

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL  
FACULDADE DE AGRONOMIA  
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DO SOLO

**FERTILIZATION AND OVERSEEDING IN THE SOUTHERN GRASSLANDS:  
IMPACTS ON PRODUCTIVITY, FERTILITY AND CARBON AND NITROGEN  
SOIL STOCKS**

**João Pedro Moro Flores  
(Tese de Doutorado)**

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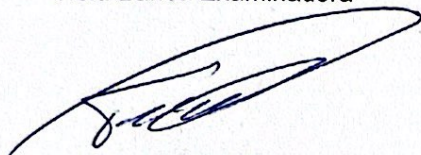
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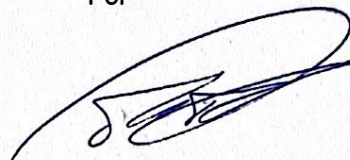
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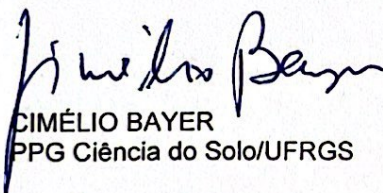


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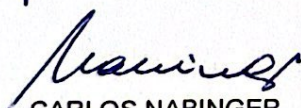
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*“Mas se não houver campo aberto  
Lá em cima quando eu me for  
Um galpão acolhedor  
De santa fé bem coberto  
Um pingo pastando perto  
Só de pensar me comovo  
Eu juro pelo meu povo  
Nem todo o céu me segura  
Retorno a velha planura  
Pra ser gaúcho de novo.”*

*Jayme Caetano Braun, “Galpão Nativo”*

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# FERTILIZAÇÃO E SOBRESSEMEADURA NOS CAMPOS SULINOS: IMPACTOS NA PRODUTIVIDADE, NA FERTILIDADE E NOS ESTOQUES DE CARBONO E NITROGÊNIO NO SOLO <sup>1</sup>

Autor: João Pedro Moro Flores

Orientador: Prof. Tales Tiecher

## RESUMO

O aumento da produtividade de forragem e da carne nas pastagens naturais do bioma Pampa é crucial para evitar a sua conversão em lavouras anuais e pastagens e florestas plantadas. Esta abordagem serve como estratégia para preservar o equilíbrio ecológico e a conservação da biodiversidade. A presente pesquisa avaliou os efeitos de diversas tecnologias de melhoramento sobre a produtividade de forragem, de animais, fertilidade e estoques de C e N do solo, bem como seus impactos na composição botânica das espécies nos campos sulinos do Brasil. As tecnologias de melhoramento incluíram calagem, fertilização com N, P e K, bem como a sobressemeadura de espécies forrageiras exóticas. Os estudos se basearam em uma meta-análise com objetivo de identificar os fatores com maior impacto na produtividade forrageira e animal nos trabalhos já publicados e avaliações de solo e forragem em cinco propriedades rurais do bioma Pampa. Na meta-análise, as tecnologias de melhoramento das pastagens naturais resultaram em 35 e 82% de aumento médio na produtividade de forragem e animal, respectivamente. A quantidade aplicada de N+P+K por hectare e a classe de solo foram os fatores que mais impactaram a produção de forragem. Nos experimentos de campo, o uso de tecnologias de melhoramento apresentou um potencial para promover o aumento médio da produtividade de forragem de 87%. Locais com solos de menor fertilidade natural e, conseqüentemente, menor produtividade das pastagens, foram mais responsivos à aplicação de tecnologias de melhoramento com um ganho de produtividade de forragem de até 191% e uma taxa anual de acúmulo de C no solo entre 2.3 e 3.6 Mg ha<sup>-1</sup> a longo prazo de adoção das tecnologias, mesmo que nos anos iniciais possam ocorrer perdas nos estoques principalmente em solos com elevados estoques iniciais de C e N. A longo prazo as tecnologias de melhoramento podem também propiciar perdas na riqueza e diversidade de espécies em locais de maior fertilidade natural.

Palavras-chave: Bioma Pampa, melhoramento, composição botânica.

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<sup>1</sup> Tese de Doutorado em Ciência do Solo. Programa de Pós-Graduação em Ciência do Solo, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul. Porto Alegre. (77 p.) Março, 2024.

# FERTILIZATION AND OVERSEEDING IN THE SOUTHERN GRASSLANDS: IMPACTS ON PRODUCTIVITY, FERTILITY AND CARBON AND NITROGEN SOIL STOCKS<sup>1</sup>

Author: João Pedro Moro Flores  
Adviser: Prof. Tales Tiecher

## ABSTRACT

Increasing forage and meat productivity in the natural grasslands of the Pampa biome is crucial to prevent their conversion into annual crops and planted pastures and forests. This approach serves as a strategy for preserving ecological balance and conserving biodiversity. This research evaluates the effects of various improvement technologies on forage and animal productivity, fertility and soil C and N stocks, as well as their impact on the botanical composition of species in the southern grasslands of Brazil. The improvement technologies included liming, fertilization with N, P and K, as well as the overseeding of exotic forage species. The studies were based on evaluations on five farms in the Pampa biome, as well as a meta-analysis aimed at identifying the factors with the greatest impact on animal and forage productivity in previously published studies. In the meta-analysis, the improvement technologies resulted in an 35% and 82% average increase in forage and animal productivity, respectively. The amount of N+P+K applied per hectare and the soil class were the factors that had the greatest impact on forage productivity. In the field experiments, the use of improvement technologies had the potential to promote an average increase in forage productivity of 87%. Sites with soils of lower natural fertility and, consequently, lower forage productivity, are more responsive to the application of improvement technologies with a gain in forage productivity of up to 191% and annual rate of C accumulation in the soil between 2.3 and 3.6 Mg ha<sup>-1</sup> over the long-term adoption of the technologies, even though in the initial years there may be losses in stocks, especially in soils with high initial C and N stocks. In the long term, improvement technologies can also lead to losses in species richness and diversity on sites with higher natural fertility.

Keywords: Pampa biome, improvement, botanical composition.

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<sup>1</sup>Doctoral thesis in Soil Science. Graduate Program in Soil Science, Faculty of Agronomy, Universidade Federal do Rio Grande do Sul. Porto Alegre. (77p.) March, 2024.



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## 1. INTRODUCTION

The central theme of this thesis is the improvement of natural grasslands through liming, fertilization and overseeding of species. In the first study, entitled “Boosting production of “Campos” natural grasslands through improving soil fertility and overseeding: a meta-analysis <sup>1</sup>” a meta-analysis was conducted to evaluate the effects of liming, fertilization and overseeding of species on animal and forage productivity in natural grasslands in southern Brazil, with the aim of providing information to guide actions to improve natural grasslands. The study aims to identify the factors that have the greatest impact on animal and forage productivity in published works on improving natural grasslands, as well as identifying the soil conditions that provide the greatest productive responses.

Secondly, data from the second study in the thesis is presented, entitled “Carbon storage in the Pampa biome: increasing productivity and conservation of natural grasslands in southern Brazil with improvement technologies <sup>2</sup>” involving forage productivity and C and N stocks in the soil. The third study involves the chemical characteristics of the soil and their relationship with forage composition and entitled “Fertilization and overseeding in natural grasslands in southern Brazil: impacts on soil fertility and the botanical composition of species”.

The aim of these studies is to determine which soil attributes and improvement strategies of natural grasslands that provide the best results in annual forage productivity and the greatest increases in soil C and N stocks under different soil and climate conditions in the Pampa biome. The second and third studies are being carried out on five farms that are members of "Alianza del Pastizal Brasil", an organization that certifies rural establishments that conserve at least 50% of their land under natural grassland, as well as promoting the sustainable use of this environment. The farms are located in the municipalities of Piratini, Lavras do Sul, Dom Pedrito, Santana do Livramento and São Pedro

do Sul, all in the Pampa biome of the state of Rio Grande do Sul. On each farm, an unimproved natural grassland environment and one or two improved natural grassland environments with different implementation times were selected.

So far, the work involving liming, fertilizing and overseeding species has been limited to short-term experiments in one place in the Pampa biome. By carrying out a joint analysis of the data already published through a meta-analysis, as well as validating these productive responses within farms with contrasting soil and climate characteristics, it is possible to obtain more concrete answers to their benefits and points of attention. It also makes it possible to understand the particularities of each natural grassland improvement practice according to the different soil and climate characteristics of the Pampa biome.

The results obtained in this thesis can serve as a basis for decision-making regarding the most appropriate natural grasslands management practices in the different regions of the Pampa biome. The practices of liming, fertilizing and overseeding species can, in addition to increasing soil fertility levels and, consequently, forage productivity for animals, provide greater contributions of carbon to the system, ensuring greater profitability and sustainability of livestock systems based on natural grassland.

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<sup>1</sup> article submitted in the *Geoderma Regional* in January 2024.

<sup>2</sup> article submitted in the *Catena* in April 2024.

## **2. LITERATURE REVIEW**

### **2.1. The natural grassland and the Pampa biome**

The natural grassland of southern Brazil encompassed approximately 13.7 million hectares, providing essential ecosystem services such as the water resource conservations, support for pollinators, and availability of genetic resources (Viglizzo & Frank, 2006; Weyland et al., 2017). Recognized as one of the few Brazilian environments capable of harmonizing livestock productivity with the preservation of native vegetation, these natural grasslands serve as the primary forage source for beef cattle farming in Southern Brazil (Viglizzo et al., 2001; Pillar et al., 2009). With more than 15 million cattle in the states of Rio Grande do Sul and Santa Catarina (IBGE, 2021), this region has sustained itself for over 300 years with minimal input usage (Viglizzo et al., 2001). This underscores that, with appropriate technology, natural grasslands can achieve heightened productivity while concurrently preserving the health of grassland ecosystems and their associated services (Nabinger et al., 2009).

Despite their economic and environmental significance, the natural grasslands in southern Brazil have undergone a substantial reduction in their original area since the 1970s, primarily attributed to conversion into annual grain crop systems (Viglizzo and Frank, 2006; Baeza and Paruelo, 2020). In the Rio Grande do Sul state, cropland in the Pampa biome increased by 57% between the summer of 2000/2001 to 2014/2015 totaling 422.600 ha (Silveira et al., 2017). The rationale for converting natural grassland areas to alternative uses (e.g., soybeans, corn, eucalyptus) is largely driven by the increase in agricultural commodity prices and their higher profitability (Modernel et al., 2016). This trend is particularly pronounced when compared to the natural grasslands with

traditional management. A traditional extensive livestock farming system based on natural grasslands faces financial challenges in competing with agricultural activities employing higher technology and production scale. Therefore, the most viable alternative to increase livestock production while preserving the forage base of natural grasslands is through the sustainable intensification of the production system (Jaurena et al., 2021). The adoption of management strategies and natural grassland improvement allow the preservation of native species in the biome and yielding economic outcomes comparable to field crops (Nabinger et al., 2009; Oliveira et al., 2015; Córdova, 2021).

Soils under natural grasslands of the Pampa biome typically exhibit low natural fertility (Hasenack et al., 2023). Consequently, the forage production in these areas faces challenges such as limited nutrient availability and low crude protein levels (Gatiboni et al., 2008). Only 4% of the natural grasslands within the Pampa biome are situated on soil classes characterized by high natural fertility (Hasenack et al., 2023), resulting in a notable enhancement in the quality of native forage composition (Ferreira et al., 2011).

## **2.2. Fertilization and overseeding in natural grasslands**

The application of limestone and fertilizers in natural grasslands (Gatiboni et al., 2008; Viégas et al., 2017), as well overseeding with cool-season species such as ryegrass (*Lolium multiflorum*) (Rizo et al., 2004), have demonstrated a positive impact on forage productivity. This impact is particularly pronounced during the winter forage gap, resulting in increased animal productivity (Ferreira et al., 2011). However, there exists variability in the response of natural grasslands to these improvement practices, ranging from no response (Gomes, 1996) and average increases of 21 to 34% (Pizzani et al., 2007; Cunha et al., 2001) to substantial increases of up to 187% in forage productivity (Sallis et al., 2000). The cause of this significant variation has not been thoroughly explored, but there are indications that it may be influenced by different soil types (Oliveira et al., 2012), botanical composition and the amount of nutrient input (Vidor et al., 1998).

Although these practices may behave differently between locations, because differently soil class result in different grassland phytofissionomies, the implementation of improvement technologies, including liming, fertilization, and

overseeding of annual exotic cool season species in natural grassland, can contribute to increasing annual forage productivity (Ferreira et al., 2011; Gatiboni et al., 2000). This enhancement in forage productivity also leads to increased carbon (C) sequestration from the atmosphere (Conant et al., 2017). This contributes positively to the current scenario of global climate change (Piñeiro et al., 2010), especially in long-term improvement natural grasslands.

The financial investment, logistics, and strategy to encompass all these aspects in a single study would be unfeasible due to the variability in combinations of soil types and climatic conditions in southern Brazil. However, this information can be obtained through a meta-analysis of previously published studies (Filippi et al., 2022; De Castro Pias et al., 2022; Tiecher et al., 2021). Understanding these particularities is pivotal for optimizing productivity and protecting the Pampa biome. In addition, a greater efficiency in the production system and fostering the development of a more sustainable livestock industry while preserving the native forage foundation.

### **2.3. Soil C and N in natural grasslands**

Increases in soil C and N contents can be promoted by overseeding winter species in natural grasslands. Changes in the proportion of cool and warm seasons species can affect root biomass, since summer species generally have a greater number of deep roots (Piñeiro et al., 2009), while winter species contribute more to root biomass in the surface layers of the soil (Bondaruk et al., 2020) because the root system is shallower than that of native species (Salvo et al., 2008; Carámbula et al., 1994). Ryegrass (*Lolium multiflorum*), white clover (*Trifolium* sp.) and Lotus (*Lotus* sp.) are the main exotic species used for overseeding natural grasslands (Rizo et al., 2004; Garagorry et al., 2008; ROSA et al., 2012).

Overseeding of exotic species in natural grasslands, especially legumes, should always be accompanied by correction of soil acidity and P availability. This practice increases the quantity and quality of forage produced (Viégas et al., 2017), especially in the long-term due to the greater accumulation of fertilizers (Gatiboni et al., 2008). These practices also benefit some native species (Oliveira et al., 2015; 2022), such as grasses of the genus *Paspalum* sp. and especially legumes such as *Desmodium* sp. (Nabinger et al., 2009).

Furthermore, incorporating legume overseeding along with phosphate fertilization can contribute to an increase in the C content and overall stock within the system (Conant et al., 2017, 2001; Poeplau et al., 2018). The higher quality (Alves et al., 2020) and diversified of residue inputs from legumes (Veloso et al., 2018) favors soil C accumulation. In addition to the increased productivity and quality of the forage produced, a possible reduction in the soil C/N ratio can contribute to a higher efficiency of C utilization by microorganisms. Consequently, this may lead to higher rates of C sequestration in the soil (Poeplau et al., 2018).

Therefore, studies evaluating the effects of natural grasslands improvement technologies on soil C and N levels and stocks are important. The contribution of these practices to increasing soil C and N stocks, and consequently C storage in natural grasslands livestock systems, has not been fully investigated. The correct allocation and implementation of these practices are fundamental not only for increasing productivity in livestock systems, but also for mitigating climate change and ensuring food security.

### **3. HYPOTHESES**

Liming, fertilization, and overseeding with cool season exotic species promote improvements on soil fertility, forage and animal productivity, and soil C and N stocks especially in situations of low natural fertility soils in natural grasslands of southern Brazil.

Liming and fertilization provide the greatest increases in forage productivity in situations of low natural fertility soils while overseeding with cool-season exotic species provides the greatest increases in forage productivity in situations of high natural fertility or soils where fertility has been improved.



#### **4. OBJECTIVES**

Identify the factors that most impact forage and animal productivity on improvement technologies used in natural grassland and to pinpoint the soil conditions that foster the highest productive responses.

Evaluate the effects of fertilization and overseeding with cool season exotic species on forage productivity, C and N content and stocks in natural grasslands in the Pampa biome.

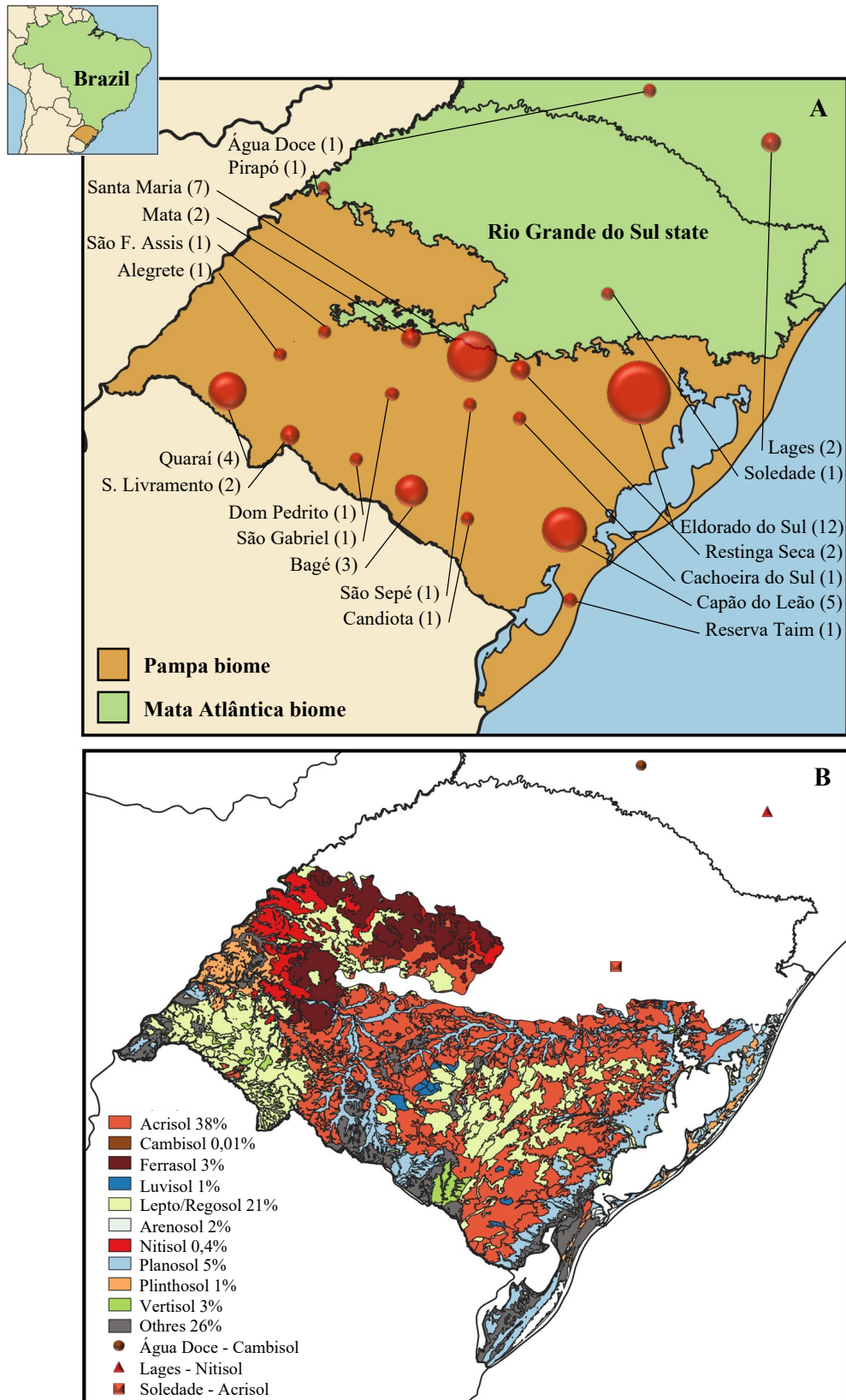
## 5. MATERIAL AND METHODS

### 5.1. Boosting production of “Campos” natural grasslands through improving soil fertility and overseeding: a meta-analysis

#### 5.1.1. Data description

The data utilized in the study were sourced from various platforms, including Scielo, Web of Science, Research Gate, PubMed, and Google Scholar. We employed specific keywords such as (ruminant\* OR bovine\* OR "beef" OR cow\* OR sheep\*) AND ("natural grassland" OR "natural pasture" OR "overseeding in natural grassland" OR "fertilization in natural grassland") AND (production OR forage OR "weight gain" OR meat) AND (grazing). Additionally, we incorporated bulletins, abstracts, and technical circulars recommended by recognized researchers in the natural grassland field. The studies were selected based on the following criteria: (i) presenting treatments with overseeding of grasses and/or legumes and/or (ii) presenting treatments with different levels of lime and/or fertilization, and (iii) total herbage and/or beef production according to the treatments. Studies were excluded from the meta-analysis if they: (i) were conducted outside Brazilian territory, (ii) lacked data on forage and/or animal production, and (iii) did not have a control treatment (grassland that did not receive fertilization and/or overseeding).

The database construction involved extracting specific details from the chosen studies, including authors, publication year, source type (article, technical note, abstract, dissertation, thesis), geographical location (**Figure 1**), and assessment year. Soil-related information gathered encompassed soil class (EMBRAPA, 2023), clay content, soil pH (water pH), soil organic matter content,



**Figure 1** Spatial distribution of studies per county (A) and spatial distribution of soil class in Pampa biome and municipalities outside the biome (B). The size of the circle and the value in parentheses in A represent the number of studies in each location. The percentage in B represents the scope of the soil class in Pampa biome in studies that evaluated the effects of improvement natural grasslands of Southern Brazil on forage and beef production.

(SOM), and initial availability levels of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and aluminum (Al). These values adhered to the CQFS-RS/SC (2016). Additionally, data on total herbage production throughout the four seasons of the year were compiled, along with corresponding beef production whenever available. Concerning natural grassland improvement technologies, information collected involved the quantity of limestone, N, P, K, and sulfur (S) added in the treatments, along with the respective nutrient sources employed. Furthermore, details regarding the grass and legume species used in overseeding were documented.

The data were extracted from tables, equations, or derived from figures. Quantities of N, P, K, and S added in the treatments were summed, as studies with the isolated use of nutrients were rare. Beef production was standardized to kilograms per hectare ( $\text{kg ha}^{-1}$  of live weight), while forage dry matter production was converted to megagrams per hectare ( $\text{Mg ha}^{-1}$  of dry matter). Detailed information about the studies and the main compiled data is presented in **Table 1**. Including repetitions, 506 pairs of observations were amassed from 50 investigating the impact (**Table 1**).

#### 5.1.2. Descriptive and statistical analyses

To quantify the increase in total forage and beef production, 506 pairs of observations were employed. Each pair consisted of a control group/treatment and a treatment with fertilization and/or overseeding of species. In each study, the natural grassland without fertilization and/or overseeding served as the control group. By contrast, the treatment observations consisted in a single or combined fertilization and/or overseeding of cool and warm seasons species in each study.

The main effects were identified using conditional inference decision trees in JMP 13 software (SAS Institute, 2016), with a significance level of  $p < 0.05$  for the response variable on the factors of variation. The effects of fertilization and overseeding of species on forage production were used as response variables and the natural were used to calculate the response effect was calculated according to **Equation 1**, where productivity of treatments = the forage and beef

production resulting from fertilization and/or overseeding; and productivity of controls = grassland that did not receive fertilization and/or overseeding (control).

$$Effect (\%) = \left[ \frac{Treatment\ production}{Control\ production} \right] \times 100 \quad Eq. 1$$

For each identified effect, bootstrap statistical analysis was performed using StatKey v.2.1.1 software, generating 5,000 observation points for each treatment. A 95% confidence interval was established for the mean of each effect in each evaluated subgroup, assuming a significant effect when the confidence intervals do not overlap with each other.

## 5.2. Carbon storage in the Pampa biome: increasing productivity and conservation of natural grasslands in southern Brazil with "improvement technologies"

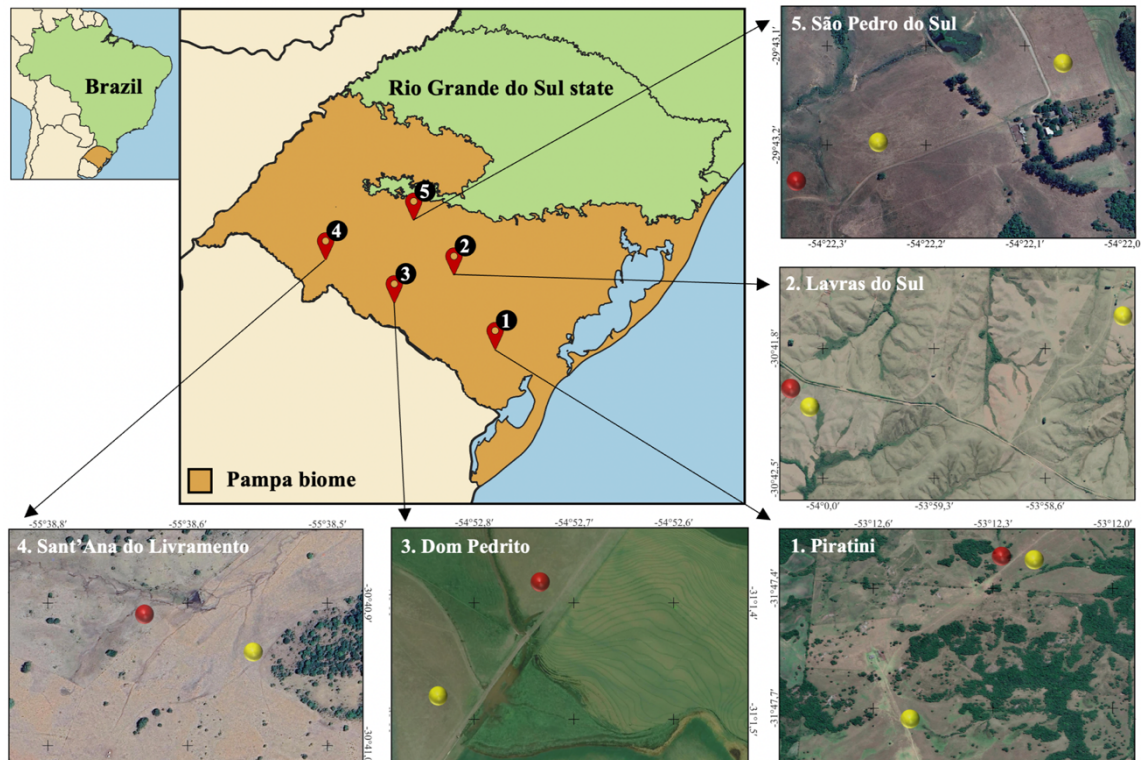
### 5.2.1. Study sites

The fields experiments were carried out in the Pampa biome region of the State of Rio Grande do Sul, in five municipalities: Piratini (PIR), Lavras do Sul (LVS), Dom Pedrito (DOP), Santana do Livramento (SLV), and São Pedro do Sul (SPS) (**Figure 1**). The climate is classified as humid subtropical (Cfa) according to the Köppen-Geiger climate classification adapted by Alvares et al. (2013), with no defined dry season and rainfall ranging from 1,500 to 1,800 mm per year.

The fields experiments were carried out on five private farms that are members of the "*Alianza del Pastizal Brasil*" (Alianza del Pastizal, 2024), an organization that certifies farms that maintain at least 50% of their land in natural grassland and promotes the sustainable use of this environment. Different production environments were tested on each farm: (i) unimproved natural grassland (NG) and (ii) improved natural grassland (NG+X, where "X" is the number of years of improvement until 2022) (**Table 1**).

There are no reports of fertilization, liming, overseeding of exotic species, or herbicide use in areas managed as NG. The NG+X areas have been managed with a combination of different improvement techniques, such as pH correction by liming, annual fertilization with N, phosphorus (P) and potassium (K) and

overseeding with cool season exotic species (ryegrass, white clover, and *Lotus* sp.) (Table 1).



**Figure 2.** Spatial distribution of studies that evaluated the effects of improved natural grasslands in southern Brazil on forage productivity, soil carbon and nitrogen stocks. The red circle represents the natural grassland study environment, and the yellow circle represents the natural grassland with fertilization and overseeding species study environment.

### 5.2.2. Soil sampling and analysis

Soil sampling was carried out in March 2022, to determine the C and N content in the soil in four layers (0-5, 5-10, 10-20, and 20-30 cm) collected with a shovel, totaling 156 samples. Samples were composed by three subsamples in each plot, approximately 30 meters apart from each other. The samples were dried in a forced-air circulation oven at 50°C, crushed, and ground to  $\leq 2.0$  mm. A sub-sample of 2.0 g was ground to  $\leq 250$   $\mu\text{m}$  on an Agate mortar to determine the total organic C and N content of the soil by dry combustion in a FlashEA® 1112 elemental analyzer. Soil C and N stocks to a depth of 30 cm were calculated using the equivalent mass method (Ellert and Bettany, 1995), which considers equal soil masses between the systems. Soil density was determined by taking

soil samples with metal rings with a volume of 113 cm<sup>3</sup> (4 × 6 cm) in each system in all soil layers sampled (0-5, 5-10, 10-20, and 20-30 cm).

**Table 1.** Characterization of environments studies that evaluated the effects of improved natural grasslands in southern Brazil.

Municipality	Soil class	Environment	Time (starting year)	Liming (Mg ha <sup>-1</sup> )	Accumulated fertilization (kg ha <sup>-1</sup> )			Overseed species
					N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	
Piratini (PIR)	Leptosol	NG	-	-	-	-	-	-
		NG+7	7 (2015)	3	155 (22)	380 (54)	-	Ryegrass
		NG+12	12 (2010)	-	105 (9)	338 (28)	-	Ryegrass
Lavras do Sul (LVS)	Chernosol	NG	-	-	-	-	-	-
		NG+4	4 (2018)	-	81 (20)	81 (20)	-	Ryegrass
		NG+14	14 (2008)	-	351 (25)	351 (25)	-	Ryegrass
Dom Pedrito (DOP)	Vertisol	NG	-	-	-	-	-	-
		NG+4	4 (2018)	-	44 (11)	104 (26)	-	Ryegrass + White clover + Lotus sp.
Santana do Livramento (SLV)	Leptosol	NG	-	-	-	-	-	-
		NG+2	2 (2020)	-	74 (37)	54 (27)	-	Ryegrass
São Pedro do Sul (SPS)	Acrisol	NG	-	-	-	-	-	-
		NG+2	2 (2020)	3	141 (71)	198 (99)	30 (15)	Ryegrass + White clover + Lotus sp.
		NG+4	4 (2018)	3	254 (64)	263 (66)	135 (34)	Ryegrass + White clover + Lotus sp.

Fertilization N, P<sub>2</sub>O<sub>5</sub> e K<sub>2</sub>O in parentheses represent the annual average applied per nutrient.

### 5.2.3. Evaluation of forage productivity

Data collection to assess forage productivity was also conducted in the selected farms (Table 1). Pasture structure was assessed by sampling vegetation in each grassland environment with a frequency of 60 days between assessments. The total dry matter productivity ( $\text{kg ha}^{-1} \text{DM}$ ) was estimated with grazing exclusion cages using the triple pairing technique (Klingman et al., 1943), obtained by adding up the forage accumulation with a frequency of 60 days.

### 5.2.4. Descriptive and statistical analyses

The data obtained were subjected to tests of normality assumptions of the mathematical model using the Shapiro-Wilk test and homogeneity of variables using the Levene test. The analysis of variance was performed using the F test and, when significant ( $p < 0.05$ ), the means were compared using the Tukey test at a 5% level of confidence using R software.

## 5.3. Fertilization and overseeding in natural grasslands in southern Brazil: impacts on soil fertility and the botanical composition of species

### 5.3.1. Study sites

The fields experiments were carried out in the Pampa biome region of the State of Rio Grande do Sul, in five municipalities: Piratini (PIR), Lavras do Sul (LVS), Dom Pedrito (DOP), Santana do Livramento (SLV), and São Pedro do Sul (SPS) (**Figure 1**). The climate is classified as humid subtropical (Cfa) according to the Köppen-Geiger climate classification adapted by Alvares et al. (2013), with no defined dry season and rainfall ranging from 1,500 to 1,800 mm per year.

The fields experiments were carried out on five private farms that are members of the “*Alianza del Pastizal Brasil*” (Alianza del Pastizal, 2024), an organization that certifies farms that maintain at least 50% of their land in natural grassland and promotes the sustainable use of this environment. Different production environments were tested on each farm: (i) unimproved natural



grassland (NG) and (ii) improved natural grassland (NG+X, where "X" is the number of years of improvement until 2022) (**Table 1**).

There are no reports of fertilization, liming, overseeding of exotic species, or herbicide use in areas managed as NG. The NG+X areas have been managed with a combination of different improvement techniques, such as pH correction by liming, annual fertilization with N, phosphorus (P) and potassium (K) and overseeding with cool season exotic species (ryegrass, white clover, and *Lotus* sp.) (**Table 1**).

### 5.2.2. Soil sampling and analysis

Soil sampling was carried out in March 2021 in four layers (0-5, 5-10, 10-20, and 20-40 cm) collected with a shovel, totaling 156 samples. Samples were composed by three subsamples in each plot, approximately 30 meters apart from each other. The samples were dried in a forced-air circulation oven at 50°C, crushed, and ground to  $\leq 2.0$  mm.

The chemical attributes used were: pH in water (1:1 v/v), available P and K extracted by Mehlich-1, exchangeable Al, Ca and Mg extracted by 1.0 mol L<sup>-1</sup> KCl, and Al saturation. All analyses were performed following the methodology described by Tedesco et al. (1995). The exchangeable Al was determined by titration with 0.0125 mol L<sup>-1</sup> NaOH solution; Ca and Mg by atomic absorption spectrometry; K by flame photometry and P by photolorimetry (Tedesco et al., 1995). The sum of bases (SB) was determined by the sum of Ca, Mg, and K. The cation exchange capacity at pH 7.0 (CEC<sub>pH7.0</sub>) was calculated by SB + (H + Al); the base saturation (V) was calculated using the relation:  $V (\%) = 100 \times SB/CEC_{pH7.0}$ ; and the Al saturation (m) was obtained by the relation:  $m (\%) = [Al / (SB + Al)] \times 100$  (CQFS-RS/SC, 2016).

### 5.2.3. Evaluation of forage productivity

Data collection to assess forage productivity was also conducted in the selected farms (**Table 1**). Pasture structure was assessed by sampling vegetation in each grassland environment with a frequency of 60 days between assessments. The total dry matter productivity (kg ha<sup>-1</sup> DM) was estimated with

grazing exclusion cages using the triple pairing technique (Klingman et al., 1943), obtained by adding up the forage accumulation with a frequency of 60 days. The triple pairing technique consists of pairing three identical and representative areas of the pasture, where one point is cut, and the harvested forage is weighed and placed in the exclusion cage at the other point, where the vegetation will be intact. A third point is also chosen, which will be the reference for the next sampling.

The botanical composition was assessed by the frequency and coverage of the species present in the experimental sites, obtained using the Botanal technique (Haydock and Shaw, 1975) in January 2022. The vegetation in the sites was analyzed based on the richness of the observed species. Species richness was based on the total number of species found in the sampled area of each site. Species diversity was estimated based on the Shannon index.

#### 5.2.4. Descriptive and statistical analyses

The data obtained were subjected to tests of normality assumptions of the mathematical model using the Shapiro-Wilk test and homogeneity of variables using the Levene test. The analysis of variance was performed using the F test and, when significant ( $p < 0.05$ ), the means were compared using the Tukey test at a 5% level of confidence using R software.

## 6. RESULTS AND DISCUSSION

### 6.1. Boosting production of “Campos” natural grasslands through improving soil fertility and overseeding: a meta-analysis

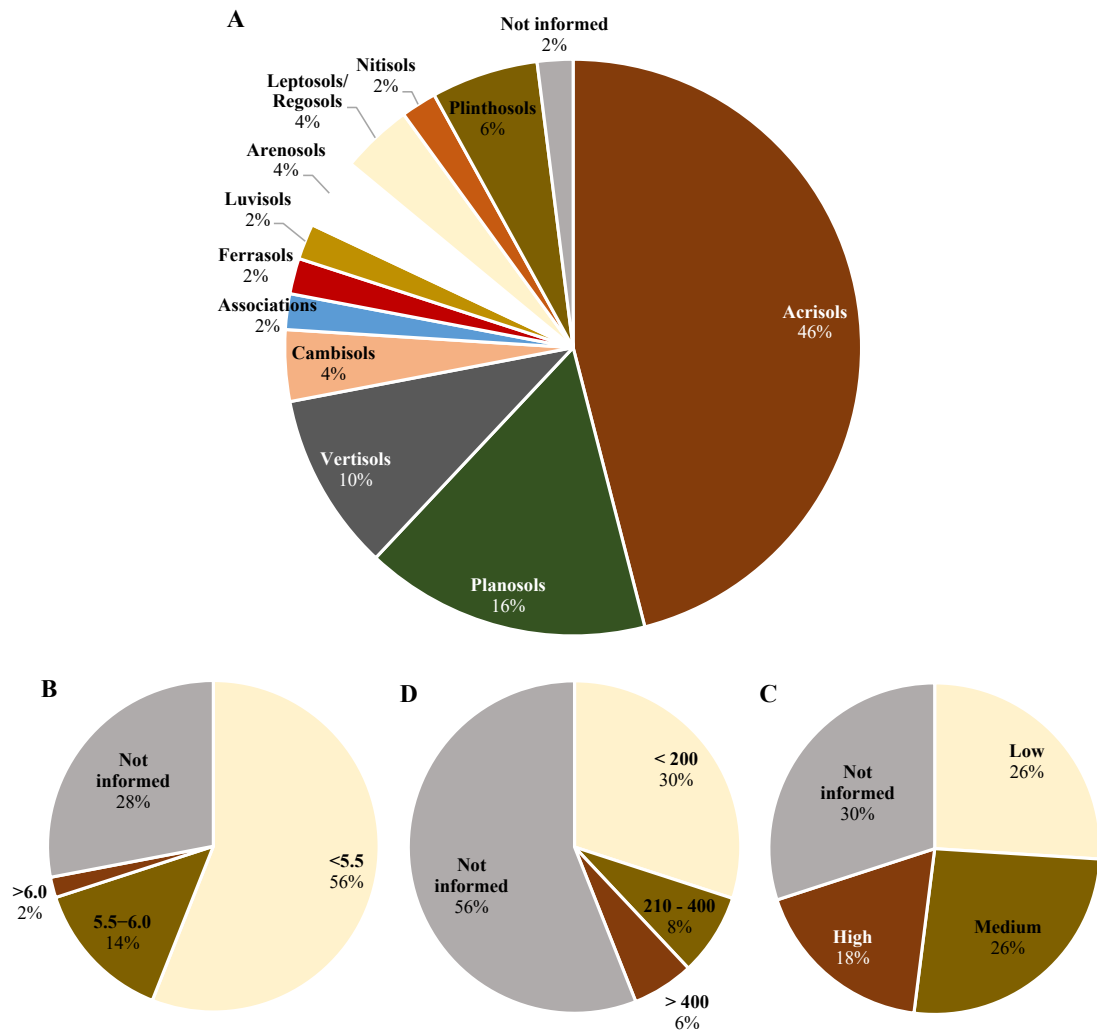
#### 6.1.1. Characterization of selected studies

The majority identified studies (90%) involving the improvement technologies of natural grasslands were conducted in the state of Rio Grande do Sul (**Figure 1**). Among these, the municipalities of Eldorado do Sul, Santa Maria, and Capão do Leão accounted for 22%, 14%, and 10%, respectively, totaling 46% related (**Figure 1**).

Regarding soil classification, 46% of the studies were conducted on Acrisols, followed by Planosol (16%), Vertisol (10%), Plinthosol (6%), Leptosol/Regosol, and Arenosol (4%) (**Figure 3a**). Studies conducted on Luvisol, Ferasol, and Nitosol account to only 2% each, while 2% of the studies did not specify a specific soil class (**Figure 3a**).

Approximately 56% of the studies were conducted in soils with low pH (below 5.5), while 14% were conducted in soils with pH between 5.5 and 6.0, and only 2% with pH above 6.0 (**Figure 3b**). Notably, 28% of the studies omitted pertinent information concerning soil pH. Around 26% of the assessed studies were conducted in soils with low SOM (<2.5%), and 26% with medium content (2.6–5.0%). Only 18% of the studies occurred in soils with more than 5.0% of SOM and 30% did not specify that information (**Figure 3c**). More than 50% of the studies did not inform the clay content of the soil (**Figure 3d**). In addition, 30% of the studies were carried out on soils with low clay content (< 200 g kg<sup>-1</sup>), and just

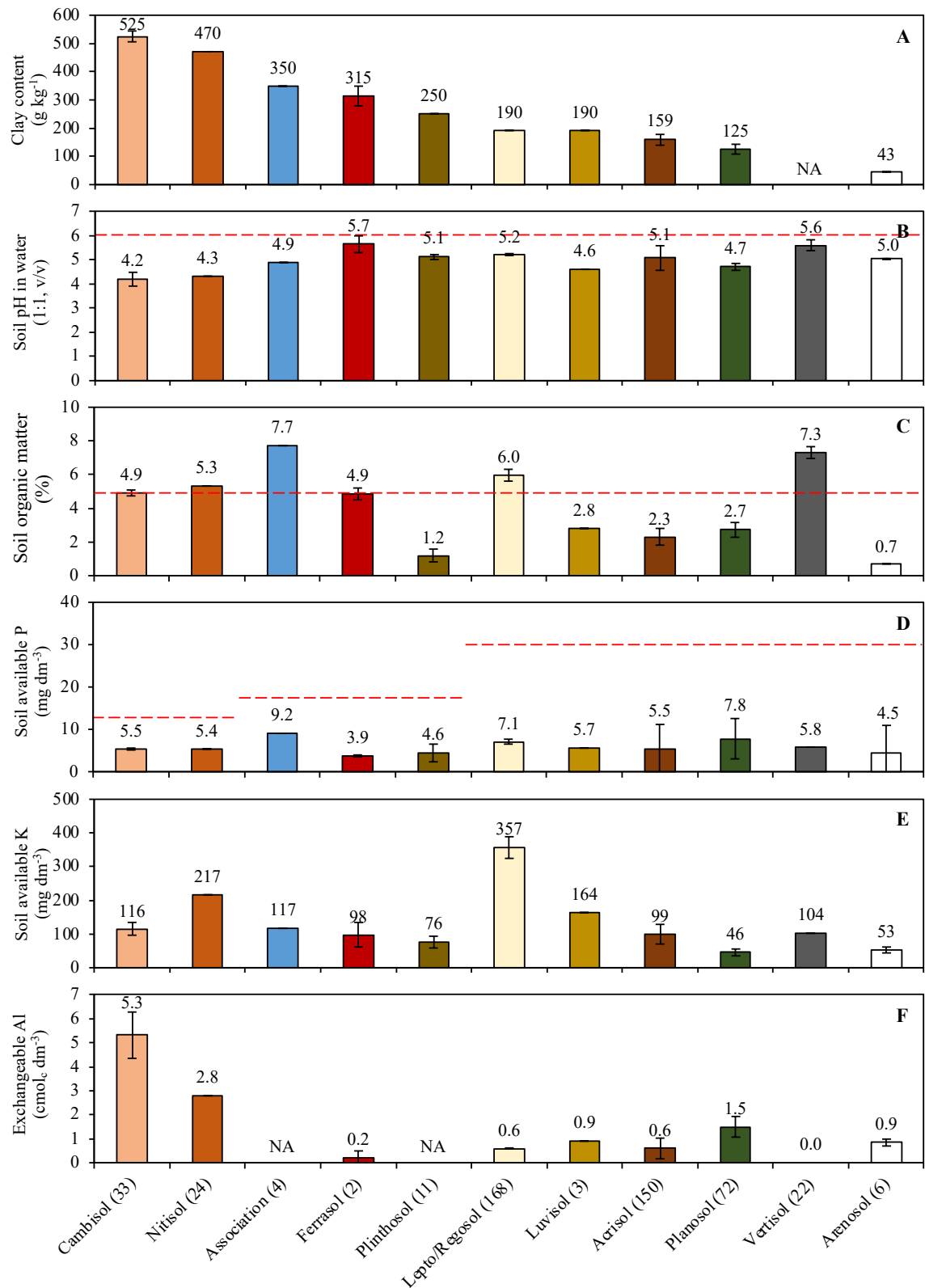
6% of them in soils with clay content higher than 400 g kg<sup>-1</sup>. The chemical and physical characterizations of each soil class are shown in **Figure 4**.



**Figure 3.** Soil class (A), soil pH in water (1:1, v/v) (B), soil organic matter content ( $\leq 2.5\%$  - Low, 2.6 – 5.0% - Medium,  $> 5.0\%$  - High) (C) and soil clay content (g kg<sup>-1</sup>) (D) at control observation in studies that evaluated the effects of improvement natural grasslands of Southern Brazil on forage and beef production. Values represent the percentage of studies included in each category.

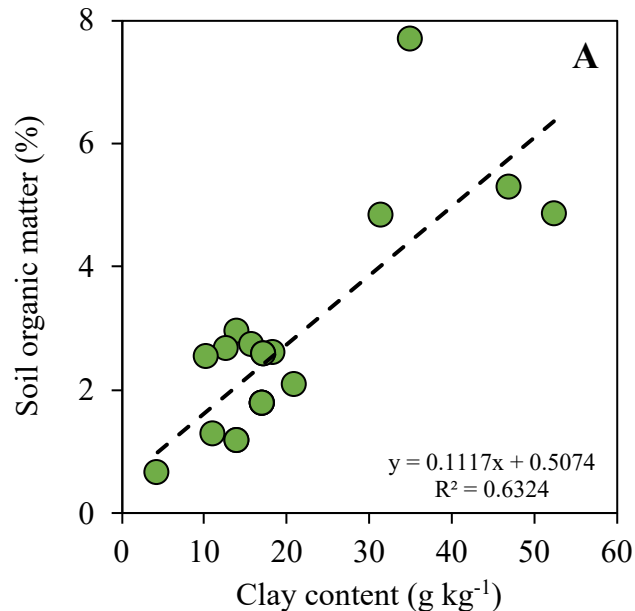
There was a positive relationship between clay content and SOM ( $p < 0.05$ ) (**Figure 5**). The clay content explained about 63 % of the SOM, increasing by 0.51 % of SOM for each increase of 100 g kg<sup>-1</sup> of clay content.

Among the natural grassland improvement technologies, fertilization alone was employed in 33% of the evaluated studies, followed by fertilization combined with overseeding (27%), lime combined with fertilization (18%), lime + fertilization



**Figure 4.** Soil clay content (A), Al exchangeable (B), soil organic matter content (SOM) (C), P available (D), K available (E), soil pH (F) according to each soil class in studies that evaluated the effects of improvement natural grasslands of Southern Brazil on forage and beef production. NA: not available. Association: Leptosol/Phaeozems/Vertisol. Red line represents the critical values according to CQFS-RS/SC, (2016). Error bars represent a 95% confidence interval of the mean.

+ overseeding (16%), overseeding alone (4%), and lime application alone (2%) (**Figure 6a**). Approximately 74% of the overseeding studies utilized ryegrass or combinations of ryegrass with other species (**Figure 6b**). More than 50% of the association with ryegrass was with clover (vesicular or white) (*Trifolium vesiculosum* and *Trifolium repens*, respectively) or *Lotus* sp. (**Figure 6c**).

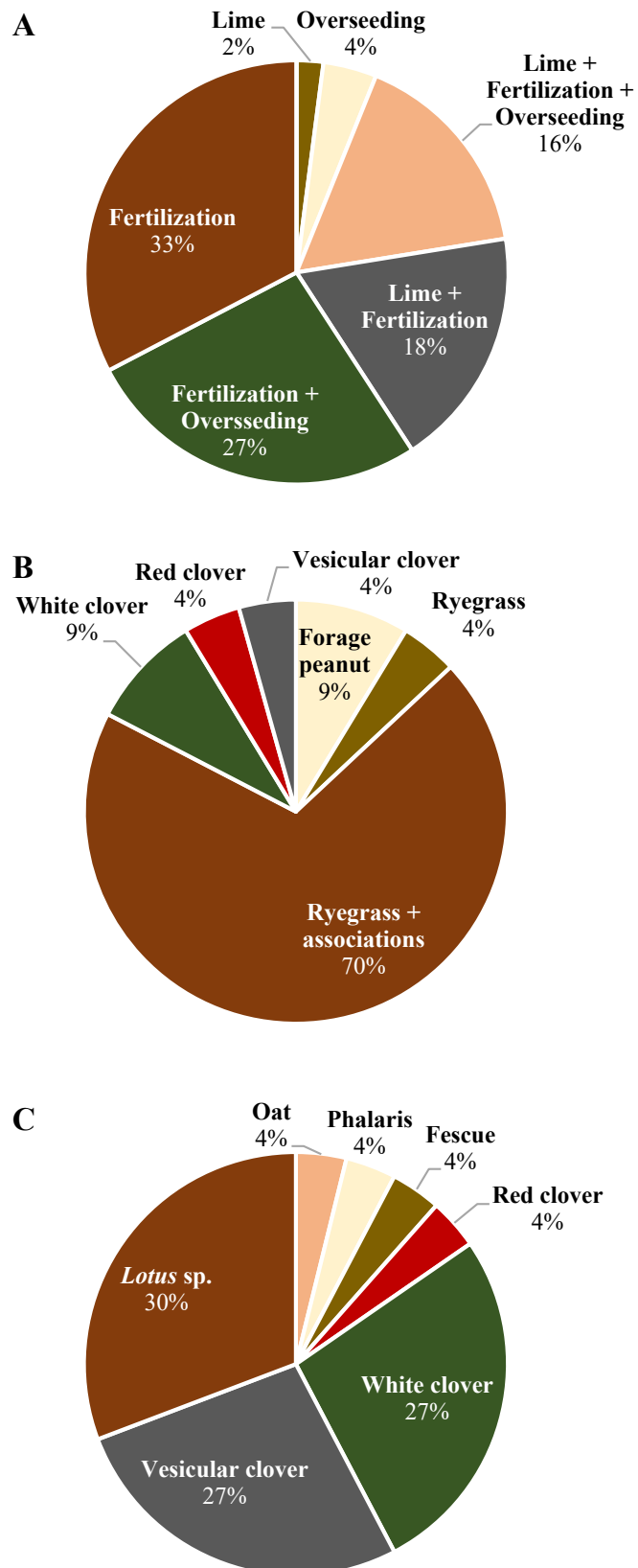


**Figure 5.** Relationship between soil clay content (g kg<sup>-1</sup>) and soil organic matter (%) in control treatment in studies that evaluated the effects of improvement natural grasslands of Southern Brazil on forage and beef production.

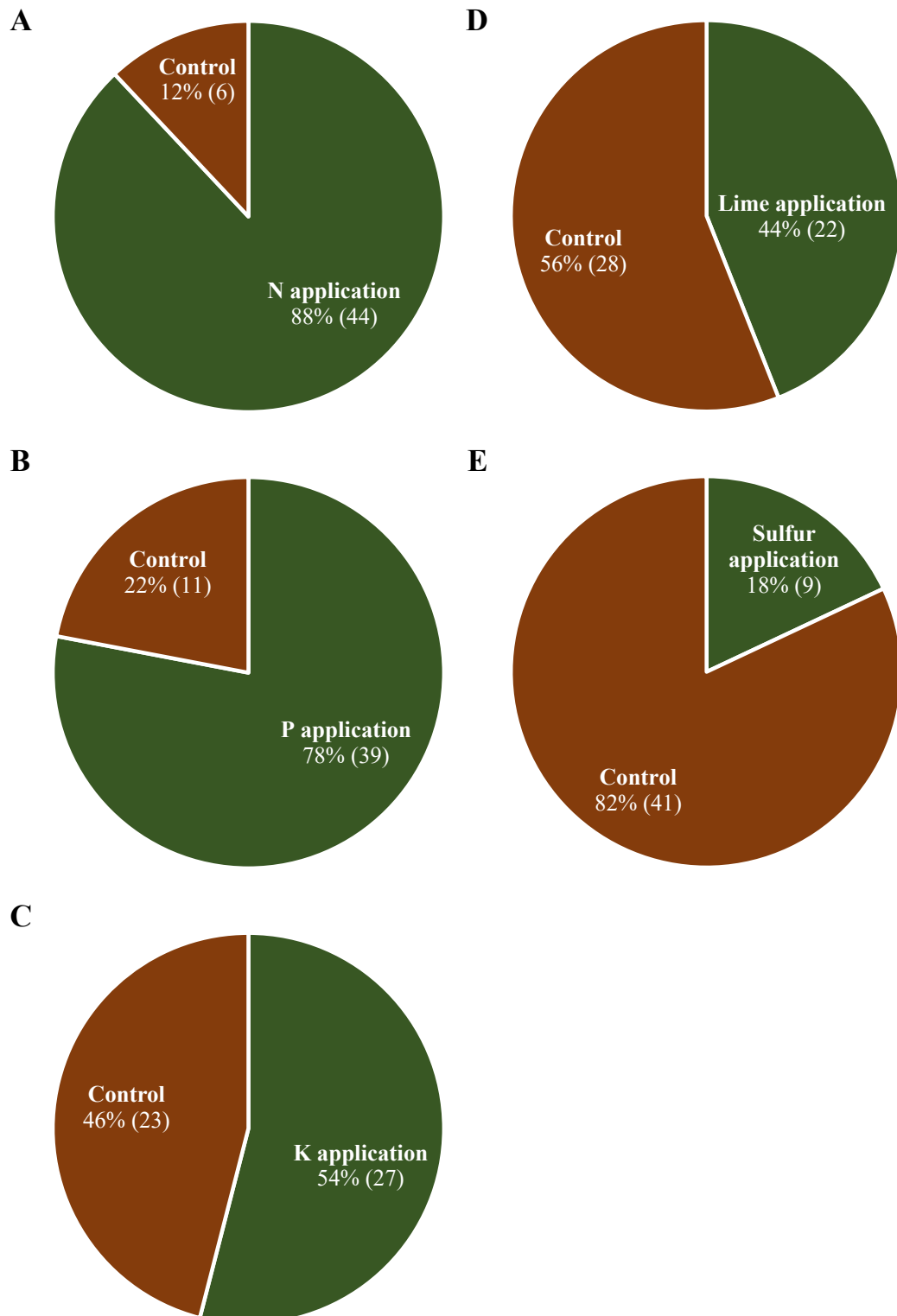
Concerning fertilization, the addition of N, P, and K occurred in 88%, 78%, and 54% of the studies, respectively (**Figure 7a, b, c**). The application of limestone and sulfur occurred in 44% and 18% of the studies, respectively (**Figure 7d, e**).

#### 6.1.2. Effect of improving natural grasslands for forage and beef production

In the overall average of the evaluated studies, liming/fertilization and overseeding of species led to a 34% increase in natural grassland forage production, ranging from 2 to 195% (**Figure 8**). In studies assessing beef productivity, liming/fertilization and overseeding of species resulted in an average increase of 44%, ranging from 13 to 131% in forage production, and up to 82%, ranging from 21 to 178% in beef production (**Figure 9**).

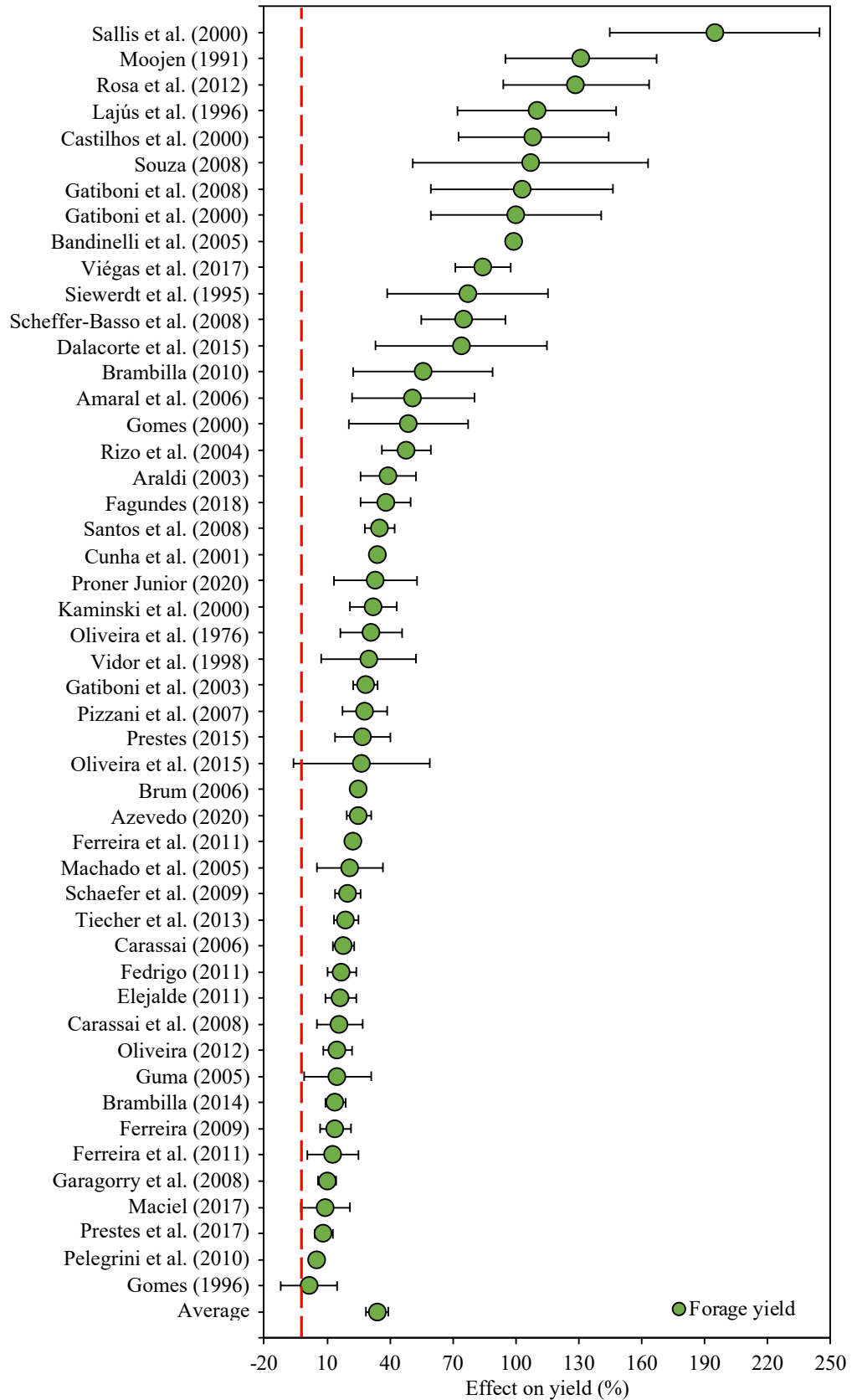


**Figure 6.** Improvement strategy (A), forage species (B), forage species associated with ryegrass (C) in studies that evaluated the effects of improvement natural grasslands of Southern Brazil on forage and beef production. Values represent the percentage of studies included in each category.



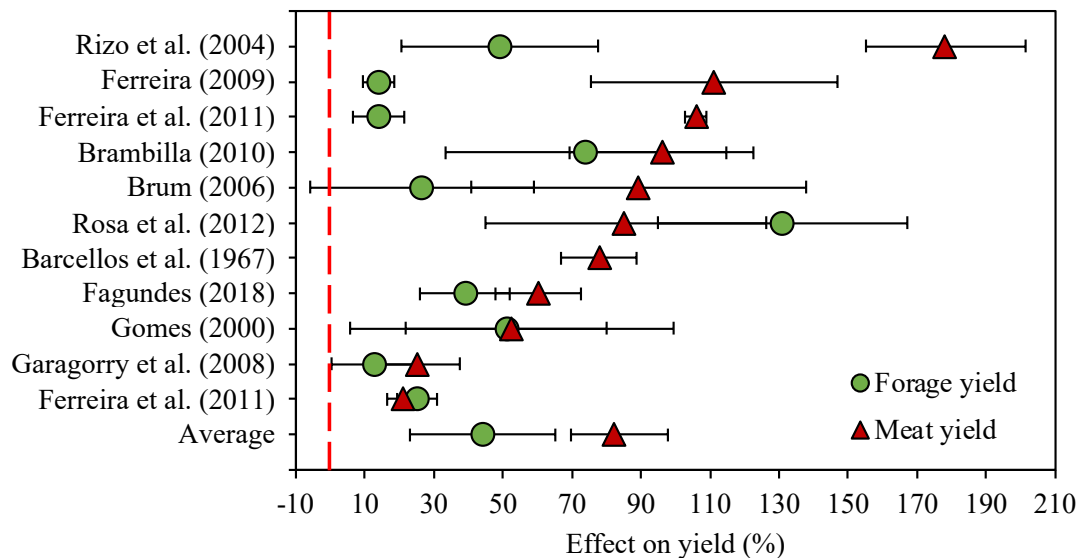
**Figure 7.** Use of nitrogen (A), phosphate (B), potassium (C), liming (D), and sulfur (E) fertilization in studies that evaluated the effects of improvement natural grasslands of Southern Brazil on forage and beef production. Values in percentage represent the studies included in each category. Values within parentheses represent the number of studies.





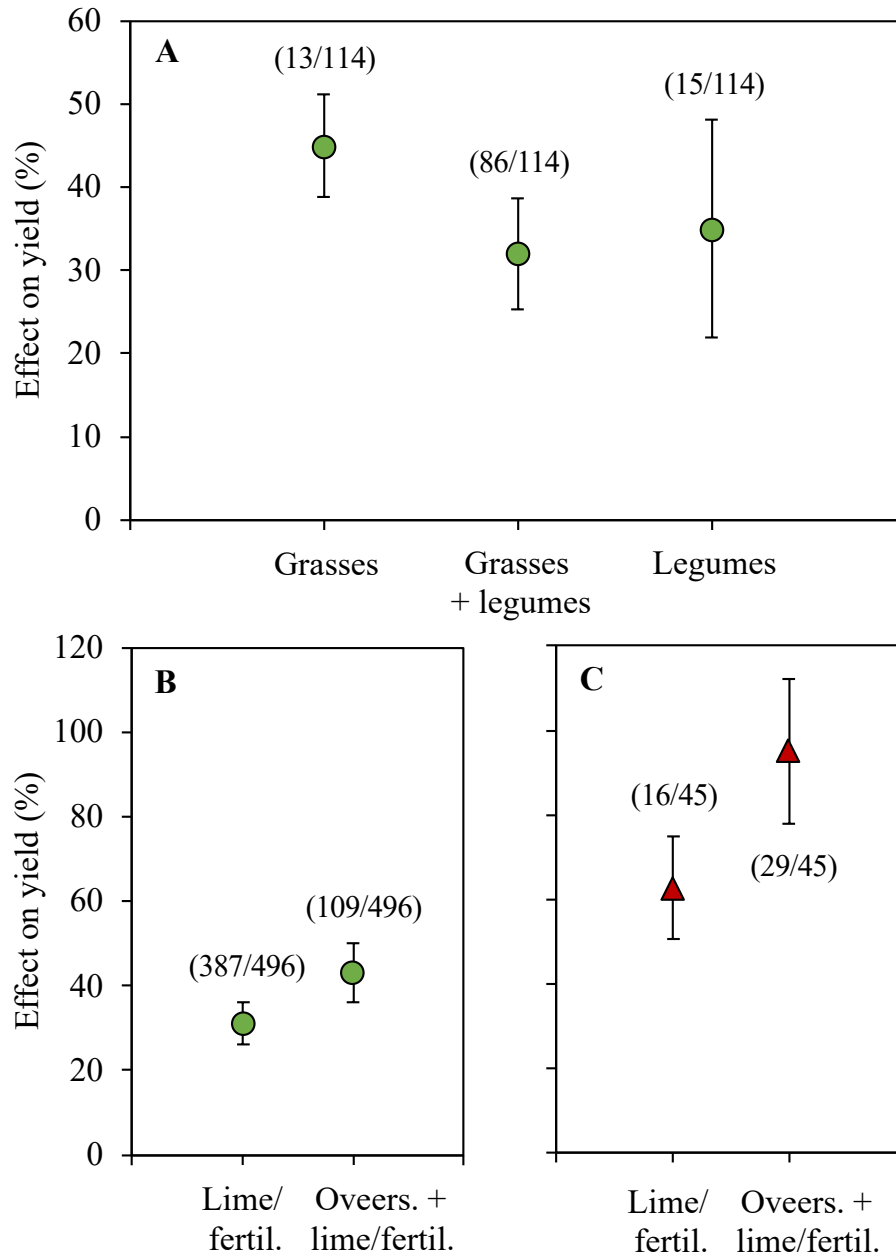
**Figure 8.** Effect of natural grassland improvement strategies on forage production (%) in each study included in the meta-analysis. Error bars represent a 95% confidence interval of the mean.

The use of overseeding with grasses, legumes, or the combination of grasses + legumes did not show differences in forage production ( $p > 0.05$ ). However, all effects were positive, ranging from 32 to 45% (**Figure 10a**). Natural grassland that received only fertilization experienced a productivity increase of 31% (26–36%), and 43% (36–50%) ( $p < 0.10$ ) when overseeding was added. Improving natural grassland with fertilization and fertilization + overseeding increased beef production by 63 (51–75%) and 95% (78–112%) ( $p < 0.10$ ), respectively (**Figure 10b, c**).



**Figure 9.** Effect of natural grassland improvement strategies on forage and beef production (%) in each primary study included in the meta-analysis. Error bars represent 95% confidence interval of the mean.

The improvement technologies increased 123% the forage production, by ranging from 2184 kg dry matter  $\text{ha}^{-1} \text{yr}^{-1}$  in the control to 5063 kg dry matter  $\text{ha}^{-1} \text{yr}^{-1}$  with lime + fertilization + overseeding (**Figure 11**). No difference was observed among the nutrients in the total forage production. Considering rates ranges with the total of N, P, K, and S applied to the natural grassland (**Figure 12a**), productivity increments started at 12% for rates up to 50  $\text{kg ha}^{-1}$  and reached 74% for rates exceeding 300  $\text{kg ha}^{-1}$ . For limestone, the greatest productivity increases were with rates between 2.1 and 4.0  $\text{Mg ha}^{-1}$ , both for soils with  $\text{pH} \leq 5.0$  (90% increase) and for soils with  $\text{pH} > 5.0$  (43% increase) (**Figure 12b, c**).

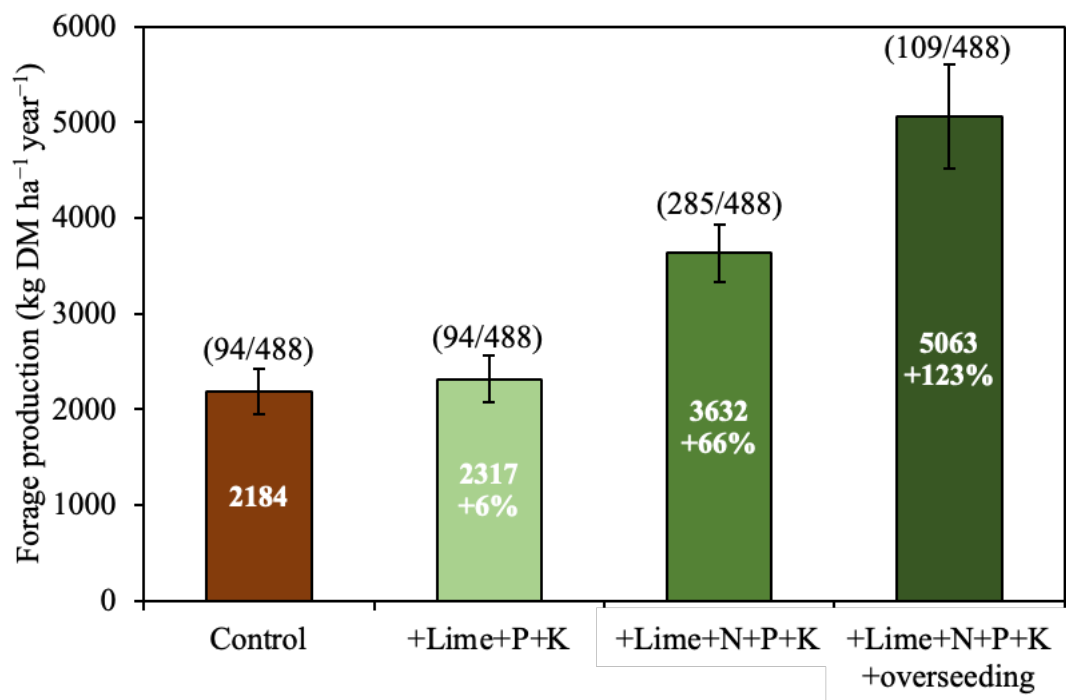


**Figure 10.** Effect of forage composition (A) and improvement technologies on forage (B) and beef production (C) in studies that evaluated the effects of improvement natural grasslands of Southern Brazil. The number of observation pairs and the total observations included in each category are shown within parentheses. Error bars represent 95% (A) and 90% (B, C) confidence interval of the mean.

### 6.1.3. Regression tree for the improvement effect on forage production

The regression tree reveals that the quantity of N+P+K+S applied and the soil type of the site explain 37% of the variation in forage production (**Figure 13**).

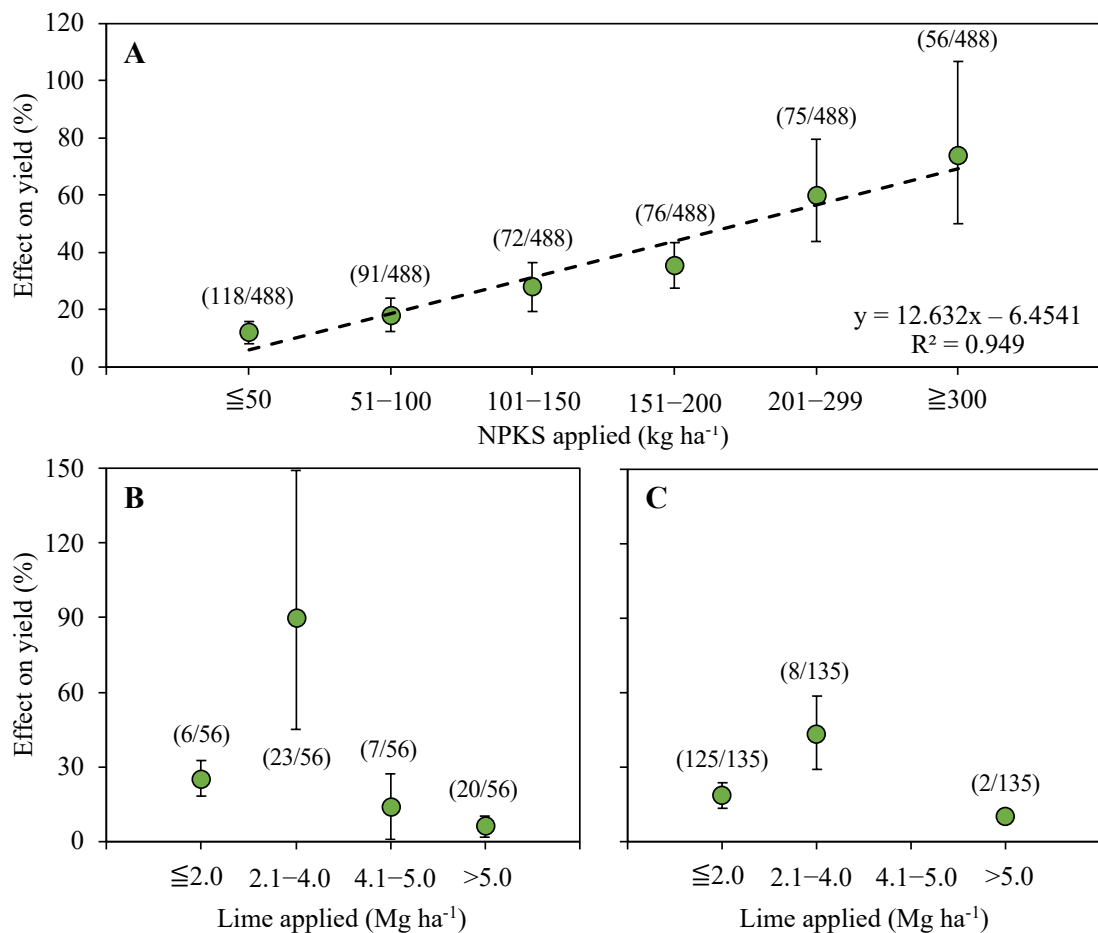
The applied amount of N+P+K+S ( $\text{kg ha}^{-1}$ ) was the factor that best discriminated the effect of improvement technologies on natural grassland forage productivity. The natural grassland shows a more pronounced response to improvement technologies when fertilized with at least  $234 \text{ kg ha}^{-1}$  of N+P+K+S, resulting in an average increase of 71% in forage production. Conversely, when the applied quantity of N+P+K+S is less than  $234 \text{ kg ha}^{-1}$ , the average forage production increase drops to only 22%.



**Figure 11.** Forage production according improvement technologies in studies that evaluated the effects of improvement natural grasslands of Southern Brazil. The number of observation pairs and the total observations included in each category are shown within parentheses. The percentage represents the increase of improvement technology compared to control. Error bars represent 95% confidence interval of the mean.

The second most influential factor in the forage production response was the soil class (**Figure 13**). In environments with fertilization of at least  $234 \text{ kg ha}^{-1}$  of N+P+K+S, the soil classes of Arenosol, Acrisol, Planosol, and Plinthosol exhibit an average increase of 98% compared to the natural grassland without improvement, whereas it is only 36% in the classes of Vertisol and Leptosol/Regosol. When the applied quantity of N+P+K+S is less than  $80 \text{ kg ha}^{-1}$ , in the soil classes of Acrisol, Planosol, and Cambisol, the average increase in

forage production was 21%, versus 6% in the soil classes of Leptosol/Regosol, Nitisol, Ferrasol, and Luvisol (**Figure 13**).



**Figure 12.** Effect of NPKS application amount (A) and lime amount in soil with pH  $\leq 5.0$  (B) and pH  $> 5.0$  (C) in forage yield in studies that evaluated the effects of improved natural grasslands of Southern Brazil on forage and meat production. The number of observation pairs and the total observations included in each category is shown within parentheses.

#### 6.1.4. Discussion

The noteworthy augmentation in forage and animal production with the implementation of natural grassland improvement technologies underscores the significance of these technologies, especially the use of liming and fertilization (**Figure 8; 9**). These improvement technologies highlight the challenges related to soil acidity, nutritional deficiencies, and the limited presence of cool-season native species in these environments (Jaurena et al., 2021; Overbeck et al., 2007). This scenario is more pronounced in natural grasslands developing on soil

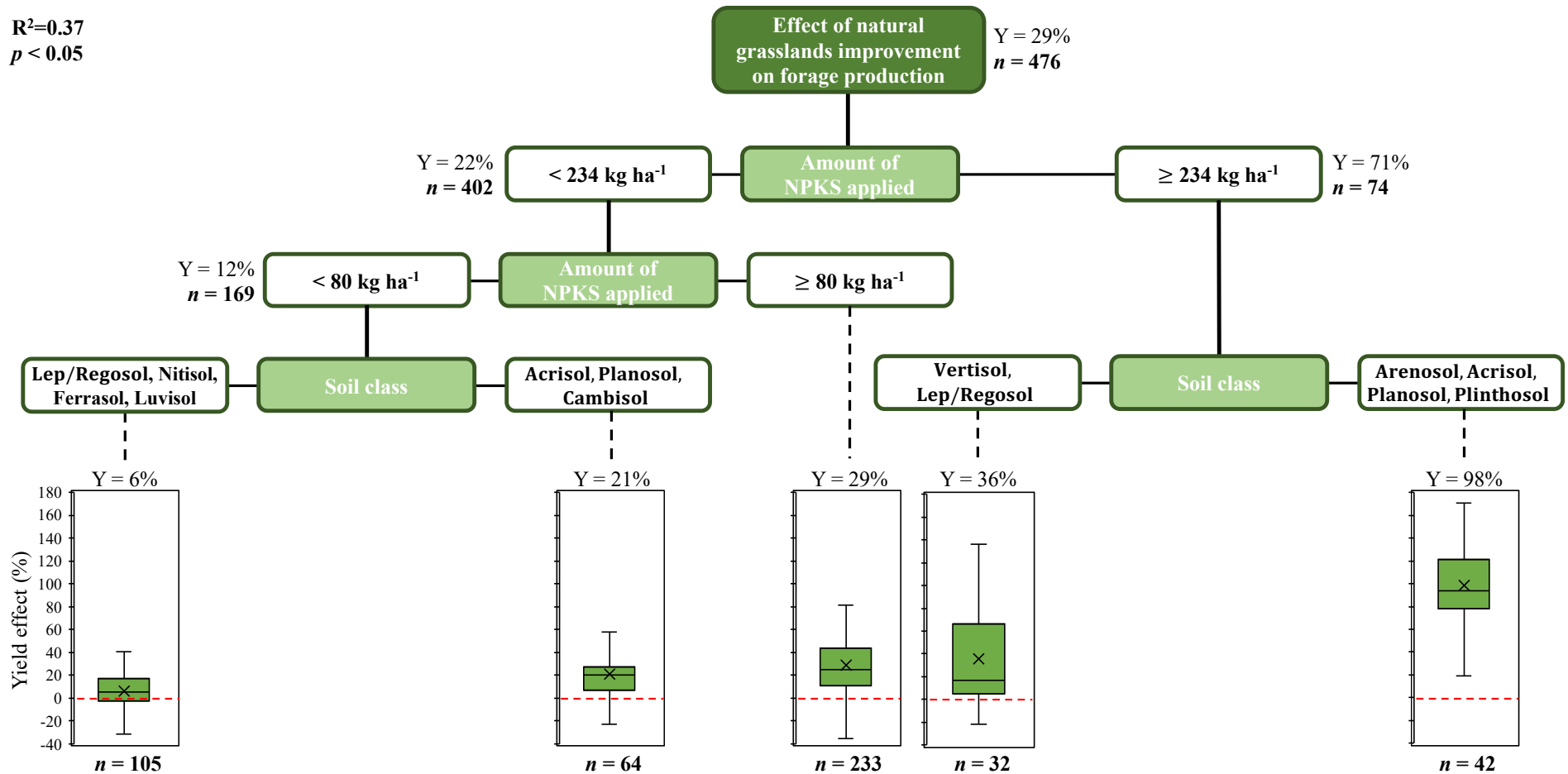
classes such as Acrisol (Gatiboni et al., 2000), Planosol (Lajús et al., 1996), and Plintosol (Castilhos et al., 2000) (**Figure 13**). These soil classes are prevalent in more than 68% of the studies evaluated here (**Figure 2**), occupying approximately 44% of the Pampa biome in the state of Rio Grande do Sul (IBGE, 2022), especially in the regions of the Central Depression and Campaign of the state (Streck et al., 2018). These areas are naturally characterized by low values of pH, low soil available P, and low content of soil organic matter (Streck et al., 2018; Pallarés et al., 2005).

The characterization data (**Figure 3b; 4b, f**) also illustrate that in at least 56% of the studies presented soil pH below 5.5. It could be a limiting factor for the establishment and production of both exotic and native leguminous species, coupled with low soil P content (**Figure 3d**). Soils with a pH below 5.5,  $Al^{+3}$  have a detrimental effect on nodulation, diminishing plant growth (Sartain and Kamprath, 1975) and root elongation (Kopittke et al., 2015), ultimately resulting in decreased water and nutrient absorption (Barcelo and Poschenrieder, 2002).

In natural grassland developed on acidic soils without the introduction of exotic species, only soil pH correction through liming and the subsequent reduction of toxic Al levels allows an increase of up to  $0.6 \text{ Mg ha}^{-1}$  in annual forage production (Poozesh et al., 2010). Furthermore, liming enhances the effect of P fertilization (Alves et al., 2022) and the absorption of N, K, Ca, Mg, and S (Goulding, 2016; Holland et al., 2018, 2019; Bossolani et al., 2021), potentially elevating annual forage production by up to  $5.5 \text{ Mg ha}^{-1}$  when combined with N+P+K+S fertilization (Lajús et al., 1996).

The higher average increase in animal production compared to forage production (**Figure 9; Figure 10b, c**) in environments subjected liming, fertilization, and overseeding of species can be elucidated by the efficient utilization of forage. This phenomenon occurs in improvement technologies environments in contrast to natural grasslands without improvement during the autumn/winter and early spring periods. Liming/fertilization and overseeding of species, in addition to fostering higher forage production, facilitate enhanced bromatological quality of the forage (Vidor and Jaques, 1998; Coelho Filho and Quadros, 1995). Meanwhile, digestibility and crude protein levels increase due to overseeding of species and liming/fertilization of the natural grassland (Gatiboni et al., 2008).

$R^2=0.37$   
 $p < 0.05$



**Figure 13.** Conditional inference tree for the effect of natural grasslands improvement on forage production (%). The central rectangle of the boxplot spans the first to third input quartile. The solid line inside the rectangle represents the median, the “x” represents the mean, and vertical lines above and below the box extend to the minimum and maximum values, respectively. The mean effect of improvement natural grasslands on forage yield (Y) is shown above the box plot, and the number of pair observations (n) used to calculate the mean effect is shown at the bottom.

The linear increase in forage production obtained with increasing ranges of N+P+K+S rates applied to natural grasslands demonstrates the significant response potential of native and overseeded exotic forage species to soil liming/fertilization (**Figure 12a, b, c**). Soil pH correction through liming (Tiecher et al., 2013) and fertilization, particularly during the winter-spring period (Oliveira et al., 2015), are practices that significantly enhance annual forage production in natural grasslands. Additionally, they mitigate the system's production seasonality (Tiecher et al., 2013) and enable higher beef production per hectare (Ferreira et al., 2011). This also enhances the competitiveness of livestock systems in natural grassland compared to alternative land uses, such as annual crops.

Concerning the implemented natural grasslands improvement technologies, fertilization and liming were evident in 94% and 36% of the studies, respectively (**Figure 6a**). In this context, environments with lower productive potential, encompassing soil classes such as Arenosol, Acrisol, Planosol, Plinthosol, and Cambisol exhibiting due to greater chemical restrictions (**Figure 4**), displayed the most pronounced effects on forage production with increasing amounts of N+P+K+S applied (**Figure 11**). Notably, the use of overseeding for natural grassland improvement should be accompanied by proper correction of soil acidity and nutrient availability. The low inherent levels of P and high  $Al^{+3}$  saturation in the soil act as limiting factors for the introduction of species with high nutritional demands (Gatiboni et al., 2000; Gatiboni et al., 2003). These species, including ryegrass, clovers, and *Lotus* sp., which constitute the primary overseeded exotic species in the examined studies (**Figure 6b, c**), are implemented easily through overseeding, without the necessity for seed incorporation into the soil, and boast rapid germination. Additionally, their substantial contribution to the forage production in the grassland (Ferreira et al., 2011) further elucidates why these species are commonly employed in natural grassland improvement technologies.

In addition to factors associated with low natural soil fertility and high acidity (Pallarés et al., 2005), natural grasslands in the Pampa biome are characterized by a prevalence of warm-season growth species. Moreover, there is lower diversity and participation of native cool-season species in the overall forage production of the system (Nabinger et al., 2009; Overbeck et al., 2007).



These deficiencies can be addressed through natural grassland improvement. Overseeding with exotic cool-season species such as ryegrass ensures high forage production during that period of the year (**Figure 11**) and allows for the maintenance of the "Campos environment".

The soil characterization data extracted from the studies presented illustrate that, on the whole, soils with higher clay content exhibit elevated levels of soil organic matter (**Figure 4a, c; Figure 5**). Sorption processes, coupled with the consequent protection of organic matter in conjunction with clay minerals constitute a pivotal mechanism for carbon stabilization in the soil, thereby diminishing its susceptibility to losses (Wattel-Koekkoek and Buurman, 2004; Feng et al., 2005). Generally, soils with higher clay content, and consequently greater specific surface area, cation exchange capacity, and Fe/Al oxide content are less prone to carbon losses through mineralization (Ransom et al., 1998; Kahle et al., 2003). This factor indicates that, although the environments with greater chemical restrictions manifested the most significant effects on forage production with improvement technology (**Figure 13**) augmenting.

However, with improvement technologies, there are temporal variations in the diversity, equitability, and species richness in natural grassland composition (Jaurena et al. 2021). Significant reductions in species richness due to fertilization over time have been reported in grasslands investigated in the Americas, Europe, and Asia (Blanck et al., 2011; Ceulemans et al., 2013; Zhao et al., 2019). In Brazil's natural grassland small variation in botanical composition with fertilization can promote species turnover, although without changing botanical indices and species richness (Somavilla et al 2021). Species turnover may lead to changes in nutrient exportation rates and such a process may affect soil nutritional levels due to changes in bromatological features of forage plants, as well as in soil organic matter accumulation.

The improvement practices delineated in this study signify technological advancements geared towards augmenting forage and animal production in natural grasslands in southern Brazil. In accordance with our findings, the incorporation of overseeding with exotic species should be concomitant with soil liming and fertilization. Soils of lesser fertility within a production system exhibit a higher potential for responsiveness to improvement technologies, and as such, these areas should be accorded priority for the application of such technologies.

Subsequent investigations in this domain should offer enhanced characterization of the study areas, given that 56% of the studies failed to report clay content, 32% omitted information on organic matter content, 28% lacked pH data, and 2% did not specify soil class. We deem this information indispensable for the judicious integration of natural grassland improvement technologies into production systems.

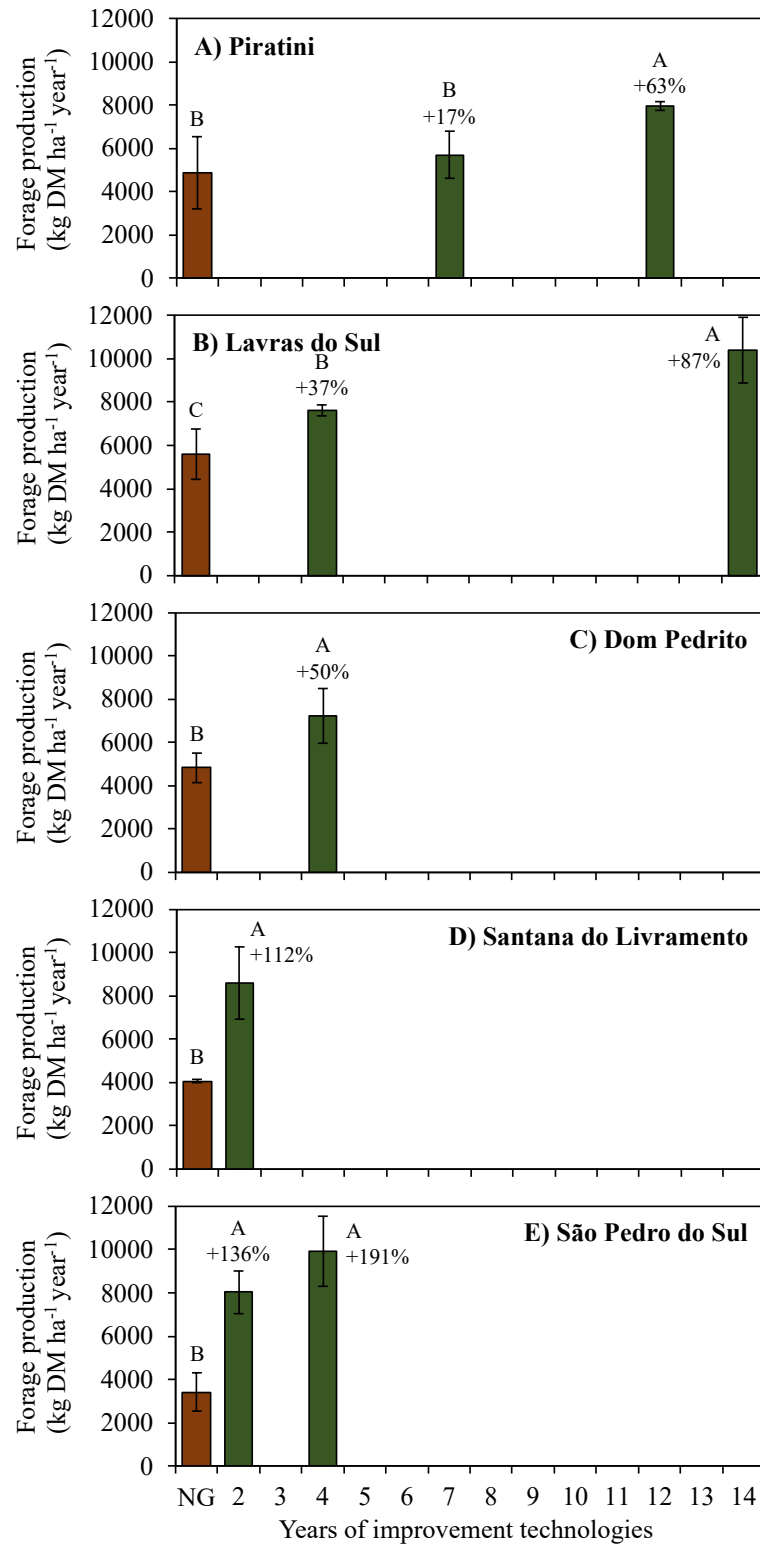
It is crucial to emphasize that the outlined improvement technologies for natural grasslands should be seamlessly integrated into the design of livestock farms, with a focus on diversifying forage supply and cautiously intensifying specific production processes, guided by technical expertise. Soil liming and must be informed by meticulous analyses of soil chemical properties. Additionally, overseeded species should undergo vigilant management throughout their development stages to avert compromising the longevity of the natural forage base in the Pampa biome, ensuring substantial productivity gains for the system.

## **6.2. Carbon storage in the Pampa biome: increasing productivity and conservation of natural grasslands in southern Brazil with "improvement technologies"**

### 6.2.1. Results

Annual forage productivity showed a significant difference between the study environment at all the sites evaluated (**Figure 14**). In the NG study environment, forage productivity ranged from 3.403 to 5.582 kg DM ha<sup>-1</sup> year<sup>-1</sup>, while in the environments with improvement technologies forage productivity ranged from 5.702 to 10.420 kg DM ha<sup>-1</sup> year<sup>-1</sup>.

The SPS, SLV and LVS productivity environments showed the highest percentages of increase in forage productivity with the use of improvement technologies (**Figure 14**). In SPS, after four years of using improvement technologies, it was possible to increase annual forage productivity by approximately 200%. In SLV, just two years after using the improvement technologies, it was possible to increase annual forage productivity by 136%, while in LVS, after 14 years, annual forage productivity was 87% higher.



**Figure 14.** Annual forage production in native grasslands with different years of improvement technologies in Piratini (A), Lavras do Sul (B), Dom Pedrito (C), Santana do Livramento (D) and São Pedro do Sul (E). NG represents the native grassland without improvement technologies. Error bars represent 95% confident interval of the mean. The percentage represents the increase of improvement technology compared to NG. Means followed by the same letter comparing the forage production between production environments are not significant according to the Tukey test  $p < 0.10$ .

The C and N content in the soil was significantly affected by the study environment and the soil layer in all the sites evaluated (**Figure 15; 16**). The 0-5 cm soil layer had the highest C and N contents in all sites and environments, when compared to the lower layers. In PIR, in the 0-5 cm layer, the average C and N content in the NG+7 and NG+12 environments was double (40.4 and 3.4 g kg<sup>-1</sup> respectively) when compared to NG (20.3 g kg<sup>-1</sup> and 1.7 g kg<sup>-1</sup> respectively) (**Figure 15a; 16a**). Also in the 0-5 cm layer, the C content in SLV and SPS in the NG+2 (**Figure 15d**) and NG+4 (**Figure 15e**) environments was 18 and 64% higher than NG and the N content was 39 and 71% higher than NG in SLV (**Figure 16d**) and SPS (**Figure 16e**), respectively.

C and N stocks in the 0-30 cm soil layer ranged from 54.5 to 158.2 Mg ha<sup>-1</sup> and 4.6 to 11.0 Mg ha<sup>-1</sup>, respectively, and were significantly affected by the productivity environment in all the sites evaluated (**Figure 17**). The PIR-NG+12 and LVS-NG+14 productivity environments showed the greatest increases in C and N stocks when compared to NG. C stocks were 49 and 34% higher in PIR-NG+12 and LVS-NG+14, respectively. For N stocks, the PIR-NG+12, LVS-NG+14 environments showed values 47 and 29% higher than NG, respectively.

Our results show that the sites with the highest natural stocks of C and N (LVS-NG and SLV-NG) showed no change in their stocks or even reduced their stocks between two and four years after the use of improvement technologies, respectively (**Figure 17b; d; g; i; Figure 18**). On the other hand, in SPS, a site with a lower initial stock of C and N, just two years of using improvement technologies was enough to increase the C stock by 15% and the N stock by 19% (**Figure 17e; j; Figure 18**).

### 6.2.2. Discussion

The increases in soil C and N contents and stocks, especially in the PIR-NG+12 and LVS-NG+14 environments, were possibly due to higher forage productivity (Salvo et al., 2008) (**Figure 14**). The higher forage productivity was due to the use of improvement technologies over time, especially on sites with more chemically limiting soil classes, as occurred in SPS (**Table 2**). The positive effects of these practices on the productivity and quality of forage produced in natural grasslands has already been widely documented (Rosa et al., 2012;

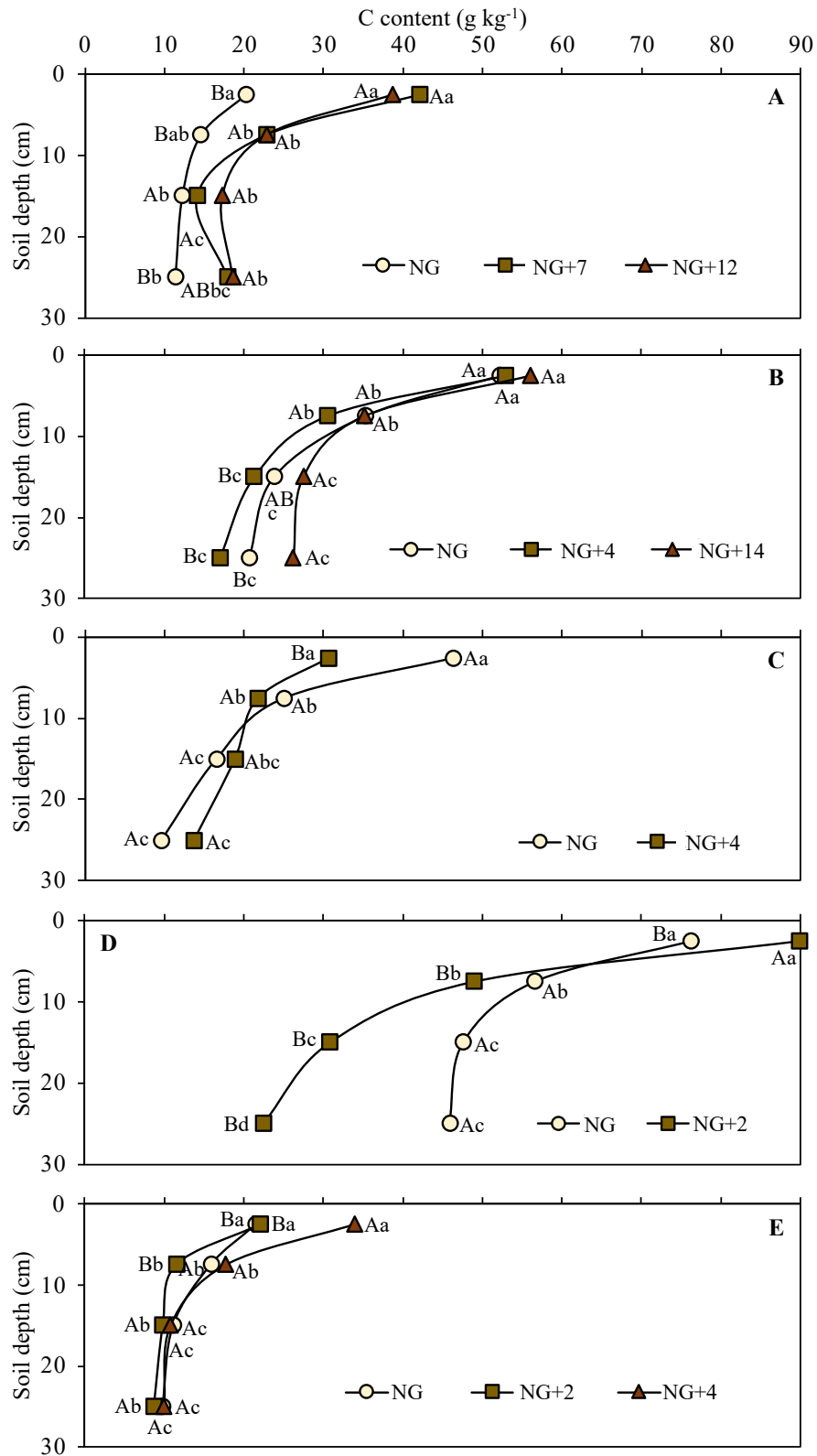
Gatiboni et al., 2008), and there are indications that they also have a positive effect on C contents and stocks in the 0-30 cm soil layer (Poeplau et al., 2018; Bondaruk et al., 2020).

**Table 2.** Soil chemical properties in natural grasslands environments studies that evaluated the effects of improved natural grasslands in southern Brazil on forage productivity, soil carbon and nitrogen stocks.

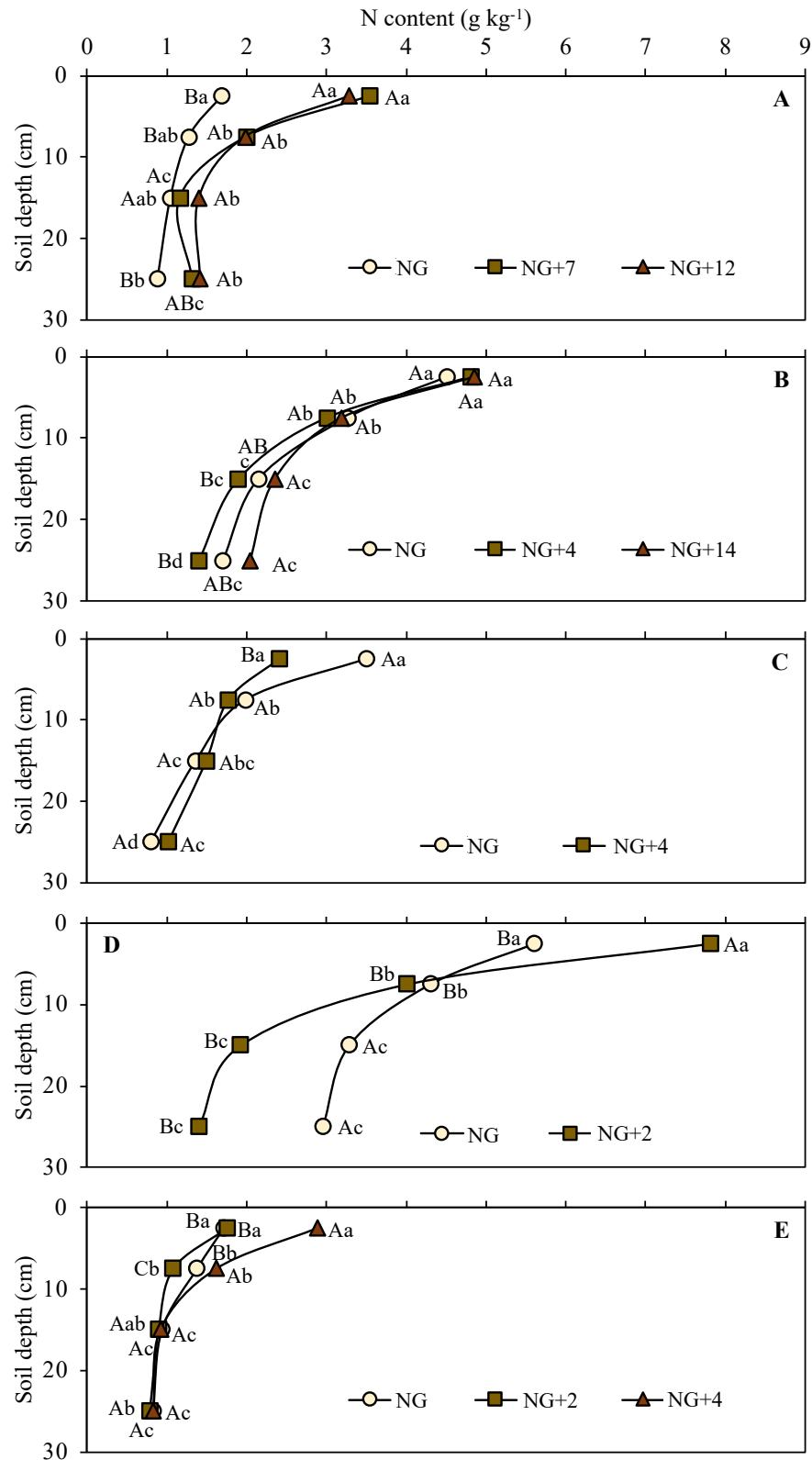
Environment	Soil layer cm	pH in water 1:1 v/v	C content g dm <sup>-3</sup>	Available P mg dm <sup>-3</sup>	Available K mg dm <sup>-3</sup>	Base saturation %	Al saturation %	CEC cmol <sub>c</sub> dm <sup>-3</sup>
PIR - NG	0-5	5.1	20.3	8.1	252	60	3	14.9
	5-10	4.9	14.6	4.8	107	42	17	12.7
	10-20	5.0	12.2	2.6	61	30	33	13.2
	20-40	5.0	11.3	2.1	63	28	38	13.7
LVS - NG	0-5	5.3	52.2	10.4	383	75	0	25.4
	5-10	5.2	35.2	5.0	280	67	2	22.0
	10-20	5.3	23.8	3.3	219	60	5	22.8
	20-40	5.4	20.7	2.3	195	64	4	24.9
DOP - NG	0-5	5.3	46.3	8.1	208	86	0	29.7
	5-10	5.2	25.0	3.6	117	89	0	33.7
	10-20	5.5	16.5	2.2	81	92	0	34.4
	20-40	6.1	9.7	1.3	75	96	0	39.2
SLV - NG	0-5	5.5	76.2	3.7	293	82	0	39.1
	5-10	5.4	56.6	2.4	163	78	0	40.7
	10-20	5.6	47.6	1.6	62	81	0	46.4
	20-40	5.6	45.9	1.3	47	85	0	45.3
SPS - NG	0-5	5.0	21.6	5.0	120	49	7	10.9
	5-10	4.9	15.8	2.7	60	33	27	10.6
	10-20	4.9	11.1	1.6	34	27	40	10.3
	20-40	5.0	9.8	1.1	28	25	45	11.6

Organic carbon (C) estimated by wet combustion (Walkley and Black method); available P and K extracted by Mehlich-1. Saturation of cation exchange capacity (CEC) with Ca + Mg + K.

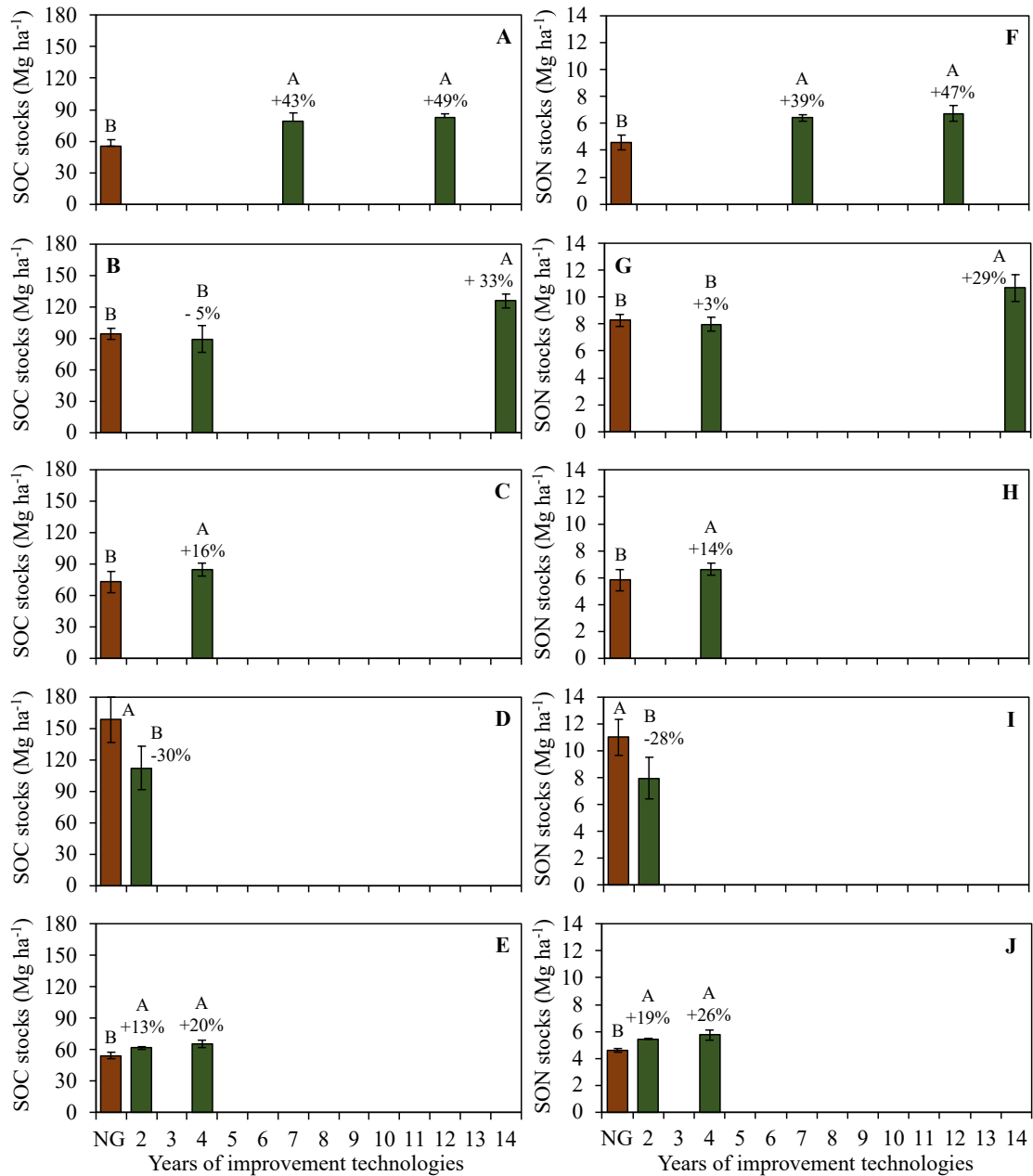
Our data suggests that the time taken to implement and use the improvement technologies is the determining factor in increasing the C and N contents and stocks in the soil. In general, the longer the improvement technologies are used, the higher the accumulated dose of N-P-K in the natural grassland tends to be (**Table 1**), the higher the annual forage productivity (**Figure 14**) and the greater the increase in C and N content and stocks (**Figure 15; 16; 17**). Although there is this trend, there are substantial differences in the accumulated doses of N-P-K between the different sites (**Table 1**), with direct repercussions on their forage productivity. In SPS-NG+4, in just 4 years, the accumulated nitrogen and phosphate fertilization was 313 and 324% higher than LVS-NG+4, respectively. This justifies the difference in the increase in forage productivity, 191% for SPS-NG+4 vs. 37% for LVS-NG+4 (**Figure 14**).



**Figure 15.** C content in the soil in native grasslands with different years of improvement technologies in Piratini (A), Lavras do Sul (B), Dom Pedrito (C), Santana do Livramento (D) and São Pedro do Sul (E). NG represents the native grassland without improvement technologies. Averages followed by the same capital letter comparing the C content between production environments in each soil layer and the same lower letter comparing the C content between depths according to each environment are not significant by Tukey's test  $p < 0.05$ .



**Figure 16.** N content in the soil in native grasslands with different years of improvement technologies in Piratini (A), Lavras do Sul (B), Dom Pedrito (C), Santana do Livramento (D) and São Pedro do Sul (E). NG represents the native grassland without improvement technologies. Averages followed by the same capital letter comparing the N content between production environments in each soil layer and the same lower letter comparing the N content between depths according to each environment are not significant by Tukey's test  $p < 0.05$ .

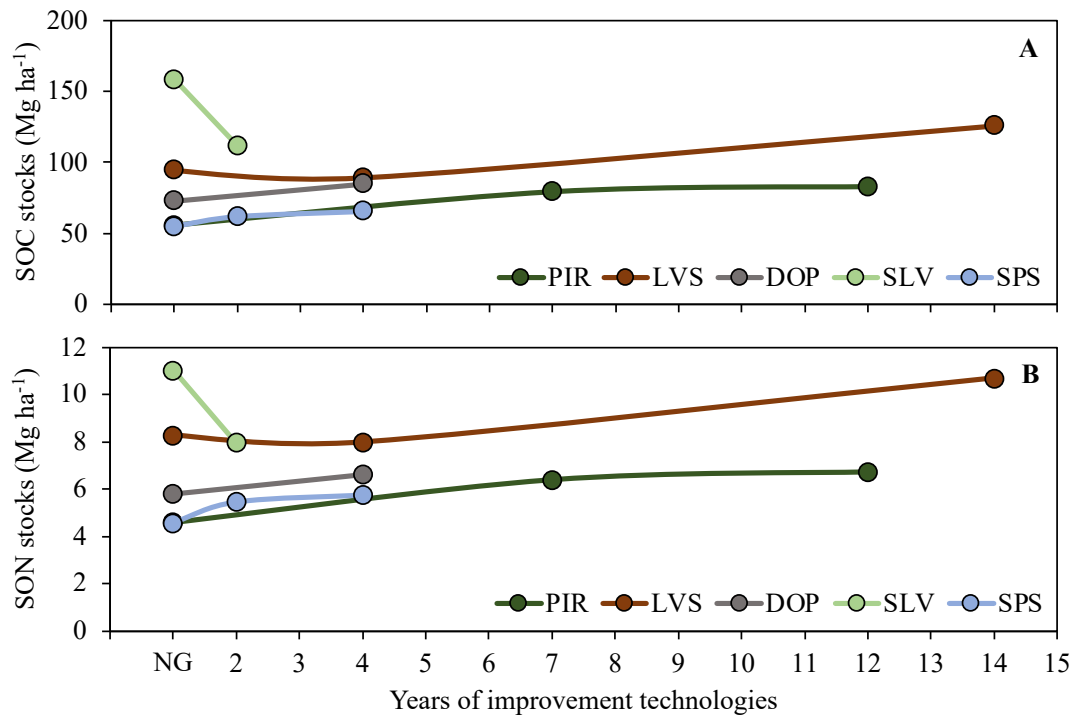


**Figure 17.** Soil organic carbon (SOC) and nitrogen (SON) stocks (0-30 cm) in native grasslands with different years of improvement technologies in Piratini (A, F), Lavras do Sul (B, G), Dom Pedrito (C, H), Santana do Livramento (D, I) and São Pedro do Sul (E, F). NG represents the native grassland without improvement technologies. Error bars represent 95% confident interval of the mean. The percentage represents the increase of improvement technology compared to NG. Means followed by the same letter comparing the SOC and SON stocks between production environments are not significant according to the Tukey test  $p < 0.05$ .

The overseeding of species, although not evaluated in isolation in the environments with improvement technologies of the present study, also contributed to the increase in C and N stocks, as suggested by Bondaruk et al.



(2020), evaluating 12 sites in Uruguay with overseeding and phosphate fertilization of natural grasslands. In addition to increasing the annual forage productivity (**Figure 14**) of native grasslands, overseeding improves the quality of the residue added to the system due to the greater presence of legumes (**Figure 19**). By promoting a reduction in the soil's C/N ratio, especially in the surface layers (**Figure 19**), there is greater efficiency in the use of C by microorganisms and, therefore, higher rates of C sequestration in the soil



**Figure 18.** Changes in soil organic carbon (SOC) (A) and nitrogen (SON) (B) stocks (0-30 cm) through the years of use of improvement technologies in natural grasslands (NG) in Piratini (PIR), Lavras do Sul (LVS), Dom Pedrito (DOP), Santana do Livramento (SLV) and São Pedro do Sul (SPS).

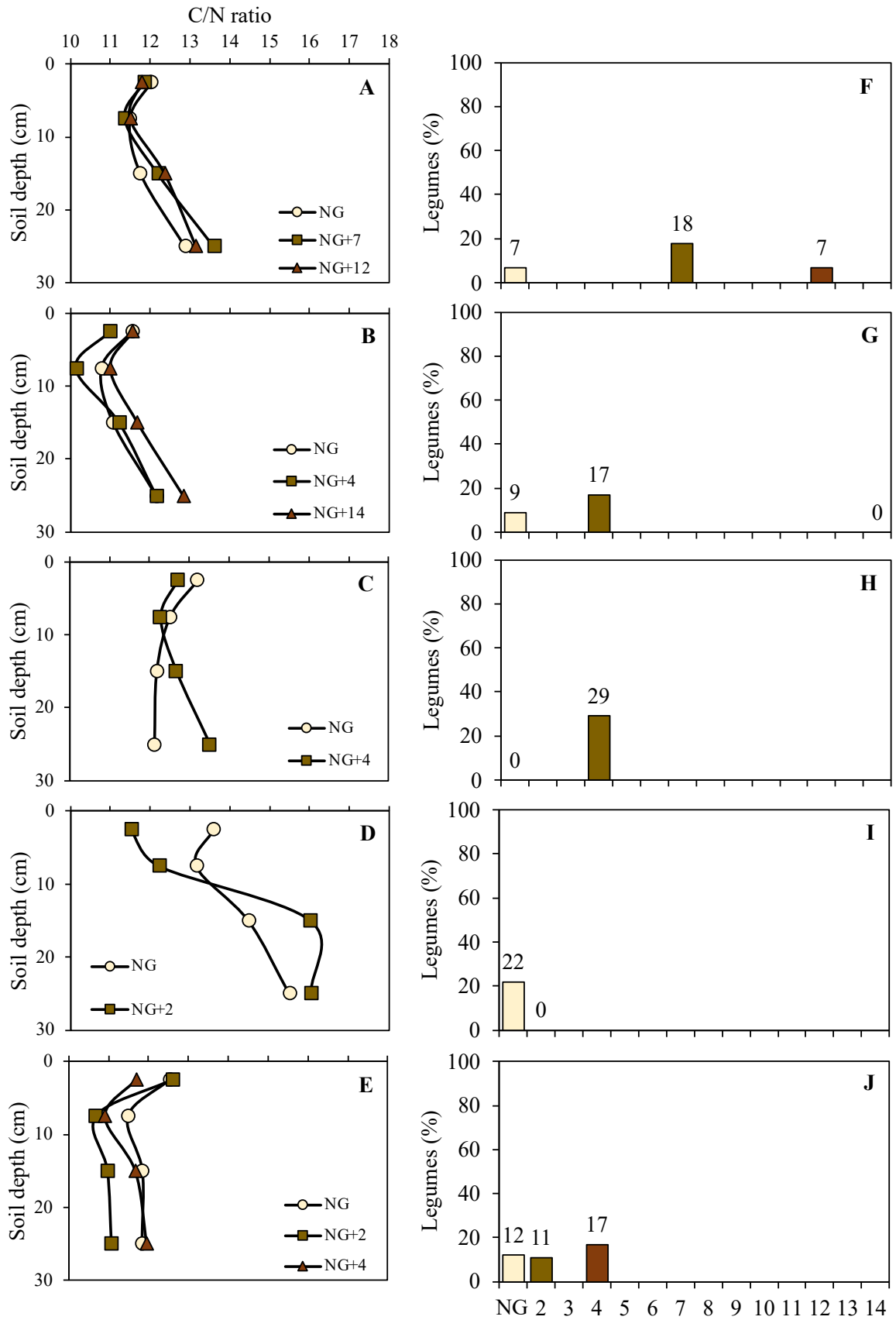
(Poeplau et al., 2018).

The significant reduction in C and N stocks in some sites, such as SLV-NG+2 when compared to NG (**Figure 17b; d; g; i**), may be caused by two main factors. In sites with naturally high C stocks (**Figure 17b; d**), what may explain the reduction in these stocks in the first few years with improvement technologies is the limitation of nitrogen in the system. The addition of N-rich organic materials to the soil can cause the "priming" effect (Kuzyakov, 2010), which occurs when the addition of organic material stimulates soil microbial activity, which, in addition to consuming all the added carbon, can also degrade the soil's native organic

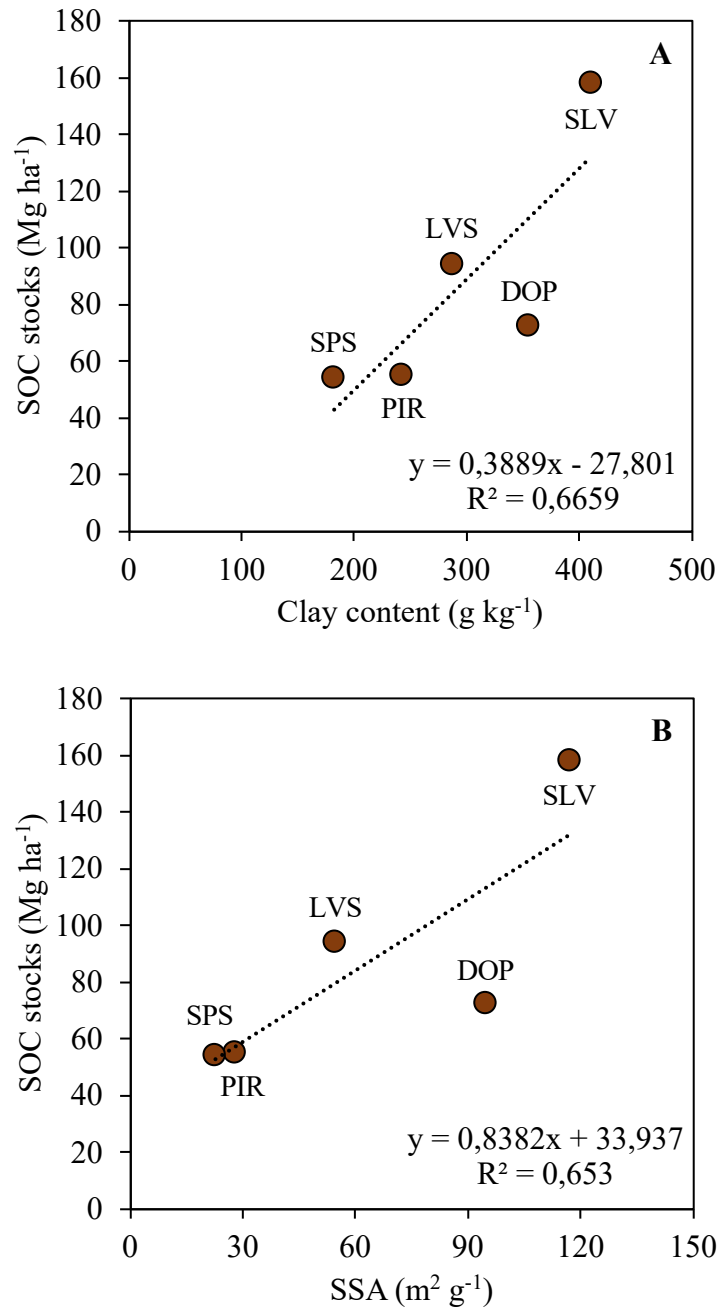
matter. In this sense, the quality of the material added to environments with improvement technologies, with a higher N content and, consequently, a lower C:N ratio than natural grassland, can promote an acceleration in the mineralization of MOS, due to the greater contribution of N to the system (Moreira and Siqueira, 2006). The data indicates that from the fourth year of use of the improvement technologies the stocks return to being positive when compared to the NG (**Figure 17; 18**). In addition, the reduction can be explained by the fact that producers generally apply a higher animal load in environments with improvement technologies (Jaurena et al., 2016). As these environments produce up to three times more forage annually than environments without improvement technologies (**Figure 14e**), the animal carrying capacity is consequently higher by the same proportion. This drastic increase in animal load can increase the availability of N to the system, since a greater amount of manure and urine is deposited in the area (da Silva et al., 2020), accelerating the mineralization of MOS.

**Table 3.** Soil clay estimated by pipette method in natural grasslands environments studies that evaluated the effects of improved natural grasslands in southern Brazil on forage productivity, soil carbon and nitrogen stocks.

		PIR	LVS	DOP	SLV	SPS
		----- g kg <sup>-1</sup> -----				
NG	0-5	205	184	293	287	152
	5-10	230	184	302	284	160
	10-20	242	210	283	321	166
	20-40	262	221	256	271	194
NG+2	0-5	-	-	-	260	104
	5-10	-	-	-	298	121
	10-20	-	-	-	303	134
	20-40	-	-	-	331	138
NG+4	0-5	-	170	308	-	77
	5-10	-	187	317	-	94
	10-20	-	206	295	-	115
	20-40	-	200	229	-	128
NG+7	0-5	209	-	-	-	-
	5-10	227	-	-	-	-
	10-20	215	-	-	-	-
	20-40	272	-	-	-	-
NG+12	0-5	194	-	-	-	-
	5-10	204	-	-	-	-
	10-20	195	-	-	-	-
	20-40	223	-	-	-	-
NG+14	0-5	-	248	-	-	-
	5-10	-	259	-	-	-
	10-20	-	290	-	-	-
	20-40	-	328	-	-	-



**Figure 19.** C/N ratio in soil and percentage of legumes in native grasslands with different years of improvement technologies in Piratini (A, F), Lavras do Sul (B, G), Dom Pedrito (C, H), Santana do Livramento (D, I) and São Pedro do Sul (E, F). NG represents the native grassland without improvement technologies.



**Figure 20.** Relationship between SOC stock and clay content (A) and soil surface area (B) in natural grasslands without improvement technologies in Brazilian Pampa biome.

Soil environments with lower clay (**Table 3**) contents and, consequently, lower C stocks (**Figure 20**) are chemically more limiting to the development of forage species in NG (**Table 2**) such as SPS. In these soils, in just two years of using improvement technologies, they have already proved effective not only in increasing forage productivity, but also in increasing C and N levels and stocks. This demonstrates the importance and responsiveness of these environments to

fertilization and, consequently, greater productivity and contribution of forage and residues to the system. This information is important because approximately 44% of the Pampa biome is under soils of low natural fertility (Hasenack et al., 2023), where soil fertility correction is one of the alternatives to not only increase forage productivity, but also favor an increase in C and N contents and stocks in these places (Bardgett et al., 2021).

At DOP, even in a soil with high natural fertility and high initial C stocks, a 16% increase in C stock was possible in 4 years (**Figure 17c**). One of the factors that may have had an impact on this result is the higher proportion of native and introduced legumes compared to the other sites (**Figure 19**). This provided a greater contribution of N to the soil via biological fixation over a longer period, reducing the "priming" effects in the first few years after the use of improvement technologies. In addition, the absence of DOP-NG+2 prevented us from seeing the effect of using improvement technologies in the short term.

Considering only the 44% of the Pampa biome with soils of low natural fertility (Hasenack et al., 2023), similar to SPS, and proposing a 20% increase in the C stocks of these environments, as shown in SPS-NG+4, it would be possible to increase the C stocks in the Pampa biome by 86 million tons. In this way, improvement technologies present themselves as alternatives for the sustainable development of livestock farming in the Pampa biome. They are economically viable practices, allowing for greater forage and, consequently, animal productivity, they are environmentally friendly given the possibility of increasing C stocks in the soil, and they are socially just, since they can be used on any size of farm.

However, the use of improvement technologies requires appropriate technical monitoring, since there is evidence of losses in the biodiversity of native species when fertilization and overseeding of species is used (Jaurena et al., 2016), and there may also be greater pressure from the population of exotic plants and weeds in the Pampa biome, which require attention. In this sense, changes in the chemical properties of the soil can be a factor directly linked to changes in its botanical composition. These negative influences of technological improvements have not yet been fully addressed and require future studies to better understand them.

### 6.3. Fertilization and overseeding in natural grasslands in southern Brazil: impacts on soil fertility and the botanical composition of species

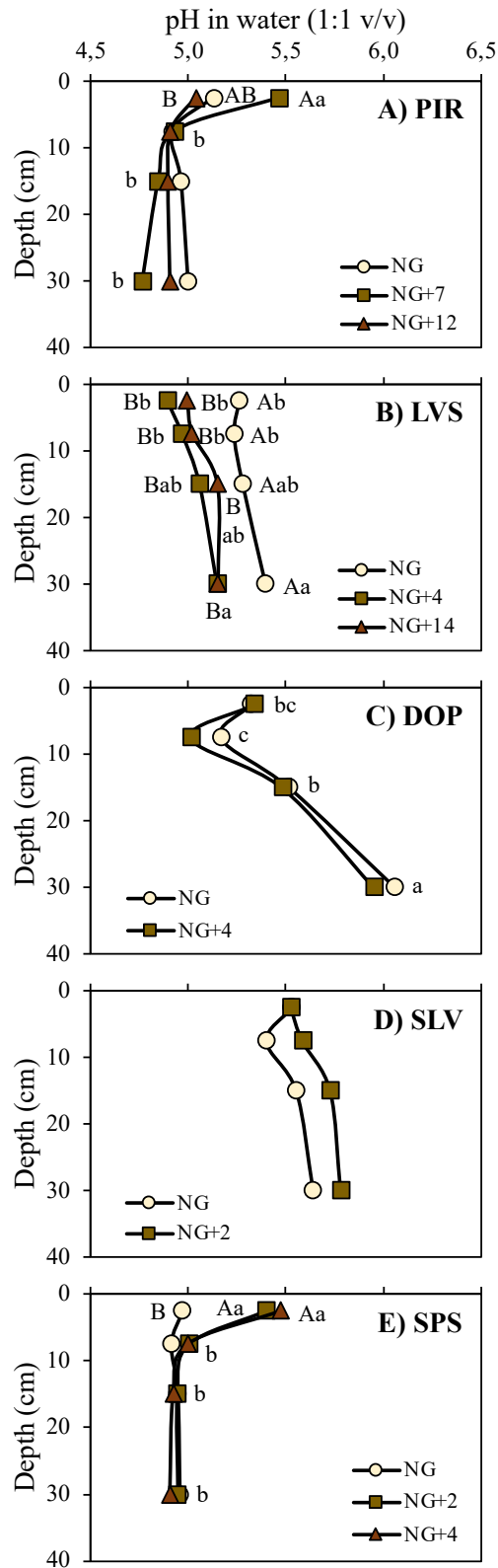
#### 6.3.1. Results and discussion

Soil pH in water was affected by the improvement technologies in three sites and affected by soil depth in four sites (**Figure 21**). In the sites where liming was performed (PIR-NG+7, SPS-NG+2, and SPS-NG+4) (**Table 1**) there was an increase in soil pH values only in the 0-5 cm soil layer. The superficial application of lime in PIR-NG+7, SPS-NG+2, and SPS-NG+4 proved to be effective in raising soil pH and Ca+Mg+K saturation in the 0-5 cm layer of these environments. The limited effect of surface liming in the first centimeters of soil is commonly reported in the literature (Alves et al., 202; Rheinheimer et al., 2018; Miotto et al., 2019), and explained by the short-term effect of the application combined with the low solubility of limestone.

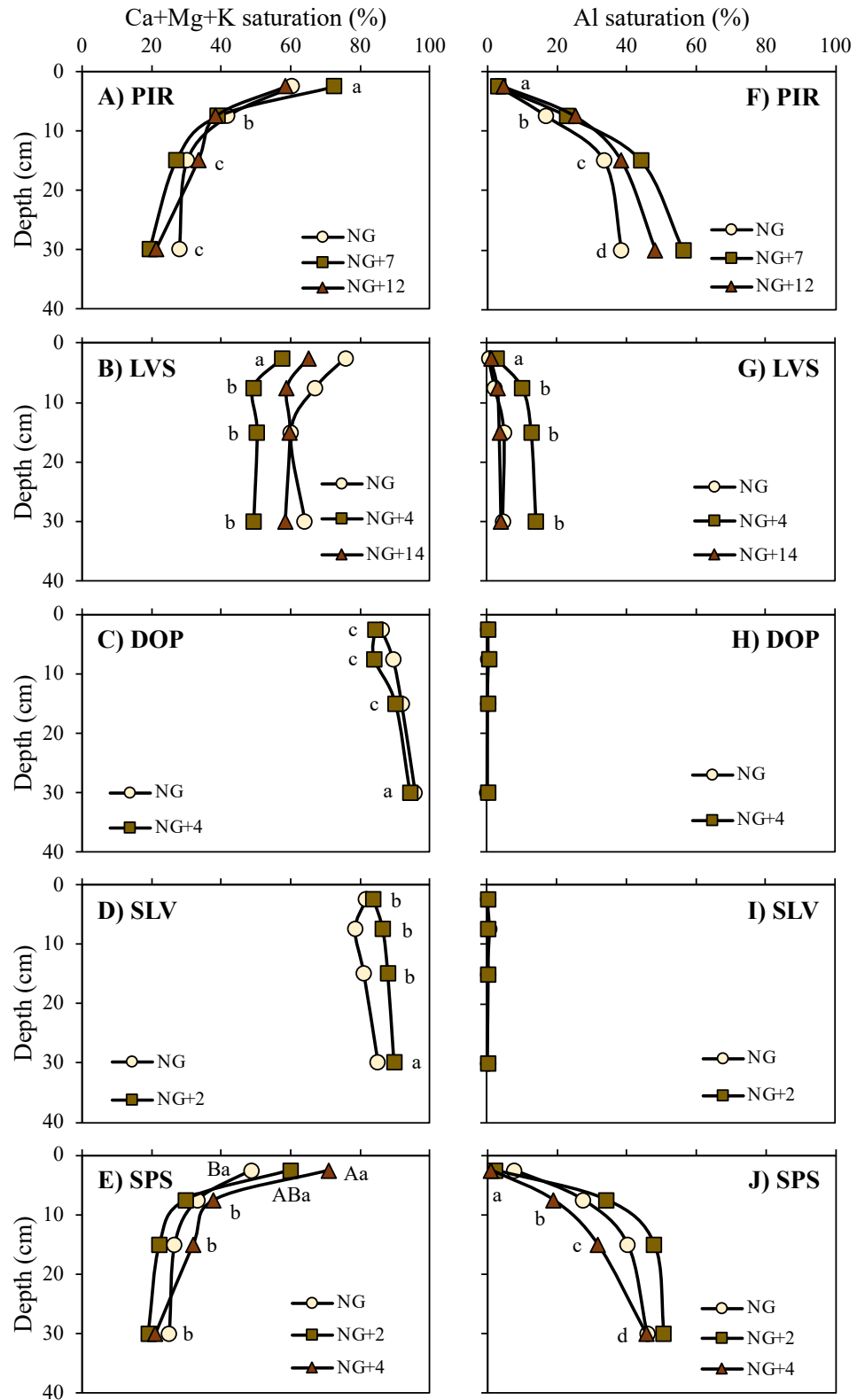
Although liming in PIR and SPS was intended to correct soil acidity, the lime rates used were lower than the recommended by the regional guidelines (CQFS-RS/SC, 2016) to raise the soil pH to 6.0 in both places. In PIR the lime rate to be applied should have been 4.8 Mg ha<sup>-1</sup> and in SPS 5.5 Mg ha<sup>-1</sup> in order to raise the pH to 6.0 (CQFS-RS/SC, 2016) and neutralize the toxic Al. Even so, the rate of 3.0 Mg ha<sup>-1</sup> applied (**Table 1**) was sufficient to raise the pH of the 0-5 cm layer to approximately 5.5 at both sites and increase Ca+Mg+K saturation in SPS-NG+4 (**Figure 22 a; e**).

In order to raise the pH of the soil in deeper layers, the lime could be incorporated mechanically, a common practice in grain crops and cultivated pastures (Auler et al., 2019). However, in order not to disturb the natural grasslands, and drastically reduce its botanical composition, the surface application used in PIR and SPS is the only alternative.

On the other hand, LVS-NG+4 and LVS-NG+14 showed reductions in soil pH values compared to NG in all soil layers up to 40 cm deep. Liming was not performed in LVS-NG+4 and LVS-NG+14, but annual applications of nitrogen fertilizers (**Table 1**) were carried out (total dose of 351 kg ha<sup>-1</sup>), leading to soil acidification up to a depth of 40 cm in both environments (**Figure 21 b**).



**Figure 21.** Soil pH in water in native grasslands with different years of improvement technologies in PIR (A), LVS (B), DOP (C), SLV (D) and SPS (E). Averages followed by the same capital letter comparing the pH between production environments in each soil layer and the same lower letter comparing the N content between depths are not significant by Tukey's test  $p < 0.05$ .



**Figure 22.** Soil Ca+Mg+K and Al saturation native grasslands with different years of improvement technologies in Piratini (A, F), Lavras do Sul (B, G), Dom Pedrito (C, H), Santana do Livramento (D, I) and São Pedro do Sul (E, J). Averages followed by the same capital letter comparing the pH between production environments in each soil layer and the same lower letter comparing the N content between depths are not significant by Tukey's test  $p < 0.05$ .

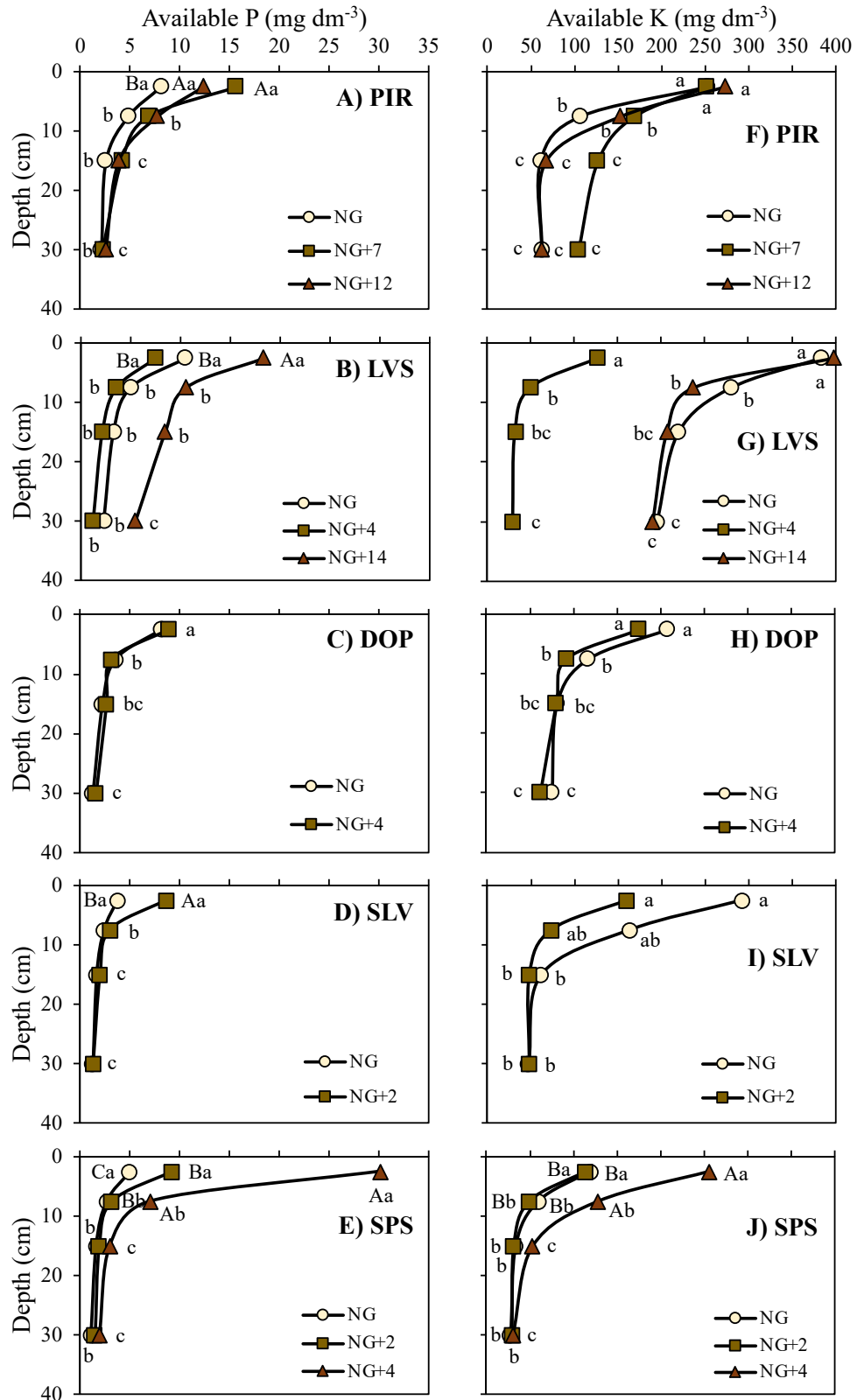


Urea acidifies the soil if nitrate is lost through leaching into the soil during the nitrification phase (transformation of  $\text{NH}_4^+$  into  $\text{NO}_3^-$ ). In this process, two  $\text{H}^+$  ions are released for each  $\text{NH}_4^+$  added to the soil (Chien et al., 2008). In general, increasing doses of N cause a decrease in pH, Ca and Mg content and an increase in exchangeable aluminum content (Schroder et al., 2011) (**Figure 22 b; g**).

Ca+Mg+K saturation only differed between production environments in the 0-5 cm layer of SPS, with SPS-NG+4 being higher than SPS-NG (**Figure 22 e**). In PIR, LVS, and SPS the surface layers of the soil showed higher values than the subsurface layers, while DOP and SLV the 20-40 cm layer showed higher values than the surface layers. For Al saturation, only PIR, LVS and SPS showed differences with soil depth (**Figure 22 f; g; j**), always being lower in the 0-5 cm layer and increasing with increasing soil depth, reaching 56 and 51% in PIR-NG+7 and SPS-NG+2, respectively, in the 20-40 cm soil layer in NG (**Figure 22 f; j**). Soil Ca and Mg exchangeable did not differ between production environments on any of the farms evaluated, only for the gradient in the soil profile, with generally higher levels in the surface layer, except for DOP and SLV (**Table 4**).

Phosphate fertilizer applications (**Table 1**) increased available P in four of the five sites evaluated (**Figure 23**), restricted to the first 5 cm of soil depth in PIR-NG+7, PIR-NG+12, LVS-NG+14, SLV-NG+2, and SPS-NG+2, while in SPS-NG+4 the increase in available P content was observed up to 10 cm of soil depth. At all sites, the 0-5 cm soil layer always had a higher available P content than the subsurface layers, even in NG.

Regarding available K levels, differences between production environments were only observed in SPS, where SPS-NG+4 showed higher levels in the 0-5 and 5-10 cm layers than the other environments (**Figure 23 j**). In all the sites and production environments, the available K levels were higher in the surface layers of the soil, and as a result there were large concentration gradients, even in NG, environments that have no reports of fertilizer and corrective inputs.



**Figure 23.** Soil available P and K in native grasslands with different years of improvement technologies in Piratini (A, F), Lavras do Sul (B, G), Dom Pedrito (C, H), Santana do Livramento (D, I) and São Pedro do Sul (E, J). Averages followed by the same capital letter comparing the pH between production environments in each soil layer and the same lower letter comparing the N content between depths are not significant by Tukey's test  $p < 0.05$ .

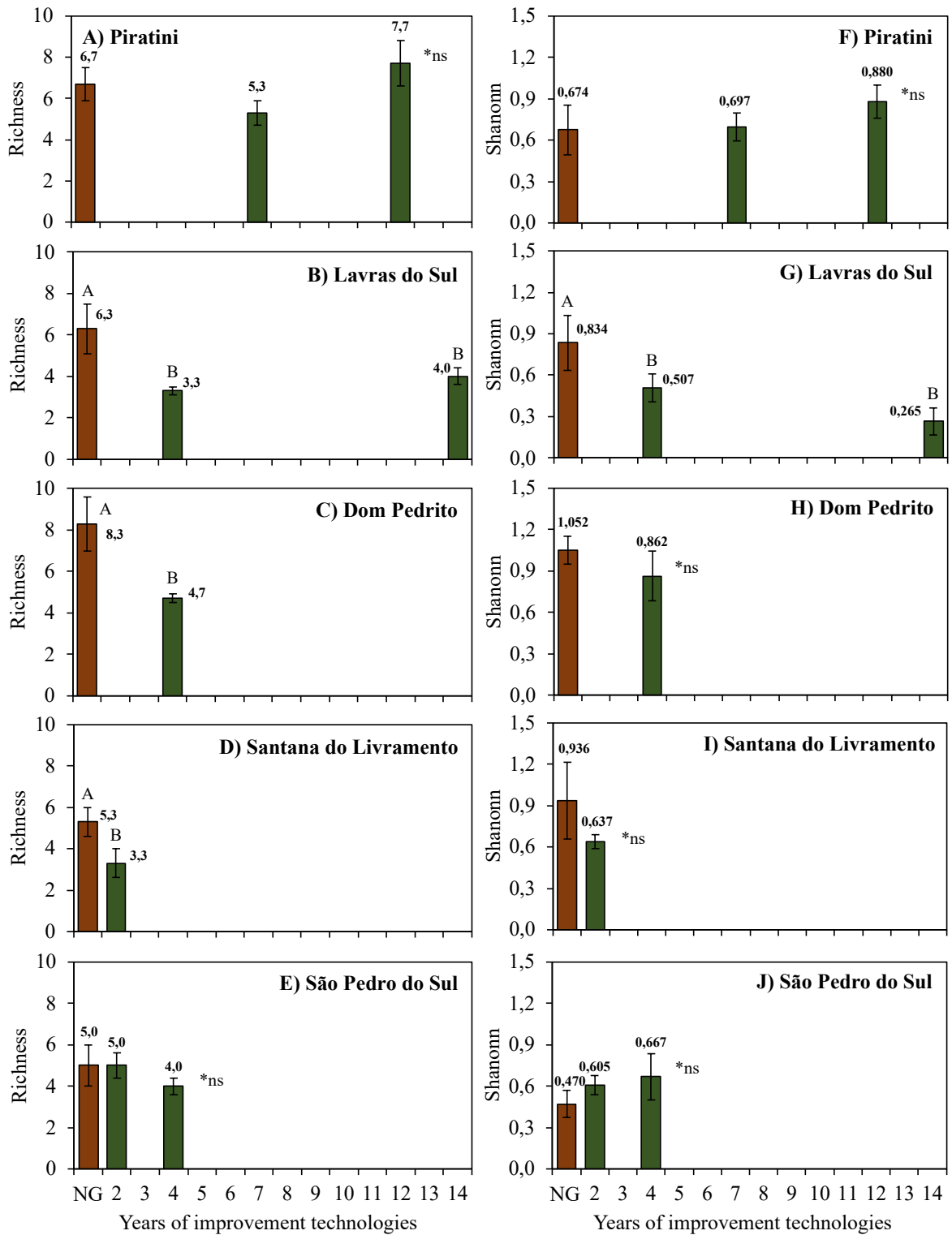
Overall, liming, phosphate, and potassium fertilization and overseeding winter species in natural grasslands raised the pH and availability of nutrients in the soil. In SPS-NG+4, the application of 263 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> from 2018 to 2022 increased the available P content in the soil by 600 and 142% for the 0-5 and 5-10 cm layers, respectively.

**Table 4.** Soil calcium (Ca) and magnesium (Mg) in natural grasslands environments studies that evaluated the effects of improved natural grasslands in southern Brazil on forage productivity, soil carbon and nitrogen stocks.

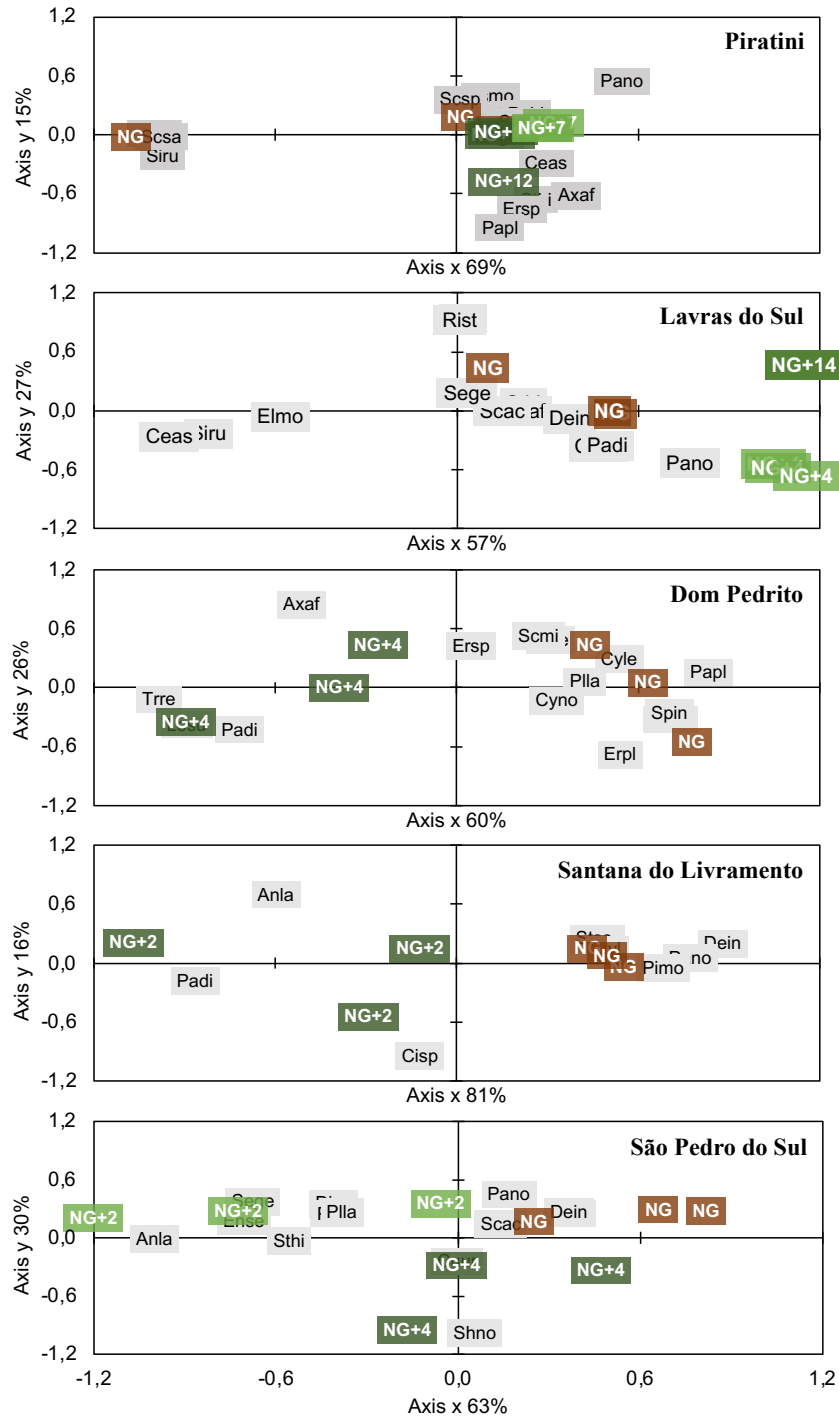
		PIR		LVS		DOP		SLV		SPS	
		Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
-----cmolc dm <sup>-3</sup> -----											
NG	0-5	5.4 a	3.0 a	12.0 a	6.3 a	19.4 b	5.7 b	23.6 c	7.5 a	3.1 a	1.9 a
	5-10	3.4 b	1.6 b	9.7 b	4.4 b	22.9 a	6.9 a	24.7 c	6.8 b	2.3 b	1.1 b
	10-20	2.7 b	1.0 c	9.3 b	3.9 b	24.0 a	7.4 a	30.0 b	7.4 b	1.8 b	0.8 b
	20-40	2.7 b	1.0 c	11.1 a	4.5 b	27.8 a	9.5 a	31.0 a	7.4 b	2.0 b	0.8 b
NG+2	0-5	-	-	-	-	-	-	23.1 c	8.5 b	4.4 a	2.5 a
	5-10	-	-	-	-	-	-	25.7 c	9.0 b	1.9 b	1.0 b
	10-20	-	-	-	-	-	-	28.5 b	9.3 b	1.8 b	0.8 b
	20-40	-	-	-	-	-	-	33.5 a	10.7 a	1.9 b	0.7 b
NG+4	0-5	-	-	6.9 a	4.2 a	18.4 b	7.0 b	-	-	6.8 a	3.3 a
	5-10	-	-	5.8 b	2.6 b	23.9 a	8.2 a	-	-	2.5 b	1.2 b
	10-20	-	-	5.9 b	2.4 b	23.6 a	7.4 a	-	-	2.1 b	0.9 b
	20-40	-	-	5.9 b	2.3 b	31.1 a	8.9 a	-	-	1.9 b	0.7 b
NG+7	0-5	7.8 a	3.2 a	-	-	-	-	-	-	-	-
	5-10	3.0 b	1.5 b	-	-	-	-	-	-	-	-
	10-20	2.0 b	0.9 c	-	-	-	-	-	-	-	-
	20-40	1.8 b	0.8 c	-	-	-	-	-	-	-	-
NG+12	0-5	4.8 a	2.4 a	-	-	-	-	-	-	-	-
	5-10	3.0 b	1.2 b	-	-	-	-	-	-	-	-
	10-20	2.6 b	1.0 c	-	-	-	-	-	-	-	-
	20-40	2.2 b	0.9 c	-	-	-	-	-	-	-	-
NG+14	0-5	-	-	11.3 a	4.9 a	-	-	-	-	-	-
	5-10	-	-	9.5 b	3.9 b	-	-	-	-	-	-
	10-20	-	-	10.2 b	4.1 ab	-	-	-	-	-	-
	20-40	-	-	11.1 a	4.6 ab	-	-	-	-	-	-

Averages followed by the same lower letter comparing the Ca and Mg between depths are not significant by Tukey's test  $p < 0.05$ .

Analyzing the number of species (**Figure 24**) and the ordering diagram (**Figure 25**) the use of improving technologies may be modifying the botanical composition of species in the natural grasslands, especially in LVS, DOP and SLV. The use of improvement technologies caused a reduction in the richness of the observed species in the long term in these environments (**Figure 24**, as also evidenced by Silva and Jacques (1993) and Castilhos and Jacques (2000)).



**Figure 24.** Richness and Shannon index in native grasslands with different years of improvement technologies in Piratini (A; F), Lavras do Sul (B; G), Dom Pedrito (C; H), Santana do Livramento (D; I) and São Pedro do Sul (E; J). NG represents the native grassland without improvement technologies. Error bars represent 95% confident interval of the mean. \*ns: not significant by Kruskal-Wallis test  $p < 0.05$ .



**Figure 25.** Ordering diagram of the native grasslands with different years of improvement technologies. Biomass of the main contributing species: *Andropogon lateralis* (Anla), *Aristida murina* (Armu), *Aspilia montevidensis* (Asmo), *Axonopus afinis* (Axaf), *Bothriochloa laguroides* (Bola), *Centella asiática* (Ceas), *Cyperus sp.* (Cisp), *Coelorhachis selloana* (Cose), *Cuphea urbaniana* (Cuur), *Cyclospermum leptophyllum* (Cyle), *Cynodon* (Cyno), *Desmodium incanum* (Dein), *Desmodium sp* (Desp), *Dichondra sericea* (Dise), *Elephantopus mollis* (Elmo), *Ervolulus sericeus* (Ense), *Eragrostis plana* (Erpl), *Eryngium sp.* (Ersp), *Eryngium horridum* (Erho), *Galium richardianum* (Gari), *Lotus subbiflorus* (Losu), *Panicum hians* (Pahi), *Paspalum dilatatum* (Padi), *Paspalum notatum* (Pano), *Paspalum plicatulum* (Papl), *Pennisetum clandestinum* (Pecl), *Piptochaestium montevidense* (Pimo), *Plantago lanceolata* (Plla), *Richardia stellaris* (Rist), *Schaptalia spicata* (Scsp), *Schevrelia acuminata* (Scac), *Schevrelia sarmentosa* (Scsa), *Schizachyrium microstachyum* (Scmi), *Schizachyrium spicatum* (Scsp), *Schizachyrium microstachyum* (Scmi), *Setaria geniculata* (Sege), *Shorgastrum notans* (Shno), *Sida rumbifolia* (Siru), *Solanum viarum* (Sovi), *Sporobolus indicus* (Spin), *Steinchisma hians* (Sthi), *Stipa setigera* (Stse), *Stylosanthes* (Styl) *Trifolium repens* (Trre), *Verbena montevidensis* (Vemo), *Vernonathura nudiflora* (Venu).

Furthermore, the use of improving technologies reduced the diversity index in LVS-NG+4 and LVS-NG+14 (**Figure 24 g**), while increasing it in PIR-NG+12. Therefore, the use of improving technologies impacts species richness and diversity differently according to the environment. PIR and SPS have naturally low-fertility soils (**Table 1; 2**) with severe limitations in P availability (**Figure 23 a; e**), low pH (**Figure 21 a; e**), and high Al saturation in the subsurface (**Figure 22 f; j**). Improvements in these soil chemical properties through liming and fertilization can maintain the level of richness and even increase species diversity in the long term (**Figure 24 f**).

While changes in soil fertility impact the environments in different ways, the shading effect of ryegrass and its competition with native species negatively impacts the richness and diversity of species in the environments in a similar way between the sites evaluated, as also observed by Bandinelli et al. (2005). This negative factor of the presence of ryegrass on the development of native species in the spring-summer period reinforces the importance of correct management of grazing height, avoiding excessive competition. It is possible to observe a greater participation of *Lotus subbiflorus* and *Trifolium repens* in DOP-NG+4, species that were overseeding when the system was implemented (**Table 1**). Similarly, *Paspalum dilatatum* showed an increase in its share of the forage mass in sites with improvement technologies in DOP and SLV, as well as *Andropogon lateralis* in SLV-NG+2 and SPS (**Figure 25**), possibly due to the greater availability of P and annual N input in the environments, as also observed by Oliveira et al. (2015; 2022).

With the use of improving technologies, improvements in the chemical characteristics of the soil allow native species that are more responsive to fertilization to increase their participation in the forage composition of the environment. Similar results were also observed by Gomes et al. (1998), who reported an increase in the participation of *Paspalum notatum* in fertilized environments.

The use of improving technologies promotes improvements in the soil's chemical characteristics and allows for better support capacity and nutrient supply for demanding species such as those used in overseeding in this study. Although the surface application used in fertilizers and liming is the only alternative, in order not to disturb the natural grasslands, this practice has

severely accentuated the gradient of nutrient concentration in the soil profile and may reduce the exploratory capacity of forage roots. Although long-term improving technologies can change the botanical composition and reduce the species richness and diversity of natural grasslands. As also observed by Bardget et al. (2021) and Jaurena et al. (2021), we believe that their use with a focus on diversifying forage supply and cautiously intensifying specific production processes and should be seamlessly integrated into the design of livestock farms. The use of improving technologies is an alternative for the sustainable development of livestock farming in the Pampa biome. Although their use may reduce the richness and diversity of species on part of the farm, it allows livestock farming based on natural grasslands to be raised to a competitive level similar to annual crops and cultivated pastures and ensures the persistence of natural grasslands in the Pampa biome.

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## 7. CONCLUSIONS

The meta-analysis of 50 studies from southern Brazil evaluating both forage and animal productivity demonstrate that the improvement technologies of natural grasslands through liming, fertilization, and overseeding of exotic forage species can result in an 35% and 82% average increase in forage and animal production, respectively. The amount of N+P+K applied per hectare and soil class were the most influential factors impacting the forage productivity of natural grasslands subjected to improvement technologies. In environments with lower productive potential, natural grassland improvement technologies show higher increments in forage productivity. For soil classes such as Arenosol, Acrisol, Planosol, or Plinthosol, forage productivity increases by up to 98%, whereas for soil classes like Leptosol/Regosol, Nitisol, Ferrasol, Luvisol, and Vertisol, the increment ranges from 6 to 36%.

In the field experiments, the use of improvement technologies had the potential to promote an average increase in forage productivity of 87%. Sites with soils of lower natural fertility and, consequently, lower forage productivity, are more responsive to the application of improvement technologies with a gain in forage productivity of up to 191% and a C accumulation rate of between 2.3 and 3.6 Mg ha<sup>-1</sup> per year. Time of use with annual fertilizer inputs and overseeding of species is a key factor in achieving the highest forage productivity and increasing nutrient levels and C and N stocks in the soil. Environments with soils with high initial stocks of C can suffer losses in their stocks in the first few years after the use of improvement technologies due to the initial nitrogen limitation in the system. Furthermore, in longer periods the improvement technologies may be losses in species richness and diversity because of competition with overseeding ryegrass and because it benefits native species that are more responsive to fertilization.

## 8. FINAL CONSIDERATIONS

Prioritizing soils with lower chemical fertility within a production system is crucial for the successful implementation of natural grassland improvement technologies. It is strongly recommended to integrate overseeding with exotic cool-season species with meticulous soil pH correction and fertility enhancements to maximize the positive outcomes in both forage and animal productivity. These findings underscore the potential of strategic improvement technologies in shaping sustainable and productive natural grassland ecosystems.

Although the use of improvement technologies in livestock farming based on natural grasslands to be raised to a competitive level like annual crops and cultivated pastures and ensures the persistence of natural grasslands in the Pampa biome. The improvement technologies can reduce the richness and diversity of native species in the Pampa biome. It is crucial to emphasize that the outlined improvement technologies for natural grasslands should be seamlessly integrated into the design of livestock farms, with a focus on diversifying forage supply and cautiously intensifying specific production processes, guided by technical expertise.

Many knowledge gaps regarding the impact of fertilization and overseeding on natural grasslands still need to be analyzed. The specific effect of each of the practices on the botanical composition of the grassland, rate response curves for liming and fertilizers, as well as the effect of irrigation on natural grasslands are examples of factors that have yet to be explored.



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## **RESUMO BIOGRÁFICO**

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