UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

FCA
FACULDADE DE CIÊNCIAS AGRONÔMICAS
UNESP | BOTUCATU

RELATÓRIO FINAL

Chamada CNPq 25/2021 - Pós-Doutorado Júnior - PDJ 2021

Processo: 150858/2022-1

Comunidade microbiana do solo e seleção rizosférica pelo milho e *Urochloa ruziziensis* em sistema consorciado com aplicações de calcário e fosfogesso

João William Bossolani

Botucatu

Agosto de 2023

JOÃO WILLIAM BOSSOLANI

COMUNIDADE MICROBIANA DO SOLO E SELEÇÃO RIZOSFÉRICA PELO MILHO E *Urochloa ruziziensis* EM SISTEMA CONSORCIADO COM APLICAÇÕES DE CALCÁRIO E FOSFOGESSO

Relatório de Pós-doutorado realizado na Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agronômicas, Botucatu.

Supervisor: Prof. Dr. Carlos Alexandre Costa Crusciol

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Processo Nº 150858/2022-1

Botucatu

2023

DETALHAMENTO DAS ATIVIDADES REALIZADAS

1) REVISÃO DE LITERATURA

A revisão bibliográfica para a elaboração dos artigos foi realizada utilizando-se trabalhos publicados em periódicos (nacionais e internacionais), além de consultas em livros relativos ao papel da calagem e gessagem nas propriedades químicas e biológicas do solo. Pela primeira vez, integramos dados de parâmetros químicos e biológicos do solo alterados pela calagem e gessagem. Portanto, a busca pelas literaturas que embasaram nosso trabalho foi intensa durante o período de condução do projeto. Ao longo dos meses subsequentes diversos novos artigos e livros foram pesquisados e incorporados às referências que foram utilizadas na elaboração do relatório final desse projeto.

2) ATIVIDADES NO LABORATÓRIO

As amostras de solo e de plantas coletadas durante todos os cultivos (2016-2021) foram processadas e analisadas no laboratório Relação Solo-Planta, do Departamento de Produção Vegetal, da Faculdade de Ciências Agronômicas/UNESP - Câmpus de Botucatu, no laboratório de Fisiologia Vegetal da Faculdade de Ciências e Engenharia/UNESP - Câmpus de Tupã, e no laboratório de Biologia Celular e Molecular (CENA/USP).

3) TABULAÇÃO DOS DADOS E ANÁLISE ESTATÍSTICA

Todos os dados obtidos foram tabulados e analisados através de análises estatísticas convencionais (paramétricas), multivariadas e bioinformática aplicada a microbiologia do solo. A bioinformática empregada nas análises microbiológicas foi baseada em métodos recentes, altamente conceituados e que são empregados por poucos institutos de pesquisa no mundo, dado sua complexidade.

4) REDAÇÃO DO RELATÓRIO CIENTÍFICO

O relatório científico apresentado a CNPq foi redigido na forma de artigo científico diretamente na língua inglesa. O presente relatório está em fase final de preparação para ser encaminhado a um periódico internacional de alto fator de impacto.

Shifts in Soil Microbial Communities and Rhizosphere selection in a Maize-Ruzigrass Intercropping System managed with Soil Amendments

João W. Bossolani[a,b]; Carlos A. C. Crusciol[a*]; Mariley Fonseca[a]; Sirlene Oliveira[a]; Márcio F. A. Leite[b]; Luiz G. Moretti[a,b]; Eiko E. Kuramae[b,c*]

[a] São Paulo State University (UNESP), College of Agricultural Sciences (FCA), 18610–034, Botucatu, São Paulo, Brazil

[b] Netherlands Institute of Ecology (NIOO–KNAW), Department of Microbial Ecology, 6708 PB, Wageningen, The Netherlands

[c] Utrecht University, Institute of Environmental Biology, Ecology and Biodiversity, Padualaan 8, 3584 CH, Utrecht, the Netherlands

[*]Corresponding authors:

C. A. C. Crusciol, Email: carlos.crusciol@unesp.br

E. E. Kuramae, Email: e.kuramae@nioo.knaw.nl

Abstract: Combining lime (L) with phosphogypsum (PG) is a strategy that plays a significant role in shaping soil chemistry and the soil microbiome. These factors have a notable impact on which microbe can be selected by the rhizospheres of co-cultivated maize and ruzigrass. Grasping this influence is vital for enhancing the growth and well-being of these paired crops. The present study aims to evaluate the effect of long-term surface application of L and/or PG on the assembly and selection of soil microbial community during maize intercropped with ruzigrass. Here, we hypothesized that in a intercropping system, plants established in soils with different levels of fertility due to application of L and PG presents different microbe associations. The following treatments were evaluated: i) control, ii) phosphogypsum (PG; 10 t ha^{-1}), iii) lime (L; 13 t ha^{-1}), and iv) combination of L with PG (LPG). Our findings indicated that the impact of L and PG on soil microbial functions depends on intricate relationships between physical changes, crop growth, and microbial reactions. Specific microorganisms were found in maize and ruzigrass root zones, differing from bulk soil. This highlights the connection between plant-microbe interactions, soil enhancements, and microbial community dynamics in agriculture. Results indicate plant-driven microbial selection, influenced mainly by soil amendments, particularly in nitrogen cycling and mycorrhizal presence due to LPG treatment. Further research is needed to fully understand intercropped plant-driven rhizosphere community selection.

Keywords: liming, soil conditioner, *Zea mays* (L.), *Urochloa ruziziensis*, soil microbiology, rhizosphere.

1. Introduction

Soil acidity is a key factor that hinders plant growth by limiting nutrient availability (Crusciol *et al.*, 2022; Wang *et al.*, 2021). Acidic soils are predominantly limited in nutrients, high levels of exchangeable aluminum (Al^{3+}) in the soil profile (Bossolani *et al.*, 2022b; Sumner & Noble, 2003) and low microbial activity (Guo *et al.*, 2019a). Acidification occurs naturally, however, this process is intensified in agricultural lands (Bossolani *et al.*, 2022a), specially with non-conservationist management.

To overcome these obstacles, the application of lime (liming; L) is one of the main practices carried out by farmers (Holland *et al.*, 2018). This management raises soil pH, increases nutrient availability, and reduces the toxic effects of Al^{3+}, restoring soil productive capacity (Bossolani *et al.*, 2021c; Caires *et al.*, 2008; Carmeis Filho *et al.*, 2017a). Another product with high potential for improving soil profile is phosphogypsum (PG), a by-product of the phosphoric acid industry, and is able to directly provide calcium (Ca^{2+}) and sulfur (S) to the system, while also neutralizing the toxic effects of Al^{3+}, without changing soil pH (Zoca & Penn, 2017).

Numerous studies have demonstrated the effectiveness of these inputs in agriculture, especially when applied in combination (Bossolani *et al.*, 2021a; Costa & Crusciol, 2016; Crusciol *et al.*, 2016, 2019); however, little is known about their effects on soil microorganisms (Bossolani *et al.*, 2021b). Soil microorganisms are very sensitive to environmental disturbances (Merloti *et al.*, 2019). Soil acidity, low nutrient availability, soil compaction and low plant diversity are some of the most important limiting factors for microbial growth and activity (Mendes *et al.*, 2014). On the other hand, its effects on soil microbial community are poorly understood, especially in tropical agricultural soils (Bossolani *et al.*, 2020). Application of L and PG alone can alter the composition of the soil microbiome and the role these microorganisms play (Li *et al.*, 2022); however, the increase in crop biodiversity growing

simultaneously can also influence the composition of these microorganisms, especially those that are selected by their rhizosphere (Mendes *et al.*, 2014).

Plant diversity is often associated as one of the main drivers responsible for changing the soil microbiome (Lupatini *et al.*, 2019; Tkacz *et al.*, 2020). However, little is known what mechanisms or environmental factors influence the recruitment of microorganisms from the reservoirs of bulk soil (microbial seedbank). These reservoirs are highly biodiverse and very sensitive to environmental disturbances (Mendes *et al.*, 2014). For this reason, it is of paramount importance to understand how the soil microbiome responds to soil fertility changes, and how plants grown within a tropical intercropped system (maize with forage) recruit specific microorganisms from bulk soil.

This study reveals the selection of microorganisms from bulk soil to the rhizosphere of maize and *Urochloa ruziziensis* (ruzigrass) plants co-cultivated in intercropping system. Here, we tried to understand whether soil fertility improved by long-term L and/or PG applications changes the soil microbial community in bulk soil, and the selection of microorganisms from bulk soil to the maize and ruzigrass rhizosphere.

2. Material and methods

2.1. Area location and experimental history

This proposal is part of a Long-Term Experiment [registered by the Global Network of Long-Term Agricultural Experiments (GLTEN), Rothamsted Research, UK; https//www.glten.org/experiments/62], conducted since 2002 and in an experimental area belonging to the São Paulo State University, Faculty of Agronomic Sciences (UNESP/FCA), located in the city of Botucatu - southeast of the state of São Paulo (22º 83’ 3’’ S, 48º 42’ 64’’ O, and 765 m above sea level). This experiment is based on surface applications of lime and/or phosphogypsum in an agricultural system managed with crop rotation under no-tillage.

According to the Brazilian Soil Classification System (Santos *et al.*, 2018), the soil of the experimental area is denominated as OXISOL, corresponding to the Typic Haplorthox, according to international classification (Soil Survey Staff, 2014). Before the beginning of the experiment (August 2002), the chemical and granulometric properties of the soil (0.0-0.2 m depth) were determined according to the methods of Kiehl, (1979) and van Raij *et al.* (2001), respectively: 4.2, soil organic matter (SOM): 21 g kg^{-1}; phosphorus (P_{resin}): 9.2 mg kg^{-1}; K^{+}: 1.2 $mmol_c$ kg^{-1}; Ca^{2+}: 14 $mmol_c$ kg^{-1}; Mg^{2+}: 5 $mmol_c$ kg^{-1}; total acidity at pH 7 (H+Al): 38.8 $mmol_c$ kg^{-1}, cation exchange capacity (CEC): 58 $mmol_c$ kg^{-1}; BS: 35%; aluminum saturation (AS): 65%; S-SO_4^{2-}: 3.65 mg kg^{-1}. Additionally, the clay content in the 0.2-0.4 m layer (used to calculate the phosphogypsum dose) was 360 g kg^{-1}. According to the climate classification of Köppen-Geiger (Alvares *et al.*, 2013), the climate of the region is of the Cwa type, presenting rainy and hot season in summer and dry in winter, with average annual precipitation (1956 to 2021) of 1,319 mm.

2.2. Experimental design, treatments and crop history

The experimental design used is a randomized block design with four treatments and four replicates. The treatments were composed of:

(i) Control [no application of lime (L) and/or phosphogypsum (P)];

(ii) Application of PG alone (10 t ha^{-1});

(iii) Exclusive application of limestone (13 t ha^{-1});

(iv) Combined application of L and PG (LPG).

Over time (2002 - 2022), treatments (L and/or PG applications) were reapplied four times (2002, 2004, 2010 and 2016). From 2002 to 2010, (Figure 1), the calculation of the L dose considered the soil depth of 0.0-0.2 m, according to the methodology proposed by van Raij et al. (1997). The doses used in the first three L applications were 2.7, 2.0 and 2.0 t ha^{-1},

respectively. In 2016, the fourth application of limestone was calculated considering the recommended dose of L in the 0.0-0.4 m layer, aiming to increase soil fertility in deep layers, and based on previous results obtained in this same experimental area by Carmeis Filho *et al.* (2017). These authors found that the classic liming recommendation based on the 0.0-0.2 m layer represents an underestimation for stable cropping systems under no-tillage with crop rotation and high input of plant residues throughout the agricultural year. Thus, the new calculation resulted in the dose of 13 t ha^{-1}, which was applied in October 2016. The reapplications were performed following the criterion that new dose of L should be applied when the soil (from the treatment with exclusive liming) reaches base saturation (V%) < 50%. To fulfill this premise, soil fertility is checked annually. The liming occurred by application of sedimentary dolomitic L, with guarantees of 233 g kg^{-1} of CaO, 175 g kg^{-1} of MgO, and effective calcium carbonate equivalent (ECCE) of 69%.

When it was necessary to reapply L, PG was also reapplied. In 2002, 2004 and 2010, PG doses were determined according to van Raij *et al.* (1997), multiplying the clay content in the 0.2-0.4 m layer by a factor of 6, resulting in the dose of 2.1 t ha^{-1}. In 2016, the most recent PG application methodology was used in tropical soils proposed by (Caires & Guimarães, 2018), which aims to increase the saturation of Ca^{2+} in the effective CEC to 60% (considering the 0.2-0.4 m layer). This new calculation resulted in the dose of 10 t ha^{-1}. In the last reapplication the PG contained the following guarantees: 280 g kg^{-1} CaO, 150 g kg^{-1} S, <1 g kg^{-1} P, and <1 g kg^{-1} of fluorine (F). Several crops were grown during the agricultural years 2002 to 2020 [See in (Bossolani *et al.*, 2022a)].

2.3. Definition of the target object and management of the experimental area

The field part of this proposal has been conducted since October 2016, when the last application of L and/or PG occurred. This experiment is based on the crop rotation model with

maize (*Zea mays* L.) intercropped with a tropical forage [*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins (Syn. *Brachiaria ruziziensis* Germ. & Evrard)] in the autumn/winter period (second crop) followed by soybean cultivation in the spring/summer period (harvest), this system of greater adoption in the producing regions of Brazil. However, the main target of this study was the intercropping of maize with ruzigrass. This crop season corresponds to the 20th agricultural year since the beginning of the experiment.

Maize (single hybrid P3707VYH; 60,000 plants ha^{-1}; DuPont, Pioneer, Johnston, IA, United States) was sown in March 2022. The basic fertilization (N-P_2O_5-K_2O), and topdressing (N), were performed according to the recommendations of Cantarella *et al.* (1997). Since the beginning of the experiment (2002), the plots consist of 14 lines of 9 m long spaced 0.45 m (56.7 m^2).

2.4. Soil sampling for chemical and microbiological analysis

The soil collection occurred into two stages. First, bulk soil was collected in the 0.0-0.1 m layer in the maize interlines [phenological stage R_1; (Ritchie *et al.*, 1993)], which was intercropped with ruzigrass. Ten individual subsamples of soil of each plot were randomly removed with a galvanized steel probe (69 mm diameter) to compose the sample. The samples were separated into two subsamples: sub-sample 1 was placed to air dry and then homogenize for further chemical analysis according to the standard methods for Brazilian tropical soils (van Raij *et al.*, 2001), while subsample 2 was stored at -20 ^{o}C for further extraction of DNA from the soil. Soil fertility analyses consisted of soil pH, P_{resin}, K^+, Ca^{2+}, Mg^{2+}, CEC, base saturation (BS), sulphur-sulphate (S-SO_4^{2-}), aluminum (Al^{3+}), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn).

The second stage of this collection occurred in the same way as the first one, however, the target this time was the rhizospheric soil of maize and forage in consortium. Ten

rhizospheric soil samples from both crops were collected per plot and then combined to form a composite sample, following the methodology proposed by DeAngelis *et al.* (2009). Briefly, the soil closely adhered to the roots of both crops was removed gently through a light brushstroke, stored in Falcon tubes and stored at -20 ^{o}C for further extraction of DNA from the soil.

2.5. DNA extraction from soil (Bulk Soil and rhizosphere)

Soil DNA extractions was performed at 250 mg of each sample using the DNeasy PowerSoil Powerlyzer Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol recommendations. The quality and concentration of DNA measurement occurred by using Nanodrop 1000 spectrophotometry (Thermo Scientific, Wilmington, DE, USA), and agarose gel electrophoresis with 1% sodium boric acid (Brody & Kern, 2004).

2.6. Amplification, sequencing and sequence processing of 16S rRNA and ITS data

The preparation of the Amplicon library and the high-throughput sequencing were performed with the DNA extracted from each soil sample (bulk soil and rhizospheres from maize and ruzigrass). Specific regions of the gene were chosen for the sequencing of bacteria (16S rRNA) and fungi (ITS). The samples were sequenced in the Illumina MiSeq system (Illumina MiSeq PE250). The sequences were deposited in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) under the accession number PRJEB40513. The bacterial rRNA 16S and fungal ITS sequences were processed, demultiplexed and classified as their quality through the DADA2 pipeline (Callahan *et al.*, 2017) in R environment (Team, 2015) with the "consensus" method to remove any chimeric and low-quality remnants from DNA sequences. Taxonomic identification (with 99% similarity) occurred using the SILVA database (v. 138) (Quast *et al.*, 2013) for bacteria and Unite (v. 8.2) (Kõljalg *et al.*, 2013) for fungal

profiles; the taxonomic matrices (of the amplicon sequence variant - ASV) were used for bioinformatic analysis.

2.8. *Statistical analysis*

GJAM *model* focused on maize and ruzigrass rhizosphere selection from bulk soil, exploring the impact of the different soil amendments on the microbial community and functions. All statistical analyses were conducted using various packages in R Studio version 1.3.959 running R version 4.02. The generalized joint attribute modeling (GJAM) package (Clark *et al.*, 2017) was used to estimate the effects of treatments on rhizosphere selection and function as a joint distribution model that allows deducing the sensitivity of input variables (covariates, categorical or continuous). A model was built with the number of simulations and cuts necessary until stability of the chains for the microbial community and functional abundance. From the final model, information were extracted on which microbials are selected according to each soil amendment treatment. The same coefficients were used in the analysis of PCA plots to explore community similarities between treatment to highlight changes induced by different treatments. In addition to the intensity of changes in the relative abundance of the soil microbiome provided by the GJAM regression coefficients, the relevance of different microbes were evaluated in terms of centered log ratio (CLR) transformed abundance (Leite & Kuramae, 2020). Functional role of microbes were estimated by using FAPROTAX and FUNGUILD databases.

3. Results

3.1. Soil chemical properties

The Ca-based soil amendments significantly changed ($P < 0.001$) all soil fertility parameters (Table 1). The direct effect of L, alone or in combination with PG, increased pH by

45-50% and reduced Al^{3+} availability by ~90% compared with the control treatment, as well as increased SOC content. The cascade effects of changes in soil pH increased the availability of all soil macronutrients; however, the combined treatment (LPG) was more efficient than L alone in increasing the availability of Ca^{2+} and S-SO_4^{2-}. The most prominent effects of the Ca-based soil amendments compared with the control were observed for Ca^{2+} availability, which increased by 4.6- and 6.2-fold when L and LPG were applied, respectively, and for Mg^{2+}, which increased ~2-fold when L or LPG was applied. The content of S-SO_4^{2-} increased by 1.5-, 1.2- and 2.4-fold compared with the control when PG, L and LPG were applied, respectively. Due to the increase in soil pH, the availability of micronutrients (Fe, Mn, Cu and Zn) decreased by 30-50% compared with the control when L or LPG was applied.

Table 1. Soil fertility (with available nutrients) of soil long-term amended with phosphogypsum (PG), lime (L) and combined lime and phosphogypsum (LPG) applied in soil surface.

Soil Fertility	units	Control	PG	L	LPG
SOC	g kg^{-1}	16.6 b	14.4 b	19.4 a	20.1 a
pH $_{(CaCl_2)}$	–	4.09 b	4.32 b	5.99 a	6.08 a
Al^{3+}	$mmol_c$ kg^{-1}	11.1 a	5.78 b	1.07 b	1.00 b
P $_{(resin)}$	mg kg^{-1}	26.7 c	35.4 b	47.5 a	50.2 a
K^+	$mmol_c$ kg^{-1}	2.55 b	2.76 b	4.27 a	4.53 a
Ca^{2+}	$mmol_c$ kg^{-1}	11.0 d	45.0 c	61.8 b	78.5 a
Mg^{2+}	$mmol_c$ kg^{-1}	8.61 b	10.4 b	25.7 a	26.6 a
S-SO_4^{2-}	mg kg^{-1}	9.40 a	21.2 b	23.6 b	32.4 a
Fe	mg kg^{-1}	38.9 a	36.1 b	19.0 c	17.3 d
Mn	mg kg^{-1}	49.5 a	38.7 b	27.9 c	26.3 c
Cu	mg kg^{-1}	3.00 a	2.77 b	1.80 c	1.52 d
Zn	mg kg^{-1}	4.12 a	3.85 b	2.96 c	2.50 d

Different lower-case letters indicate significant differences between treatments by LSD test at $p \leq 0.05$.

3.2. Microbe selection by crop rhizospheres

Using GJAM regression coefficients from microbial taxa, we constructed a principal coordinate analysis (PCA) to identify similarities in the shifts between the treatments and the fractions of biodiversity (bulk soil and rhizosphere from maize and ruzigrass) (Fig. 1). Our

analysis indicated a clear segregation of the treatments in the first two axes (68% of the total variation). L- and LPG-amended soils clustered together (right side), whereas PG-amended and control soils were positioned in the left and far from the center of the PCA. In terms of distance, PG positioned close to the control treatment and both distant from L and LPG. Looking at the diversity compartments of each treatment, the microbial taxa from bulk soil of the control and PG treatments presented microbiome close to each other and with the rhizosphere of ruzigrass, whereas the microbiome of the rhizosphere of maize in the control and PG treatments presented similar microbiome between they. Opposite to the control and PG treatments, the microbiome from L and LPG bulk soils clustered together and close to the microbes selected by the rhizosphere of the maize and ruzigrass cultivated in LPG treatment. The microbial taxa selected by the maize rhizosphere grown in L-amended soil was the most distant from the microbial seedbank present in their respective bulk soil, whereas the microbial taxa selected by the rhizosphere of ruzigrass established in L-amended soil was an intermediate between the maize rhizosphere and bulk soil.

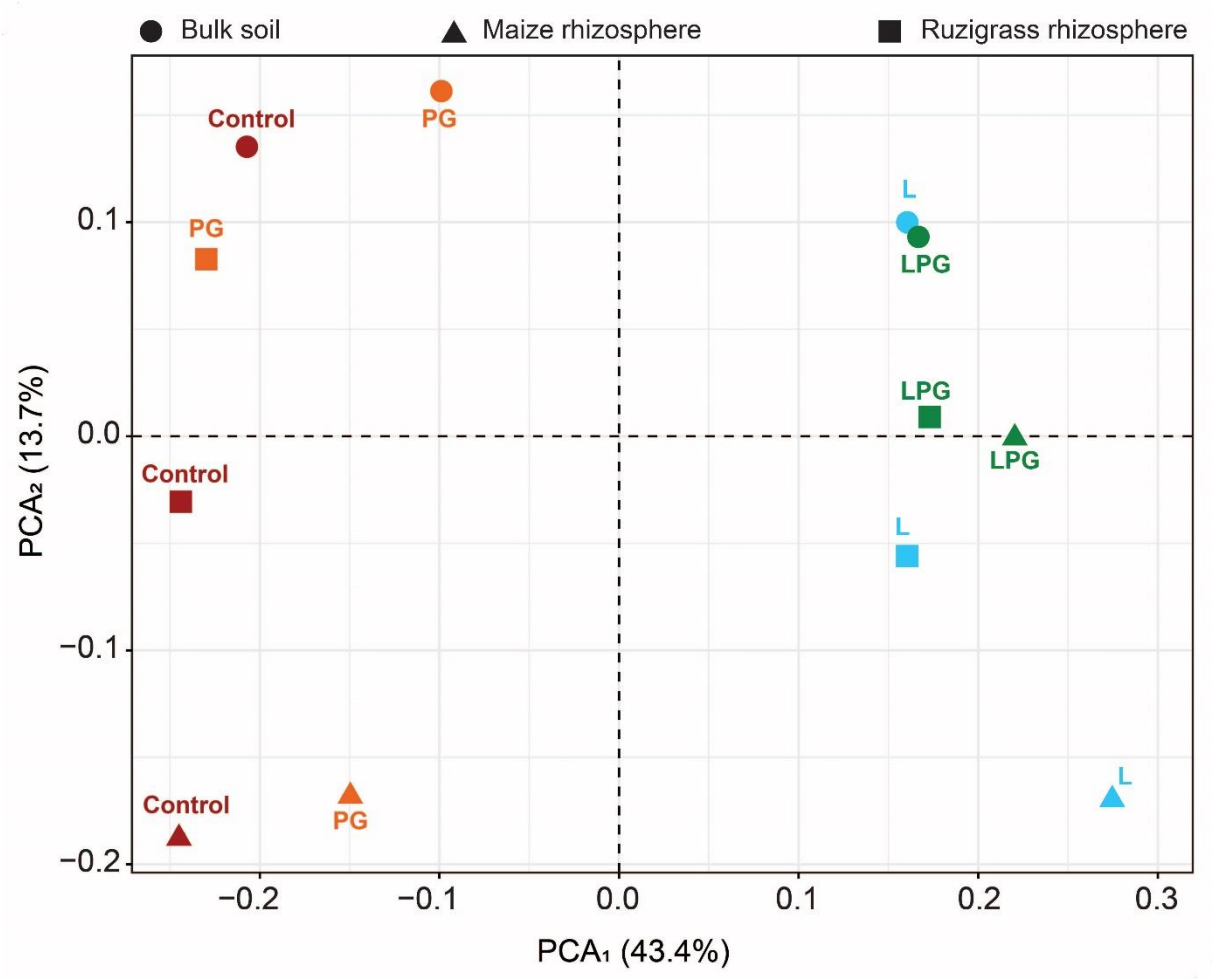


Fig. 1. Principal Component Analysis (PCA) of regression coefficients of bacterial and fungal amplicon sequence variant (ASVs), according to the soil amendments.

The utilization of CLR-transformed data facilitated the assessment of microbial relevance (both bacteria and fungi) and elucidated shifts in microbial community structure across distinct soil diversity compartments (Fig. 2). These graphical representations depict the directional changes (both positive and negative) observed within the principal taxa as influenced by specific treatments, relative to the baseline soil community (control treatment). The horizontal axis delineates the bulk soil, while the vertical y-axis portrays alterations resulting from long-term adding soil amendments. The directional arrows represents the significant selection of microorganisms from the microbial seedbank (bulk soil) by the

rhizospheres of maize and ruzigrass. Our findings identified a distinct rhizosphere-driven selection in microbial community composition when compared with bulk soil matrix. Specifically, the microbial community structure within the rhizosphere exhibited notable dissimilarity from that found in the bulk soil, regardless of the treatment.

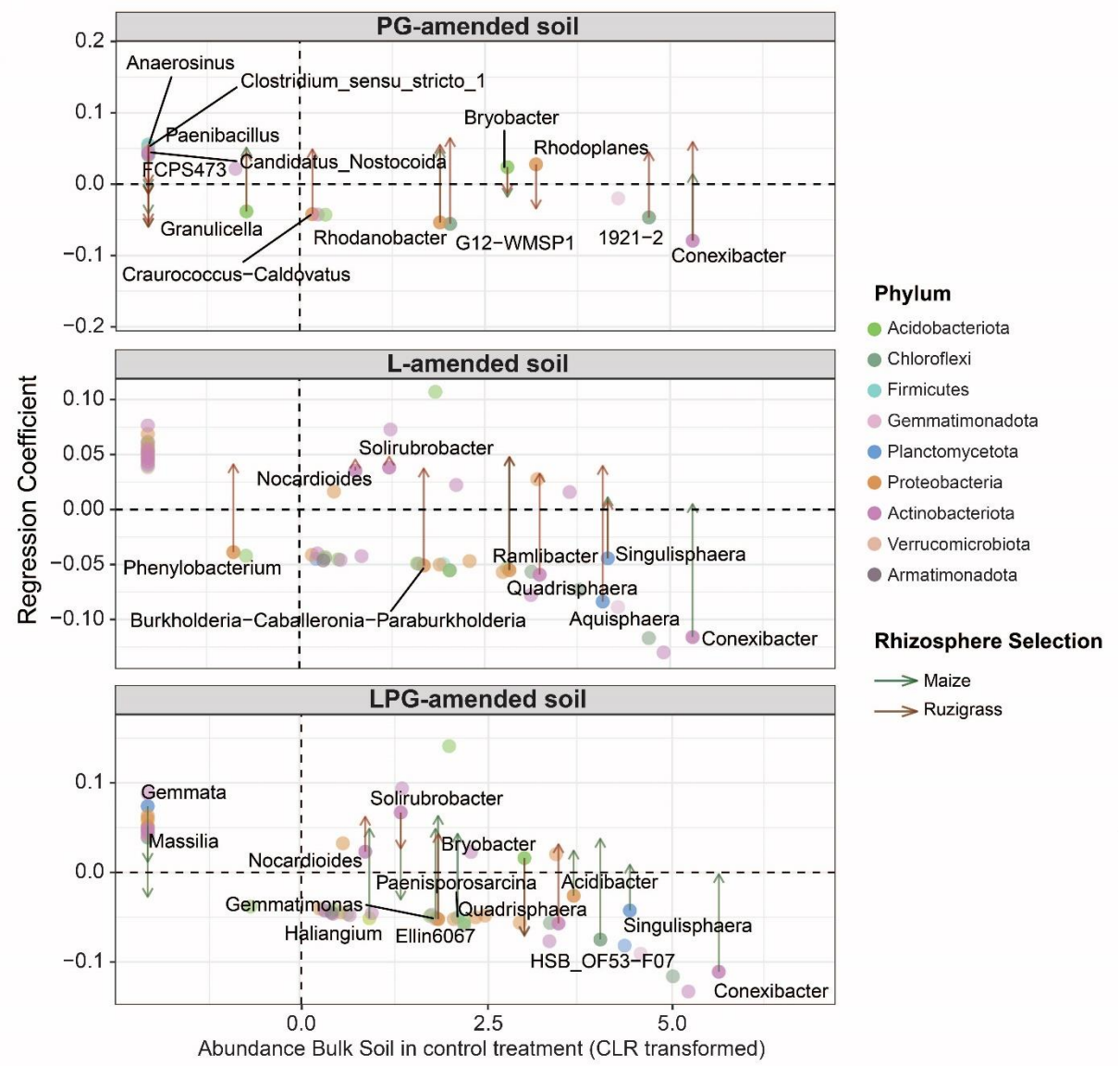


Fig 2. Impact of soil amendments addition in the soil bacteria community and in the rhizosphere selection of maize (green arrow) and ruzigrass (brown arrow) plants. Horizontal axis represents the CLR transformed abundance of the different taxa (genus level) and the vertical axis represents the regression coefficients for the taxa responding to the soil amendment as given by the GJAM model. The green and brown arrows represents the taxa that are significantly selected by the different rhizosphere (maize and ruzigrass, respectively). The length and direction of the arrow indicates the value of the regression coefficient and its value for the specific case of each rhizosphere.

The application of soil amendments elicited an increase in the number of significant taxa demonstrating substantial shifts within the bulk soil compartment (Table 2). Particularly, within the bulk soil, the most prominent impact was observed in the LPG treatment, which exhibited 46 positive and 46 negative shifts (cumulative bacteria and fungi) in soil microbial composition (Table 2). This was closely followed by L-amended soil, wherein 42 positive and 42 negative shifts were recorded, and subsequently, PG-amended soil with 19 positive and 19 negative shifts. Among the taxa significantly affected in the bulk soil, maize and ruzigrass plants exerted discernible preferences for certain microorganisms through their rhizospheres (Figure 2 and 3). Here, the maize rhizosphere within PG-amended soil manifested a propensity to select three bacterial genera (*Rhodanobacter*, *Granulicella* and *Conexibacter*) and 2 fungal genera (*Pseudopithomyces* and *Penicillium*) from the bulk soil, while simultaneously inhibiting the abundance of 6 bacterial genera (*Bryobacter*, *FCPS473*, *Paenibacillus*, *Clostridium_sensu_stricto_1*, *Anaerosinus* and *Candidatus_Nostocoida*) and 1 fungal genus (*Xepicula*) within its rhizosphere. In L-amended soil, the maize rhizosphere preferentially selected 3 bacterial (*Singulisphaera*, *Ramlibacter* and *Conexibacter*) and 2 fungal genera (*Dichotomopilus* and *Cyphellophora*), while repelling 5 fungal genera (*Clonostachys*, *Claviceps*, *Paracremonium*, *Roussoella* and *Penicillium*). Notably, the LPG-amended soil exhibited an even more pronounced effect, with the maize rhizosphere selectively promoting 8 bacterial (*HSB_OF53-F07*, *Paenisporosarcina*, *Gemmatimonas*, *Haliangium*, *Singulisphaera*, *Ellin6067*, *Massilia* and *Acidibacter*) and 6 fungal (*Acremonium*, *Penicillifer*, *Hebeloma*, *Solicoccozyma*, *Scolecobasidium* and *Cyphellophora*) genera, and concurrently repressing 4 bacterial (*Bryobacter*, *Gemmata*, *Conexibacter* and *Solirubrobacter*) and 2 fungal genera (*Saitozyma* and *Penicillium*).

Table 2. Shifts in soil microbiome within each soil diversity compartment as affected by soil amendments.

Soil amendments	Diversity compartment		Shifts	Taxa of Bacteria	Taxa of Fungi
PG	Microbial seedbank	Bulk soil	Positive	12	7
			Negative	9	10
	Rhizosphere selection	Maize rhizosphere	Positive	3	2
			Negative	6	1
		Ruzigrass rhizosphere	Positive	6	5
			Negative	6	4
L	Microbial seedbank	Bulk soil	Positive	28	14
			Negative	32	10
	Rhizosphere selection	Maize rhizosphere	Positive	3	2
			Negative	0	5
		Ruzigrass rhizosphere	Positive	8	1
			Negative	0	6
LPG	Microbial seedbank	Bulk soil	Positive	28	18
			Negative	35	11
	Rhizosphere selection	Maize rhizosphere	Positive	8	6
			Negative	4	2
		Ruzigrass rhizosphere	Positive	4	2
			Negative	1	6

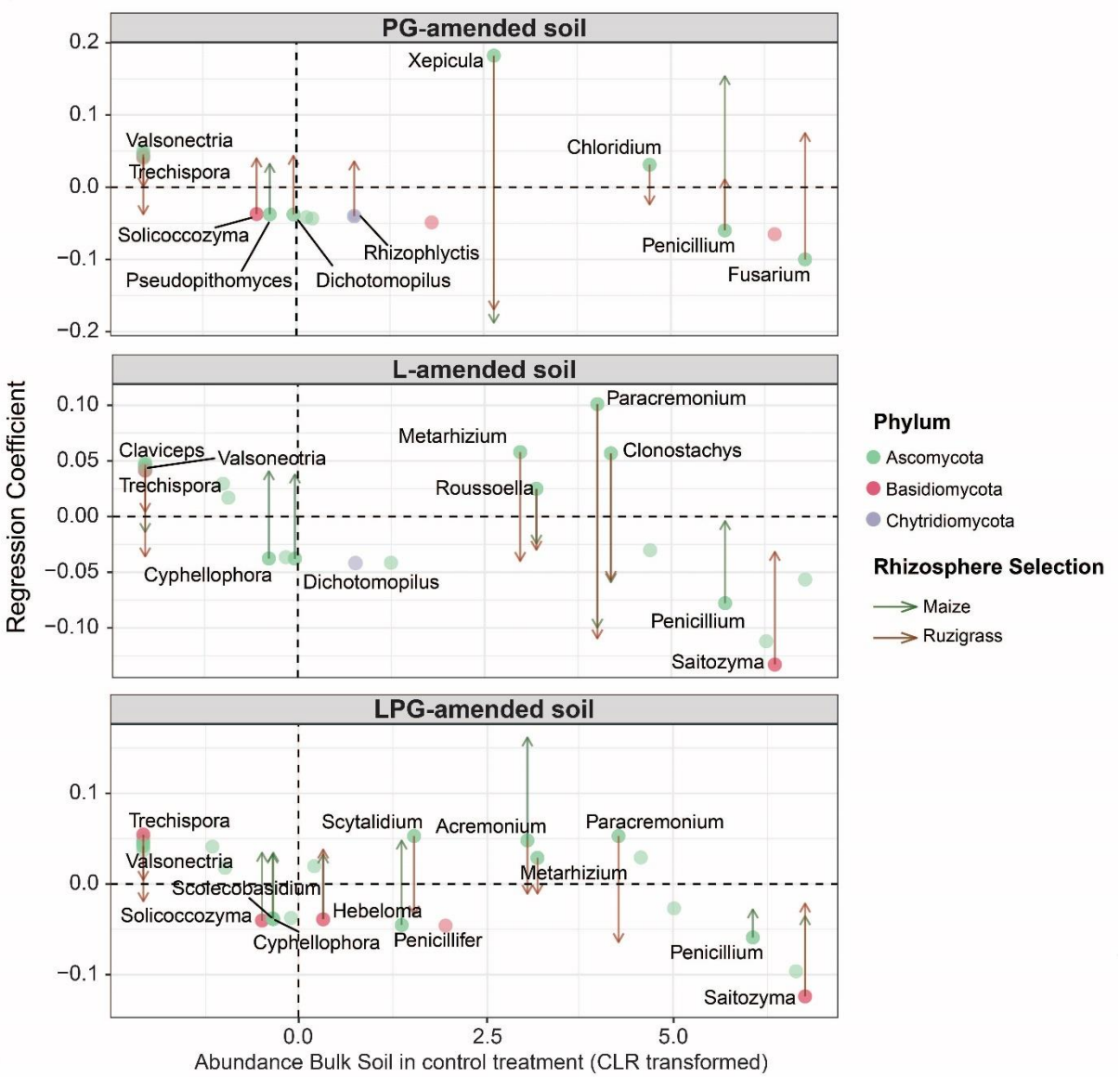


Fig 3. Impact of soil amendments addition in the soil fungi community and in the rhizosphere selection of maize (green arrow) and ruzigrass (brown arrow) plants. Horizontal axis represents the CLR transformed abundance of the different taxa (genus level) and the vertical axis represents the regression coefficients for the taxa responding to the soil amendment as given by the GJAM model. The green and brown arrows represents the taxa that are significantly selected by the different rhizosphere (maize and ruzigrass, respectively). The length and direction of the arrow indicates the value of the regression coefficient and its value for the specific case of each rhizosphere.

Additionally, the ruzigrass rhizosphere in PG-amended soil displayed a tendency to select 6 (*1921-2*, *G12-WMSP1*, *Craurococcus-Caldovatus*, *Rhodanobacter*, *Granulicella* and *Conexibacter*) bacterial genera and concomitantly inhibit 6 other bacterial genera (*Bryobacter*, *Paenibacillus*, *Clostridium_sensu_stricto_1*, *FCPS473*, *Anaerosinus* and *Rhodoplanes*); for

fungal rhizosphere community ruzigrass selected 5 (*Fusarium, Dichotomopilus, Solicoccozyma, Rhizophlyctis* and *Penicillium*) and repelled another 4 genera (*Chloridium, Xepicula, Trechispora* and *Valsonectria*). In contrast, ruzigrass rhizosphere within L-amended soil exhibited selection of 8 bacterial (*Aquisphaera, Singulisphaera, Phenylobacterium, Burkholderia-Caballeronia-Paraburkholderia, Ramlibacter, Quadrisphaera, Nocardioides* and S*olirubrobacter*) and 1 fungal (*Valsonectria*) genera, coupled with inhibition of 6 fungal genera (*Clonostachys, Metarhizium, Paracremonium, Trechispora, Saitozyma* and *Roussoella*). Notably, the LPG amendment created favorable conditions for the ruzigrass rhizosphere to preferentially select 4 genera of bacteria (*Ellin6067, Quadrisphaera, Nocardioides* and *Solirubrobacter*) and 2 genera of fungi (*Valsonectria* and *Hebeloma*) from bulk soil, while discouraging the presence of 1 bacterial genus (*Bryobacter*) and 6 fungal genera (*Metarhizium, Acremonium, Paracremonium, Trechispora, Saitozyma* and *Scytalidium*). In summary, bulk soil typically presented greater microbial abundance than rhizosphere.

3.3. Shifts in soil microbial function and ecological guilds

Long-term application of Ca-based soil amendments resulted in significant changes in the ecosystem functions of the soil microbiome within a maize and ruzigrass intercropping system (Figure 4). Our investigation systematically quantified the impacts on the taxonomic composition of bacterial and fungal communities in relation to their potential functions and ecological guilds under different treatment regimes. Consistent with the shifts observed in the soil microbiome composition (Fig. 4 and 5), the assessment of soil microbial functions demonstrated higher activity in the bulk soil compared with rhizosphere (Table 3; Fig. 4 and 5). Notably, the fungal functional attributes displayed a greater susceptibility to perturbations induced by the soil amendments compared to bacterial counterparts.

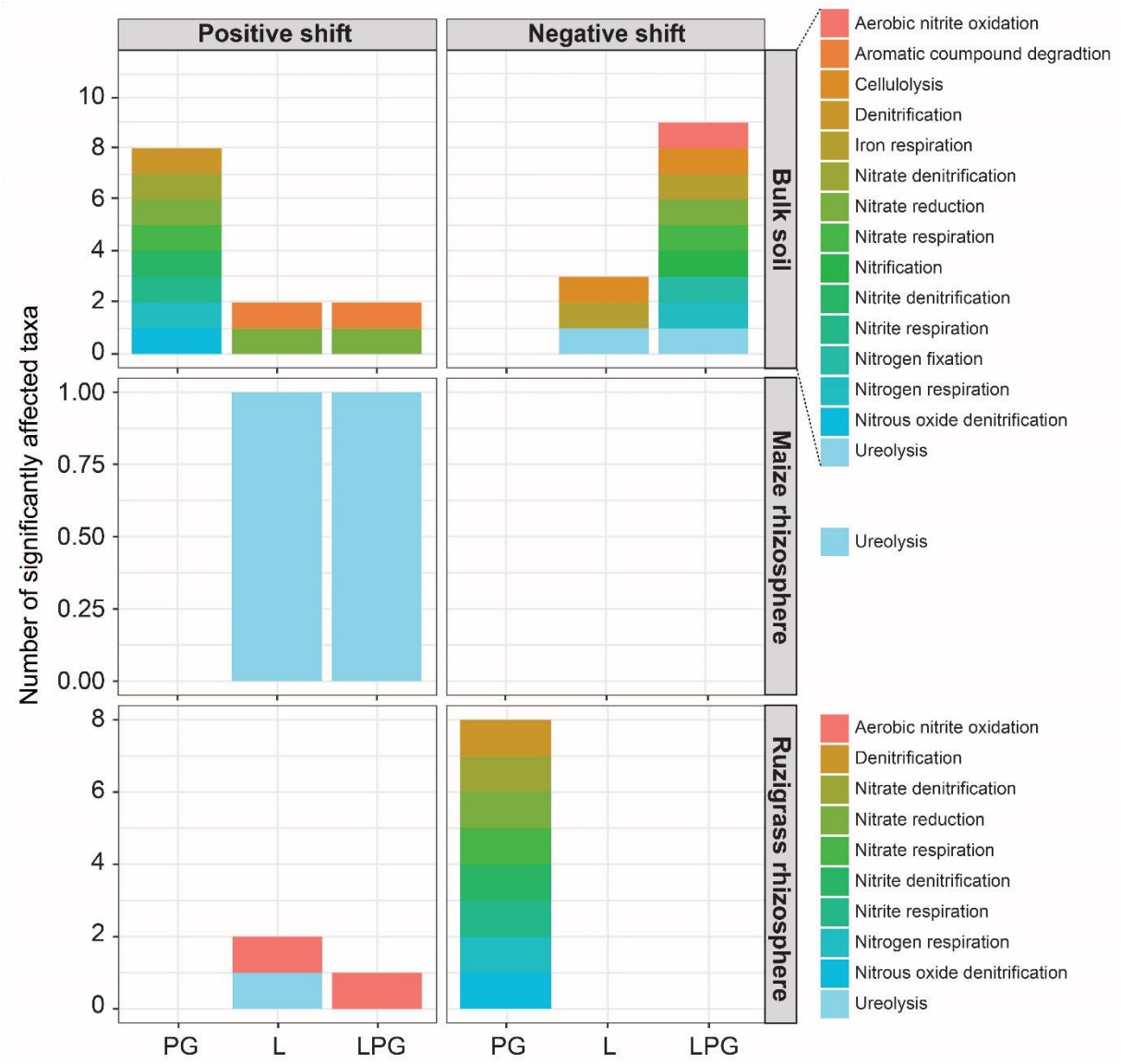


Fig 4. Functional role as estimated by FAPROTAX for the microbes that are significantly impacted by the soil amendments.

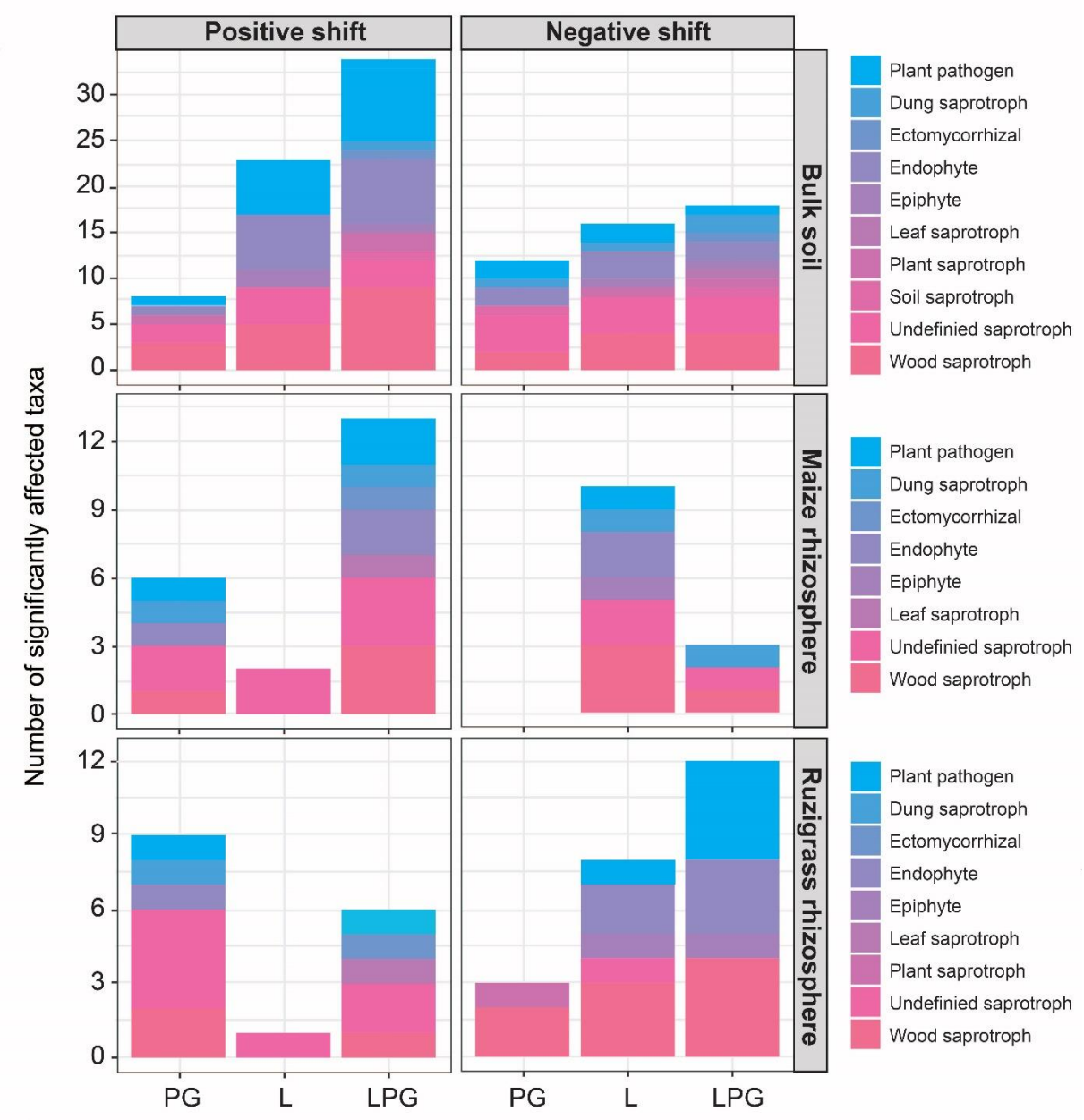


Fig 5. Functional role as estimated by FUNGUILD for the microbes that are significantly impacted by the soil amendments.

In total, 184 taxa associated with pivotal fungal functions in agricultural soils exhibited alterations in response to the treatments, whereas a comparatively 37 bacterial taxa were significantly modified in relation to the original community (control treatment) (Fig. 5). Overall, within the LPG treatment, the bulk soil exhibited the most pronounced response for bacteria (positive shifts = 2; negative shifts = 9) and mainly for fungi community (positive shifts = 34; negative shifts = 18). Furthermore, 10 functional roles demonstrated positive shifts while

18 roles displayed negative shifts. Subsequently, the L-amended soil displayed positive shifts for 2 taxa of bacteria (2 functional roles), along with 23 taxa and 5 functions exhibiting negative shifts for fungi community. In addition, negative shifts occurred for 3 taxa of bacteria (3 functional roles) and 16 taxa of fungi (7 functional roles). Lastly, the PG-amended soil treatment led to 8 positive shifts for bacterial taxa (8 functional roles) and also for 8 fungi taxa (4 functional roles). Negative shifts occurred only for fungi community (12 taxa and 6 functional roles) . These findings underscore the varying impacts of different soil amendment treatments on the taxonomic and functional dynamics of the soil microbiome within the intercropped system studied.

Table 3. Shifts in the main taxa associated with bacterial functional potential and fungal ecological groups within each soil diversity compartment as affected by soil amendments.

Soil amendments	Diversity compartment		Shifts	Taxa of Bacteria	Taxa of Fungi	Function of Bacteria	Function of Fungi
PG	Microbial seedbank	Bulk soil	Positive	8	8	8	4
			Negative	0	12	0	6
	Rhizosphere selection	Maize rhizosphere	Positive	0	6	0	5
			Negative	0	0	0	0
		Ruzigrass rhizosphere	Positive	0	9	0	5
			Negative	8	3	8	2
L	Microbial seedbank	Bulk soil	Positive	2	23	2	5
			Negative	3	16	3	7
	Rhizosphere selection	Maize rhizosphere	Positive	1	2	1	1
			Negative	0	10	0	6
		Ruzigrass rhizosphere	Positive	2	1	2	1
			Negative	0	8	0	5
LPG	Microbial seedbank	Bulk soil	Positive	2	34	2	9
			Negative	9	18	9	10
	Rhizosphere selection	Maize rhizosphere	Positive	1	13	1	7
			Negative	0	3	0	3
		Ruzigrass rhizosphere	Positive	1	6	1	5
			Negative	0	12	0	5

Among the comprehensive array of bacterial and fungal taxa evaluated, a subset demonstrated noteworthy changes under the influence of the treatments in maize and ruzigrass intercropped rhizospheres. Moreover, the impact of soil amendments on the ecological role of fungi surpassed that on bacteria. Particularly, soil amended with LPG demonstrated the highest occurrence of taxa displaying substantial shifts, closely associated with the principal functional roles of bacteria and fungi. Out of the 15 selected bacterial soil functions, the maize rhizosphere from the LPG treatment displayed a single taxa with a positive shift, notably linked to ureolysis function in the soil. Conversely, 13 taxa of fungi exhibited positive shifts, aligned with 7 distinct soil functional roles, namely Dung saprotroph, Ectomycorrhizal, Endophyte, Leaf saprotroph, Plant pathogen, Undefined saprotroph, and Wood saprotroph. In addition, three taxa exhibited negative shifts, associated with three functions of fungi (Dung saprotroph, Undefined saprotroph, and Wood saprotroph). In maize rhizosphere from soils treated with L, a sole positive shift emerged in the ureolysis function, while two fungi taxa displayed positive shifts linked to Undefined saprotroph, and six taxa presented negative shifts associated with Dung saprotroph, Endophyte, Plant pathogen, Undefined saprotroph, and Wood saprotroph. Notably, maize rhizosphere from soil amended with PG showed a complete absence of taxa or functions meeting the selection criteria based on quantifiable significant changes utilizing GJAM regression coefficients, in contrast to the bulk soil. Conversely, the maize rhizosphere exhibited discernible alterations in five distinct ecological guilds (Dung saprotroph, Endophyte, Plant pathogen, Undefined saprotroph, and Wood saprotroph), encompassing a total of six taxa that demonstrated positive shifts in their relative abundances.

In the present study, we observed distinct rhizosphere dynamics in response to ruzigrass and maize co-cultivation in soils subjected to lime and phosphogypsum amendments. Notably, the forage rhizosphere exhibited differential behavior compared to maize, even when sharing the same soil environment. Long-term application of L and LPG amendments resulted in a

diminished presence of taxa with discernible positive shifts. Among these, two taxa in bacterial communities from L-amended soil demonstrated associations with the degradation of aromatic compounds and ureolysis, while a singular taxa in the LPG-treated group was also linked to aromatic compound degradation. Conversely, no bacterial taxa exhibited negative shifts under these treatments. Notably, the rhizosphere of ruzigrass in PG-treated soil displayed a substantial negative shift in eight bacterial taxa, primarily linked to nitrogen cycling processes encompassing denitrification, nitrate denitrification, nitrate reduction, nitrate respiration, nitrite denitrification, nitrogen respiration, and nitrous oxide denitrification.

Among the kingdoms, the most pronounced differences among treatments were evident within the fungal community and functions. In the ruzigrass rhizosphere subjected to LPG treatment, six taxa displayed positive shifts: Dung saprotroph, Ectomycorrhizal, Leaf saprotroph, Undefined saprotroph, and Wood saprotroph. Simultaneously, 12 taxa exhibited negative shifts, representing roles such as endophytic associations, epiphytic existence, plant pathogenicity, and wood saprotroph. In comparison, the rhizosphere of ruzigrass exposed to L treatment displayed a single taxa with a positive shift, characterized as an undefined saprotroph, while five taxa underwent negative shifts, associated with endophytic interactions, epiphytic existence, plant pathogenicity, undefined saprotroph, and wood saprotroph. Lastly, examination of fungi taxa within the ruzigrass rhizosphere cultivated in PG-amended soil underscored five taxa with positive shifts encompassing functions like dung saprotroph, endophytic associations, plant pathogenicity, undefined saprotroph, and wood saprotroph, alongside two taxa undergoing negative shifts linked to plant saprotroph and wood saprotroph.

4. Discussion

The interactions between soil microbes, their environment and plants growing at the same time play a pivotal role in shaping soil health and nutrient cycling dynamics (Lammel *et al.*, 2018; Zhang *et al.*, 2021). The present study compared the effects of long-term application

of L combined or not with PG on the soil fertility and microbiome changes. In addition, our study is the first to show how the rhizospheres of both maize and ruzigrass co-cultivated in intercropping system select microbes from bulk soil towards their rhizospheres. The present study also aims to elucidate if the functions of soil microbes (bacteria and fungi) are changed by soil amendments in each soil diversity compartment (bulk soil and rhizospheres).

4.1. Effects of soil amendment-based management on soil fertility and microbial community structure

In our study, after seventeen years of managing soil with amendments in a no-till intercrop system, remarkable changes occurred on soil fertility. The application of lime (L), both singly and when combined with PG (LPG treatment), displayed the most substantial capacity for neutralizing soil acidity components (pH and Al^{3+}), which have a strong potential to reduce crop development (Crusciol *et al.*, 2022), as well as negatively affecting the growth and activity of soil microbes (Bossolani *et al.*, 2021b; Li *et al.*, 2022). In addition, lime application triggered soil buffering processes (Fageria & Nascente, 2014) that boosted the availability of Ca^{2+}, Mg^{2+}, K^{+}, P_{resin} and $S\text{-}SO_4^{2-}$, and over time, increased total organic carbon (TOC) levels. On the other hand, employing only PG led to marginal shifts in soil fertility levels compared to the untreated control. Although PG is not traditionally recognized as an acid neutralizing soil amendment (Zoca & Penn, 2017), its synergy with L exploits the acidity correction ability of L to alleviate soil acidity (Crusciol *et al.*, 2019). In addition, PG demonstrates as effective to mitigate Al^{3+} toxicity and increase Ca^{2+} in the soil. This cascade of effects becomes notably significant in agricultural soils marked by limited Ca^{2+} availability, a common attribute of extensively weathered tropical soils. Notably, surface application of L can decrease the concentration of essential micronutrients in the soil (Fe, Mn, Cu, Zn) by increasing soil pH (Pegoraro *et al.*, 2006), as occurred in our study. This further reinforces the important role of PG as a soil improver that enriches the soil with Ca^{2+} without excessively altering soil

pH, thus avoiding potential reduction in crop yields. In summary, the judicious integration of L and PG emerges as a promising strategy for enhancing soil fertility, reducing toxicity of Al^{3+}, without disrupting crop yield.

Despite our results highlighting the improvement of soil fertility by applying soil amendments, crop productivity gains are not totally dependent on the physical and chemical quality of the soil. Recent studies have emerged demonstrating the impact of L (Guo *et al.*, 2019b; Li *et al.*, 2022), PG (Costa *et al.*, 2023), and their combination (Bossolani *et al.*, 2020, 2021b) applications on soil microbial communities and activity. It is well known that sol pH influences the growth and diversity of prokaryotes (Lammel *et al.*, 2018); however, pH also impacts various other soil properties that can indirectly affect microbial communities (Holland *et al.*, 2018), which also play a vital role in soil microbial ecology, such as SOC content, and nutrient availability that covariate with soil pH (Lammel *et al.*, 2018). It is noteworthy that the majority of studies have focused on C, N, and P neglecting the important roles of other elements in the composition and activity of microorganisms. Nevertheless, high fertile soils (L and LPG treatments) presented high abundance of soil microbes than in poorly soils (control and PG treatments), which presented high levels of Al^{3+}, Fe, Mn and Cu, that are potentially toxic to microbial growth (Tian *et al.*, 2019).

Our findings reinforce the fact that fertile soils alter soil microorganism growth dynamics. Soils treated with L and LPG showed higher rates of bacteria and fungi with significant shifts (positive and negative) compared to soil treated with PG, and both compared with the original community (control treatment), as also demonstrated by (Bossolani *et al.* (2021b). On the other hand, very little is known about how plants select soil microorganisms based on their rhizospheres, that is, the "effort" they perform to select microorganisms that may be of interest for their full development, based on the available seedbank.

Our PCA was constructed by using the regression coefficients provided by GJAM modelling. Treatments and soil compartments was segregated in two groups. However, within each of these groups, the soil compartments showed different patterns. In group 1 (control and PG), maize rhizospheres were grouped as similar but separate from the bulk soil and ruzigrass rhizosphere. In group 2 (L and LPG), only the maize rhizosphere community was segregated from the other samples. In general, plants with different genetic backgrounds have a distinct composition of root-associated microbiomes (Fitzpatrick *et al.*, 2018), which justifies the separation of the maize rhizosphere from the other soil compartments. Here, we hypothesized that rhizospheres exerted discernible preferences for certain microorganisms, while "repulsing" other microorganisms that were probably not of interest to plants. The rhizosphere selection is based on the organisms present in the microbial seedbank, demonstrating the importance of managements that aim to increase the species richness and diversity of soil microorganism, as shown by the greater number of affected taxa in high fertile soils (L and, specially LPG). From there, the plants secreted organic exudates through their roots attracting soil microorganisms to their rhizospheres. Root exudates provide a favorable environment for the growth of specific microbes, leading to increased abundance (Coskun *et al.*, 2017). Interestingly, the quantity and quality of exudates released by the roots are influenced by the photosynthetic capacity of the plants. Bossolani *et al.* (2021a) demonstrated that maize plants established in fertile soils by the application of L alone or combined with PG presented greater photosynthetic activity (gas exchange, photosynthetic pigments and RuBisCO activity). These authors reported that greater availability of nutrients in the soil is the main driver of improved plant physiology. These results are in line with the fact that maize and ruzigrass plants from L and LPG treatments presented a higher number of taxa selected from the microbial seedbank.

4.2. Selection and functioning of the rhizobacterial community affected by soil amendments

Our results shed light on the responsiveness of soil microbiomes to changes in soil fertility (resource availability), and the consistent selection of bacterial groups from soil to the rhizospheres of intercropped maize with ruzigrass. In comparison to the original bacterial soil community (control), L and LPG treatments displayed the most pronounced changes (either increases or decreases in abundance) in the bacterial community of bulk soil. The historical legacy created by long-term application of soil amendments on soil physicochemical (Bossolani *et al.*, 2020, 2022a) and straw return (Carmeis Filho *et al.*, 2017b) to the soil led to the creation of a more resource-rich environment for bacterial proliferation. When a crop is cultivated in these soils, they end up exerting a great influence over the rhizosphere bacteriome, mostly by providing root-exudates and other labile chemical resources (Bakker *et al.*, 2012). As plant roots grow, they significantly affect the amount and chemical identity of C resources available to soil microbes (Ling *et al.*, 2022). In addition, the absorption of nutrients by the roots also alters the microenvironment of the rhizosphere, further regulating the efflux of exudates in the soil (Bakker *et al.*, 2015).

This complex network of processes leads to a bacterial community that differs markedly from the source communities in bulk soil (Zhang *et al.*, 2021). In line with that, proportionally to the number of total shifts in taxa from bulk soil, PG exhibited the highest number of positive shifts in bulk soil compared to the other treatments. However, the opposite trend emerged for both maize and ruzigrass rhizospheres, where greater enrichment rates were observed in treatments with L and LPG. In general, the rhizosphere microbiota is a subset of the community in bulk soil, but the groups of microorganisms can differ between species grown in the same soil (Yan *et al.*, 2017). In contrast, Ling *et al.* (2022) demonstrated in their meta-analysis study that even when genotypic and environmental differences were considered, there were certain similar traits in several plants studied. In our study, we detected that some microorganisms in common were selected by the rhizospheres of the maize and ruzigrass cultivated in each

treatment, as for example in the PG treated soil (low fertility), where *Conexibacter* (Actinobacteriota), *Rhodanobacter* (Proteobacteria) and *Granilucella* (Acidobacteriota) were selected by both rhizospheres. Also, in both rhizospheres there was depletion of *Clostridium* (Firmicutes) and *Anaerosinus* (Firmicutes), *Paenbacillus* (Firmicutes) and *Briobacter* (Actinobacteriota). In high fertile soil, such as in L-amended soil, *Ramlibacter* (Proteobacteria) and *Singulisphaera* (Planctomycetota) were shared by the rhizospheres of maize and ruzigrass, whereas in LPG-amended soil *Ellin6067* (Proteobacteria) was selected and *Solirubrobacter* (Actinobacteriota) was depleted. Although both plants share similarities in the recruitment of bacteria within the same treatment, the patterns of recruitment were different between treatments. These findings offer practical evidence for the logical concept that the initial composition of the microbial community available to a plant can fundamentally restrict the outcomes of selection in the rhizosphere (Bakker *et al.*, 2015), and this is highly dependent on soil fertility levels (Pang *et al.*, 2019).

Considering the phylum of the enriched and depleted bacteria in the rhizospheres, our data are partially consistent with the findings of (Ling *et al.*, 2022). These authors reported that in the wide range of rhizospheres from different plants and soil, the Actinobacteriota and Proteobacteria phyla were enriched in rhizosphere environments, whereas other dominant phyla are depleted from rhizospheres, including Firmicutes, Chloroflexi and Gemmatimonadota, corroborating with our findings. Actinobacteriota and Proteobacteria are oligotrophs and, therefore, generally adapted to C-rich environments (i.e., rhizosphere), favoring their growth and spread, as well as their metabolic activity (Ling *et al.*, 2022). In PG-treated soil Firmicutes was consistently depleted in both rhizospheres, confirming that these microorganisms are abundant in soils with low nutrient availability (Zhang *et al.*, 2022), and which are depleted from grasses rhizospheres (Ling *et al.*, 2022).

As a consequence in bacterial changes in both bulk soil and rhizospheres after soil amendments management, the potential bacterial function was also impacted. Compared with control treatment, PG presented high number of bacterial taxa with positive shifts linked with N processes in bulk soil, whereas the opposite occurred for LPG-amended soil, which presented a higher number taxa with negative shifts for these same N functional roles. L- and LPG amended soil presented positive shifts for taxa linked with aromatic compound degradation and nitrate reduction, as well as negative shifts for ureolysis (L and LPG) and for many other N functional roles. Here, our results point to a greater potential for aromatic compound degradation in L- and LPG amended soils. Several bacteria use these substances as carbon and energy sources for growth (Ahmad *et al.*, 2020). Beforehand, combining soil acidity correction with ruzigrass cultivation could increase the bacterial biodegradation pathways for these persistent organic pollutants are of great importance, given the harm to organisms (Ahmad *et al.*, 2020).

Looking at the rhizosphere compartment, this region harbors a diverse and plentiful diversity bacteria that significantly influence the C and N cycles in the soil (Ling *et al.*, 2022). In our study, maize rhizosphere presented significant positive shift for a taxon related to ureolysis when the soil was treated with L (L and LPG treatments). Ureolysis is related to ureolytic bacteria that use N and other nutrients For hydrolyzing urea (Ma *et al.*, 2020). Ureolysis is favored in soils with a high presence of SOC and pH closer to alkalinity, with peaks between pH_{H_2O} 6 to 8 (Hasan, 2000). This result points to good N fertilization practices with urea, very common in maize cropping, since the potential for N loss can be greater in these soils when urea as fertilizer is used.

Ruzigrass rhizosphere also presented positive shifts for ureolysis (L) and aerobic nitric oxidation (L and LPG). Contrary to what occurred in bulk sol, ruzigrass rhizosphere from PG treatment presented several negative shifts for taxa linked to the N cycle, especially those

involved in nitrification. Although nitrification primarily occurs in aerobic conditions, the rhizosphere typically experiences a lack of oxygen due to the higher oxygen consumption by roots and microorganisms compared to the surrounding soil (Lecomte *et al.*, 2018). Other factors that may influence the reduction of functions linked to nitrification processes are (a) competition for N between microorganisms and ruzigrass and (b) potential reduced nitrifying activity as a consequence of low nitrification rates (inhibition of nitrification) induced by tropical forage grasses (Coskun *et al.*, 2017; Subbarao *et al.*, 2015), such as ruzigrass.

4.3. Selection and functioning of the rhizofungi community affected by soil amendments

Contrary to numerous studies that have documented decreased fungal activity in soils with corrected pH levels (Holland *et al.*, 2018), in our study we noticed a higher number of significant taxa and functions of these microorganisms in soils treated with L and, specially, LPG. The greater abundance of affected fungi taxa could potentially be elucidated by the substantial SOC content in these treatments resulting from the prolonged implementation of a no-till cropping system (Carmeis Filho *et al.*, 2017b; Inagaki *et al.*, 2017). Tropical agricultural systems are based on crop rotation, which increases the availability of straw in the system (Carmeis Filho *et al.*, 2017b), which is often linked to an increased fungal activity (Arcand *et al.*, 2016). Straw contributes to the formation of microenvironments conducive to fungal growth, in addition to crop residues providing a diverse food source for fungi, despite differences in soil fertility (Arcand *et al.*, 2016).

Interestingly, the fungal community in the rhizosphere of maize and ruzigrass plants also showed different patterns between treatments. In general, most of the fungi that were significantly altered by the treatments belong to the phylum Ascomycota. Among them, the genus Penicillium, which is potentially pathogenic, was selected by the maize rhizosphere in all treatments, but the shift intensity was lower in the LPG-treated soil, followed by L and PG. Interestingly, only in the PG treatment ruzigrass selected this fungus genus. Although plants

can actively modify their root exudate property to recruit their specific rhizosphere microbial communities (Bakker *et al.*, 2015), the "choice", that is, the attraction to root exudates is made according to the preferences of the microorganisms themselves (Zhalnina *et al.*, 2018). Still, the rhizosphere selection of the microbiome is dependent on the composition and the abundance of microbes of the bulk soil.

Nevertheless, ruzigrass in the PG-amended soil also recruited fungus of the genus *Fusarium*, another potentially pathogenic fungus, whereas the same did not occurred in the other treatments. These results are in line with several studies that prove that the use of L can reduce the establishment of *Fusarium* in agricultural soils (Pegg *et al.*, 2019). Fusarium wilt diseases are usually most severe in sandy soils with a low pH. Thus, raising soil pH with L has been used as an efficient tool to reduce wilt development in a range of crops (Pegg *et al.*, 2019). The relationship of the high pH value with the reduction of fusariosis is linked to a reduction in the availability of micronutrients to the fungus, as well as enhancing the activity of a competing microbial flora (Jones & Woltz, 1970).

In addition, improvements in soil fertility in LPG-amended soil facilitated the proliferation of a diverse array of saprophytic fungi (including those associated with soil, wood, and plant saprotrophs) responsible for the breakdown of organic matter (Buscot & Varma, 2019). The heightened microbial activity on the soil surface is attributed to the accumulation of crop residues (Córdova *et al.*, 2018). Our findings indicate that the continued utilization of LPG on tropical agricultural soils encouraged fungal communities that augment the potential for carbon mineralization. As a result, this enhances the decomposition of readily degradable compounds into stable carbon molecules, ultimately refining the quality of soil organic matter (Bossolani *et al.*, 2021b).

Within the broad category of fungi there has been significant interest in the influence of soil amendments on beneficial groups such as mycorrhizal fungi and plant growing promoters

(Holland *et al.*, 2018). In our study, *Hebeloma* (mycorrhiza) was consistently enriched in the rhizospheres of maize and ruzigrass in the LPG-amended soil. Mycorrhizal fungi are often associated with mycotrophic crops as annual grasses (e.g., maize) and forage grasses (e.g., ruzigrasss) (Méndez *et al.*, 2019), because these crops presents a greater amount of spread, fine and fibrous roots, as well as high photosynthetic capacity and high P uptake (Ganugi *et al.*, 2019). Regarding to soil changes, mycorrhizal root colonization typically rises as the soil reaches pH_{H_2O} levels between 5.5-6.5 (Ganugi *et al.*, 2019). However, it decreases as soil alkalinity increases. Additionally, it has been observed that liming promotes the production of Mycorrhizal fungal spores (Wang *et al.*, 1993). This result is of particular significance as mycorrhizas have critical roles in uptake of plant-limiting resources, such as P and water (Johnson *et al.*, 2005). Enrichment in endophytic and epiphytic fungi also occurred in the rhizospheres of maize and ruzigrass growing in LPG-amended soil. These fungi, most belonging to the phylum Ascomycota, are linked with increase plant resilience, improving plant nutrition, stress tolerance and defense (Pozo *et al.*, 2021).

5. Conclusions

In conclusion, the influence of lime (L) and phosphogypsum (PG) on soil microbial functions is a complex interplay of physicochemical changes, crop cultivation and microbial responses. Furthermore, our investigation has unveiled a nuanced selection of microbial taxa within the rhizospheres of maize and ruzigrass, distinctly diverging from the bulk soil composition. These findings underscore the intricate interplay between plant-microbe interactions and soil amendment strategies, yielding insights into the dynamics of microbial community structure, selection, and functioning in agricultural soils. Our results suggest that maize and ruzigrass selects a specific microbial community from soil reservoir based on functional traits, which may be related to benefits to the plant; but the soil amendments proved

to be the main modulator of the soil microbiome, and that rhizosphere selection by cultivated plants is dependent on the changes caused by soil amendments. Important results were identified related to nitrogen cycle responses, in particular greater urealysis in soils treated with L and LPG, as well as the presence of mycorrhizae in high fertile soils treated with LPG. Lastly, further analysis is required to gain a better grasp the mechanisms by which the intercropped plants select the rhizospheric community.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by National Council for Scientific and Technological Development (CNPq) (Grant150858/2022-1), by São Paulo Research Foundation (FAPESP) (Grant 2018/11063-7 and 2019/12764-1), and The Netherlands Organization for Scientific Research (NWO) grant 870.15.022. In addition, the second author would like to thank the National Council for Scientific and Technological Development (CNPq) for an award for excellence in research. Publication number XXXX of the Netherlands Institute of Ecology (NIOO-KNAW).

References

Ahmad F, Zhu D, Sun J. 2020. Bacterial chemotaxis: a way forward to aromatic compounds biodegradation. *Environmental Sciences Europe* **32**. DOI: 10.1186/s12302-020-00329-2

Alvares CA, Stape JL, Sentelhas PC, de Moraes Gonçalves JL, Sparovek G. 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* **22**: 711–728. DOI: https://doi.org/10.1127/0941-2948/2013/0507

Arcand MM, Helgason BL, Lemke RL. 2016. Microbial crop residue decomposition dynamics in organic and conventionally managed soils. *Applied Soil Ecology* **107**: 347–359. DOI: 10.1016/j.apsoil.2016.07.001

Bakker MG, Chaparro JM, Manter DK, Vivanco JM. 2015. Impacts of bulk soil microbial community structure on rhizosphere microbiomes of Zea mays. *Plant and Soil* **392**: 115–126. DOI: 10.1007/s11104-015-2446-0

Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanco JM. 2012. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant and Soil* **360**: 1–13. DOI: 10.1007/s11104-012-1361-x

Bossolani JW, Crusciol CAC, Garcia A, Moretti LG, Portugal JR, Rodrigues VA, Fonseca M de C da, Calonego JC, Caires EF, Amado TJC, Reis AR dos. 2021a. Long-term lime and phosphogypsum amended-soils alleviates the field drought effects on carbon and antioxidative metabolism of maize by improving soil fertility and root growth. *Frontiers in Plant Science* **12**: 1437. DOI: 10.3389/fpls.2021.650296

Bossolani JW, Crusciol CAC, Leite MFA, Merloti LF, Moretti LG, Pascoaloto IM, Kuramae EE. 2021b. Modulation of the soil microbiome by long-term Ca-based soil amendments boosts soil organic carbon and physicochemical quality in a tropical no-till crop rotation system. *Soil Biology and Biochemistry* **156**: 108188. DOI: 10.1016/j.soilbio.2021.108188

Bossolani JW, Crusciol CAC, Merloti LF, Moretti LG, Costa NR, Tsai SM, Kuramae EE. 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. *Geoderma* **375**: 114476. DOI: 10.1016/j.geoderma.2020.114476

Bossolani JW, Crusciol CAC, Momesso L, Portugal JR, Moretti LG, Garcia A, Fonseca M de C da, Rodrigues VA, Calonego JC, Andre. 2022a. Surface liming triggers improvements in subsoil fertility and root distribution to boost maize crop physiology , yield and revenue. *Plant and Soil* **472**: 1–23. DOI: 10.1007/s11104-022-05432-2

Bossolani JW, Crusciol CAC, Moretti LG, Garcia A, Portugal JR, Bernart L, Vilela RG, Caires EF, Amado TJC, Calonego JC, dos Reis AR. 2022b. Improving soil fertility with lime and phosphogypsum enhances soybean yield and physiological characteristics. *Agronomy for Sustainable Development* **42**. DOI: 10.1007/s13593-022-00765-9

Bossolani JW, Crusciol CAC, Portugal JR, Moretti LG, Garcia A, Rodrigues VA, Fonseca M de C da, Bernart L, Vilela RG, Mendonça LP, Reis AR. 2021c. Long-term liming improves soil fertility and soybean root growth, reflecting improvements in leaf gas exchange and grain yield. *European Journal of Agronomy* **128**: 126308. DOI: 10.1016/j.eja.2021.126308

Brody JR, Kern SE. 2004. Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis. *BioTechniques* **36**: 214–216. DOI: https://doi.org/10.2144/04362BM02

Buscot F, Varma A. 2019. *Mineralization and Humification Pathways*. *Microorganisms in Soils: Roles in Genesis and Functions*. DOI: 10.1017/CBO9781107415324.004

Caires EF, Garbuio FJ, Churka S, Barth G, Corrêa JCL. 2008. Effects of soil acidity amelioration by surface liming on no-till corn, soybean, and wheat root growth and yield. *European Journal of Agronomy* **28**: 57–64. DOI: 10.1016/j.eja.2007.05.002

Caires EF, Guimarães AM. 2018. A novel phosphogypsum application recommendation method under continuous no-till management in Brazil. *Agronomy Journal* **110**: 1987–1995. DOI: 10.2134/agronj2017.11.0642

Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal* **11**: 2639–2643. DOI: 10.1038/ismej.2017.119

Cantarella H, RAIJ B van, Camargo CEO. 1997. Adubação de cereais. *RAIJ, B. van et al., eds. Recomendações de adubação e calagem para o Estado de São Paulo* **2**: 43–50

Carmeis Filho ACA, Crusciol CAC, Castilhos AM. 2017a. Liming demand and plant growth improvements for an Oxisol under long-term no-till cropping. *Journal of Agricultural Science* **155**: 1093–1112. DOI: 10.1017/S0021859617000235

Carmeis Filho ACA, Penn CJ, Crusciol CAC, Calonego JC. 2017b. Lime and phosphogypsum impacts on soil organic matter pools in a tropical Oxisol under long-term no-till conditions. *Agriculture, Ecosystems and Environment* **241**: 11–23. DOI: 10.1016/j.agee.2017.02.027

Clark JS, Nemergut D, Seyednasrollah B, Turner PJ, Zhang S. 2017. Generalized joint attribute modeling for biodiversity analysis: Median-zero, multivariate, multifarious data. *Ecological Monographs*. DOI: 10.1002/ecm.1241

Córdova SC, Olk DC, Dietzel RN, Mueller KE, Archontouilis S V., Castellano MJ. 2018. Plant litter quality affects the accumulation rate, composition, and stability of mineral-associated soil organic matter. *Soil Biology & Biochemistry* **125**: 115–124. DOI: 10.1016/j.soilbio.2018.07.010

Coskun D, Britto DT, Shi W, Kronzucker HJ. 2017. How Plant Root Exudates Shape the Nitrogen Cycle. *Trends in Plant Science* **22**: 661–673. DOI: 10.1016/j.tplants.2017.05.004

Costa CHM, Crusciol CAC. 2016. Long-term effects of lime and phosphogypsum application on tropical no-till soybean-oat-sorghum rotation and soil chemical properties. *European Journal of Agronomy* **74**: 119–132. DOI: 10.1016/j.eja.2015.12.001

Costa RF, Firmano RF, Bossolani JW, Alleoni LRF. 2023. Soil Chemical Properties, Enzyme Activity and Soybean and Corn Yields in a Tropical Soil Under No-till Amended with Lime and Phosphogypsum. *International Journal of Plant Production* **17**: 235–250. DOI: 10.1007/s42106-023-00233-8

Crusciol CAC, Bossolani JW, Portugal JR, Moretti LG, Momesso L, Campos M, Costa NR, Volf MR, Calonego JC, Rosolem CA. 2022. Exploring the synergism between surface liming and nitrogen fertilization in no-till system. *Agronomy Journal* 1–16. DOI: 10.1002/agj2.20988

Crusciol CAC, Marques RR, Carmeis Filho ACA, Soratto RP, Costa CHM, Ferrari Neto J, Castro GSA, Pariz CM, Castilhos AM, Franzluebbers AJ. 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. *Nutrient Cycling in Agroecosystems* **115**: 347–372. DOI: 10.1007/s10705-019-10017-0

Crusciol CAC, Marques RR, Filho ACAC, Soratto RP, Costa CHM, Neto JF, Castro GSA, Pariz CM, de Castilhos AM. 2016. Annual crop rotation of tropical pastures with no-till soil as affected by lime surface application. *European Journal of Agronomy* **80**: 88–104. DOI: 10.1016/j.eja.2016.07.002

DeAngelis KM, Brodie EL, DeSantis TZ, Andersen GL, Lindow SE, Firestone MK. 2009. Selective progressive response of soil microbial community to wild oat roots. *The ISME Journal* **3**: 168–178. DOI: 10.1038/ismej.2008.103

Fageria NK, Nascente AS. 2014. *Management of soil acidity of South American soils for*

sustainable crop production. *Advances in Agronomy*. Elsevier. DOI: 10.1016/B978-0-12-802139-2.00006-8

Fitzpatrick CR, Copeland J, Wang PW, Guttman DS, Kotanen PM, Johnson MTJ. 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences of the United States of America* **115**: E1157–E1165. DOI: 10.1073/pnas.1717617115

Ganugi P, Masoni A, Pietramellara G, Benedettelli S. 2019. A review of studies from the last twenty years on plant–arbuscular mycorrhizal fungi associations and their uses for wheat crops. *Agronomy* **9**. DOI: 10.3390/agronomy9120840

Guo A, Ding L, Tang Z, Zhao Z, Duan G. 2019a. Microbial response to CaCO3 application in an acid soil in southern China. *Journal of Environmental Sciences* **79**: 321–329. DOI: 10.1016/j.jes.2018.12.007

Guo A, Ding L, Tang Z, Zhao Z, Duan G. 2019b. Microbial response to CaCO 3 application in an acid soil in southern China. *Journal of Environmental Sciences (China)* **79**: 321–329. DOI: 10.1016/j.jes.2018.12.007

Hasan HAH. 2000. Ureolytic microorganisms and soil fertility: A review. *Communications in Soil Science and Plant Analysis* **31**: 2565–2589. DOI: 10.1080/00103620009370609

Holland JE, Bennett AE, Newton AC, White PJ, McKenzie BM, George TS, Pakeman RJ, Bailey JS, Fornara DA, Hayes RC. 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. *Science of the Total Environment* **610**–**611**: 316–332. DOI: 10.1016/j.scitotenv.2017.08.020

Inagaki TM, de Moraes Sá JC, Caires EF, Gonçalves DRP. 2017. Why does carbon increase in highly weathered soil under no-till upon lime and gypsum use? *Science of the Total Environment* **599**–**600**: 523–532. DOI: 10.1016/j.scitotenv.2017.04.234

Johnson D, Leake JR, Read DJ. 2005. Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland. *Plant and Soil* **271**: 157–164. DOI: 10.1007/s11104-004-2267-z

Jones JP, Woltz SS. 1970. Fusarium wilt of tomato: interaction of soil liming and micronutrient amendments on disease development. *Phytopathology* **60**: 812–813. DOI: 10.1094/Phyto-60-812

Kiehl EJ. 1979. Edaphology manual: soil-plant relationship. *Agronômica Ceres. São Paulo, Brazil: Piracicaba*

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson KH. 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277. DOI: 10.1111/mec.12481

Lammel DR, Barth G, Ovaskainen O, Cruz LM, Zanatta JA, Ryo M, de Souza EM, Pedrosa FO. 2018. Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. *Microbiome* **6**: 7–9. DOI: 10.1186/s40168-

018-0482-8

Lecomte SM, Achouak W, Abrouk D, Heulin T, Nesme X, Haichar F el Z. 2018. Diversifying anaerobic respiration strategies to compete in the rhizosphere. *Frontiers in Environmental Science* **6**: 1–16. DOI: 10.3389/fenvs.2018.00139

Leite MFA, Kuramae EE. 2020. You must choose, but choose wisely: Model-based approaches for microbial community analysis. *Soil Biology & Biochemistry* **151**. DOI: 10.1016/j.soilbio.2020.108042

Li S, Liu J, Yao Q, Yu Z, Li Y, Jin J, Liu X, Wang G. 2022. Short-term lime application impacts microbial community composition and potential function in an acid black soil. *Plant and Soil* **470**: 35–50. DOI: 10.1007/s11104-021-04913-0

Ling N, Wang T, Kuzyakov Y. 2022. Rhizosphere bacteriome structure and functions. *Nature communications* **13**: 836. DOI: https://doi.org/10.1038/s41467-022-28448-9

Lupatini M, Korthals GW, Roesch LFW, Kuramae EE. 2019. Long-term farming systems modulate multi-trophic responses. *Science of the Total Environment* **646**: 480–490. DOI: 10.1016/j.scitotenv.2018.07.323

Ma L, Pang AP, Luo Y, Lu X, Lin F. 2020. Beneficial factors for biomineralization by ureolytic bacterium Sporosarcina pasteurii. *Microbial Cell Factories* **19**: 1–12. DOI: 10.1186/s12934-020-1281-z

Mendes LW, Kuramae EE, Navarrete AA, Van Veen JA, Tsai SM. 2014. Taxonomical and functional microbial community selection in soybean rhizosphere. *ISME Journal* **8**: 1577–1587. DOI: 10.1038/ismej.2014.17

Méndez DFS, de Paula AM, Ramos MLG, Busato JG. 2019. Maize productivity, mycorrhizal assessment, chemical and microbiological soil attributes influenced by maize-forage grasses intercropping. *Brazilian Archives of Biology and Technology* **62**: 1–14. DOI: 10.1590/1678-4324-2019170737

Merloti LF, Mendes LW, Pedrinho A, de Souza LF, Ferrari BM, Tsai SM. 2019. Forest-to-agriculture conversion in Amazon drives soil microbial communities and N-cycle. *Soil Biology and Biochemistry* **137**: 107567. DOI: 10.1016/j.soilbio.2019.107567

Pang Z, Tayyab M, Kong C, Hu C, Zhu Z, Wei X, Yuan Z. 2019. Liming positively modulates microbial community composition and function of sugarcane fields. *Agronomy* **9**: 1–17. DOI: 10.3390/agronomy9120808

Pegg KG, Coates LM, O'Neill WT, Turner DW. 2019. The Epidemiology of Fusarium Wilt of Banana. *Frontiers in Plant Science* **10**: 1–19. DOI: 10.3389/fpls.2019.01395

Pegoraro RF, Da Silva IR, Novais RF, De Sá Mendonça E, De Oliveira Gebrim F, Moreira FF. 2006. Diffusive flux and bioavailability of micronutrients in soils: Influence of liming, soil texture and green manure. *Revista Brasileira de Ciencia do Solo* **30**: 859–868. DOI: 10.1590/S0100-06832006000500012

Pozo MJ, Zabalgogeazcoa I, Vazquez de Aldana BR, Martinez-Medina A. 2021. Untapping the potential of plant mycobiomes for applications in agriculture. *Current Opinion in Plant Biology* **60**: 102034. DOI: 10.1016/j.pbi.2021.102034

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-

based tools. *Nucleic Acids Research* **41**: 590–596. DOI: 10.1093/nar/gks1219

Ritchie SW, Hanway JJ, Benson GO. 1993. How a corn plant develops. Special Report n. 48. Iowa State University of Science and Technology. *Cooperative Extension Service. Ames, IA, USA*

Santos HG, Jacomine PKT, Dos Anjos LHC, De Oliveira VA, Lumbreras JF, Coelho MR, De Almeida JA, de Araújo Filho JC, De Oliveira JB, Cunha TJF. 2018. *Sistema brasileiro de classificação de solos.* Brasília, DF: Embrapa, 2018.

Soil Survey Staff. 2014. Keys to Soil Taxonomy. USDA - Natural Resources Conservation Service: Washington, DC, 360

Subbarao GV, Yoshihashi T, Worthington M, Nakahara K, Ando Y, Sahrawat KL, Rao IM, Lata JC, Kishii M, Braun HJ. 2015. Suppression of soil nitrification by plants. *Plant Science* **233**: 155–164. DOI: 10.1016/j.plantsci.2015.01.012

Sumner ME, Noble AD. 2003. Soil acidification: the world story. *Handbook of soil acidity. Marcel Dekker, New York* 1–28

Team RC. 2015. R: A Language and environment for statistical computing. *R Foundation for Statistical Computing*

Tian J, Dungait JAJ, Lu X, Yang Y, Hartley IP, Zhang W, Mo J, Yu G, Zhou J, Kuzyakov Y. 2019. Long-term nitrogen addition modifies microbial composition and functions for slow carbon cycling and increased sequestration in tropical forest soil. *Global Change Biology* **25**: 3267–3281. DOI: 10.1111/gcb.14750

Tkacz A, Bestion E, Bo Z, Hortala M, Poole PS. 2020. Influence of plant fraction, soil, and plant species on microbiota: A multikingdom comparison. *mBio* **11**: 1–17. DOI: 10.1128/mBio.02785-19

van Raij B, Cantarella H, Quaggio JA, Furlani AMC. 1997. *Recomendações de adubação e calagem para o Estado de São Paulo*. Instituto Agronômico/Fundação IAC Campinas

van Raij B, Quaggio JA, Cantarella H, Abreu CA. 2001. Os métodos de análise química do sistema IAC de análise de solo no contexto nacional. *Análise química para avaliação da fertilidade de solos tropicais* 5–39

Wang GM, Stribley DP, Tinker PB, Walker C. 1993. Effects of pH on arbuscular mycorrhiza I. Field observations on the long-term liming experiments at Rothamsted and Woburn. *New Phytologist* **124**: 465–472. DOI: 10.1111/j.1469-8137.1993.tb03837.x

Wang Y, Yao Z, Zhan Y, Zheng X, Zhou M, Yan G, Wang L, Werner C, Butterbach-Bahl K. 2021. Potential benefits of liming to acid soils on climate change mitigation and food security. *Global Change Biology* **27**: 2807–2821. DOI: 10.1111/gcb.15607

Yan Y, Kuramae EE, De Hollander M, Klinkhamer PGL, Van Veen JA. 2017. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *ISME Journal* **11**: 56–66. DOI: 10.1038/ismej.2016.108

Zhalnina K, Louie KB, Hao Z, Mansoori N, Da Rocha UN, Shi S, Cho H, Karaoz U, Loqué D, Bowen BP. 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature microbiology* **3**: 470–480. DOI: https://doi.org/10.1038/s41564-018-0129-3

Zhang R, Rong L, Zhang L. 2022. Soil nutrient variability mediates the effects of erosion on

soil microbial communities: results from a modified topsoil removal method in an agricultural field in Yunnan plateau, China. *Environmental Science and Pollution Research* **29**: 3659–3671. DOI: 10.1007/s11356-021-15894-z

Zhang Z, Chai X, Tariq A, Zeng F, Li X, Graciano C. 2021. Intercropping Systems Modify Desert Plant-Associated Microbial Communities and Weaken Host Effects in a Hyper-Arid Desert. *Frontiers in Microbiology* **12**. DOI: 10.3389/fmicb.2021.754453

Zoca SM, Penn C. 2017. An Important Tool With No Instruction Manual: A Review of Gypsum Use in Agriculture. *Advances in Agronomy* **144**: 1–44. DOI: 10.1016/bs.agron.2017.03.001