European Journal of Agronomy 149 (2023) 126907

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Long term co-application of lime and phosphogypsum increases 15 N recovery and reduces 15 N losses by modulating soil nutrient availability, crop growth and N cycle genes

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ARTICLE INFO

Keywords:
Soil amendments
Crop rotation
^{15}N-labeled
Microbial functional genes
Nitrification
Denitrification

ABSTRACT

In no-tillage rotation systems, the recovery of nitrogen (N) fertilizer in the soil–plant system is affected by soil fertility and biological changes caused by the surface application of lime (L) and phosphogypsum (PG). Here we assessed the effect of surface-applied L and/or PG on the fate of ^{15}N-labeled fertilizer, soil chemical properties, microbial gene copy number (16 S rRNA of prokaryotes and genes of N cycle) and grain yield of maize (*Zea mays* L. intercropped with ruzigrass) in rotation with soybean [*Glycine max* (L.) Merrill] during two growing seasons. We found that applying L improved soil fertility, particularly when combined with PG (LPG treatment), resulting in higher grain yield. Moreover, compared with the control, the recovery of ^{15}N-labeled ammonium sulfate [$(^{15}NH_4)_2SO_4$] increased in maize and ruzigrass dry matter but decreased in soybean grown on the residue of the first growing season in two treatments (L and LPG). The losses of ^{15}N-labeled fertilizer were highest in the control and PG treatments. A large amount of ^{15}N-labeled fertilizer was found in the deep layers of PG-amended soil, indicating leaching of fertilizer-derived ^{15}N. Conversely, the analysis of soil microbial N cycle genes revealed that the abundances of denitrifiers were highest in the control (no correctives applied), suggesting that the N fertilizer remaining in the soil increased denitrification rates. Surface application of a combination of L and PG is clearly a feasible strategy for increasing soil fertility, ^{15}N recovery from fertilizer, and grain yield while reducing environmental pollution associated with nitrification and denitrification.

1. Introduction

Soil acidity limits tropical agricultural production (Holland et al., 2018). This acidification is the result of natural processes (*e.g.*, weathering of source material and nutrient cycling) and anthropogenic activities, including inappropriate agricultural management practices (Fageria and Nascente, 2014). Low soil pH causes a chain of negative effects on soil biogeochemistry (Holland et al., 2018). These detrimental effects can impair root deepening, nutrient and water uptake, and consequent lower yields (Bossolani et al., 2021a). For hundreds of years, dolomitic lime ($CaCO_3$ + $MgCO_3$) has been applied to ameliorate soil acidity (Li et al., 2019). Lime application increases soil pH and base saturation, thereby increasing soil fertility and complexing exchangeable aluminum (Al^{3+}), which is toxic to plants (Crusciol et al., 2019).

The common practice is lime application as incorporated material by plowing and harrowing in the conventional tillage (Santos et al., 2018). However, in systems under no-tillage, no incorporation is the main practice when lime is applied on the soil surface. Action of this product becomes restrict to the small soil layer around the lime particles themselves (Soratto and Crusciol, 2008), limiting its effectiveness in

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https://doi.org/10.1016/j.eja.2023.126907
Received 10 March 2023; Received in revised form 26 June 2023; Accepted 29 June 2023
Available online 5 July 2023

correcting acidity to the topsoil (Bossolani et al., 2022b; Costa et al., 2018). Subsoil effects of liming can occur but depend on the soil texture, lime rate, application over time, and no-till system management (Bossolani et al., 2020b; Carmeis Filho et al., 2017a; Soratto and Crusciol, 2008). An emerging strategy to increase the efficiency of surface liming is to combine lime with phosphogypsum (Bossolani et al., 2022a,b, 2021a,b,c; Soratto and Crusciol, 2008). Phosphogypsum from phosphoric acid industry is a primarily composed residue of calcium sulfate ($CaSO_4{\cdot}2\ H_2O$) and characterized by approximately 200 times more soluble than lime (Zoca and Penn, 2017). Calcium (Ca^{2+}) and sulfate (SO_4^{2-}–S) are products released by phosphogypsum dissociation in the soil (Caires and Guimarães, 2018). Sulfate binds Al^{3+}, making it unavailable for plant uptake and favoring root deepening. The decomposition of these deeper roots creates biopores that increase lime mobility throughout the soil layers (Tiritan et al., 2016). Thus, phosphogypsum complements lime with both important actions in the soil profile (Bossolani et al., 2021a). Additionally to improving soil chemical properties, the combination of lime and phosphogypsum strongly benefits physical an biological proprieties such as soil structure (Carmeis Filho et al., 2017b), composition and function of microbial communities (Bossolani et al., 2021b, 2020a). Lime and phosphogypsum can also alter the efficiency of N fertilization since soil amendments affect microbially mediated N reactions and N dynamics in the interactions of soil–plant system (Shi et al., 2017). The rise in soil pH following liming also has cascading reaches reaction of N cycle (J. Liu et al., 2018; X.Y. Liu et al., 2018).

The reaction of ammonium-based fertilizers can lead to soil acidification as a result of nitrification (Yang et al., 2021). An way to neutralize acidifying effect of the ammonium-based fertilizers is the lime and phosphogypsum applications, stimulate nitrification in acidic soils and improve N fertilizer uptake by crops (Garbuio et al., 2011). To mitigate N losses by leaching, volatilization and N_2O emission from the agricultural system, N recovery by plants should be maximized. Applying lime and phosphogypsum enhances root growth in deeper layers where fertilizer-derived N can be vertically displaced (Weligama et al., 2010). After harvest, the crop residues become a temporary N pool that can be accessed by subsequent crops through residue decomposition and mineralization, thus decreasing N fertilizer losses (Yang et al., 2021).

Research on the dynamics of N in tropical agricultural systems, especially those based on crop rotation of grasses and legumes, is in the early stages. Moreover, N supplied to maize intercropped with forage grass has an unclear recovery of residual N fertilizer by soybean [*Glycine max* (L.) Merrill], especially when soil amendments are associated with N fertilizers. Here we hypothesized that crops (maize + ruzigrass in rotation with soybean) recover more ^{15}N-labeled fertilizer when established in soils broadcast with lime and phosphogypsum than when established in acidic soils, as the former results in less N loss to the environment. Over the course of 18 years conducting this experiment, this study is the first demonstrating the improvement of N fertilizer recovery (based on ^{15}N labeled fertilizer) by the innovative combination of lime and phosphogypsum. Moreover, it highlights the significant role played by soil fertility and microorganisms in shaping these dynamic processes. To test this hypothesis, we examined the effects of the surface application of lime alone or in combination with phosphogypsum in a maize (intercropped with ruzigrass)–soybean rotation system on (i) soil fertility, (ii) crop biomass and grain yield, (iii) ^{15}N recovery in the entire system (soil and plant), and (iv) the microorganism in N cycle genes.

2. Material and methods

2.1. Site location and description

The study of 18-years field experiment is registered by the Global Long Term Agricultural Experiments Network (GLTEN), Rothamsted Research, UK; https://www.glten.org/experiments/62] established in Botucatu, southern of São Paulo State, Brazil (22°83′3′ S, 4°42′64′ W, 765 m a.s.l.). In this long-term experiment, lime (L) and/or phosphogypsum (PG) were surface applied in an agricultural system with crop rotation under no-tillage. The climate in this region is classified as mesothermic (Cwa) and humid subtropical, with dry winters, and hot and wet summers according to the Köppen–Geiger climate classification system. Climatic data during the experimental period are illustrated in Fig. 1A. The soil is a sandy clay loam kaolinitic thermic Typic Haplorthox (Soil Survey Staff, 2014). Prior to the establishment of the study in 2002, the initial soil properties were determined at a depth of 0–20 cm according to van Raij et al. (2001) (Table S1). Detailed description of this study are found in Bossolani et al. (2022a).

2.2. Experimental design and crop management

The field study has been managed with a randomized complete block design composed by four treatments and four replicates tested since 2002. Plot sizes were 57 m^2 (9.0 × 6.3 m). The treatments tested were (*i*) no soil amendments applied, as a control treatment; (*ii*) application of phosphogypsum (PG); (*iii*) application of lime (L); and (*iv*) application of L + PG (LPG).

The experimental field received treatment reapplications in four years (2002, 2004, 2010, and 2016) during the 18 years of agricultural cultivation. Reapplications cropped up based on the results of annual base saturation (BS) determinations recommended by van Raij et al. (1997). These recommendations consider BS and cation exchange capacity (CEC) values and were used. From 2002–2010, the calculation was based on the results obtained in the 0–20 cm soil layer. However, the calculation for the last reapplication considered the 0–40 cm soil layer. This change was adopted because Carmeis Filho et al. (2017a) showed that a lime dose considering the 0–20 cm layer underestimates the ideal dose of lime in the same field experiment for intensive tropical agricultural systems. The revised calculation resulted in 13 Mg ha^{-1} of lime to increase the BS to 70 %. When liming occurred, phosphogypsum was also reapplied. For the first three applications of phosphogypsum, the dose was calculated by multiplying the clay content in the 20–40 cm depth by 6 as a factor, which resulted in a dose of 2.1 Mg ha^{-1} (van Raij et al., 1997). Posteriorly, a new method was suggested for calculating the phosphogypsum dose established by enhancing the saturation of Ca^{2+}in the effective CEC (ECEC) to 60 % in the 20–40 cm depth (Caires and Guimarães, 2018). Thus, the new dose applied was10 Mg ha^{-1} of phosphogypsum in 2016.

This study investigates the direct residual effects of soil amendments application in the third year after the last L and or PG reapplication in 2016, and the indirect effects of the first applications (2002, 2004 and 2010) and the long-term cultivation of crops performed since 2002. The cropping history from 2002 to 2020 and the details of the previous treatment applications are given in Table S2.

2.3. Crop management

The chronological details of the crop management events are illustrated in Fig. 1A and B. Maize (hybrid P3707VYH, DuPont Pioneer, Rio Grande do Sul, Brazil) was planted in March 2019. The grain crop was intercropped (same row) with ruzigrass (*Urochloa ruziziensis* cv. Comum)]. For ruzigrass, was used a seed density of 10 kg ha^{-1}. Each plot received 42.8 kg P ha^{-1} and 46.5 kg K ha^{-1} at planting time. Nitrogen fertilizer was carried out in two applications, with 28 kg N ha^{-1} at seeding and sidedressed 100 kg N ha^{-1} at phenological stage V_5 of maize (Ritchie et al., 1993). Maize harvest occurred in July 2019, and the maize stover fractions (stalk, leaves, sheaths, tassel, core cob, and straw cob) was left in the soil surface. After the maize harvest, the ruzigrass continued growing until October 2019, when it was chemically terminated using glyphosate (2.5 kg ha^{-1} a.i.). In November 2019, soybean (cultivar TMG 7062 IPRO, TMG – Tropical Melhoramento & Genética, Paraná, Brazil) was sown over the ruzigrass residue. At planting time of soybean, initial fertilization was performed with 26.2 kg P ha^{-1} and

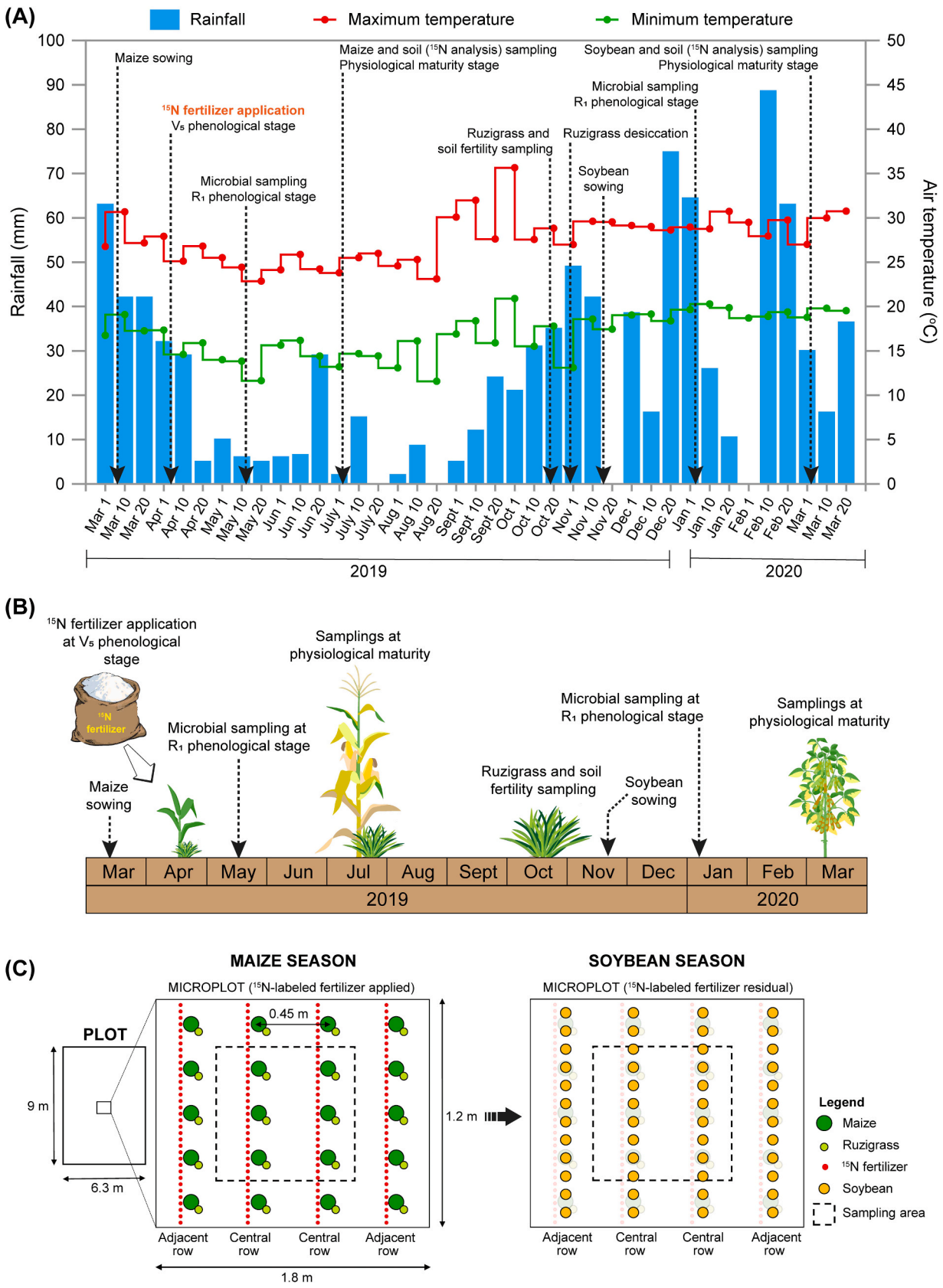

Fig. 1. Weather conditions (A), main activities during the experimental period (A and B), and schematic representation of the plot, ^{15}N–labeled fertilizer microplot, as well as the sampling protocol (C). The average (1956–2022) annual precipitation is ~1360 mm, and the mean annual air temperature range is 15.3–26.1 °C.

49.8 kg K ha^{-1} and seed was inoculated with *Bradyrhizobium* sp. The soybean was harvested in March 2020.

2.4. ^{15}N microplot establishment

Unconfined microplots (1.8 × 1.2 m) were set up in each treatment during the maize + ruzigrass season (Fig. 1C). All crop management occurred in the main plots were also used in the microplots. Maize microplots consisted of four rows plants, with five plants per row (~4.2 plants m^{-1}). ^{15}N-labeled ammonium sulfate [$(^{15}NH_4)_2SO_4$] fertilizer enriched at 6.31 atom % ^{15}N excess (Sigma–Aldrich Inc., St. Louis, MO, USA) was side-dressed (100 kg N ha^{-1}) when maize plants reached the V_5 phenological stage, as occurred for the main plots. The microplots received only ^{15}N-labeled fertilizer, whereas the main plot received unlabeled ammonium sulfate. The maize stover was returned to the microplot after lab processing to ensure the cycling of the ^{15}N present in the plant residues. After maize harvesting, the microplot remained marked to evaluate the recovery of residual fertilizer labeled with ^{15}N by soybean in crop rotation.

2.5. ^{15}N-labeled material sampling and isotopic analyses

At penological stage R_6 (maize physiological maturity), six plants from central part of each microplot were cut to ground level. Maize plants were separated into vegetative fractions (stalk, leaves, sheaths, tassel, core cob, and straw cob) and grains. All vegetative fractions from each microplot were mixed (herein defined as stover), chopped with a forage grinder, and oven-dried at 60 °C to obtain the dry weight. The dry weight of the grain fraction was obtained after a similar procedure. The subsamples of dried stover and grains were ground in a Wiley mill (0.50-mm sieve). The remaining stover was returned to the microplots.

The ruzigrass biomass was sampled in October 2019 before chemical desiccation. In each microplot, an area of 0.25 m^2 was collected (at ground level). The ruzigrass biomass samples were oven-dried, and a subsample of the dry material was ground in a Wiley mill. The remaining biomass was returned to the microplots. For soybean, at the beginning of physiological maturity [R_7 phenological stage; (Fehr and Caviness, 1977); March 2020], before leaf senescence, six plants were sampled and separated into vegetative fractions [stem, leaves (including petiole), and pods] and grains. All vegetative fractions were pooled and termed soybean stover. The same drying and grinding procedures described for maize were carried out. Plants from each main plot (maize stover and grains, ruzigrass, and soybean stover and grains) were also subjected to the same procedure to assess the natural ^{15}N abundance. All milled plant tissue was used to measure the total N concentration and ^{15}N.

Soil was sampled at seven depths (0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm) using a core sampler. Six soil samples were collected per microplot and combined into one sample per depth per microplot. Three sample points were located in the maize rows that received ^{15}N-labeled fertilizer, and the three other points were collected between the maize rows. The soil samples were oven-dried, ground in a ball mill, and homogenized through a 100-mesh sieve (0.15-mm sieve). These soil samples were used to measure the total N concentration and ^{15}N. The natural ^{15}N abundance in the soil was also measured. To estimate the soil N accumulation for each soil depth and treatment, volumetric ring method was used to evaluate the soil bulk density during the ruzigrass season (Blake and Hartge, 1986). Total N concentration and ^{15}N abundance from soil and all plant tissue (maize, ruzigrass, and soybean) were analyzed using an automatic elemental analyzer (Flash EA, Thermo Scientific, Germany) interfaced with an isotope ratio mass spectrometer (CF–IRMS, Delta V, Thermo Scientific, Germany).

2.6. ^{15}N calculations

A number of variables were calculated to determine ^{15}N recovery, including N derived from fertilizer (Ndff), maize + ruzigrass and soybean ^{15}N recovery, soil ^{15}N retention in each soil layer and across all soil depths (down to 100 cm), and unrecovered ^{15}N in the soil–plant system. In these calculations, maize + ruzigrass was considered the first season, while soybean was the second season. ^{15}N recovery was determined according to the following equations:

$$Ndff \quad (kgha^{-1}) = (x/y \quad) \times TN \tag{1}$$

$$15N \quad recovery \quad (\%) = (Ndff/NFR) \times 100 \tag{2}$$

$$15N \quad \text{unrecovered}_{FS} \quad (\%) = 100 - \quad 15N \quad recovery_{FS} \tag{3}$$

$$15N\text{remaining}(\%) = \quad 15N \quad recovery_{FS} - \quad 15N \quad recovery_{maize \quad grain} \tag{4}$$

$$15N \quad \text{unrecovered}_{SS} \quad (\%) = \quad 15N \quad remaining - \quad 15N \quad recovery_{SS} \tag{5}$$

where *Ndff* represents the amount of N derived from fertilizer; *x* and *y* represents the ^{15}N abundance (atom % ^{15}N excess) in the crops (maize/ruzigrass/soybean) and their respective fractions (stover and grain) or soil, and in the fertilizer, respectively (a natural abundance of 0.368 atom % ^{15}N was considered in the calculations); *TN* represents the N accumulated in the plant fractions or in the soil (kg ha^{-1}); ^{15}N *recovery* represents the recovery of the labeled ^{15}N fertilizer; *NFR* represents the dose (kg ha^{-1}) of the N fertilizer applied; ^{15}N $unrecovered_{FS}$ and ^{15}N $unrecovered_{SS}$ represents the percentages of potential losses of N fertilizer (^{15}N unaccounted) after maize + ruzigrass (^{15}N fertilizer applied during maize + ruzigrass season) and after soybean (^{15}N fertilizer residual in the second season); ^{15}N $recovery_{FS}$ represents the percentage of the total N recovery, considering the sum of maize stover and grain, ruzigrass, and soil in the maize + ruzigrass crop season; ^{15}N *remaining* represents the amount of ^{15}N available in the system before soybean was grown; ^{15}N $recovery_{maize\ grain}$ represents the amount of ^{15}N exported in maize grains; ^{15}N $recovery_{SS}$ represents the percentage of the total N recovery, considering the sum of soybean stover, grains and soil.

2.7. Soil sampling and chemical analyses

To measure soil fertility status, composite soil samples ($n = 8$) were taken from the 0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm layers of each plot. The sampling procedure was carried out in October 2019, before ruzigrass desiccation (3 years after the last application of L and PG and 17 years after the beginning of the study). The soil was air-dried, homogenized and ground (2-mm sieve) for chemical analysis. Soil pH, SOC, exchangeable Al^{3+}, Ca^{2+} and Mg^{2+} and SO_4^{2-}S were analyzed according to van Raij et al. (2001).

2.8. Soil sampling, DNA extraction and qPCR analyses

Soil was sampled from the 0–10 cm layer for microbiological analysis after the maize and soybean harvests. Bulk soil samples (*i.e.*, six individual samples to compose one sample) were collected between rows at maize and soybean R_1 stages. A sample with 0.25 g of soil was used to determine the total DNA [DNeasy Power Lyzer Power Soil DNA Isolation Kit (Qiagen, Hilden, Germany)]. The DNA extraction procedure was carried out following the manufacturer's instructions. The NanoDrop 1000 spectrophotometer equipment (Thermo Scientific, Wilmington, DE, USA) was used to assess the soil DNA quality and concentration. After DNA extraction, the samples were stored at − 20 °C for further analysis.

The abundances of 16S rRNA gene (total bacteria and archaea), nitrifiers (*AmoA-AOB*, ammonia monooxygenase gene of bacteria; *AmoA-AOA,* ammonia monooxygenase gene of archaea), and denitrifiers (*nirK*, nitrite reductase gene; *nosZ,* nitrous oxide reductase gene) were assessed by qPCR (StepOnePlus Real-Time PCR System; Applied Biosystems,

Foster City, CA). A 10-fold serial dilution (10^8 to 10^3) was used to create the standard curves based on target gene (previously amplified by PCR). The reaction conditions for each gene amplification are shown in Table S3. All analyses were performed in triplicate and obtained gene abundance calculated as number of DNA copies per g of fresh soil.

2.9. Dataset and statistical analysis

All dataset were tested for normality by using Anderson–Darling test and homoscedasticity through Levene's test. When significant, means were analyzed using Fisher's protected least significant difference (LSD) test, at $p \leq 0.05$. Redundancy analysis (RDA) was used to verify the correlations between soil fertility and ^{15}N recovery, between soil fertility and N cycle genes, and between N cycle genes and ^{15}N recovery by maize and soybean. RDA triplots were generated using Canoco (version 4.5, Biometrics, Wageningen, Netherlands). Monte Carlo permutation test (999 random permutations) was performed to verify the significance of soil fertility, ^{15}N recovery, and N cycle gene responses. One-way PERMANOVA (permutational multivariate analysis of variance) was performed to group treatments by similarity.

3. Results

3.1. Soil profile fertility

Contrasting levels of soil fertility were observed three years after the last soil amendment reapplication (Table 1). Soil acidity was reduced across the entire soil profile in both L and LPG compared with the control and PG. As expected, PG did not efficiently alter soil pH compared with the control. SOC content was higher in LPG than in L and in PG than in the control at depths $\leq$ 20 cm. At all depths, Ca-based amendments of soil (PG, L, and LPG) increased the Ca^{2+} concentration compared with the control, following the trend: LPG > L > PG. L and LPG also increased the Mg^{2+} concentration throughout the soil profile, but at depths > 40 cm, the Mg^{2+} concentration was higher in LPG than in L. L and LPG were equally efficient in reducing the Al^{3+} concentration in the soil profile, especially in the surface layers (*i.e.*, $\leq$ 40 cm). PG also reduced the Al^{3+} concentration compared with the control but to a lesser extent than L and LPG. The most notable effect of PG was an increase in the concentration of SO_4^{2-}–S, but the concentration of SO_4^{2-}–S was highest in LPG across the soil profile. The SO_4^{2-}–S concentration did not differ between L and PG at depths $\leq$ 10 cm but was higher in PG in subjacent layers.

3.2. Aboveground dry matter yield

On average, in the first growing season, maize aboveground (shoot) dry matter (stover + grain) production was 71 % higher in L and LPG than in the control and PG (Fig. 2A). Ruzigrass biomass followed the same pattern. In the second growing season, soybean aboveground dry matter production was, on average, 16 %, 70 %, and 108 % higher in LPG than in L, PG, and the control, respectively (Fig. 2B; Table S4).

3.3. ^{15}N recovery in the soil–plant system

^{15}N recovery in maize fractions (stover and grain) and ruzigrass was higher in L and LPG (Fig. 3 A; Table S5). ^{15}N recovery did not differ between L and LPG and was ~48 % higher than in the control and PG. By contrast, ^{15}N recovery in maize grain and ruzigrass biomass was highest in LPG. In general, ~75 % of the ^{15}N fertilizer found in maize shoots was exported by the grain. LPG increased grain ^{15}N recovery by 232 %, 150 %, and 16 % compared with the control, PG, and L, respectively. A similar trend was observed in ruzigrass: ^{15}N recovery was 55 %, 37 %, and 13 % higher in LPG than in the control, PG, and L, respectively. Soil retention (down to 100 cm) of ^{15}N fertilizer was highest in PG (23 kg ha^{-1}), followed by L (19 kg ha^{-1}), the control (16 kg ha^{-1}), and LPG (11 kg ha^{-1}). The pattern of unrecovered ^{15}N was opposite that of ^{15}N recovery by maize and ruzigrass plants: the highest losses of fertilizer-derived ^{15}N occurred in the control (46 %), followed by PG (35 %), L (14 %) and LPG (11 %).

At the end of the maize + ruzigrass season, the amount of ^{15}N in the soil–plant system was denoted as ^{15}N remaining (*i.e.*, ^{15}N recovered by plants and soil retention, excluding the amount exported by grains;

Table 1
Soil fertility in response to soil amendments [control (no soil amendments applied), phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)] at seven stratified soil layers up to 100 cm depth.

Soil depth (cm)	SA‡	pH ($CaCl_2$)	SOC (g kg^{-1})	Ca^{2+} (mmol$_c$ kg^{-1})	Mg^{2+} (mmol$_c$ kg^{-1})	Al^{3+} (mmol$_c$ kg^{-1})	SO_4^{2-}–S (mg kg^{-1})
0 – 5	Control	4.08 b†	15.9c	11.0 d	8.61 b	4.45 a	9.4c
	PG	4.32 b	17.1 b	45.0c	10.4 b	2.32 b	23.6 b
	L	6.00 a	17.8 ab	61.7 b	25.7 a	0.16c	21.2 b
	LPG	6.08 a	18.3 a	78.5 a	26.6 a	0.06c	32.4 a
5 – 10	Control	3.68 b	14.3c	9.90 d	7.75 b	6.68 a	8.45c
	PG	3.89 b	15.4 b	40.5c	9.35 b	3.47 b	21.3 b
	L	5.40 a	16.0 ab	55.5 b	23.1 a	0.24c	19.0 b
	LPG	5.48 a	16.5 a	70.6 a	23.9 a	0.08c	29.2 a
10 – 20	Control	4.01 b	11.9 d	9.11 d	5.00 b	14.9 a	12.1 d
	PG	4.11 b	13.1c	19.0c	6.04 b	13.0 b	32.6 b
	L	5.01 a	14.4 b	35.0 b	16.2 a	1.06c	25.6c
	LPG	5.17 a	15.3 a	44.6 a	16.7 a	1.08c	42.5 a
20 – 40	Control	3.98 b	10.8 b	8.81 d	2.77 b	15.5 a	15.6c
	PG	4.03 b	11.2 b	13.6c	4.29 b	12.9 a	40.8 b
	L	4.53 a	13.0 a	30.2 b	12.7 a	2.85 b	31.2c
	LPG	4.62 a	14.0 a	38.3 a	12.6 a	2.95 b	59.4 a
40 – 60	Control	3.83 b	10.2c	5.71 d	2.84c	16.7 a	16.3c
	PG	3.87 b	11.1 bc	8.38c	3.47c	15.4 a	43.6 b
	L	4.14 a	11.9 ab	17.5 b	11.1 b	9.40 b	37.3c
	LPG	4.27 a	12.5 a	22.2 a	13.0 a	7.58 b	69.8 a
60 – 80	Control	3.76 b	9.22c	1.92c	2.30c	20.5 a	21.8 d
	PG	3.88 b	10.1 bc	5.61 b	2.03c	19.0 a	54.1 b
	L	4.13 a	10.6 ab	11.9 a	10.1 b	11.3 b	43.5c
	LPG	4.16 a	11.5 a	12.2 a	14.7 a	9.09 b	72.6 a
80 – 100	Control	3.75 b	8.60 b	1.42 d	1.35c	20.9 a	23.7 d
	PG	3.88 b	8.77 b	3.83c	1.18c	19.4 a	64.4 b
	L	4.06 a	9.31 ab	7.21 b	2.34 b	11.7 b	31.7c
	LPG	4.05 a	9.92 a	9.17 a	4.07 a	9.45 b	72.8 a

† Different lowercase letters for each soil layer indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$; ‡ Soil amendments (SA)

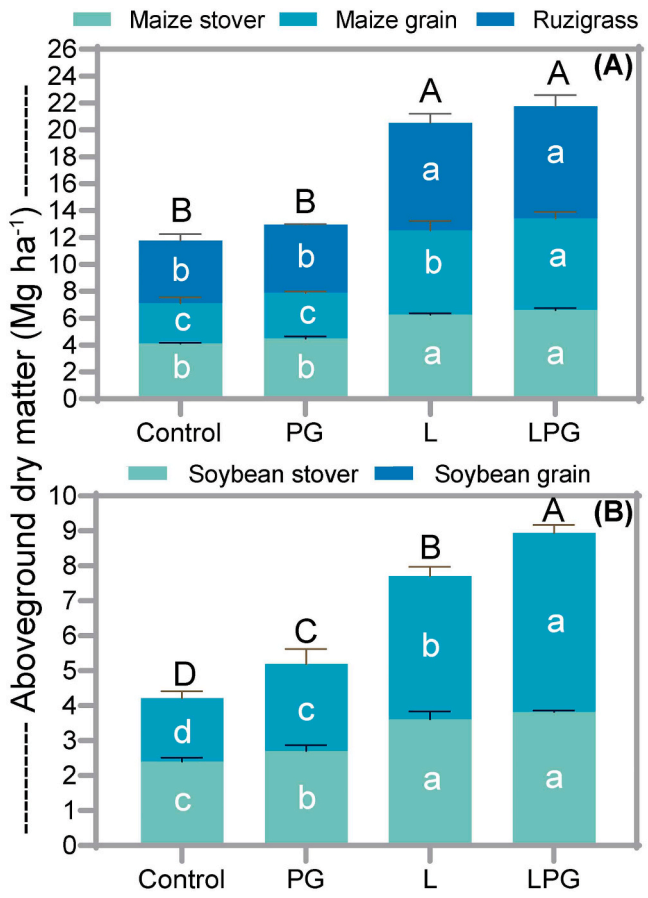

Fig. 2. Aboveground (stover + grain) dry matter yield in the first (maize and ruzigrass) (A), and second (B; soybean) growing seasons in response to soil amendments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. Different lowercase or capital letters indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$. Error bars express the standard error of the mean (n = 4).

Fig. 3B; Table S5). On average, the ^{15}N remaining in L and LPG was ~27 % higher than that in the control and 9% higher than that in PG. Overall, ~52 % of the ^{15}N fertilizer applied to maize remained in the soil–plant system before soybean planting.

At soybean harvest, the trends of ^{15}N recovery in stover and grain were opposite those of maize (Fig. 3C; Table S5). Compared with the average of the control and PG, ^{15}N recovery in stover and grain was 63 % lower in L and 33 % lower in LPG. In addition, the average ^{15}N recovery in soybean shoots was 92 % lower than that in maize shoots. Soil retention of ^{15}N after soybean was lowest in the control treatment (32 %); the average of the other treatments was 40 %. The amount of unrecovered ^{15}N following soybean was also affected by the soil amendments. The highest loss occurred in L (16 %), whereas the average of PG and LPG was 10 %. At the end of the experiment, the total amount of unrecovered ^{15}N (sum of the both growing seasons) decreased in the following order: control (55 %) > PG (45 %) > L (29 %) > LPG (22 %) (Fig. 3D; Table S5).

Based on the values of total ^{15}N recovery from each season and dry weight production, we calculated the yield-scaled ^{15}N recovery (Fig. 3E and F; Table S5). In the maize + ruzigrass season, the use of ^{15}N fertilizer for each Mg ha^{-1} of biomass produced was greater in LPG (3.5 kg ^{15}N Mg^{-1} biomass) than in the other treatments (average of 3.2 kg ^{15}N Mg^{-1} biomass; Fig. 3E), whereas the opposite pattern was observed in the soybean season (Fig. 3 F). The accumulation of fertilizer-derived ^{15}N in biomass production in the soybean season ranged from 0.39 kg ^{15}N Mg^{-1} biomass (average of control and PG) to 0.14 kg ^{15}N Mg^{-1} biomass (average of L and LPG).

3.4. ^{15}N retention along the soil profile

The treatments influenced ^{15}N retention along the soil profile after the maize and soybean harvests (Fig. 4). After the maize harvest, ^{15}N fertilizer retention in the uppermost soil layers (0–20 cm) was highest in L and PG, whereas in deeper layers (20–100 cm), ^{15}N retention was highest in PG (Fig. 4A). After the soybean harvest, ^{15}N retention at depths ≤ 40 cm was highest in LPG, followed by L (Fig. 4B). Conversely, similar to the pattern observed after the maize harvest, ^{15}N retention at depths > 60 cm was highest in PG.

3.5. Microbial analysis in the soil

During the maize season, the total bacterial abundance (16 S rRNA gene) in L and LPG was ~20% higher than that in PG and ~25 % higher than that in the control (Fig. 5A). The total bacterial abundance was also highest in LPG during the soybean season. Total archaeal abundance was more sensitive to changes caused by the treatments (Fig. 5B). For both crops, L and mainly LPG increased the abundance of total archaea compared with the control and PG. Compared with PG and the control, the average total archaeal abundance between L and LPG increased by 81 % and 265 %, respectively, during the maize cultivation, and by 18 % and 58 %, respectively, during the soybean cultivation.

Functional marker genes were quantified by qPCR to assess soil microbial communities related to the N cycle. The treatments affected the abundance of the bacterial *amoA-AOB* gene only during the maize season; the abundance of this gene was ~14 % higher in LPG than in the other treatments (Fig. 5C). By contrast, the pattern of the abundance of the archaeal *amoA-AOA* gene was similar to that of total archaea in both crop seasons. In the maize season, the *amoA-AOA* gene abundance was highest in L and LPG and was significantly higher in LPG than in PG and the control (Fig. 5D).

The abundances of the *nirK* and *nosZ* genes, which are both related to denitrification, were lower in soils amended with L and LPG than in PG and the control, especially during maize cultivation (Fig. 5E and F). The exception was *nosZ* during the soybean season, which was not altered by the treatments. During the maize season, the gene abundances of both *nirK* and *nosZ* were higher in the control than in L and LPG. During the soybean season, the abundance of *nirK* was lowest in L. Interestingly, during maize season, LPG presented the highest ratio between *nirK* and *nosZ* gene abundances, whereas during soybean season, control and PG presented the highest values (Fig. S1).

3.6. Linking plant–soil–N cycle data

The relationship between soil fertility and ^{15}N recovery in the soil–plant system of both growing seasons was presented in the first RDA (Fig. 6A). The sum of the two axes (RDA_1 = main variation; RDA_2 = remaining variation) explained ~88% of all variation in ^{15}N recovery by crops. PERMANOVA separated the four soil amendments into distinct groups. Soil pH and Ca^{2+} concentration were the main soil properties responsible for the variation in ^{15}N recovery. The vectors also showed that ^{15}N recovery by maize and ruzigrass was highest in L and LPG, whereas ^{15}N recovery by soybean was highest in the control. The loss of ^{15}N fertilizer in the maize season was highest in the control.

The second RDA examined the relationship between soil fertility and soil microbial gene abundance. PERMANOVA again distinguish the treatments into four groups, although the control and PG showed a certain degree of similarity. The sum of the axes indicated that soil properties explained 70 % of all variation in the abundance of soil microbial genes. The main soil characteristics responsible for this variation were soil pH and SOC content (Fig. 6B). The abundances of denitrifying genes (*nirK* and *nosZ*) correlated positively with the control and PG and with the *amoA-AOA* gene during the soybean season. On the other hand,

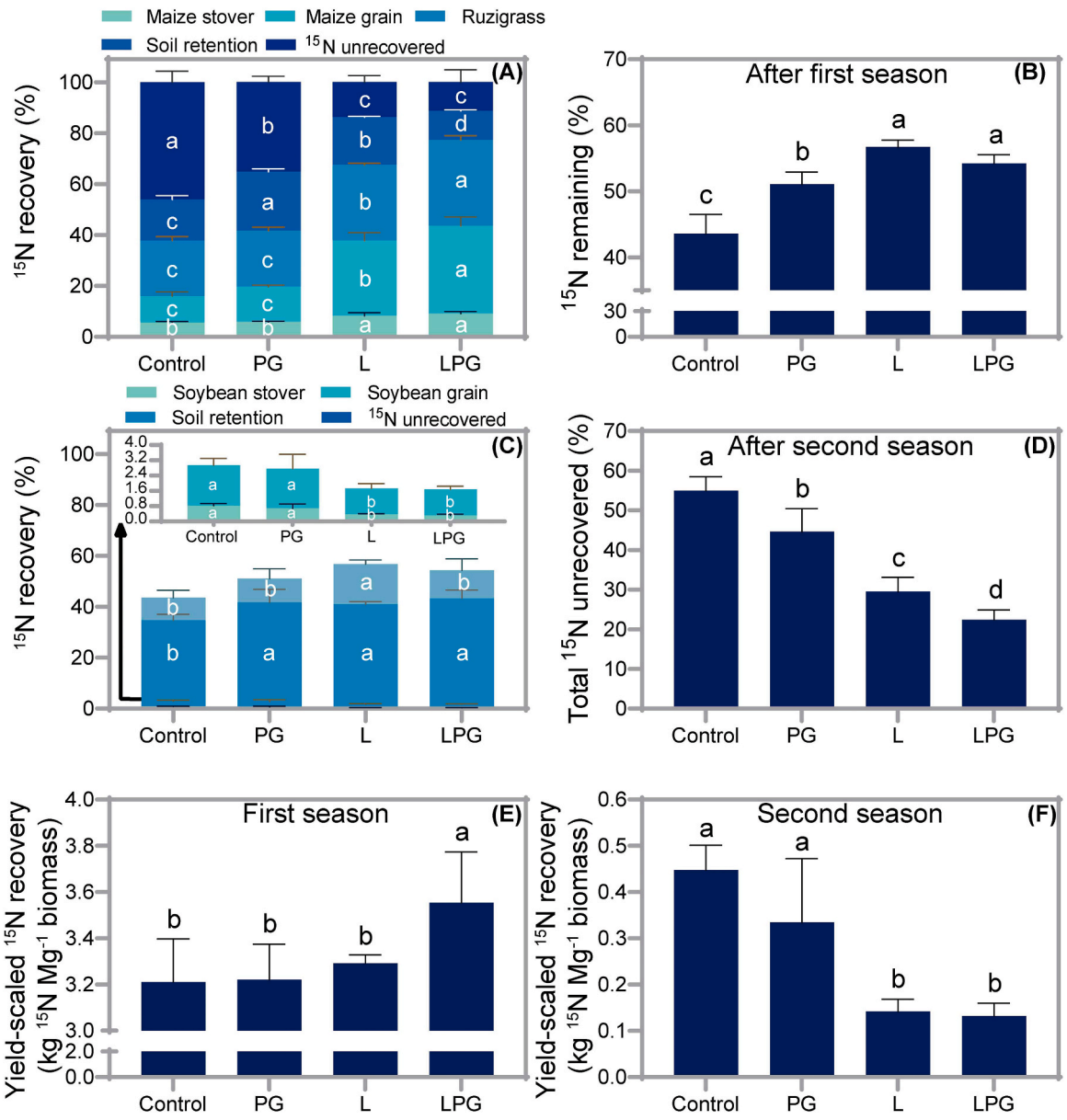

Fig. 3. ^{15}N recovery in each compartment (plant, soil or unrecovered) from the first (A), and second (C) growing seasons; ^{15}N–fertilizer remaining after the first growing season (B), total ^{15}N unrecovered (first + second growing seasons; D); and yield–scaled ^{15}N recovery related to the total biomass production in the first (E), and second (F) growing seasons in response to soil amendments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. Different lowercase letters indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

the abundances of total bacteria and total archaea were greater in the treatments with higher soil fertility (L and LPG). Soil fertility was also linked to the abundances of ammonia-oxidizing microbial genes (*amoA*-AOB and *amoA*-AOA) during the maize season.

The third RDA revealed the role of soil microbial genes in modulating ^{15}N recovery in the maize + ruzigrass season (Fig. 6C). Axis 1 explained 93 % of the variation. PERMANOVA segregated the treatments into four distinct groups. The highest ^{15}N losses in the maize + ruzigrass season were positively correlated with denitrification genes in the control treatment. In addition, the highest abundances of total bacteria, total archaea and nitrifying genes were positively correlated with the highest ^{15}N recovery by maize and ruzigrass in L and especially LPG. Total archaea, *amoA*-AOB, *amoA*-AOA, *nirK*, and *nosZ* were the main soil microbial genes that modulated ^{15}N fertilizer utilization during the maize + ruzigrass season.

The final RDA combined soil microbial genes and ^{15}N recovery by soybean in the second season. PERMANOVA indicated that the treatments were different from each other and that the sum of the axes explained 62 % of all variation. In this analysis, only the *nirK* gene participated significantly in the modulation of ^{15}N recovery responses, especially in the control and PG. ^{15}N recovery by soybean was also highest in these treatments. By contrast, the largest losses of ^{15}N fertilizer in the second season occurred in L and LPG.

4. Discussion

4.1. Soil profile fertility/nutrient availability

The surface application of amendments under no-tillage is a feasible practice for reducing subsoil acidity and improving soil fertility over time. Here, we present the long-term construction of subsoil fertility after four applications of L and/or PG amendment over 17 years. The results of this study refer to the third year after the last reapplication of these soil amendments. Subsoil acidity was sharply reduced by liming (L

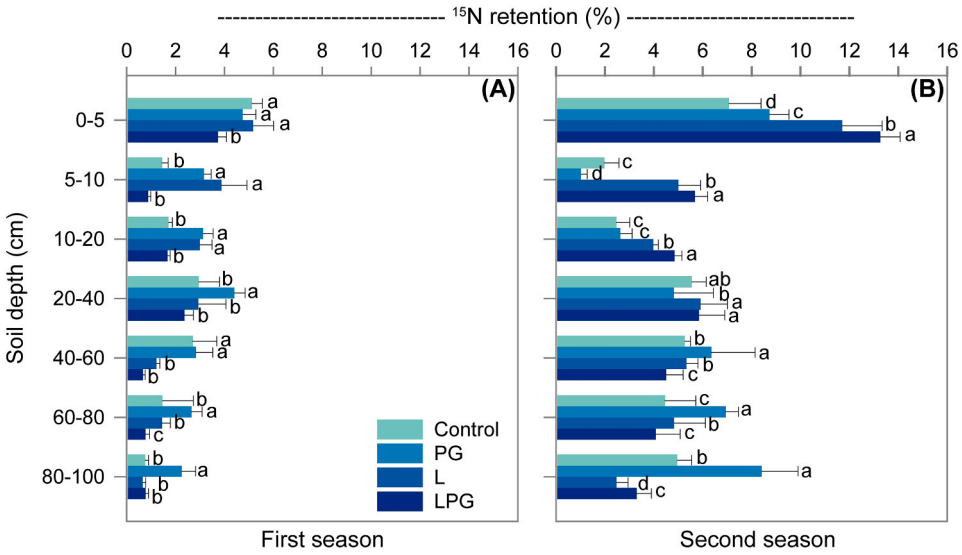

Fig. 4. ^{15}N retention at seven stratified soil layers down to 100 cm after the first (A) and second (B) growing seasons in response to soil amendments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. Different lowercase letters for each soil layer indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 4$).

and LPG); however, combining L with PG (LPG) increased Ca^{2+} concentrations in all soil layers, especially at greater depths. An adequate exchangeable Ca^{2+} concentration in deeper layers is essential for deep rooting to increase nutrient and water uptake (Bossolani et al., 2021c) and for reducing the risk of water deficit during dry spells (Bossolani et al., 2021a).

Sole application of phosphogypsum did not change the soil pH, although it increased the Ca^{2+} concentration in the soil profile compared with the control. The effects of phosphogypsum alone are small compared with those of liming, but phosphogypsum potentiates the effects of liming when applied together (Crusciol et al., 2019). When phosphogypsum is applied to soil, it quickly dissociates into SO_4^{2-} and Ca^{2+} (Zoca and Penn, 2017). In addition to its fertilizing effect (S and Ca supply), phosphogypsum reduces Al^{3+} toxicity (Tiecher et al., 2018). $SO4^{2-}$ also forms ionic bonds with exchangeable cations (K^+, Ca^{2+}, Mg^{2+}, NH_4^+), displacing them to deeper layers (Tiecher et al., 2018; Zoca and Penn, 2017). The exchangeable Mg^{2+} concentration also increased along the soil profile after liming (L and LPG), regardless of the application of phosphogypsum. Low Mg^{2+} availability is limiting for crop development, but fertilization programs do not include this nutrient in conventional NPK formulas. For this reason, Mg^{2+} is often considered a "forgotten element" (Cakmak and Kirkby, 2008). Acidic soils are poor in Mg^{2+}, which is essential for numerous metabolic plant (Jaghdani et al., 2021) and microbial processes. Liming is the most viable form of Mg^{2+} fertilization to make Mg^{2+} less limiting in intensive crop production systems (Li et al., 2019). The ability of SO_4^{2-} to create ionic pairs with Mg^{2+} (Zoca and Penn, 2017) increased the concentration of Mg^{2+} in deep layers in LPG compared with L. The long-term effects of L and LPG also impacted SOC content in all soil layers. Soils with adequate fertility management provide greater above- and belowground crop residues (Bossolani et al., 2021a; Costa et al., 2018), leading to increases in SOC over time (Inagaki et al., 2017).

4.2. Aboveground dry matter yield and fate of ^{15}N-labeled fertilizer

The improvement in soil fertility by broadcasting L and LPG also increased the aboveground dry matter of maize, ruzigrass, and soybean plants. In general, these crops have a low tolerance of acidic soils with high Al^{3+} concentrations (Rao et al., 1993). Numerous studies have demonstrated the benefits of liming, especially when combined with phosphogypsum, for increasing biomass and grain yield (Bossolani et al., 2022b, 2021a; Costa et al., 2018; Crusciol et al., 2019; Soratto and Crusciol, 2008). Although straw production did not differ between L and LPG, maize and soybean grain yields increased in LPG. The improvement in these yields was due to improvements in soil fertility (*i.e.*, higher SOC, Ca^{2+}, Mg^{2+}, and SO_4^{2-}–S concentrations) and lower Al^{3+} concentrations, as supported by RDA, which showed positive correlations of stover and grain yields with soil fertility parameters. Increased Ca^{2+} levels in deeper layers are strongly linked to rooting depth, which improves the uptake of soil resources by crops (Costa et al., 2018; Ritchey et al., 1982). Our results emphasizes the importance of combining liming and phosphogypsum (LPG) in annual crop rotations to obtain high straw (for no-tillage system maintenance) and grain yields.

The cascading effects of the soil amendments on soil fertility and crop growth and yield impacted the fate of ^{15}N. LPG increased not only ^{15}N recovery by maize grains but also ^{15}N accumulation in ruzigrass biomass. While the ^{15}N in ruzigrass biomass and maize stover act as N pools that can potentially be returned to the system (^{15}N remaining data) through crop residue decomposition, N in grains is exported after harvest. Any N that is contained in plant tissues is less susceptible to losses to the environment by denitrification or leaching (Zhou et al., 2020). Increasing the recovery of N fertilizer by crops guarantees higher crop yields as well as environmentally safe agriculture. This link is reinforced by the results for unrecovered ^{15}N after maize growth. Soils managed with L and LPG had the lowest N losses, whereas the control (no amendments applied) had the highest ^{15}N losses.

The soybean–maize (intercropped with forage grasses) rotation system is widely used in tropical regions, particularly in Brazil (Ceccon et al., 2013). Interestingly, the trend of ^{15}N recovery by maize and ruzigrass was opposite that of soybean cultivated in rotation. The yield-scale ^{15}N recovery for each season reinforces this pattern. Approximately 90% of the total N accumulated by soybean is a supply from biological N fixation (BNF) (Freitas et al., 2022), but ^{15}N recovery was higher in the control and PG treatments. These results reflect (*i*) the smaller amount of ^{15}N fertilizer remaining in the soil in L and LPG and/or more likely (*ii*) increased BNF efficiency due to liming (Alves et al., 2021; Andrade et al., 2002), which reduced the plants' dependence on soil fertilizer-derived ^{15}N. Several studies have shown that liming increases soil pH, leading to changes in the compositions of soil Rhizobia species (Andrade et al., 2002; Zhalnina et al., 2013), as the abundance of N-fixing Rhizobia species is strongly reduced in highly acidic soil.

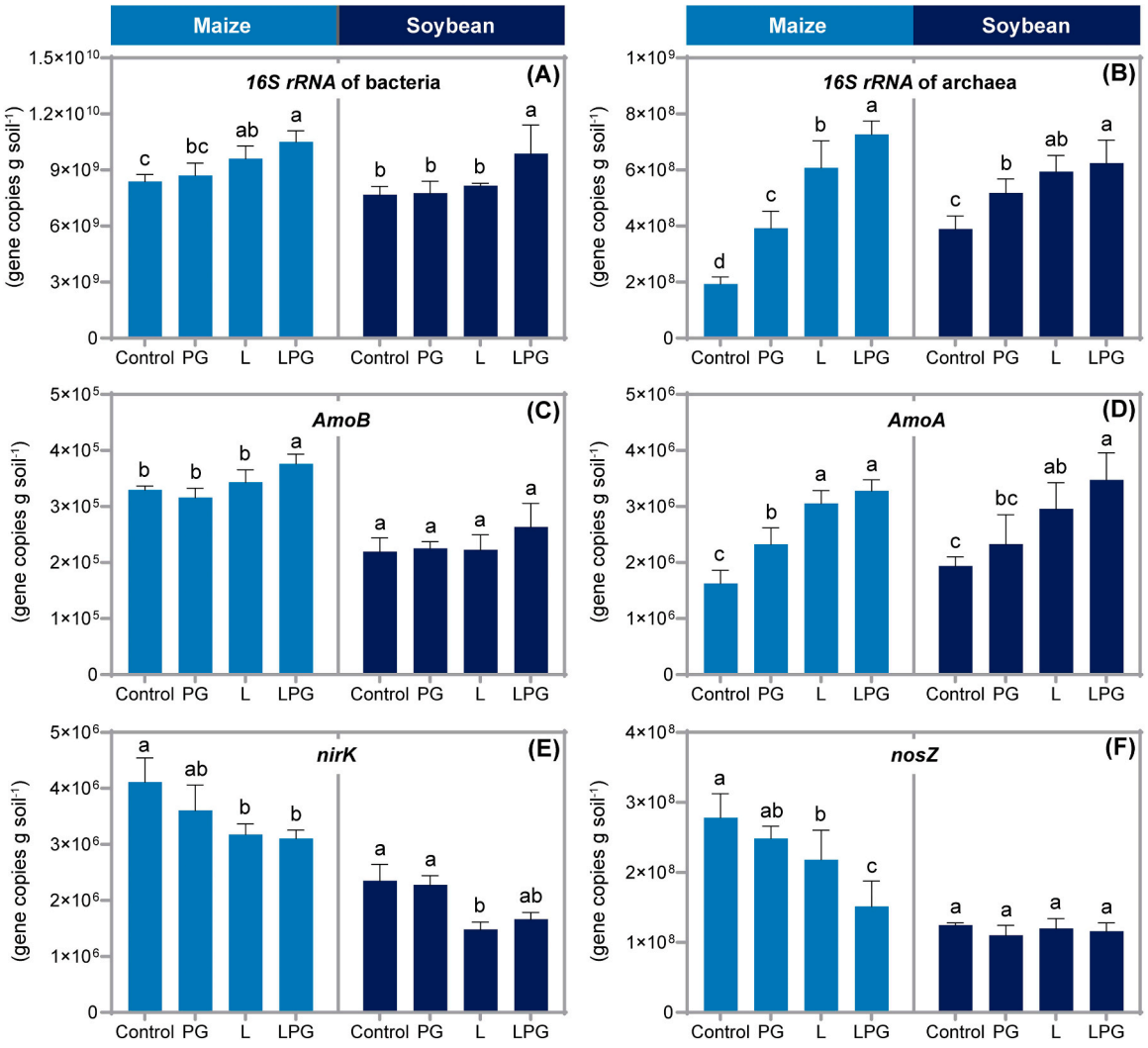

Fig. 5. Copy numbers of phylogenetic (16 S rRNA) marker genes of bacteria (A) and archaea (B), and functional marker genes related to the N cycle as *amoB* (C), *amoA* (D), *nirK* (E) and *nosZ* (F) during maize and soybean seasons in response to soil amendments [control, phosphogypsum (PG), lime (L), and lime + phosphogypsum (LPG)]. Different lowercase letters indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$. Error bars express the standard error of the mean ($n = 12$).

4.3. Distribution of ^{15}N fertilizer along the soil profile and soil microbial genes

The soil retention data indicated a high concentration of ^{15}N fertilizer in deep layers in PG, whereas the smallest amounts of ^{15}N-labeled fertilizer were observed in L and LPG. Liming catalyzes numerous soil biological processes (Guo et al., 2019; Liu et al., 2018). The cascading effects of liming on soil fertility can dramatically affect soil N transformation processes by microorganisms (Bossolani et al., 2020a), modulating the release of plant-available N and N losses through groundwater and/or to the atmosphere (Holland et al., 2018; Yang et al., 2021).

Liming, especially in combination with phosphogypsum (LPG), had positive effects on total bacterial and archaeal gene abundances. Increased soil fertility is often correlated with changes in soil microbial communities (Bossolani et al., 2021b; Holland et al., 2018). In response to the increases in the bacterial and archaeal populations, the abundance of N cycle genes increased (Fig. S2). High abundances of ammonia-oxidizing microbial genes (*AmoA*-AOB and especially *AmoA*-AOA) were found in L and LPG. Archaea are the most abundant microorganisms responsible for nitrification in agricultural soils with low NH_4^+-N content, and when NH_4^+-N fertilization is high, nitrifying bacteria outcompete archaea despite the low abundance of the former (Rütting et al., 2021). Our third RDA revealed a strong influence of archaea (16S rRNA and *AmoA*-AOA abundances) during the maize season on the ^{15}N recovery response. Despite the greater *AmoA*-AOA gene abundance, numerous reports suggest that *AmoA*-AOB may have a predominant role in regulating N cycling in acidic soils (Kunhikrishnan et al., 2016). In addition, the increase in nitrifying microorganisms due to liming suggests that fertilizer-derived NH_4^+ is rapidly converted to NO_3^- (Beeckman et al., 2018). Numerous studies have reported an increase in the abundance of genes of nitrifying microorganisms in soils managed with liming (Bossolani et al., 2020a,b; J. Liu et al., 2018; X.Y. Liu et al., 2018). When there are no biotic or abiotic impediments to plant growth, NO_3^- generated by nitrification is taken up by crops. However, when uptake rates are low by plants (*e.g.*, incipient root system) or region has an excessive rainfall and water promotes the NO_3^- leaching (Holland et al., 2018). The high biomass and grain yields in L and LPG, combined with the low amount of ^{15}N fertilizer in deep layers, indicate intense N uptake by the crops. However, high amounts of ^{15}N were found in the soil in PG, probably due to a reduction in the nitrification rate because of the acidic conditions (Beeckman et al., 2018). Additionally, the SO_4^{2-} generated from PG dissociation in the soil may have formed an ion pair with NH_4^+, resulting in zero-charged ammonium

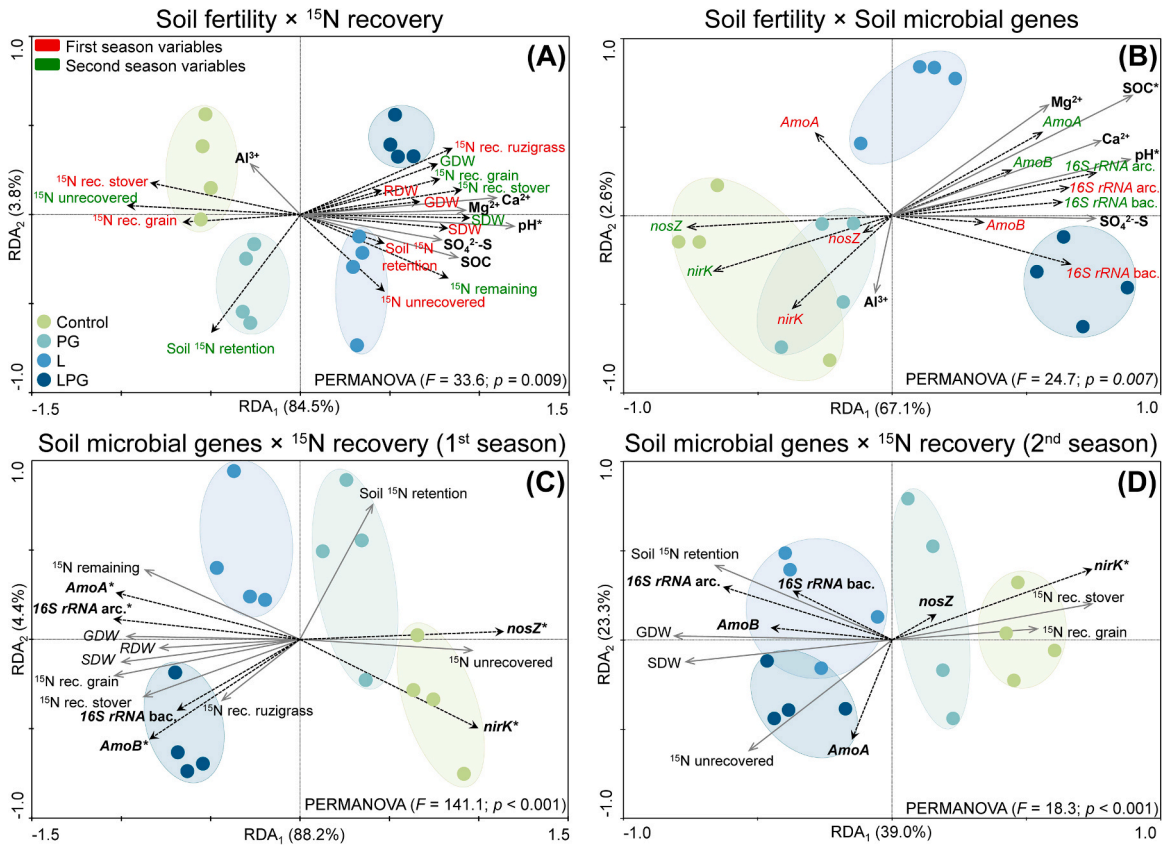

Fig. 6. Redundancy analysis (RDA) triplot showing the relationship between the soil fertility × 15 N recovery from both first and second growing seasons (A), soil fertility × N microbial genes from both first and second growing seasons (B), soil microbial genes × 15 N recovery form first (C) and second (D) growing seasons. Canonical axes are labeled with percentage of total variance (RDA1 = main variation; RDA2 = remaining variation). Arrows indicate correlations between variables. The significance of these correlations was assessed by Monte Carlo permutation test with 999 permutations. Significant variables ($p \leq 0.05$) are indicated by an asterisk. Color dashed lines indicate significant ($p \leq 0.05$) clusters by permutation analysis (PERMANOVA, $p \leq 0.05$). Maize + ruzigrass season variables are represented by red color, whereas second season are represented by green color (A and B). SOC = soil organic C; 15 N rec. = 15 N recovery; SDW = stover dry weight; GDW = grain dry wight; RDW = ruzigrass biomass dry weight; 16 S rRNA bac. = 16 S rRNA bacteria; 16 S rRNA arc. = 16 S rRNA archaea.

sulfate [$(NH_4)_2SO_4$], which may leach into deeper soil layers before being absorbed by crops. Bossolani et al. (2021a) showed that root growth was shallower in the control and PG than in L and LPG.

Leaching to layers beyond the root zone makes N unavailable to plants, and the N is eventually carried to groundwater. Interestingly, the levels of ^{15}N fertilizer in deep layers were lower in the control than in PG, and the abundances of the *AmoA*-AOB and *AmoA*-AOA genes were lower in the control than in the limed treatments (L and LPG). Although these results indicate low nitrification rates, the control presented the highest abundance of denitrification genes. Although not all genes associated with the denitrification cycle were assessed, the abundance of two crucial genes (*nirK* and *nosZ*) linked to this pathway increased, as supported by the third and fourth RDAs. A lower N uptake rate by crops increases the availability of mineral N (NH_4^+ and NO_3^-), thus providing substrates for denitrifying populations and favoring conditions for N_2O and N_2 production (Zhou et al., 2020). Nevertheless, our results showed an increase in the *nirK/nosZ* ratio in LPG-amended soil during maize season, indicating a greater potential for N loss *via* N_2O (Kunhikrishnan et al., 2016). However, it is certain that most of the NO_3^- generated by nitrification was uptaken by maize and ruzigrass. On the other hand, during soybean season, L and LPG treatments showed a lower ratio of *nirK/nosZ* abundance, indicating a greater tendency in converting N_2O into N_2, which suggests that these treatments could be a N_2O sink (Merloti et al., 2019). Additionally, Barton et al. (2008) suggested that increasing soil pH by liming may decrease N_2O emissions by restricting the availability of NO_2^- for reduction to N_2O. This hypothesis corroborates the data from the present study, in which the higher rate of nitrification that occurs in L- and LPG-amended soils is compensated by the greater uptake by plants (supported by ^{15}N recovery data), preventing NO_3^- from being converted back into NO_2^- and/or leached.

Finally, our multifaceted approach combining different sets of variables indicated that soil pH and SOC were the main soil properties responsible for increasing the abundances of total bacteria and archaea, as well as the genes of nitrifying microorganisms, while reducing the abundance of denitrifiers. Numerous studies have shown that *AmoA*-AOB are favored in nutrient-rich environments with high SOM content (Kunhikrishnan et al., 2016). By changing the soil pH, liming triggers soil buffering processes that increase soil nutrient availability (Holland et al., 2018), as reflected in the increased yield capacity of crops (Costa et al., 2018). The high return of plant residues in fertile soils leads to increased plant growth (roots and shoots), which usually results in higher SOC content in the long term (Inagaki et al., 2017).

In summary, the final N balance in the tropical system managed with lime and phosphogypsum indicates that soils with higher pH and rich in nutrients tend to increase the abundance of genes linked to nitrification. Our results suggest that plants established in fertile environments are more able to absorb the NO_3^- from N fertilizer nitrification, preventing its loss to the environment (regulatory mechanism of N loss). On the other hand, in low fertile soils (control and PG), despite the lower abundance of nitrifying genes, it suggests that a good part of the NO_3^- generated in nitrification (even if potentially lower than in corrected soils) is not fully absorbed by plants. This leads to greater potential for N losses in the soil-plant-atmosphere system. In conclusion, liming resulted in the largest changes in soil nutrient availability (fertility) and crop yields, but

phosphogypsum further potentiated these effects. Therefore, soil amendments such as LPG are an essential tool to trigger synergistic effects among high soil quality, crop yield, N fertilizer use efficiency and potential N_2O mitigation and increase agricultural system sustainability.

CRediT authorship contribution statement

João William Bossolani: Conceptualization, Methodology, Data curation, Software, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Carlos Alexandre Costa Crusciol:** Supervision, Project administration, Funding acquisition. **Eduardo Mariano:** Data curation, Software, Formal analysis. **Mariley Fonseca, Luiz Gustavo Moretti, Letusa Momesso and José Roberto Portugal:** Visualization, Data curation, Writing - review & editing. **N í dia Raquel Costa** and **Juliano Carlos Calonego:** Methodology, Data curation and Software. **Eiko Eurya Kuramae:** Supervision, Project administration, Writing - review & editing. All authors provided critical feedback on the manuscript and gave final approval for publication.

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.

Data availability

Data will be made available on request.

Acknowledgments

This study was supported by the São Paulo Research Foundation (FAPESP; grant numbers 2018/11063–7 and 2019/12764–1), the National Council for Scientific and Technological Development (CNPq; Universal Research Project: 421637/2018–8) and fellowship research productivity from the CNPq (310535/2021–2) to CACC.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2023.126907.

References

Alves, L.A., Ambrosini, V.G., Denardin, L.G., de, O., Flores, J.P.M., Martins, A.P., Filippi, D., Bremm, C., Carvalho, P.C., de, F., Farias, G.D., Ciampitti, I.A., Tiecher, T., 2021. Biological N2 fixation by soybeans grown with or without liming on acid soils in a no-till integrated crop-livestock system. Soil Tillage Res. 209. https://doi.org/10.1016/j.still.2020.104923.

Andrade, D.S., Murphy, P.J., Giller, K.E., 2002. Effects of liming and legume/cereal cropping on populations of indigenous rhizobia in an acid Brazilian Oxisol. Soil Biol. Biochem. 34, 477–485. https://doi.org/10.1016/S0038-0717(01)00206-1.

Barton, L., Kiese, R., Gatter, D., Butterbach-Bahl, K., Buck, R., Hinz, C., Murphy, D.V., 2008. Nitrous oxide emissions from a cropped soil in a semi-arid climate. Glob. Change Biol. 14, 177–192.

Beeckman, F., Motte, H., Beeckman, T., 2018. Nitrification in agricultural soils: impact, actors and mitigation. Curr. Opin. Biotechnol. 50, 166–173. https://doi.org/10.1016/j.copbio.2018.01.014.

Blake, G.R., Hartge, K.H., 1986. Bulk density. Methods soil Anal. Part 1 Phys. Mineral. methods 5, 363–375.

Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G., Costa, N.R., Tsai, S.M., Kuramae, E.E., 2020a. 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. https://doi.org/10.1016/j.geoderma.2020.114476.

Bossolani, J.W., Santos, F.L., Meneghette, H.H.A., Sanches, I.R., Moretti, L.G., Parra, L.F., Lazarini, E., 2020b. Soybean in crop rotation with maize and palisade grass intercropping enhances the long-term effects of surface liming in no-till system. J. Soil Sci. Plant Nutr. 20, 1–12. https://doi.org/10.1007/s42729-020-00347-2.

Bossolani, J.W., Crusciol, C.A.C., Garcia, A., Moretti, L.G., Portugal, J.R., Rodrigues, V. A., Fonseca, M., de, C., da, Calonego, J.C., Caires, E.F., Amado, T.J.C., Reis, A.R. 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. Front. Plant Sci. 12, 1437. https://doi.org/10.3389/fpls.2021.650296.

Bossolani, J.W., Crusciol, C.A.C., Leite, M.F.A., Merloti, L.F., Moretti, L.G., Pascoaloto, I. M., Kuramae, E.E., 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 Biol. Biochem. 156, 108188 https://doi.org/10.1016/j.soilbio.2021.108188.

Bossolani, J.W., Crusciol, C.A.C., Portugal, J.R., Moretti, L.G., Garcia, A., Rodrigues, V. A., Fonseca, M., de, C., da, Bernart, L., Vilela, R.G., Mendonça, L.P., Reis, A.R., 2021c. Long-term liming improves soil fertility and soybean root growth, reflecting improvements in leaf gas exchange and grain yield. Eur. J. Agron. 128, 126308 https://doi.org/10.1016/j.eja.2021.126308.

Bossolani, J.W., Crusciol, C.A.C., Momesso, L., Portugal, J.R., Moretti, L.G., Garcia, A., Fonseca, M., de, C., da, Rodrigues, V.A., Calonego, J.C., Andre, 2022a. Surface liming triggers improvements in subsoil fertility and root distribution to boost maize crop physiology, yield and revenue. Plant Soil 472, 1–23. https://doi.org/10.1007/s11104-022-05432-2.

Bossolani, J.W., Crusciol, C.A.C., Moretti, L.G., Garcia, A., Portugal, J.R., Bernart, L., Vilela, R.G., Caires, E.F., Amado, T.J.C., Calonego, J.C., dos Reis, A.R., 2022b. Improving soil fertility with lime and phosphogypsum enhances soybean yield and physiological characteristics. Agron. Sustain. Dev. 42 https://doi.org/10.1007/s13593-022-00765-9.

Caires, E.F., Guimarães, A.M., 2018. A novel phosphogypsum application recommendation method under continuous no-till management in Brazil. Agron. J. 110, 1987–1995. https://doi.org/10.2134/agronj2017.11.0642.

Cakmak, I., Kirkby, E.A., 2008. Role of magnesium in carbon partitioning and alleviating photooxidative damage. Physiol. Plant 133, 692–704. https://doi.org/10.1111/j.1399-3054.2007.01042.x.

Carmeis Filho, A.C.A., Crusciol, C.A.C., Castilhos, A.M., 2017a. Liming demand and plant growth improvements for an Oxisol under long-term no-till cropping. J. Agric. Sci. 155, 1093–1112. https://doi.org/10.1017/S0021859617000235.

Carmeis Filho, A.C.A., Crusciol, C.A.C., Guimarães, T.M., Calonego, J.C., Mooney, S.J., 2017b. Impact of amendments on the physical properties of soil under tropical long-term no till conditions. PLOS One 12, 27959897. https://doi.org/10.1371/journal.pone.0173011.

Ceccon, G., Staut, L.A., Sagrilo, E., Machado, L.A.Z., Nunes, D.P., Alves, V.B., 2013. Legumes and forage species sole or intercropped with corn in soybean-corn succession in midwestern Brazil. Rev. Bras. Ciência do Solo 37, 204–212. https://doi.org/10.1590/s0100-06832013000100021.

Costa, C.H.M., Carmeis Filho, A.C.A., Crusciol, C.A.C., Soratto, R.P., Guimarães, T.M., 2018. Intensive annual crop production and root development in a tropical acid soil under long-term no-till and soil-amendment management. Crop Pasture Sci. 69, 488–505. https://doi.org/10.1071/CP17233.

Crusciol, C.A.C., Marques, R.R., Carmeis Filho, A.C.A., Soratto, R.P., Costa, C.H.M., Ferrari Neto, J., Castro, G.S.A., Pariz, C.M., Castilhos, A.M., Franzluebbers, A.J., 2019. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutr. Cycl. Agroecosyst. 115, 347–372. https://doi.org/10.1007/s10705-019-10017-0.

Fageria, N.K., Nascente, A.S., 2014. Management of soil acidity of South American soils for sustainable crop production, Advances in Agronomy. Elsevier. https://doi.org/10.1016/B978–0-12–802139-2.00006–8.

Fehr, W.R., Caviness, C.E., 1977. Stages of soybean development.

Freitas, V.F., de, Cerezini, P., Hungria, M., Nogueira, M.A., 2022. Strategies to deal with drought-stress in biological nitrogen fixation in soybean. Appl. Soil Ecol. 172, 104352 https://doi.org/10.1016/j.apsoil.2021.104352.

Garbuio, F.J., Jones, D.L., Alleoni, L.R.F., Murphy, D.V., Caires, E.F., 2011. Carbon and nitrogen dynamics in an oxisol as affected by liming and crop residues under no-till. Soil Sci. Soc. Am. J. 75, 1723–1730. https://doi.org/10.2136/sssaj2010.0291.

Guo, A., Ding, L., Tang, Z., Zhao, Z., Duan, G., 2019. Microbial response to CaCO3 application in an acid soil in southern China. J. Environ. Sci. 79, 321–329. https://doi.org/10.1016/j.jes.2018.12.007.

Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: a review. Sci. Total Environ. 610–611, 316–332. https://doi.org/10.1016/j.scitotenv.2017.08.020.

Inagaki, T.M., de Moraes Sá, J.C., Caires, E.F., Gonçalves, D.R.P., 2017. Why does carbon increase in highly weathered soil under no-till upon lime and gypsum use. Sci. Total Environ. 599–600, 523–532. https://doi.org/10.1016/j.scitotenv.2017.04.234.

Jaghdani, S.J., Jahns, P., Tränkner, M., 2021. Mg deficiency induces photo-oxidative stress primarily by limiting CO_2 assimilation and not by limiting photosynthetic light utilization. Plant Sci. 302 https://doi.org/10.1016/j.plantsci.2020.110751.

Kunhikrishnan, A., Thangarajan, R., Bolan, N.S., Xu, Y., Mandal, S., Gleeson, D.B., Seshadri, B., Zaman, M., Barton, L., Tang, C., Luo, J., Dalal, R., Ding, W., Kirkham, M.B., Naidu, R., 2016. Functional relationships of soil acidification, liming, and greenhouse gas flux. Adv. Agron. 139, 1–71. https://doi.org/10.1016/bs.agron.2016.05.001.

Li, Y., Cui, S., Chang, S.X., Zhang, Q., 2019. Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. J. Soils Sediment. 19, 1393–1406. https://doi.org/10.1007/s11368-018-2120-2.

Liu, J., Liu, M., Wu, M., Jiang, C., Chen, X., Cai, Z., Wang, B., Zhang, J., Zhang, T., Li, Z., 2018. Soil pH rather than nutrients drive changes in microbial community following long-term fertilization in acidic Ultisols of southern China. J. Soils Sediment. 18, 1853–1864. https://doi.org/10.1007/s11368-018-1934-2.

Liu, X.Y., Rezaei Rashti, M., Esfandbod, M., Powell, B., Chen, C.R., 2018. Liming improves soil microbial growth, but trash blanket placement increases labile carbon and nitrogen availability in a sugarcane soil of subtropical Australia. Soil Res. 56, 235–243. https://doi.org/10.1071/SR17116.

Merloti, L.F., Mendes, L.W., Pedrinho, A., de Souza, L.F., Ferrari, B.M., Tsai, S.M., 2019. Forest-to-agriculture conversion in Amazon drives soil microbial communities and N-cycle. Soil Biol. Biochem. 137, 107567 https://doi.org/10.1016/j.soilbio.2019.107567.

Rao, I.M., Zeigler, R.S., Vera, R., Sarkarung, S., 1993. Selection and breeding for acid-soil tolerance in crops. Bioscience 43, 454–465.

Ritchey, K.D., Silva, J.E., Costa, U.F., 1982. Calcium deficiency in clayey B horizons of savanna oxisols. Soil Sci. 133, 378–382.

Ritchie, S.W., Hanway, J.J., Benson, G.O., 1993. How a corn plant develops. Special Report n. 48. Iowa State University of Science and Technology. Coop Ext Serv, Ames, IA, USA.

Rütting, T., Schleusner, P., Hink, L., Prosser, J.I., 2021. The contribution of ammonia-oxidizing archaea and bacteria to gross nitrification under different substrate availability. Soil Biol. Biochem. 160, 108353 https://doi.org/10.1016/j.soilbio.2021.108353.

Santos, D.R., Tiecher, T., Gonzatto, R., Santanna, M.A., Brunetto, G., da Silva, L.S., 2018. Long-term effect of surface and incorporated liming in the conversion of natural grassland to no-till system for grain production in a highly acidic sandy-loam Ultisol from South Brazilian Campos. Soil Tillage Res 180, 222–231. https://doi.org/10.1016/j.still.2018.03.014.

Shi, P., Zhang, Y., Hu, Z., Ma, K., Wang, H., Chai, T., 2017. The response of soil bacterial communities to mining subsidence in the west China aeolian sand area. Appl. Soil Ecol. 121, 1–10. https://doi.org/10.1016/j.apsoil.2017.09.020.

Soil Survey Staff, 2014. Keys to soil taxonomy. USDA - Natural Resources Conservation Service, Washington, DC, p. 360.

Soratto, R.P., Crusciol, C.A.C., 2008. Dolomite and phosphogypsum surface application effects on annual crops nutrition and yield. Agron. J. 100, 261–270. https://doi.org/10.2134/agronj2007.0120.

Tiecher, T., Pias, O.H., de, C., Bayer, C., Martins, A.P., Denardin, L.G., de, O., Anghinoni, I., 2018. Crop response to gypsum application to subtropical soils under no-till in Brazil: a systematic review. Rev. Bras. Cienc. do Solo 42, 1–17. https://doi.org/10.1590/18069657rbcs20170025.

Tiritan, C.S., Büll, L.T., Crusciol, C.A.C., Carmeis Filho, A.C.A., Fernandes, D.M., Nascente, A.S., 2016. Tillage system and lime application in a tropical region: Soil chemical fertility and corn yield in succession to degraded pastures. Soil Tillage Res. 155, 437–447. https://doi.org/10.1016/j.still.2015.06.012.

van Raij, B., Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1997. Recomendações da adubação e calagem para o Estado de São Paulo. Instituto Agronômico/Fundação IAC Campinas.

van Raij, B., Quaggio, J.A., Cantarella, H., Abreu, C.A., 2001. Os métodos de análise química do sistema IAC de análise de solo no contexto nacional. Análise Quím. Para. Aval. da Fertil. solos Trop. 5–39.

Weligama, C., Sale, P.W.G., Conyers, M.K., Liu, D.L., Tang, C., 2010. Nitrate leaching stimulates subsurface root growth of wheat and increases rhizosphere alkalisation in a highly acidic soil. Plant Soil 328, 119–132.

Yang, F., Zhang, Z., Barberán, A., Yang, Y., Hu, S., Guo, H., 2021. Nitrogen-induced acidification plays a vital role driving ecosystem functions: insights from a 6-year nitrogen enrichment experiment in a Tibetan alpine meadow. Soil Biol. Biochem. 153 https://doi.org/10.1016/j.soilbio.2020.108107.

Zhalnina, K., De Quadros, P.D., Gano, K.A., Davis-Richardson, A., Fagen, J.R., Brown, C. T., Giongo, A., Drew, J.C., Sayavedra-Soto, L.A., Arp, D.J., Camargo, F.A.O., Daroub, S.H., Clark, I.M., McGrath, S.P., Hirsch, P.R., Triplett, E.W., 2013. Ca. nitrososphaera and bradyrhizobium are inversely correlated and related to agricultural practices in long-term field experiments. Front. Microbiol. 4, 1–13. https://doi.org/10.3389/fmicb.2013.00104.

Zhou, W., Jones, D.L., Hu, R., Clark, I.M., Chadwick, D.R., 2020. Crop residue carbon-to-nitrogen ratio regulates denitrifier N2O production post flooding. Biol. Fertil. Soils. https://doi.org/10.1007/s00374-020-01462-z.

Zoca, S.M., Penn, C., 2017. An important tool with no instruction manual: a review of gypsum use in agriculture. Adv. Agron. 144, 1–44. https://doi.org/10.1016/bs.agron.2017.03.001.