 | 4546.3 |
| AP-3-W | 7360.1 | 3517.3 | 3158.3 | 2426.3 | 2822.9 |

APPENDIX B6 - Samples fitting to theoretical ecological models, based on Akaike information criterion (AIC) for rank abundance distributions of OTUs of rara taxa communities. Across pasture system: Natural Grassland (NG); Improved Natural grassland (IG); Perennial Cultivated Pasture (PP) and Annual Cultivated Pasture (AP) in the summer (S) and winter (W) season. The Rank abundance models based on corrected AIC value from Poisson distributions using maximum likelihood estimation. The lowest AIC value for each sample represented the best-fitted model for general community's assembly. The ZSM (Zero Sum Multinomial) and Broken-stick are null models regarding to theoretical neutral assembly while Preemption (Preemp), Lognormal (LogN) and Zipf are niche-based models regarding to deterministic assembly.

| Sample | **Akaike information criterion (AIC)** | | | | |
|---|---|---|---|---|---|
| | **Rare** | | | | |
| | **Neutral** | | **Niche-based** | | |
| | ZSM | Null | Preemp | Lognor | Zipf |
| NG-1-S | 917.1 | 352.6 | 239.3 | 228.8 | 241.0 |
| NG-2-S | 1776.1 | 765.2 | 521.3 | 500.4 | 542.6 |
| NG-3-S | 1194.4 | 429.7 | 319.7 | 310.1 | 335.8 |
| NG-4-S | 1602.4 | 682.4 | 456.0 | 436.8 | 463.6 |
| NG-1-W | 1570.6 | 596.8 | 431.8 | 420.7 | 454.9 |
| NG-2-W | 1184.1 | 488.0 | 324.9 | 311.8 | 333.5 |
| NG-3-W | 1382.2 | 537.6 | 375.6 | 368.0 | 395.7 |
| NG-4-W | 887.1 | 293.5 | 224.1 | 217.9 | 231.6 |
| IG-1-S | 2027.2 | 764.2 | 550.4 | 550.1 | 605.7 |
| IG-2-S | 1746.1 | 720.3 | 480.3 | 457.1 | 487.5 |
| IG-3-S | 1847.8 | 656.6 | 486.1 | 478.7 | 520.5 |
| IG-4-S | 1565.8 | 619.5 | 419.8 | 404.9 | 435.6 |
| IG-1-W | 1540.9 | 633.6 | 419.3 | 403.6 | 432.6 |
| IG-2-W | 1112.5 | 426.9 | 290.4 | 277.3 | 298.0 |
| IG-3-W | 1899.8 | 719.0 | 513.9 | 498.9 | 540.1 |
| IG-4-W | 2297.1 | 901.5 | 652.9 | 644.9 | 720.4 |
| PP-1-S | 2063.6 | 825.3 | 564.1 | 536.5 | 577.6 |
| PP-2-S | 2511.2 | 977.2 | 685.8 | 667.1 | 717.0 |
| PP-3-S | 2072.3 | 795.4 | 553.6 | 529.0 | 559.2 |
| PP-4-S | 1727.7 | 625.7 | 452.7 | 442.2 | 485.2 |
| PP-1-W | 1913.7 | 662.8 | 492.5 | 481.5 | 520.9 |
| PP-2-W | 1910.1 | 676.3 | 495.5 | 491.8 | 535.1 |
| PP-3-W | 2211.8 | 857.1 | 595.7 | 582.2 | 630.0 |
| PP-4-W | 3097.5 | 1296.6 | 909.1 | 878.7 | 964.1 |
| AP-1-S | 1596.6 | 640.4 | 434.2 | 415.4 | 448.2 |
| AP-2-S | 1823.6 | 739.2 | 502.3 | 481.1 | 515.8 |
| AP-3-S | 1710.6 | 626.5 | 455.0 | 442.9 | 482.1 |
| AP-4-S | 2221.1 | 875.4 | 619.9 | 604.2 | 660.2 |
| AP-1-W | 2541.0 | 986.4 | 708.3 | 687.5 | 738.3 |
| AP-2-W | 2454.4 | 1013.4 | 704.7 | 687.3 | 749.9 |
| AP-3-W | 1711.1 | 661.0 | 462.0 | 449.3 | 489.5 |

## APPENDIX C – CHAPTER IV

APPENDIX C- Samples sequencing through 16S rRNA gene and ITS region. Sample name, Identification (ID), Number of sequencing reads, sequence length, and percentages after quality control on Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST) pipeline. Samples: Improved-natural grassland (IG) and Annual-cultivated pasture (AP), in Rhizosphere (R) and bulk soil (B).

| Target | Grassland | Sample | ID MG-RAST | Number of sequences reads | Mean sequence length |
|---|---|---|---|---|---|
| 16S | IG Bulk | IG_B1 | b3d490b6f96d676d343936353230342e33 | 30,075 | 452 ± 13 bp |
| | | IG_B2 | 821a9073456d676d343936353230352e33 | 32,895 | 452 ± 12 bp |
| | | IG_B3 | 8c761892676d676d343936353139392e33 | 27,669 | 452 ± 12 bp |
| | IG_Rhizo | IG_R1 | 923af22c466d676d343936353230362e33 | 31,798 | 450 ± 13 bp |
| | | IG_R2 | 97e7c2cfe16d676d343936353230302e33 | 31,284 | 451 ± 13 bp |
| | | IG_R3 | 065f82f2db6d676d343936353230332e33 | 33,392 | 451 ± 13 bp |
| | AP_Bulk | AP-B1 | e6d1603de46d676d343936353230392e33 | 31,577 | 453 ± 13 bp |
| | | AP_B2 | 821f641c616d676d343936353230372e33 | 33,263 | 455 ± 13 bp |
| | | AP_B3 | 12dd68434f6d676d343936353231302e33 | 30,574 | 453 ± 13 bp |
| | AP_Rhizo | AP_R1 | e936613b2b6d676d343936353230382e33 | 20,065 | 452 ± 12 bp |
| | | AP_R2 | 9a6721ec766d676d343936353230312e33 | 32,467 | 452 ± 12 bp |
| | | AP_R3 | 236e1dfe7b6d676d343936353230322e33 | 36,096 | 452 ± 13 bp |
| ITS | IG Bulk | F-IG_B1 | 532b9b7e8f6d676d343936353631332e33 | 32,632 | 288 ± 47 bp |
| | | F-IG_B2 | | | |
| | | F-IG_B3 | | | |
| | IG_Rhizo | F-IG_R1 | 51e5569a2b6d676d343936353630372e33 | 32,815 | 283 ± 34 bp |
| | | F-IG_R2 | 65d2bd130d6d676d343936353631322e33 | 34,461 | 279 ± 33 bp |
| | | F-IG_R3 | a35bb0c34b6d676d343936353630362e33 | 32,195 | 277 ± 38 bp |
| | AP_Bulk | F-AP-B1 | ed2101d12a6d676d343936353630352e33 | 30,604 | 275 ± 39 bp |
| | | F-AP_B2 | | | |
| | | F-AP_B3 | 697c2f7dce6d676d343936353631302e33 | 33,010 | 279 ± 38 bp |
| | AP_Rhizo | F-AP_R1 | 82f3fb19176d676d343936353631312e33 | 27,952 | 281 ± 33 bp |
| | | F-AP_R2 | 14ab6ec4ca6d676d343936353630382e33 | 35,289 | 278 ± 30 bp |
| | | F-AP_R3 | b4cb51c2726d676d343936353630392e33 | 33,249 | 278 ± 32 bp |

APPENDIX C2- Relative abundance of de all phyla found by 16S RNA gene sequencing for bulk soil (BS) and rhizosphere (R). Soil sample collected Improved-natural grassland (IG), and Annual-cultivated pasture (AP). Means ± standard deviation.

| | **Grassland systems and soil compartments** | | | |
|---|---|---|---|---|
| **Bacteria Phyla** | **IG_BS** | **IG_R** | **AP_BS** | **AP_R** |
| Proteobacteria | 29.56±1.56 | 32.46±1.30 | 30.71±0.26 | 29.90±3.14 |
| Actinobacteria | 14.20±1.19 | 23.73±3.67 | 16.55±2.86 | 29.87±1.74 |
| Firmicutes | 1.72±0.38 | 1.03±0.26 | 2.16±1.20 | 3.51±0.80 |
| Unclassified | 0.84±0.07 | 0.66±0.19 | 0.66±0.09 | 0.54±0.19 |
| Acidobacteria | 22.95±2.95 | 15.78±2.78 | 20.57±2.65 | 9.05±2.58 |
| Verrucomicrobia | 12.57±1.69 | 5.50±1.42 | 8.00±0.91 | 5.51±0.45 |
| Chloroflexi | 6.60±0.07 | 5.01±1.16 | 6.66±1.02 | 4.78±1.38 |
| Bacteroidetes | 2.75±0.67 | 7.59±0.54 | 3.91±0.67 | 7.29±1.62 |
| Gemmatimonadetes | 1.21±0.05 | 0.59±0.12 | 2.94±0.23 | 2.26±0.72 |
| Planctomycetes | 5.10±0.13 | 3.86±0.53 | 4.95±0.15 | 3.41±0.44 |
| Latescibacteria | 0.61±0.06 | 0.050±0.04 | 0.88±0.32 | 0.04±0.02 |
| Cyanobacteria | 0.08±0.04 | 0.37±0.09 | 0.080±0.04 | 0.40±0.07 |
| Patescibacteria | 0.62±0.07 | 2.32±0.81 | 0.90±0.25 | 2.73±1.0 |
| Nitrospirae | 0.26±0.09 | 0.03±0.03 | 0.20±0.11 | 0.07±0.01 |
| Armatimonadetes | 0.13±0.07 | 0.52±0.11 | 0.09±0.04 | 0.40±0.04 |
| Rokubacteria | 0.15±0.06 | 0.00±0.00 | 0.27±0.11 | 0.00±0.00 |
| GAL15 | 0.06±0.02 | 0.00±0.00 | 0.02±0.01 | 0.00±0.00 |
| WPS-2 | 0.19±0.09 | 0.08±0.10 | 0.03±0.03 | 0.00±0.00 |
| Elusimicrobia | 0.17±0.016 | 0.13±0.02 | 0.17±0.05 | 0.09±0.02 |
| Entotheonellaeota | 0.04±0.04 | 0.01±0.01 | 0.02±0.02 | 0.00±0.00 |
| FBP | 0.00±0.00 | 0.07±0.01 | 0.01±0.01 | 0.10±0.04 |
| Fibrobacteres | 0.01±0.01 | 0.05±0.03 | 0.02±0.02 | 0.01±0.01 |
| BRC1 | 0.01±0.01 | 0.03±0.03 | 0.012±0.02 | 0.01±0.01 |
| Chlamydiae | 0.13±0.06 | 0.05±0.02 | 0.06±0.06 | 0.10±0.01 |
| WS2 | 0.01±0.01 | 0.04±0.02 | 0.04±0.02 | 0.01±0.01 |
| Dependentiae | 0.06±0.01 | 0.02±0.01 | 0.06±0.01 | 0.00±0.00 |
| Euryarchaeota | 0.00±0.00 | 0.01±0.01 | 0.01±0.01 | 0.00±0.00 |
| Deferribacteres | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.01±0.01 |
| Epsilonbacteraeota | 0.00±0.00 | 0.01±0.01 | 0.00±0.00 | 0.00±0.00 |
| Deinococcus-Thermus | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.01±0.01 |

APPENDIX C3- Relative abundance of de all phyla found by ITS rRNA gene sequencing for bulk soil (BS) and rhizosphere (R). Soil sample collected Improved-natural grassland (IG), and Annual-cultivated pasture (AP). Means ± standard deviation

| **Fungal Phyla** | **Grassland system and soil compartment** | | | |
|---|---|---|---|---|
| | **IG_BS** | **IG_R** | **AP_BS** | **AP_R** |
| Ascomycota | 29.24±8.56 | 68.94±8.96 | 50.89±10.43 | 79.52±1.80 |
| Mortierellomycota | 13.31±3.48 | 3.02±0.18 | 20.58±2.78 | 4.74±0.54 |
| Basidiomycota | 13.66±9.16 | 10.29±2.39 | 8.64±6.29 | 7.34±4.60 |
| Chytridiomycota | 0.24±0.14 | 1.19±0.29 | 0.85±0.52 | 1.21±1.33 |
| Kickxellomycota | 0.61±0.44 | 0.94±0.84 | 0.52±0.59 | 0.15±0.18 |
| Rozellomycota | 0.65±0.47 | 0.18±0.16 | 0.62±0.38 | 0.015±0.02 |
| Olpidiomycota | 0.00±0.00 | 0.01±0.02 | 0.06±0.10 | 0.00±0.00 |
| Glomeromycota | 0.18±0.15 | 0.06±0.07 | 0.13±1.72 | 0.02±0.02 |
| Mucoromycota | 0.01±0.01 | 0.03±0.06 | 0.00±0.00 | 0.00±0.00 |
| Zoopagomycota | 0.00±0.00 | 0.00±0.00 | 0.05±0.06 | 0.01±0.01 |
| Entorrhizomycota | 0.00±0.00 | 0.00±0.00 | 0.01±0.02 | 0.00±0.00 |
| unidentified | 0.72±0.81 | 0.64±0.77 | 0.65±0.35 | 0.19±0.10 |
| unidentified1 | 41.38±10.42 | 14.71±5.96 | 17.00±5.85 | 6.81±2.22 |

APPENDIX C4- Relative abundance of de bacteria potential function by FAPROTAX (16S) for bulk soil (BS) and rhizosphere (R). Soil sample collected Improved-natural grassland (IG), and Annual-cultivated pasture (AP). Means ± standard deviation.

| Function | Grassland systems and soil compartments | | | |
|---|---|---|---|---|
| | IG_BS | IG_R | AP_BS | AP_R |
| Aerobic_chemoheterotrophy | 33.14±1.84 | 42.06±3.01 | 39.08±1.22 | 42.63±1.23 |
| Chemoheterotrophy | 33.99±1.34 | 43.14±2.30 | 40.44±0.23 | 44.10±1.03 |
| Animal_parasites_or_symbionts | 12.88±3.28 | 0.91±0.82 | 3.39±1.54 | 0.70±0.39 |
| Cellulolysis | 5.88±1.07 | 0.85±0.78 | 3.01±0.98 | 0.76±0.27 |
| ,Predatory_or_exoparasitic | 4.11±0.51 | 2.59±0.94 | 4.21±0.78 | 1.09±0.22 |
| Methylotrophy | 0.30±0.42 | 0.22±0.31 | 0.00±0.00 | 0.02±0.02 |
| Nitrification | 0.45±0.20 | 0.06±0.06 | 0.65±0.34 | 0.13±0.02 |
| Iron_respiration | 2.04±0.80 | 0.33±0.36 | 2.32±1.58 | 0.00±0.00 |
| Aromatic_compound_degradation | 1.74±0.88 | 5.20±0.42 | 3.44±098 | 5.33±0.59 |
| Respiration_of_sulfur_compounds | 1.18±0.43 | 0.14±0.06 | 1.01±0.04 | 0.00±0.00 |
| Chitinolysis | 0.16±0.22 | 0.00±0.00 | 0.46±0.44 | 0.28±0.25 |
| Dark_hydrogen_oxidation | 0.00±0.00 | 0.02±0.02 | 0.00±0.00 | 0.00±0.00 |
| Nitrogen_fixation | 0.30±0.41 | 0.04±0.05 | 0.00±0.00 | 0.00±0.00 |
| Fermentation | 0.31±0.13 | 0.73±0.49 | 0.44±0.21 | 0.81±0.10 |
| Human_pathogens_all | 0.43±0.30 | 0.27±0.20 | 0.02±0.03 | 0.58±0.47 |
| Aromatic_hydrocarbon_degradation | 0.13±0.19 | 0.27±0.21 | 0.11±0.15 | 0.18±0.13 |
| Hydrocarbon_degradation | 0.43±0.36 | 0.48±0.38 | 0.11±0.16 | 0.18±0.13 |
| Sulfite_respiration | 0.00±0.00 | 0.00±0.00 | 0.03±0.04 | 0.00±0.00 |
| Nitrate_reduction | 0.82±0.31 | 0.64±0.22 | 0.30±0.25 | 0.98±0.45 |
| Nitrogen_respiration | 0.43±0.30 | 0.24±0.20 | 0.00±0.00 | 0.41±0.49 |
| Intracellular_parasites | 0.89±0.28 | 0.40±0.22 | 0.62±0.30 | 0.78±0.14 |
| Methanogenesis | 0.00±0.00 | 0.02±0.02 | 0.00±0.00 | 0.00±0.00 |
| Chloroplasts | 0.27±0.16 | 0.31±0.16 | 0.16±0.08 | 0.49±0.12 |
| Photoautotrophy | 0.03±0.04 | 0.50±0.29 | 0.10±0.07 | 0.29±0.14 |
| Photoheterotrophy | 0.03±0.04 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| Phototrophy | 0.06±0.04 | 0.50±0.29 | 0.01±0.07 | 0.29±0.14 |
| Ureolysis | 0.00±0.00 | 0.08±0.10 | 0.00±0.00 | 0.01±0.02 |