



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

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ABSTRACT

Unsustainable agricultural management practices such as non-conservationist tillage and overuse of fertilizers result in soil acidity and, in turn, soil degradation due to reduced carbon (C) concentrations and nutrient availability and increased aluminum toxicity. Application of lime (L) and phosphogypsum (PG) can overcome these constraints and improve soil quality, but the long-term effects of these amendments on both abiotic and biotic soil properties are not known, particularly when applied in combination. Here, we evaluated the effects of L (acidity corrective), PG (soil conditioner), and their combination (LPG) on soil organic matter (SOM) transformations, soil chemical and physical properties, and microbiome assembly in a long-term experiment under a no-till crop rotation system in a tropical soil. The Ca-based soil amendments increased C concentrations (labile and stable fractions), improved soil physicochemical properties, and changed the associations between several bacterial and fungal groups. Contrary to expectations, the acidic soil amended with PG exhibited greater number of significant shifts in the bacterial community than soil amended with L or LPG, as well as higher soil bulk density. By contrast, the fungal community underwent greater shifts in soil amended with L or LPG, which had higher macroporosity. L and LPG amendment shaped the fungal community and rearranged the SOM fractions at similar rates, suggesting an essential role of the altered fungi in SOM transformation. In addition, combining L with PG increased the relevance of many low-abundance microorganisms, especially fungi, compared with the control, indicating an increase in their ecological role in the soil. Finally, by applying general joint attribute modeling and sensitivity analysis, we determined that soil fertility increased most in LPG-amended soil, as the ensuing changes in the bacterial and fungal communities resulted in improved SOM fractions, soil physical characteristics and, ultimately, soil quality.

1. Introduction

The processes that govern soil acidification are global drivers of soil degradation (low productive capacity) (von Uexküll and Mutert, 1995; Gibbs and Salmon, 2015; Meng et al., 2019). Soil acidification is a natural phenomenon resulting from the weathering of parent material with low exchangeable bases [e.g., calcium (Ca^{2+}) and magnesium (Mg^{2+})] but is worsened by unsustainable soil management practices

such as non-conservationist tillage, overuse of ammonium-based fertilizers, removal of cations by crop harvesting, and release of organic acids from decomposing crop residues (Bian et al., 2013; Tiritan et al., 2016; Pulido-Moncada et al., 2018). The same agricultural mismanagement practices that worsen soil acidity and fertility also negatively impact soil organic matter (SOM) (Fageria and Baligar, 2008; Carmes Filho et al., 2017b). Low SOM levels in soils promote leaching of cations, further contributing to soil acidification and severely restricting crop

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production (Evans et al., 2012; Castellano et al., 2015; Paradelo et al., 2015; Carmeis Filho et al., 2017b).

In addition to deteriorating abiotic soil properties (chemical and physical), soil acidity disrupts soil microbial community structure, abundance, activities and functions (Kuramae et al., 2010, 2012; Navarrete et al., 2013). The soil microbiome is one of the main reservoirs of diversity in the biosphere and plays an essential role in agroecosystem functioning, including SOM decomposition and nutrient cycling (Kušliene et al., 2014). Low soil pH reduces carbon (C) concentrations and nutrient availability and increases the toxicity of certain elements [e.g., aluminum (Al^{3+}) and manganese (Mn)], thus directly affecting microbial growth (Bowman et al., 2008; Tian et al., 2019). Additionally, the high concentration of hydrogen (H^+) in acidic soils likely reduces microbial biomass and physiology (Meng et al., 2019; Tian et al., 2019).

Alleviating these deleterious effects requires sustainable agricultural practices that increase soil productive capacity while providing important ecosystem services, such as increasing the stock of nutrients and C in the soil and improving activities in belowground processes (De Tender et al., 2019). Liming is a traditional practice to mitigate the negative effects of acidity on soil and crops, particularly in tropical soils (Carmeis Filho et al., 2017a; Crusciol et al., 2019; Bossolani et al., 2020a, 2020b). Liming increases nutrient availability and provides extra nutrient inputs (i.e., Ca^{2+} and Mg^{2+}) (Bossolani et al., 2020b), thereby improving soil fertility and resources for plant and microbial growth (Liu et al., 2018a; Guo et al., 2019; Bossolani et al., 2020a; Jha et al., 2020). Thus, beyond neutralizing acidic soils, liming benefits soil health (Holland et al., 2018) to promote the grain and biomass yield of arable and grassland crops. Due to its low solubility, lime (L) is typically applied to the soil and subsequently incorporated to increase the reaction efficiency (Bossolani et al., 2018; Crusciol et al., 2019; Fontoura et al., 2019). However, the emergence of no-till systems (NTS) has led to the introduction of superficial liming (lime applied to the soil surface, without subsequently incorporation through plowing and harrowing), the effects of which on soil acidity are unclear. To circumvent the limitations of superficial liming, a few recent studies have evaluated the application of phosphogypsum (PG, composed primarily of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), a by-product of phosphoric acid production, in combination with L (Carmeis Filho et al., 2017b; Crusciol et al., 2019; Bossolani et al., 2020a). Unlike L, PG does not correct soil acidity but is more soluble in soil and can potentially reduce Al^{3+} concentrations and increase Ca^{2+} availability. Thus, PG is useful as a supplement to improve soil quality under NTS (Zoca and Penn, 2017; Bossolani et al., 2018).

The main challenge in using Ca-based soil amendments is achieving a balance between crop productivity and soil quality that provides a sustainable production system (Yun et al., 2016; Holland et al., 2018; Liu et al., 2018b). The impacts of Ca-based soil amendments on agriculture are wide-ranging, and previous work has focused on isolated effects on soil properties and short-term impacts. Short-term research is insufficient to assess ecosystem properties, such as soil structure, C stocks, and microbiota, that may take decades to stabilize, and results obtained before 15 years have elapsed are susceptible to misinterpretation (Cusser et al., 2020). The few studies that have examined long-term agricultural management with L and PG have shown increases in soil physicochemical quality (Briedis et al., 2012; Carmeis Filho et al., 2018). However, we still lack an understanding of how combined Ca-based soil amendments (L + PG) affect soil chemical and physical factors, C sequestration, and microbial communities and how these soil factors are associated with and affect the soil microbiome.

To address this gap, we investigated the legacy of long-term L and PG amendments alone or in combination in a no-tillage intercropping system by answering the following two questions:

- 1) How did 17 years of amendment with lime, phosphogypsum or both change the abiotic properties (chemical and physical) and biotic communities (bacteria and fungi) of the soil?

- 2) How were the changes in microorganisms associated with changes in soil chemical and physical properties?

We hypothesized that the type of soil amendment would affect soil fertility, soil physical structure and soil organic matter fractions and these factors would drive different associations of bacterial and fungal groups.

2. Material and methods

2.1. Field description

The experiment was conducted in a 17 years-old long-term experimental field (Soratto and Crusciol, 2008) in Brazil (22° 83' 3" S, 48° 42' 64" W, elevation 765 m above sea level). The soil is a sandy clay loam kaolinitic and thermic Typic Haplorthox (USDA, 2014). The soil physicochemical characteristics prior the experiment (2002) were soil pH (0.01 M CaCl_2 suspension): 4.2, soil organic matter (SOM): 21 g kg^{-1} ; K^+ : 1.2 mmolc kg^{-1} ; Ca^{2+} : 14 mmolc kg^{-1} ; Mg^{2+} : 5 mmolc kg^{-1} ; total acidity at pH 7 (H + Al): 38.8 mmolc kg^{-1} , cation exchange capacity (CEC): 58 mmolc kg^{-1} ; BS: 35%; aluminum saturation (AS): 65% (see the complete physicochemical characterization in Table S1). According to the Köppen-Geiger's climatic classification, the region corresponds a mesothermic type (Cwa) with a humid subtropical dry winter and hot summer (Alvares et al., 2013). The long-term average (1956–2019) annual temperature is 26.1 °C maximum and 15.3 °C minimum with a mean annual precipitation of 1359 mm (Unicamp, 2020).

2.2. Experimental setup, treatments and cropping history

The experimental design was a randomized complete block with four treatments and four replications. The treatments were (i) natural soil conditions (control treatment with no lime and no phosphogypsum amendment), (ii) lime (L) (13 Mg ha^{-1}); (iii) phosphogypsum (PG) (10 Mg ha^{-1}); (iv) combined L plus PG (LPG). Fertilizer inputs (N-P₂O₅-K₂O) occurred in all plots, including the control treatment, in all crops established in this experiment over the 17 years. A schematic graph representing the crop system, soil amendment application and sampling is illustrated in Fig. 1. During the study period (2002–2019), the treatments were applied four times (2002, 2004, 2010 and 2016), and different crops were cultivated in season and off season from 2002 to 2019. The treatments were applied whenever the base saturation (BS) of the standard treatment (exclusive L) reached value $\leq 50\%$ in the soil (0.00–0.40 m). Soil fertility was monitored through annual sampling. This study had maize (*Zea mays* L.) intercropped with ruzigrass [*Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins (Syn. *Brachiaria ruziziensis* Germ. & Evrard)] sown in agricultural year of 2019. Details of the previous crop sequences and in each reapplication, as well as the characteristics of the applied amendments are shown in Table S2. Since the experiment was initiated, the treatments were performed superficially, except for the first application in October 2002, when no-tillage system was initiated.

The water content of L and PG amendments were determined for the calculations of the correct rate to be applied on soil, which calculations considering both amendments with 0 g kg^{-1} of water content. The composition of amendments applied in last reapplication were measured: effective calcium carbonate equivalent (ECCE): 69%, CaO: 310 g kg^{-1} and MgO: 140 g kg^{-1} for L and CaO: 280 g kg^{-1} , S: 150 g kg^{-1} for PG. All the calculations of recommended rate of L and PG are shown in the Supplementary Material and Methods.

The bulk soil samples were collected between maize + ruzigrass rows at 0.00–0.10 m layer from a no-till maize intercropped with ruzigrass system. The samples were collected at V₁₀ phenological maize stage (Ritchie et al., 1993) in June 2019. For physicochemical and organic matter fractionation, samples were air-dried and sieved (2 mm sieve) and for molecular analysis, aliquots of 80 g fresh soil were stored at

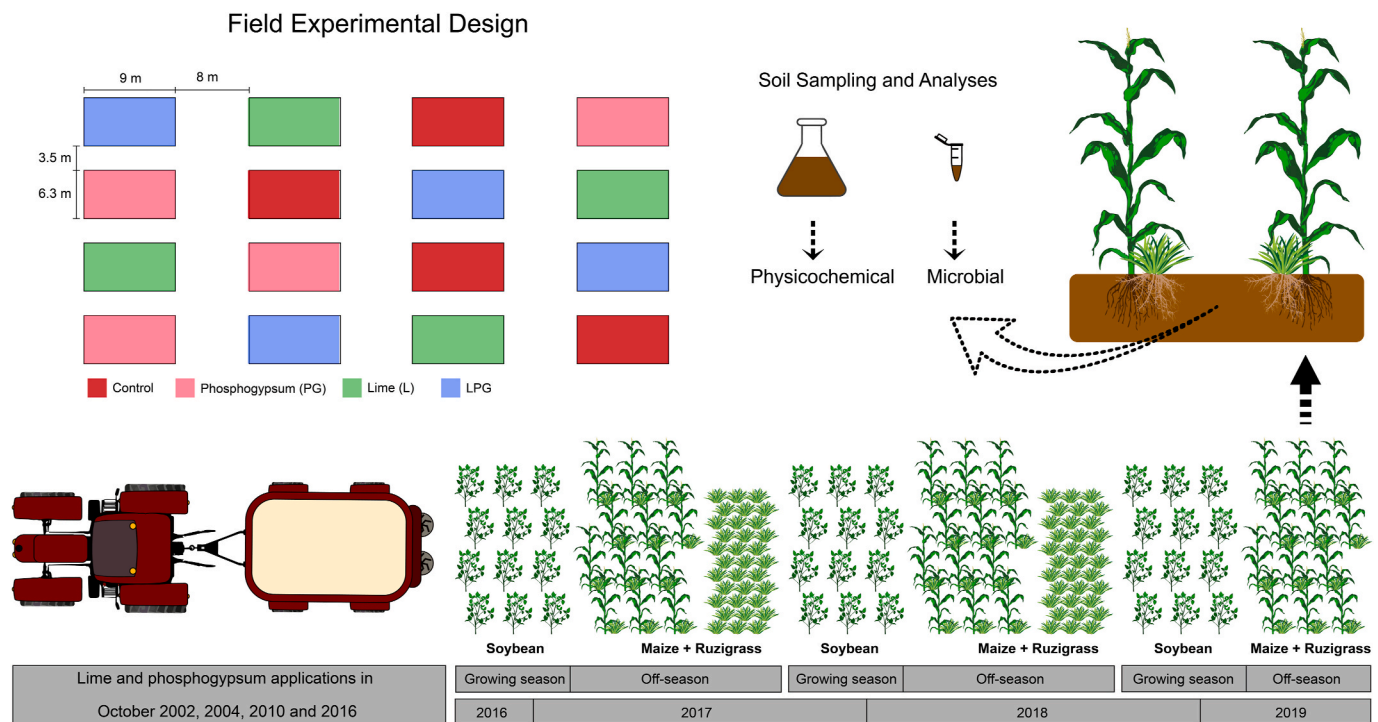


Fig. 1. Schematic graph representing the field experimental design, crop system, soil amendment application and sampling.

–20 °C for subsequent DNA extraction.

2.3. Soil sampling and analysis

2.3.1. Soil physicochemical properties

Soil samples were processed according to the standard methods for Brazilian tropical soils (van Raij et al., 2001). Seventeen years after treatment establishment, ten individual soil subsamples of each plot were randomly taken at 0.00–0.10 m depth with a galvanized-steel probe (69-mm-diameter) to compose the sample. The samples were separated into two subsamples: subsample 1 to air dry and then homogenized for further chemical analysis (except NO_3^- and NH_4^+) and subsample 2 to store at –20 °C for 15 days until NO_3^- and NH_4^+ determination could be performed. The soil pH values were measured in 0.01 M CaCl_2 (the most stable pH used in soil analysis) (van Raij et al., 2001). The exchangeable cations (K^+ , Ca^{2+} , and Mg^{2+}) and available P-phosphate were extracted using ion exchange resins and, in the extract, P was determined calorimetrically, and cations by atomic absorption spectrometry (Shimadzu AA-7000) (van Raij et al., 2001). Soil sulfur-sulfate (S-SO_4^{2-}) extraction were performed by calcium phosphate extraction at 0.01 mol L^{-1} in a 1:2.5 soil/solution ratio and later determined by the turbidimetric method using BaSO_4 (Bardsley and Lancaster, 1960). The cationic micronutrients (Fe, Mn, Cu and Zn) were extracted in a mixture DTPA 0.005 M + TEA 0.1 M + CaCl_2 0.01 M, and determined by atomic absorption spectrometry (van Raij et al., 2001). The total acidity at pH 7.0 (H^+ + Al) was estimated by the Shoemaker-McLean-Pratt buffer solution method (Shoemaker et al., 1961). The exchangeable Al^{3+} was extracted with neutral 1 mol L^{-1} KCl at 1:10 soil/solution ratio and determined by titration with a 0.025 mol L^{-1} NaOH solution. After extraction of KCl solution, the content of ammonium (NH_4^+) and nitrate (NO_3^-) was also determined by colorimetry by sodium salicylate method and vanadium chloride reduction respectively (Mulvaney et al., 1996). Total inorganic nitrogen (Ni) was obtained by summing the measured concentrations of mineral forms (NH_4^+ and NO_3^-).

For the soil water-stable aggregate (WSA) analysis, undisturbed soil monolith samples (~200 g each sample) were collected and air dried,

slightly sieved in an 8.0-mm sieve, and retained on the 4.0-mm sieve. A 25-g aliquot of the retained aggregate fraction (air-dried) was gently wetted by a spray-bottle with water taken to wet-sieving for 15 min using a nest of sieve with 4.00, 2.00, 1.00, 0.50, 0.25, and 0.105-mm mesh sizes coupled to mechanical equipment adjusted to 31 vertical oscillations min^{-1} (Yoder, 1936). The fractions obtained (retained on each sieve) were dried in a forced-air oven at 42 °C for 72 h and weighed afterwards. Through the initial moisture content of each soil fraction and its respective weight, the values were adjusted to 0 g kg^{-1} of water (dry soil weight). These results were used to calculate the mean weight diameter (MWD) according to the methodologies proposed by Kemper and Chepil (2015). In addition, the undisturbed soil samples were placed to gradually saturate with water over 48 h. Then, all saturated samples were weighed and taken to Richard's pressure chamber on porous plates under –0.006 MPa tension (Klute, 1986) until they stabilized. Subsequently, the samples were weighed and dried in a forced-air oven at 105 °C for 60 h. The total porosity was calculated by the difference between the weights of the water-saturated and dried samples. Macroporosity was determined as the difference between the water content of the water-saturated samples and after being subjected to the Richard's pressure chamber at –0.006 MPa tension. Microporosity was calculated as the difference between the total porosity and macroporosity (Smith and Mullins, 1991).

2.3.2. Soil organic matter physicochemical fractionation

In the same soil samples used for chemical analysis, the total organic carbon (TOC), particulate organic carbon (POC) and mineral-associated organic carbon (MOC) were also determined. The TOC was measured by an elemental analyzer (LECO-TruSpec1 CHN, Leco Corp., St. Joseph, MI, USA). Physical fractionation of soil organic matter was performed according to Cambardella and Elliot (1994) method. A 20 g of each soil sample was homogenized in 80 mL of $(\text{NaPO}_3)_6$ solution at 5 g L^{-1} . During 16 h, the samples were horizontally shaken and then sieved through 0.053-mm mesh using deionized-water. The remaining material was dried at 40 °C in a forced-air oven and grounded with a porcelain mortar and pestle for homogenization. The POC (defined as C content in

the 0.053–2 mm soil fraction) were measured via dry combustion with an elemental analyzer. Mineral-associated organic carbon was calculated using the difference between total organic carbon and particulate organic carbon.

Humic substances were obtained by chemical fractionation of organic matter (Ciavatta et al., 1990), in which Supelite DAX-8 resin was used to separate non-humified substances from humic fractions. In 10 g of each soil sample, the humic substances were extracted with 100 mL of 0.1 mol NaOH plus 0.1 mol Na₄P₂O₇ solution and N₂ bubbling for 2 min. The samples were shaken for 2 h at 160 oscillations min⁻¹ followed by centrifugation for 25 min at 14,000 RPM. The suspension was filtered in a 0.45-mm Millipore filter and added the humin fraction. The remaining fraction (humin and minerals) were stored for subsequent C analysis. The humic acid (HA) fraction was obtained by adding 0.5 mol H₂SO₄ to the filtered solution until it reached pH ≤ 2.0, followed by centrifugation at 9000 RPM for 25 min; then, the precipitate was eluted in 20 mL of 0.3 mol NaOH and the C content was determined. The fulvic acid (FA) fraction was determined through the previously filtered supernatant into a plastic column containing Supelite DAX-8 resin. The retained fraction was eluted with 20 mL of 0.5 mol NaOH and taken to C determination. The organic carbon of humin (C-HU), humic acids (C-HA) and fulvic acids (C-FA) was quantified by dichromate oxidation and colorimetric determination of reduced chromium, following Heanes (1984), using sucrose (4.754 g L⁻¹) containing 2 mg organic C mL⁻¹. The organic carbon was measured by spectrophotometer at 600 nm.

2.3.3. Soil DNA extraction

Total soil DNA was extracted from 250 mg of each replicate soil sample using the PowerLyzer Soil DNA Isolation Kit (MOBIO laboratories, Inc.), according to manufacturer's protocol. A control DNA extraction (no soil) was carried out to check possible contamination from soil DNA extraction kit reagents. Soil DNA quantities and qualities were determined using an ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The extracted DNA was also visualized on 1% (w/w) sodium boric acid agarose gel electrophoresis. The purified DNA was stored at -80 °C until further analyses.

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

Amplicon library preparation and high-throughput sequencing were performed using the DNA extracted from each soil sample. Specific regions of the gene were chosen for the sequencing of bacteria and fungus. For bacteria, we used the variable V3-V4 region amplified with forward primer 515F (5'-GTGC CAGCMGCCGCGTAA-3') and the reverse primer 806R (5'-G GACTACHVGGGTWTCTAAT-3') with sizes of ~300–350 bp. For fungi, primers (5'-CTTGGTCATTAGAGGAAGTAA-3') as forward and ITS2 (5'-GCTGCGTTCCTCATCGATGC-3') as reverse were used to amplify the internal transcribed spacer region (ITS). For bacteria and fungi sequencing, primers contained multiplex tags for sample identification. The samples were sequenced on the Illumina MiSeq System (Illumina MiSeq PE250) at McGill University and Génome Québec Innovation Center (Montréal, Québec, Canada). The sequences were deposited in the European Nucleotide Archive (ENA; <https://www.ebi.ac.uk/ena>) under the accession number PRJEB40513. The bacterial 16S rRNA and fungal ITS sequences were processed, demultiplexed and quality control using DADA2 pipeline (Callahan et al., 2017) in R environment (Team, 2015) with the “consensus” method, to remove any remaining chimeric and low-quality sequences. The taxonomic identification (with 99% of similarity) was performed using SILVA database (v. 138) (Quast et al., 2013) for bacterial and unite (v. 8.2) (Köljal et al., 2013) for fungal profiles; the taxonomic matrices (from amplicon sequence variant – ASV) generated were used for further statistical analyses.

2.4. Statistical analyses

All statistical analyses were conducted in R Studio version 1.3.959 running R version 4.02 (Team, 2015) using different packages. Generalized Joint Attribute Modeling (GJAM) package (Clark et al., 2017) was used to estimate the effects of Ca-based soil amendments. From the model we extracted the regression coefficients for each treatment to identify shifts in the microbial community (bacteria and fungi) and soil factors in different treatments. Model diagnosis evaluated the Markov Chain Monte Carlo (MCMC) to check when the estimated coefficients reached a stable value (after 10,000 simulations). The regression coefficients obtained were visualized via clusters tree using WARD distance. Same coefficients were used on PCA plot analysis to explore communities' similarities between treatments plot to highlight shifts as induced by the different Ca-based soil amendments. Besides the intensity of the shifts in the relative abundance of the soil microbiome provided by the GJAM regression coefficients, the relevance of the different microbes for the soil community was evaluated in terms of the centered log-ratio (CLR) transformed abundance (Leite and Kuramae, 2020). CLR transformation informs the relevance of the different microbial groups (in our case both bacteria and fungi) as a proportion of the sample's average. We used this transformation to classify the soil microbes as originally high abundant or low abundant based on the log-fold differences in relation to the average, which in the CLR-transformed data corresponds to the zero value.

3. Results

3.1. Soil physicochemical parameters

The Ca-based soil amendments significantly changed ($P < 0.001$) all soil fertility parameters (Table 1; Table S3). The direct effect of L, alone or in combination with PG, increased pH by 45–50% and reduced Al³⁺ availability by ~90% compared with the control treatment. 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 NO₃⁻, NH₄⁺, Ca²⁺ and S-SO₄²⁻. The most prominent effects of the Ca-based soil amendments compared with the control were observed for Ca²⁺ availability, which increased by 4.6- and 6.2-fold when L and LPG were applied, respectively, and for Mg²⁺, which increased ~2-fold when L or LPG was applied. The content

Table 1

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

Soil parameters ^a		units	Control	PG	L	LPG
Soil fertility	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
	NO ₃ ⁻	mg kg ⁻¹	22.9 c	24.5 c	30.0 b	32.5 a
	NH ₄ ⁺	mg kg ⁻¹	15.2 c	16.9 c	23.6 b	30.0 a
	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
	MWD	mm	2.10 b	2.26 b	2.57 a	2.70 a
Soil physics	SBD	kg dm ⁻³	1.87 a	1.71 b	1.60 c	1.54 c
	mP	%	30.7 a	29.2 a	32.6 a	32.0 a
	MP	%	9.60 bc	8.07 c	10.6 b	13.8 a

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

^a Mean weight diameter of soil aggregates (MWD), soil bulk density (SBD), microporosity (mP) and macroporosity (MP).

of $S\text{-SO}_4^{2-}$ 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.

The Ca-based soil amendments also changed soil physical attributes (Table 1, Table S3), except microporosity (mP). Compared with the control, applying L (alone or with PG) increased the mean weight diameter (MWD) of soil aggregates by 22–28% and decreased the soil bulk density (SBD) by 14–18%. Additionally, the soil macroporosity (MP) was 44% higher compared with the control when LPG was applied, while L increased MP by ~11% and PG had no significant effect.

3.2. Labile and stable C fractions and aboveground dry matter production by maize and ruzigrass

The different lability fractions of soil organic carbon (SOC) changed significantly ($P < 0.001$) (Fig. 2; Table S3) after application of the Ca-based soil amendments four times over 17 years. Compared with control, LPG-amended soil presented the greatest increases in SOM physical fractions. Carbon present in particulate organic carbon (POC) increased by 35%, whereas in mineral-associated organic carbon (MOC) increased by 43%, and in total organic carbon (TOC) increased by 42% (Fig. 2A–C). Regarding to the SOM chemical fractions, humic acid (HA), fulvic acid (FA) and humin (HU) also changed significantly ($P < 0.001$) in response to Ca-based soil amendments (Fig. 2; Table S3). Similar increases in the C concentration from the HA and FA fractions were

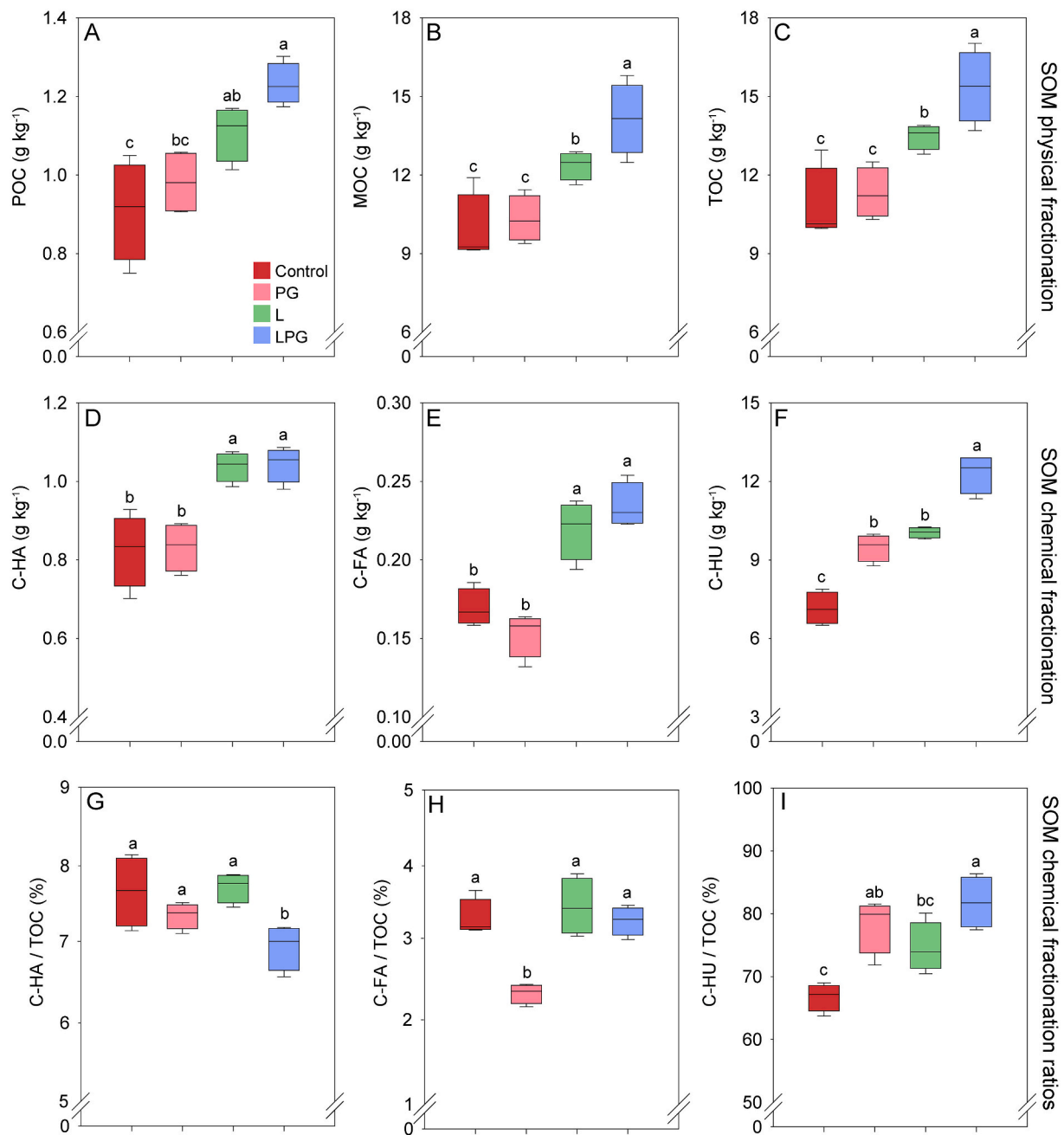


Fig. 2. Soil organic carbon concentration from physic fractions (A) particulate organic carbon (POC), (B) mineral-associated organic carbon (MOC), (C) total organic carbon (TOC), and from chemical fractions (D) humic acid (C-HA), (E) fulvic acids (C-FA), (F) humin (C-HU), C-HA/TOC ratio (G), C-FA/TOC ratio (H), and C-HU/TOC ratio (I) of the long-term soil with amendments (lime; PG, phosphogypsum; LPG, lime + phosphogypsum) applied in soil surface.

observed in L and LPG, whereas PG and the control treatments exhibited similar lower values of C concentration from these fractions (Fig. 2D and E). The C content from HU, was higher when PG was added to L (LPG), suggesting a synergetic effect of these two Ca-based soil amendments on SOM quality (Fig. 2F). Compared with the control, the C content from the HA increased by 27% in LPG and by 26% in L treatments. Carbon in FA fraction increased by 38% in LPG and 30% in L, whereas the C content in HU fraction, increased by 72% in LPG and 40% in L treatment. Interestingly, the ratios between SOM chemical fractions and TOC showed that the share of the HA fraction in TOC was lower in LPG treatment than in other treatments (Fig. 2G). The FA fraction showed lower values in PG-amended soil, while in the other treatments, the values were similar (Fig. 2H). On the other hand, the largest share of the HU fraction occurred in LPG treatment, followed by PG and L (Fig. 2I). Control treatment presented the lowest contribution of the HU fraction in TOC.

Aboveground dry matter (ADM) production of maize and ruzigrass

increased in amended soils (Fig. S1). Maize ADM increased as follows: LPG > L > PG > control (Fig. S1A); ruzigrass produced the same amount of ADM when L and LPG was applied, both higher than that obtained in control and PG treatments, which did not differ from each other (Fig. S1B).

3.3. Impact of Ca-based soil amendments on the soil microbiome

Sensitivity analysis was applied to determine the relative impacts of each treatment on different groups of dependent variables: bacterial and fungal communities, SOM physical fractionation (SOM-PF), SOM chemical fractionation (SOM-CF), soil fertility and soil physical characteristics (Fig. 3A). In general, the treatments had the greatest impact on soil fertility, followed by the microbiome (bacteria and fungi), SOM-CF, soil physical characteristics, and SOM-PF. In addition, among all groups, the sensitivity values were highest in the control and LPG treatments.

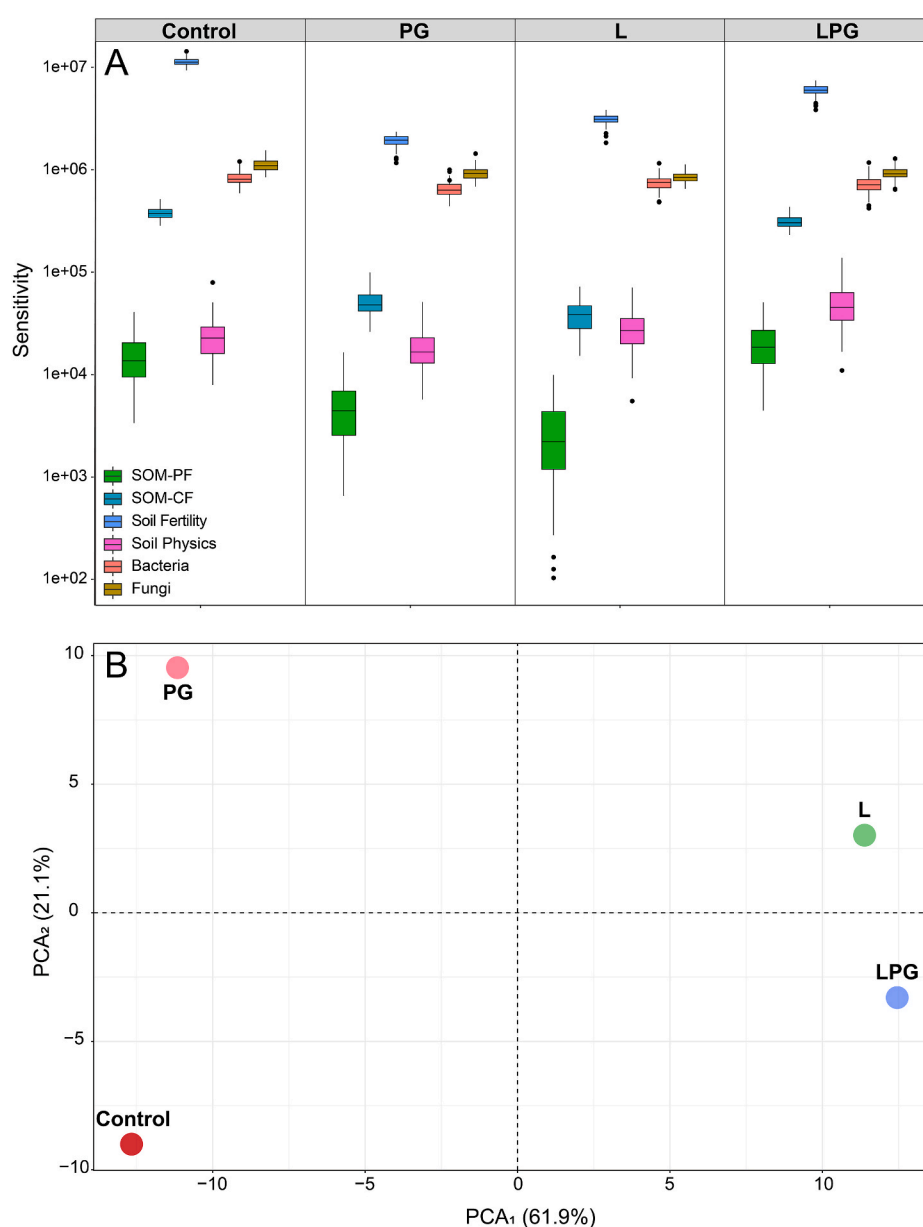


Fig. 3. Sensitivity analysis (A), and PCA of regression coefficients (B) of bacterial and fungal amplicon sequence variant (ASVs), SOM physical (SOM-PF) and chemical (SOM-CF) fractions, soil fertility, and soil physics to the environment, according to the soil amendments (B). Control (C), phosphogypsum (PG), lime (L), lime + phosphogypsum (LPG).

We also constructed a principal coordinate analysis (PCA) integrating the regression coefficients from all of the variable groups to identify similarities between the treatments in shifts in soil physicochemical parameters and the microbiome (Fig. 3B). Our analysis indicated a clear segregation of the treatments in the first two axes (83% of the total variation). L- and LPG-amended soils clustered together, whereas PG-amended and control soils were positioned far from each other and from the center of the PCA. In terms of distance from the control, we observed higher proximity for PG than for L and LPG. The control and LPG showed similar levels of sensitivity (Fig. 3A), but L and

LPG exhibited high similarity in the PCA analysis (Fig. 3B), most likely because they produced similar effects on the groups of variables corresponding to SOM fractions and soil physicochemical attributes (Fig. 2 and Table 1).

For soil fertility, the differences in regression coefficients were greatest between the control and LPG treatments for Ca^{2+} and Mg^{2+} (Fig. 4). In PG-amended soil, Ca^{2+} increased, but Mg^{2+} decreased; in L-amended soil, both Ca^{2+} and Mg^{2+} increased. Similarly, we observed contrasting shifts between the control and LPG treatments in P, NH_4^+ , pH, S-SO_4^{2-} , NO_3^- and K^+ . Interestingly, the opposite pattern was



Fig. 4. Regression coefficients of only the significant responses of soil factors (physical and chemical fractions, soil fertility, and soil physics), bacterial and fungal communities according to the soil amendments. Coefficients are significant when their 95% confidence interval do not overlap with 0. Unclassified (un).

observed for micronutrients and Al^{3+} . Between the control and LPG treatments, the greatest shifts occurred in Al^{3+} , Fe, Mn, Cu, and Zn.

Within the SOM fractions, the changes in the chemical fractions (HA, FA and HU) were greater than those in the physical fractions (POC, MOC and TOC). Here again, contrasting effects of the control and LPG treatments were observed for HU, FA, HA, POC, MOC, and TOC. For soil physical characteristics, the regression coefficients for the MP and MWD of soil aggregates increased in L- and, most notably, LPG-amended soils. In these same treatments, SBD decreased. Between the control and LPG treatments, the following shifts were obtained: MWD, SBD, MP and mP.

The relative abundances of the microbes also responded to long-term soil amendment. Overall, the treatments influenced the relative abundances of 55 different genera of bacteria (Table S4 and S5). More than half of these bacterial genera (61.8%) belonged to the phyla Proteobacteria (19 genera; 34.5%) and Actinobacteria (15 genera; 27.3%); intermediate responses of Acidobacteriota (6 genera; 10.9%), Chloroflexi (5 genera; 9.1%), Planctomycetota (4 genera; 7.3%), and Firmicutes (3 genera; 5.5%) were observed. Finally, in each of the phyla Armatimonadota, Gemmatimonadota and Verrucomicrobiota, only one bacterial genus (1.8%) was affected by the treatments. The relative abundances of 25 bacterial genera increased and 6 genera decreased in the control. Significant changes were observed in 21 genera in PG (20 positive shifts and 1 negative shift), 19 in L (14 positive shifts and 5 negative shifts), and 14 in LPG (10 positive shifts and 4 negative shifts). Apart from the phylum Proteobacteria, which accounted for the largest number of affected genera considering all treatments (19 genera), the same number of genera were affected in the control and LPG treatments (7 genera, 36.8%). Among the remaining phyla, the largest number of genera affected by the control treatment belonged to the phylum Actinobacteria (10 genera; 66.7%), followed by the phyla Acidobacteriota (4 genera, 66.7%), Chloroflexi (4 genera, 80%) and Planctomycetota (3 genera; 75%). In LPG-amended soil, the same dominant phyla were affected (mostly positively), but the numbers of represented genera were smaller (Actinobacteria = 4 genera, 26.7%; Acidobacteriota = 1 genus, 16.7%; Chloroflexi = 1 genus, 20%; Planctomycetota = 1 genus, 25%). The different Ca-based soil amendments also had unique impacts on the soil microbiome. Two phyla, Firmicutes and Gemmatimonadota, were significantly affected only by PG and the control. The control treatment positively affected one genus of Firmicutes, whereas PG positively affected the relative abundances of 3 different genera of Firmicutes. Within Gemmatimonadota, only one bacterial genus showed significant changes as a result of the two treatments. The phylum Armatimonadota was detected only in the control treatment (represented by one genus). Likewise, the phylum Verrucomicrobiota was exclusive to the PG treatment.

Compared with bacteria, more fungal representatives were affected by the treatments. Overall, the treatments influenced the relative abundances of 57 genera and 75 species of fungi (Tables S4 and S6). Almost all of these fungal species (96%) belonged to the phyla Ascomycota (63 species; 84%) and Basidiomycota (9 species; 12%). The phyla Mortierellomycota (2 species; 2.7%) and Chytridiomycota (1 species; 1.3%) exhibited the smallest changes in abundance in our study. Interestingly, the LPG treatment had the strongest impact on the fungal community. In LPG-amended soil, the abundances of 24 species of fungi increased, and the relative abundances of 8 other fungal species decreased. The control treatment had the next largest impact, with changes in 28 fungal species (18 positive shifts and 10 negative shifts), followed by the PG and L treatments, which exhibited changes in abundance of 24 species each (PG = 13 positive shifts and 11 negative shifts; L = 19 positive shifts and 5 negative shifts). LPG amendment mainly affected the phyla Ascomycota (26 species) and Mortierellomycota (2 species). The phylum Chytridiomycota was found only in the control treatment.

Based on the regression coefficients, the cluster analysis highlighted groups of variables with similar responses to the treatments, thus allowing us to identify which microbes followed the shifts in soil

physicochemical properties (Fig. 5). First, we observed that the Ca-based soil amendments strongly affected most soil fertility variables and HU, clearly segregating them into distinct clusters (C1a-c), in agreement with the sensitivity analysis (Fig. 3A). In addition, we found clustering of SOM physical fractions (POC, MOC and TOC) with many microbes (especially fungi) in the C5 cluster and clustering of SOM chemical fractions (FA and HA), MWD of soil aggregates and K^+ in the C3 cluster, suggesting positive associations between the microbes in these clusters and these soil properties. Although there was no clear segregation between bacteria and fungi, the C2 cluster showed a greater proportion of bacteria (59%) than fungi (39%), both of which were associated with SBD. In addition, we observed a higher proportion of fungi than bacteria in the C3 cluster (bacteria = 37%; fungi = 48%) associated with FA, HA, MWD of aggregates, and K^+ ; the C4 cluster (bacteria = 37%; fungi = 61%) associated with MP; the C5 cluster (bacteria = 19%; fungi = 71%) associated with MOC, POC, TOC, and mP; and the C6 cluster (bacteria = 30%; fungi = 70%) without associations with soil factors.

In addition to examining the strengths of shifts in relative abundance in the microbial community, our analysis identified the relevance of the microbes affected by each soil amendment compared with the original soil community (control treatment) (Fig. 6). Overall, the treatments influenced the relative abundances of 33 fungal species (belonging to 29 genera) and 15 bacterial genera. In PG-amended soil, the relative abundances of 6 microbes increased, among which the abundances of four were originally low in the control treatment (2 bacterial and 2 fungal). PG also reduced the abundances of 10 microbes, all fungal, of which 4 were originally low in abundance and 6 were abundant in the control treatment. In L-amended soil, the abundances of 11 microbes increased (4 bacterial genera and 5 fungal species with low abundance originally and 2 abundant fungal species), whereas 4 other bacterial genera and 6 fungal species that were originally abundant in the control decreased. Finally, LPG amendment increased the abundances of 13 microbes, represented by 2 bacterial genera and 9 fungal species originally with low abundance and 2 abundant fungal species, and decreased the relative abundances of 11 microbes, represented by 1 bacterial genus and 1 fungal species originally with low abundance and 3 bacterial genera and 6 fungal species that were originally abundant in the control treatment. Overall, the different Ca-based soil amendments markedly altered the patterns and relevance of microbial community members in bulk soil. We observed a trend towards reducing the relevance of originally abundant microorganisms and increasing the relevance of low-abundance ones. For example, the relevance of *Acidothermus* and *Aquasphaera* increased in PG, but the relevance of *Acidothermus* decreased over time in the treatments that received L (L alone and LPG). In addition, PG increased the relevance of *Arthrobotrys dactyloides* and *Chrysosporium fluviale*, species with low abundance in the control. Notably, *Mortierella*, which was originally low in abundance, gained relevance in LPG but lost relevance in PG (negative regression coefficient). L- and LPG-amended soils showed similarities in the reduction of the relative abundances of *Acidothermus* and *Chloroflexi* 1921-2 bacteria and the fungal species *Saitozyma podzolica*, *Humicola olivacea*, and *Fusarium acutatum*. Interestingly, LPG reduced the relevance of *Nitrobacter* (originally abundant), *Bradyrhizobium* (bacterial genus originally low in abundance) and *Hebeloma crustuliniforme* (fungus originally low in abundance) but increased the relevance of *MND1* and *Pedomicrobium*, both of which were low-abundance bacteria in the control treatment.

4. Discussion

4.1. Ca-based soil amendments change soil physicochemical attributes and shape soil chemical and physical carbon fractions

Seventeen years of soil management with amendments in a no-till intercrop system changed the soil physicochemical parameters as well as the amount and quality of SOC. Amendment with L (combined or not with PG) provided the greatest neutralization of soil acidic components

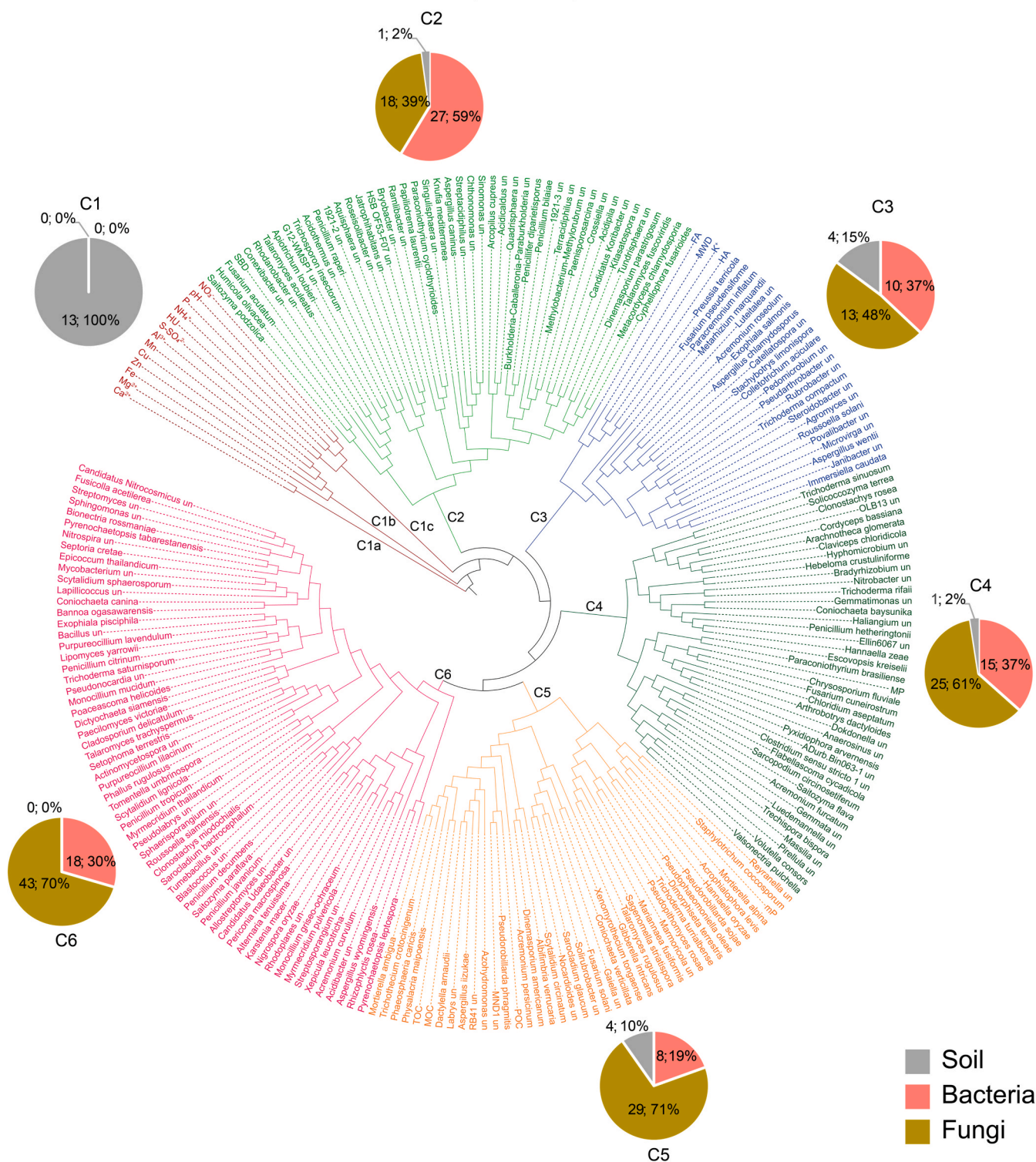


Fig. 5. Similarity dendrogram between regression coefficients from soil factors, bacterial and fungal communities to the soil amendments. Colors from circular plots represents the variable groups. Particulate organic carbon (POC), mineral- associated organic carbon (MOC), total organic carbon (TOC), humic acid (HA), fulvic acid (FA), humin (HU), mean weight diameter (MWD), soil bulk density (SBD), microporosity (mP) and macroporosity (MP), unclassified (un). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(pH and Al^{3+}) and increased the availability of macronutrients (NO_3^- , NH_4^+ , P, K^+ , Ca^{2+} , Mg^{2+} and S-SO_4^{2-}), whereas LPG amendment efficiently increased N (NO_3^- and NH_4^+), Ca^{2+} and S-SO_4^{2-} availability.

Applying PG alone resulted in a small change in soil physicochemical properties compared with the control treatment. Although PG is not

considered an acid-neutralizing compound (Zoca and Penn, 2017; Bossolani et al., 2018), combining L and PG synergistically exploits L's ability to correct soil acidity and PG's ability to promote Al^{3+} neutralization and increase Ca^{2+} input in the soil. Such effects are particularly relevant under the conditions of low Ca^{2+} availability found in highly

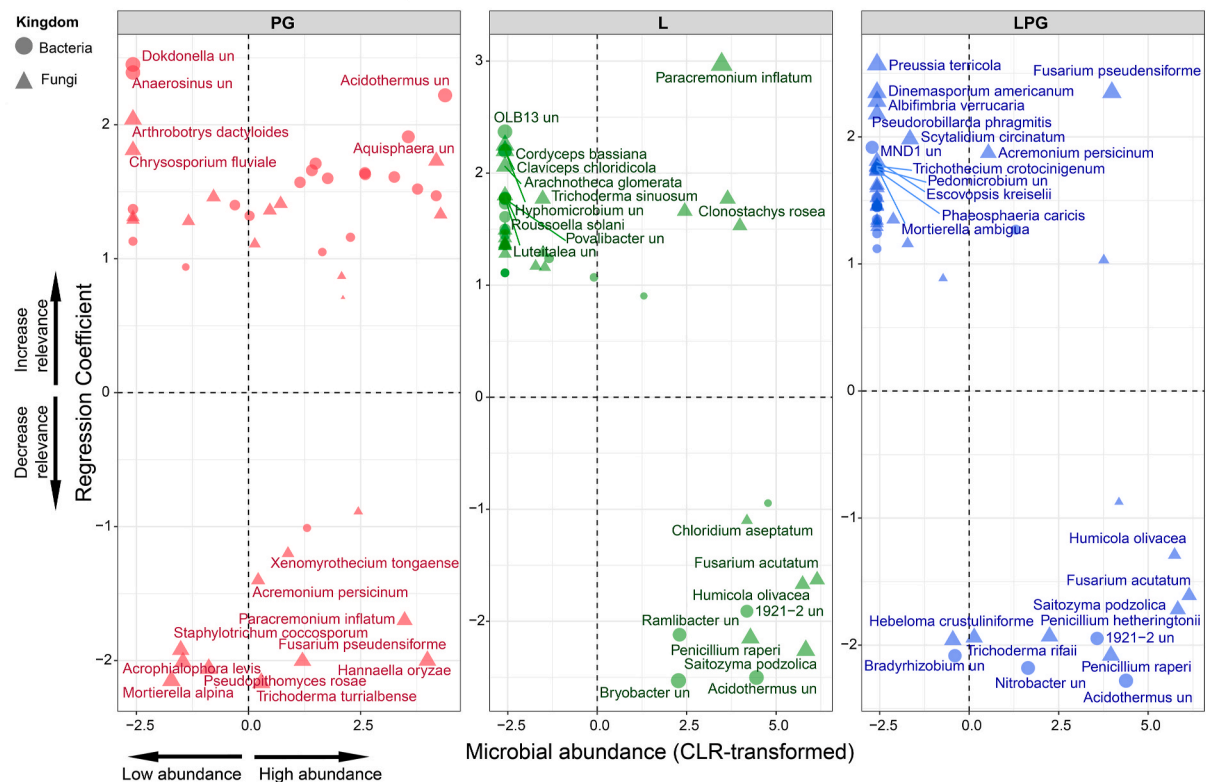


Fig. 6. Relationship between the regression coefficient and the abundance (CLR transformed data) of top 30 most negative and most negative shifts from bacterial and fungal taxa in relation to the control treatment. Unclassified (un).

weathered tropical soils (Carmeis Filho et al., 2017b; Crusciol et al., 2019; Bossolani et al., 2020a). High L inputs to the soil surface can drastically reduce the availability of soil micronutrients (Fe, Mn, Cu and Zn) by increasing pH (Pegoraro et al., 2006; Dhaliwal et al., 2019), as observed in our study, and thus PG is an efficient amendment that provides Ca^{2+} to the soil without further changing the soil pH and reducing crop yield (Costa and Crusciol, 2016; Bossolani et al., 2020a).

The primary effects of L on soil fertility trigger cascading effects on soil quality and crop responses. We showed increases of grain and biomass productivity (in the form of aboveground residues and below-ground root biomass) in the current study as well as in our previously studies, in the same experimental area (Carmeis Filho et al., 2017b; Bossolani et al., 2020a) by L alone or in combination with PG. These improvements are positively correlated with increases in SOM. We observed increases in SOM fractions in soils amended with L or, most notably, LPG. Biomass-C input by aboveground dry matter production and high Ca^{2+} availability in amended soils are important for increasing C concentrations in the soil and also indicate a greater capacity for atmospheric C sequestration in fertile systems managed with Ca-based soil amendments (Inagaki et al., 2016; Carmeis Filho et al., 2017a).

Higher biomass production from crop residues in amended soils is the main source of C contributing to increased SOC concentrations (Inagaki et al., 2016; Carmeis Filho et al., 2017b), as observed in our results. Additionally, Ca^{2+} availability is an important ionic bridge between clay particles and SOM, resulting in greater soil aggregation and consequent C protection (Briedis et al., 2012; Aye et al., 2016). Thus, Ca^{2+} availability has high potential as an indicator of SOC accumulation in NTS (Briedis et al., 2012). Our results demonstrate synergism between L and PG, two Ca-based soil amendments that act via distinct mechanisms to increase nutrient availability without abruptly increasing pH.

The long-term Ca-based soil amendments also affected the physical characteristics of the soil, with the formation of larger stable aggregates (soil aggregate MWD), higher MP, lower SBD and, consequently,

improved soil particle structure. The higher Ca^{2+} availability in the L-amended soils (with or without PG) may have played an important role in the formation of stable soil aggregates, as this element is essential for root growth and consequent formation of colloids by the mechanical action of roots and the release of root exudates (Carmeis Filho et al., 2018). Additionally, Ca^{2+} , Fe, and Al^{3+} have been described as “persistent soil aggregating agents” that are responsible for binding between SOM and clay particles and the formation of organo-mineral complexes (Tisdall and Oades, 1982; Fornara et al., 2011). As shown here and in our previous work (Carmeis Filho et al., 2017b), these organo-mineral complexes protect SOM-derived C against decomposition processes over time.

Lime has been reported to increase microbial activity and the mineralization of labile organic matter (Inagaki et al., 2016), but these reports were based on studies carried out in agricultural systems managed with soil disturbance operations, such as plowing and harrowing (Aye et al., 2016; Joris et al., 2016), and not the application of L on the surface of tropical soils in long-term no-till crop rotation systems. In addition, studies associating L with higher soil respiration rates and thus consumption of SOM pools by microorganisms (Holland et al., 2018) considered only short-term periods. Under long-term effects of Ca-based soil amendments in no-till crop systems, the return of biomass-C over time is high (Carmeis Filho et al., 2017b), balancing the system with positive C turnover. During the 17-year period of the present study, only 4 applications of PG, L or LPG were carried out based on the results of soil chemical analyses performed annually as described in the Material and Methods section.

Plant residues and organic compounds in soil are transformed by microbes (Waksman and Gerretsen, 1931; Gregorich et al., 1996; Bending et al., 2002; Hilscher et al., 2009; Kuramae et al., 2013; Suleiman et al., 2019), which together with soil physicochemical quality, strongly influence the stabilization of SOM fractions (Castellano et al., 2015). The fractions of labile organic carbon, i.e., POC, and stable

organic carbon, i.e., MOC, were highest in LPG-amended soil, whereas the SOM chemical fractions HA, FA and HU did not differ between soil amended with L alone and with LPG. Our results suggest that the enhancement of SOM mineralization by L and LPG favors the decomposition of non-recalcitrant compounds, the synthesis of more stable molecules and improved SOM quality (Stevenson, 1994). By contrast, the content of easily microbial degradable SOM compounds (POC) in LPG-amended soil can further act as major available nutrients and C sources for microbial metabolic activity. Interestingly, PG increases the share of the HU fraction in TOC, however, it reduces FA. LPG-amended soil also increased the HU fraction. In both treatments, there is a considerable addition of Ca^{2+} , which favor in the process of stabilizing SOM and reducing the biochemical evolution of young organic compounds (Tisdall and Oades, 1982; Fornara et al., 2011).

4.2. Linking the soil microbiome with the legacy of Ca-based soil amendments for soil quality

The vast majority of studies of agricultural management practices are limited to examining single factors such as soil tillage (Fuentes et al., 2006; Babin et al., 2019), fertilizers (Dai et al., 2018; Babin et al., 2019; Tian et al., 2019), amendments (Liu et al., 2018b; Guo et al., 2019; Pang et al., 2019) or the behavior of select groups of bacteria or fungi. By contrast, our study integrated generalized joint attribute modeling (GJAM) and data on SOM quality (SOM physical and chemical fractions), soil chemical attributes (soil fertility), soil physical attributes (soil physics) and the soil microbiome (bacterial and fungal communities). This study is the first to investigate how the surface application of Ca-based soil amendments (acidity corrective and/or soil conditioner) in a tropical no-till intercrop system over 17 years influences soil physicochemical properties and bacterial and fungal communities. By going beyond isolated effects of the Ca-based soil amendments on each of the soil attributes and the microbiome, our joint data analysis provides strong evidence on how Ca-based soil amendments shift soil factors that modulate the microbiome, which in turn plays a crucial role in shaping the chemical fractions of SOM, soil physical characteristics and soil physical fractions to ultimately increase soil quality.

The sensitivity analysis revealed how each group of variables was affected by the treatments. The amendments strongly affected soil parameters, especially soil fertility, and LPG had the greatest impact, consistent with the results of the soil physicochemical analysis and the regression coefficients in GJAM. Our control treatment represented 17 years of standard agriculture with fertilizers and biomass-C inputs in all cropping seasons but without addition of L and PG amendments. This agricultural model has its own effects on soil chemical parameters and leads to strong acidification and nutrient displacement (e.g., N, P, Ca^{2+} and Mg^{2+}) in the weathered soil of this tropical region (Fageria and Baligar, 2008).

LPG amendment strongly impacted the soil C concentration and nutrient availability over the 17 years of the experiment. Interestingly, the sensitivity analysis showed that the responses of the bacterial and fungal communities were similar among the treatments. After soil fertility, microbes were the group of variables most strongly affected by the amendments. This result demonstrates that long-term agriculture without management measures improving soil quality (i.e., without Ca-based soil amendments) impacts the soil microbiome, selecting specific microorganisms and consequently impacting the soil ecosystem services delivered by microbes. In addition, PCA confirmed that the changes in the soil microbiome over the 17-year period were greatest in the control and LPG treatments and that the treatments that received L (as L or LPG) were most similar in terms of changes in soil physicochemical parameters and the soil microbiome. Despite the similar levels of impact (sensitivity) on the microbiome among the treatments, distinct groups of microorganisms were selected in each treatment, demonstrating the capacity of each soil amendment to modulate and rearrange distinct soil microbial communities compared to the control.

The low-fertility soils in the control and PG treatments favored the largest number of members within the phyla Actinobacteria (i.e., *Acidothermus*, *Crossiella* and *Jatrophihabitans*), Acidobacteriota (i.e., *Terracidiphilus* and *Acidipila*) and Chloroflexi. These bacterial genera are known to be oligotrophic, with high resistance to adverse conditions (Rodrigues et al., 2013), such as acidic environments with low nutrient availability (Kielak et al., 2016; Kuramae and Costa, 2019) and the presence of heavy metals (Hemmat-Jou et al., 2018; Wang et al., 2018). These results are in accordance with the analyses of the soil in the control and PG treatments, that had the lowest pH values and nutrient availability (e.g., N, P, Ca^{2+} and Mg^{2+}) and highest values of Al^{3+} , Fe, Mn, Cu and Zn.

We also observed positive shifts in the relative abundances of several genera of the phylum Proteobacteria in all treatments, although the specific genera differed in each treatment. In the control, these genera included *Acidicaldus* and *Rhodanobacter*, which are characteristic of acidic and oligotrophic environments and play a role in the cycling of metals such as Fe (Hedrich et al., 2011), and other genera related to nitrification (i.e., *Ellin6067*) (Ye et al., 2016). Interestingly, the genus *Methylobacterium-Methylobacterium*, which was previously described as a plant-associated microorganism with beneficial roles in plant growth via changes in auxin and cytokinin balance (Abanda-Nkwatt et al., 2006), was recently associated with parasitic potential rather than benefits in soybean plants, as it competes for nitrogen compounds (allantoin and allantoinic acid) from biological nitrogen fixation (BNF) as a N source (Minami et al., 2016). At our study site, maize is intercropped with ruzigrass and soybean in crop rotation, and therefore the increase of this microorganism may have negative effects on soybean performance; further studies are needed to confirm this hypothesis.

In LPG-amended soil, interesting positive shifts were observed in the relative abundances of *Microvirga*, *Labrys* and *Pedomicrobium*, organisms that are notably copiotrophic, while negative shifts were observed in *Bradyrhizobium* and *Nitrobacter*, commonly denoted as oligotrophic (Msaddak et al., 2017; Yao et al., 2017; Moretti et al., 2018). These changes can be partially explained by the soil trophic level (Holland et al., 2018). Agricultural management with Ca-based soil amendments increases the availability of C and soil nutrients in three main ways: i) increased soil pH results in an increase in negatively loaded bridges in colloids, thereby preventing nutrient displacement, ii) direct input of nutrients from the Ca-based soil amendments; and iii) increased biomass-C yield from crop residues and nutrient cycling over time (Carneiro Filho et al., 2017b). Thus, Ca-based soil amendments reduce the selective pressure of nutrient availability on microorganisms (Dai et al., 2018; Leff et al., 2015), as observed for *Bradyrhizobium* and *Nitrobacter* in LPG-amended soil. The decomposition and recycling of organic matter is largely carried out by soil microorganisms capable of converting organic components into nutrients available to plants (Steinberger and Shore, 2009). In our previous work at the same site, we observed an increase in the abundance of the *nifH* gene in soils amended with both L and PG (Bossolani et al., 2020a), and therefore greater relevance of *Bradyrhizobium* in this treatment was expected. Instead, reduced relevance of *Bradyrhizobium* was observed in LPG-amended soil, suggesting that the *nifH* gene found previously may be derived from microorganisms other than *Bradyrhizobium*. Future studies should sequence specific regions of DNA related to biological nitrogen fixation (BNF) to further elaborate this finding. In addition, fertile soils increase plants' capacity to compete with microorganisms for soil resources, thereby reducing available substrates for microbial growth and partially explaining the reduction of the role of *Nitrobacter* in LPG-amended soil. Another explanation is that nitrification is lower in intercropping systems with grain crops and tropical forage grasses (Coskun et al., 2017; Bossolani et al., 2020a) like the one in the present study. Subbarao et al. (2015) suggested that forage grasses release a compound known as braquilactone that is capable of suppressing nitrification.

Interestingly, the changes in soil fertility due to LPG amendment fostered increases in a wide range of saprophytic fungi (soil, wood, and

plant saprotrophs) that decompose organic matter (i.e., *Preussia terricola*, *Albifimbria verrucaria*, *Phaeosphaeria caricis*, *Escovopsis kreiselii* and *Scytalidium circinatum*). The activity of microorganisms on the soil surface is high due to the deposition of crop residues (Córdova et al., 2018). Our results showed that the long-term application of LPG on weathered tropical soils promoted fungal groups that increase C mineralization potential, thereby favoring the decomposition of non-recalcitrant compounds into stable C molecules and improving SOM quality. Contrary to many studies that have reported reduced fungal activity in soils with higher pH (Bothe, 2015; Holland et al., 2018), we observed an increase in the participation of these microorganisms in soils amended with L and, in particular, LPG. The positive relationship between the abundance of these fungi and the amendments might be explained by the high SOC in these treatments obtained by the long-term no-till cropping system. Furthermore, remarkably, the increase in fungi was also related to the formation of larger aggregates (higher MWD), which made LPG-amended soil less dense (lower SBD values) and more porous (higher MP). Additionally, fungi had a greater role in aggregate formation than bacteria, corroborating the results of Bossuyt et al. (2001) for a different soil type and conditions. Fungi can produce metabolites (e.g., polysaccharides, glomalin and lipids) that increase aggregate formation (Mukerji et al., 2006).

The locations of bacteria and fungi within soil pores are critical determinants of the survival and activity of these microorganisms; fungi have a preference for growing in macroaggregates, while bacteria prefer small pores (Ding et al., 2015). Our results showed greater shifts in bacteria in the control and PG-amended soils but greater shifts in fungi in L- and LPG-amended soils. Accordingly, the control and PG-amended soils had the highest SBD, whereas the L- and LPG-amended soils had the highest MP.

Finally, we observed changes in the relevance of specific microorganisms in each soil amendment compared with the control. Overall, the Ca-based soil amendments reduced the relevance of abundant microorganisms (bacteria and fungi) and increased the relevance of low-abundance microorganisms. Interestingly, L and, in particular, LPG amendment increased the relevance of several microorganisms (mostly fungi) that were not abundant in the control treatment, suggesting an increase in the ecological role of low-abundance microorganisms. As expected, the nitrogen fixer *Bradyrhizobium* and the ectomycorrhizal fungus *Hebeloma crustuliniforme*, both of which were naturally low in abundance in the control, became even less relevant in LPG-amended soil, probably due to the greater availability of nutrients in this treatment. Similarly, *Nitrobacter*, an abundant genus in the control, lost relevance in LPG-amended soil, suggesting reduced nitrification as observed previously by Bossolani et al. (2020a). With regard to fungi, increasing the relevance of less-abundant species can benefit the agricultural system, such as *Arthrobotrys dactyloides*, a nematode-trapping fungus (Strom et al., 2019, 2020), in PG-amended soil and *Mortierella ambigua*, an opportunistic microbe of arable soils with N-rich organic residues in the early stages of decomposition (De Tender et al., 2019) that can parasitize nematode eggs (Lupatini et al., 2019), in LPG-amended soil. Interestingly, the bacteria genera *Pedomicrobium* and *Proteobacteria MND1*, both of which were low in abundance in the control but had greater relevance in the LPG treatment, have been reported to use hydrocarbon contaminants as a C source to oxidize and retain Fe and Mn in soil environments (Orcutt et al., 2011). In addition, Yao et al. (2017) found strong correlations of *Pedomicrobium* with pH, TOC and N in long-term biochar-amended soils. The enrichment of low-abundance microorganisms by Ca-based soil amendments can be beneficial for agricultural systems by promoting beneficial bacteria and fungi that increase soil functionality (De Tender et al., 2019; Lupatini et al., 2019; Suleiman et al., 2019). Most previous studies have focused on dominant taxa and minimized the importance of low-abundance taxa. However, recent studies have reported that these low-abundance taxa are major determinants of soil multifunctionality (Jiao et al., 2019; Wagg et al., 2019; Zhu et al., 2020). Low-abundance bacteria and

fungi are now recognized as drivers of key functions in agroecosystems that serve as an adverse pool to enhance both resilience and resistance under environmental disturbances (Jiao et al., 2019). Under favorable conditions, these microorganisms can eventually play important roles and provide unique functional traits (Jiao et al., 2019; Wagg et al., 2019). Thus, low-abundance microbial taxa could be a genetic reservoir that is activated under appropriate conditions for growth.

5. Conclusions

Our findings suggest that, in a long-term no-till intercropping system, amendments that neutralize acidity of the soil play a crucial role in increasing C concentrations (labile and stable fractions) and soil physicochemical attributes, especially considering the synergism between L and PG (LPG amendment). Our model showed that LPG amendment increased soil fertility by promoting changes in the soil microbiome to improve SOM fractions and soil physical characteristics. The effects of L and LPG on the fungal community resulted in similar changes in SOM fractions (physical and chemical), suggesting important roles of these fungi in the transformation of SOM compounds. Finally, we observed that over 17 years, the Ca-based soil amendments (especially LPG) increased the relevance of low-abundance microorganisms and reduced the relevance of other microorganisms that were originally abundant compared with the control treatment, suggesting an increased role of these low-abundance microorganisms in ecosystem functioning in these soils.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2021.108188>.

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