



Higher lime rates for greater nitrogen recovery: A long-term no-till experiment labeled with ^{15}N

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ABSTRACT

Context or problem: Soil acidity limits crop growth and yield all over the world. Low grain yields is usually associated with poor soil fertility; however, little attention has been given to the nitrogen-based fertilizer use efficiency in soils managed with lime.

Objective: Given the current scenario of uncertainties regarding the availability and prices of fertilizers, our study aimed to understand how maize intercropped with ruzigrass and soybean plants develop in long-term soils managed with lime rates, and what the fate of the ^{15}N -labeled ammonium sulfate [$(^{15}\text{NH}_4)_2\text{SO}_4$] applied in the soil-plant system.

Methods: The treatments consisted of four dolomitic lime rates applied to the soil surface [control, half the recommended lime rate ($\frac{1}{2}$ RLR), full recommended lime rate (1 RLR) and double the recommended lime rate (2 RLR)].

Results: The higher lime rate (2 RLR) improved fertility, carbon and nitrogen stocks in the soil profile, and grain and/or stover production of maize, ruzigrass and soybean. As a consequence, maize and ruzigrass recovered a high amount of ^{15}N -fertilizer. On the other hand, soybean recovered less ^{15}N -fertilizer, regardless of treatment, but a greater amount was found in acidic soils. At the end of the maize and soybean growth cycles, our results showed that in 2 RLR-amended soil, the ^{15}N unrecovered was 71% lower than control. Finally, our results suggested that the use of low lime rates ($\frac{1}{2}$ RLR) may increase the ^{15}N losses potential to deep layers, whereas low amounts of ^{15}N were found in the subsoil when higher lime rates were applied.

Conclusions and implications: Soil acidity management through higher lime rates leads, over time, to increased soil fertility, resulting in a favorable environment for plant growth and the use of nitrogen fertilizers. In this way, it is possible to obtain a more productive and less costly agricultural system, and with less potential to pollute the environment.

1. Introduction

Lime application is an important practice for ameliorating soil acidity (Meng et al., 2019; Sjaudinis et al., 2020), a common issue in many tropical regions around the world (Li et al., 2019; Patra et al., 2021). Standard lime application practices have been developed for long-term conservation systems in which there is no soil disturbance, such as no-tillage systems (NTSs) (Tiritan et al., 2016; Carmeis Filho

et al., 2017a; Bossolani et al., 2021b). In addition to correcting soil acidity, liming improves soil fertility by supplying calcium (Ca^{2+}) and magnesium (Mg^{2+}), reducing toxic aluminum (Al^{3+}) levels, and increasing soil organic matter (SOM) content over time (Briedis et al., 2012; Bossolani et al., 2022a). As a consequence of soil improvements, the crop root system can grow to deep layers, leading to higher uptake of soil resources (water and nutrients) (Crusciol et al., 2019; Bossolani et al., 2021b, 2022a), and greater fertilizer use efficiency (Fageria and

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Nascente, 2014; Crusciol et al., 2022b).

For maize (*Zea mays* L.) and soybean (*Glycine max*) crops, nitrogen (N) is the most important nutrient (Bender et al., 2013, 2015). Biological nitrogen fixation (BNF) provides practically all necessary N for soybean (Freitas et al., 2022). However, for maize, N fertilizers are required, which significantly impacts production costs (Lu et al., 2021), particularly in the current scenario of rising fertilizer prices (Schnitkey et al., 2022). Furthermore, the complex dynamics of N in the soil–plant system can cause losses by volatilization, denitrification and leaching of nitrate (NO_3^-) in the soil profile (Zhou et al., 2021). Interestingly, leaching accounts for the majority of N losses (Tamagno et al., 2022). Nevertheless, N-fertilizer not absorbed by crops and present in soil surface layers (biologically active layers), can be lost by denitrification (Bossolani et al., 2020a). Agricultural practices that improve soil chemical characteristics can favor crop root growth, thereby allowing the exploitation of a greater volume of soil and reducing NO_3^- leaching and denitrification (Caires et al., 2016).

In NTSs, soil chemical attributes at deeper soil layers can be improved by applying higher rates of lime on the soil surface (Carneiro Filho et al., 2017a; Bossolani et al., 2022a), which can be enhanced by including tropical forage grasses in the system, particularly intercropped with maize (Ceccon et al., 2013; Costa et al., 2021). Tropical forage grasses, such as ruzigrass, present abundant and aggressive root growth, occupying a large volume of soil (Baptistella et al., 2020). When decomposing, these roots form biopores in the soil (Rosolem et al., 2017), facilitating the translocation of suspended particles from the lime to deeper soil layers (Tiritan et al., 2016; Bossolani et al., 2020b). Under these conditions, a soil acidity correction front forms in deeper layers down to 1.0 m, favoring greater growth of the subsequent crop root system, and thus increasing the possibility of NO_3^- absorption (Calonego and Rosolem, 2010), and reducing their losses to the environment (Rosolem et al., 2017).

In the present study, we hypothesized that the surface application of double the recommended lime rate under long-term NTS based on maize intercropped with ruzigrass followed by soybean would: i) improve chemical attributes in the soil profile; ii) increase biomass production; iii) increase the recovery of ^{15}N -fertilizer by maize, ruzigrass and soybean crops; and iv) reduce ^{15}N loss by potential leaching. To test these hypotheses, we evaluated the effects of surface application of different lime rates in a long-term experiment on soil fertility down to a depth of 100 cm; soil C and N stocks; maize, ruzigrass and soybean aboveground biomass production; the recovery of ^{15}N fertilizer by the crops; and the stratified soil ^{15}N down to 100 cm depth.

2. Material and methods

2.1. Site description and crop management

This study used a long-term (18 years) field experiment [registered by the Global Long Term Agricultural Experiments Network (GLTEN), Rothamsted Research, UK; <https://www.gltten.org/experiments/62>] established in Botucatu, São Paulo State, Brazil. This experiment was based on surface applications of lime in an agricultural system managed under long-term no-till. All geographical, climate and soil attributes are summarized in Table 1, and the climatic conditions during the experimental period are illustrated in Fig. 1A.

The chronological details of the crop management are detailed in Fig. 1A and B and Table 1. The maize grain crop was intercropped (same row) with ruzigrass (*Urochloa ruziziensis*). The maize stover (stalk, leaves, sheaths, tassel, core cob, and straw cob) was left in the field. After the maize harvest, live ruzigrass remained until October 2019, when it was chemically terminated using glyphosate (2.5 kg ha^{-1} a.i.). Soybean was then sown over the ruzigrass residue. For all crops, phytosanitary treatments were performed as necessary and recommended for maize and soybean.

Table 1

Geographic coordinates, climate and physical-chemical attributes, and crop management of the experimental field area. Botucatu, Brazil.

Site Description	Value	Unit	
Geographical coordinates			
Latitude	22° 83' 3' S	°	
Longitude	48° 42' 6' W	°	
Sea level	765	m	
Climate attributes			
Climate classification ^a	Mesothermal climate	Cwa	
Annual precipitation ^b	~1360	mm	
Air temperature (minimum)	15.3	°C	
Air temperature (maximum)	26.1	°C	
Initial soil physical attributes^c (0–20 cm)			
Soil type ^d	Typic Haplorthox	–	
Clay	347	g kg ^{–1}	
Silt	108	g kg ^{–1}	
Sand	545	g kg ^{–1}	
Bulk density	1.19	g cm ^{–3}	
Initial soil chemical attributes^e (0–20 cm)			
pH (CaCl ₂)	4.2	–	
Soil organic C (SOC)	12.2	g kg ^{–1}	
Phosphorus–available (P _{resin})	9.2	mg kg ^{–1}	
Calcium (Ca ²⁺ _{resin})	14.0		
Magnesium (Mg ²⁺ _{resin})	5.0	mmol _c kg ^{–1}	
Potassium (K ⁺ resin)	1.2	mmol _c kg ^{–1}	
Total acidity (H+Al) (at pH 7.0)	37.0	mmol _c kg ^{–1}	
Aluminum saturation (AS)	65.0	%	
Base saturation (BS)	35.0	%	
Cation exchange capacity (CEC _{pH 7.0})	57.0	%	
Crop management			
	Maize	Ruzigrass	Soybean
Sowing	Mar. 2019	Mar. 2019	Nov. 2019
Cultivar	Hybrid P3707VYH	Common	TMG 7062 IPRO
Row spacing (m)	0.45	–	0.45
Plant density (plants ha ^{–1})	65,000	10 kg seeds	280,000
Base fertilization (kg ha ^{–1})	28 N; 98 P ₂ O ₅ ; 56 K ₂ O	–	0 N; 70 P ₂ O ₅ ; 70 K ₂ O
Top dressing (N; kg ha ^{–1})	100	–	–
Harvest/desiccation	Jul. 2019	Oct. 2019	Mar. 2020

^a Alvares et al. (2013). ^bUnicamp (2020). ^cDonagema et al. (2017). ^dSoil Survey Staff (2014). ^ePrior to establishment of the study (2002), the initial soil properties were determined at a depth of 0–20 cm according to van Raij et al. (2001).

2.2. Experimental design and field management

A randomized complete block design involving four different treatments with four replicates each was used. Each plot was 57 m² (9.0 × 6.3 m). The treatments were (i) control (no liming); (ii) half the recommended lime rate ($\frac{1}{2}$ RLR); (iii) recommended lime rate (1 RLR) and (iv) twice the recommended lime rate (2 RLR). Over the 18-year experimental period, the treatments were applied four times (2002, 2004, 2010, and 2016). Reapplications were based on the results of annual assessments of base saturation. The lime rate was defined following the recommendations of van Raij et al. (1997) for fertilization and liming for the state of São Paulo, Brazil, considering base saturation (BS) and cation exchange capacity (CEC). Over the time, liming occurred in 2002 (beginning of the experiment; 1 RLR = 2.7 Mg ha^{-1}), 2004 (1 RLR = 2.0 Mg ha^{-1}), 2010 (1 RLR = 2.0 Mg ha^{-1}), and 2016 (1 RLR = 6.5 Mg ha^{-1}). The residual effects of liming was characterized in 2019, the third year after the last soil amendment reapplication (2016). The cropping history from 2002 to 2020 and the details of previous treatment applications (including the lime rates applied in each year) are given in Table S1.

2.3. 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

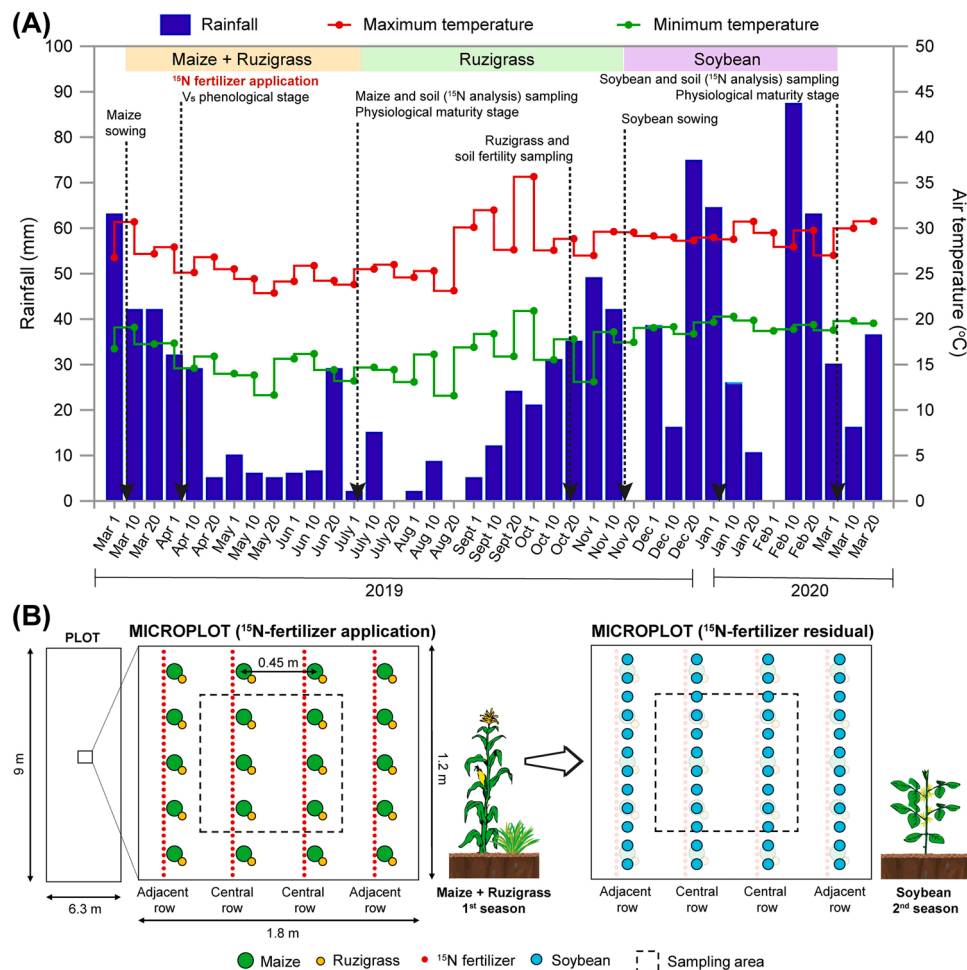


Fig. 1. (A) Weather conditions during the experimental period and (B) schematic representation of the plot, ¹⁵N-labeled fertilizer microplot, and sampling area.

layers of each plot using a soil push probe with an inner diameter of 50 mm. Sampling was performed in October 2019, before ruzigrass desiccation (3 years after the last application of lime rates and 17 years after the onset of the experiment). The soil was air-dried and ground to pass through a 2 mm sieve for chemical analysis according to (van Raij et al., 2001). Total organic carbon (C) and total N were analyzed by an elemental analyzer (LECO-TruSpec CHNS) using 0.2 g of soil. The samples were tested for the presence of CO₃²⁻-C according to the method described by (Nelson and Sommers, 1996). As no CO₃²⁻-C was found in the soil (last liming occurred in 2016 and soil samplings occurred in 2019), we assume that all C is present in organic forms and therefore designate this main reservoir as organic C (OC). Then, when C and N values were multiplied by the soil bulk density at each soil depth, originated the C and N stocks. Total C and N stocks were calculated by summing all soil layers.

2.4. Non-isotopic nitrogen use index

The non-isotopic N use index were calculated only for maize and ruzigrass crops. Soybean performs biological N fixation, which can lead to misinterpretations of the conventional calculations used here. Nitrogen usage index (NUI) and N uptake efficiency (NUpE) were calculated according to the following equations:

$$NUI \text{ (kg DMkg}^{-1}\text{N}_{ac}) = \frac{\text{total aboveground DM}}{\text{total N}_{ac}} \quad (1)$$

$$NUpE \text{ (kgN}_{ac} \text{ kg}^{-1} \text{N}_{ap}) = \frac{N_{ac}}{N_{dose}} \quad (2)$$

were DM is the total dry matter accumulated on aboveground of maize or ruzigrass; N_{ac} is the total N accumulated on total DM; and N_{ap} is the applied N dose (28 kg ha⁻¹ at sowing + 100 kg ha⁻¹ at topdressing).

2.5. Establishment of ¹⁵N microplots

Unconfined microplots (1.8 × 1.2 m) were set up in each treatment during the maize + ruzigrass season (Fig. 1 C). All agricultural management in the microplots matched those of the main plots. Each microplot consisted of four rows of five maize plants each (~4.2 plants m⁻¹). ¹⁵N-labeled ammonium sulfate [(¹⁵NH₄)₂SO₄] fertilizer with an abundance of 6.31 atom % ¹⁵N excess (Sigma-Aldrich Inc., St. Louis, MO, USA) was applied as topdressing (100 kg ha⁻¹ N) at the V₅ maize stage. The microplots received only ¹⁵N-labeled fertilizer, whereas the main plots received unlabeled ammonium sulfate. After lab processing, the maize stover was returned to the microplot to ensure the cycling of the ¹⁵N present in the plant residues. After the maize harvest, the recovery of residual ¹⁵N-labeled fertilizer by soybean in the microplot was assessed.

2.6. Sampling procedure of ¹⁵N-labeled material and isotopic analyses

At maize physiological maturity (R₆ stage), six maize plants were sampled from each microplot. The maize plants were partitioned into vegetative fractions (stalk, leaves, sheaths, tassels, 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 65 °C to constant weight to obtain the dry weight. The

same procedure was used to dry the grain fraction. Subsamples of the dried stover and dried grain 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 m² was collected (at ground level). The ruzigrass biomass samples were oven-dried, and a subsample of the dry plant material was ground in a Wiley mill. Similar to maize, the remaining biomass was returned to the microplots. At the beginning of soybean physiological maturity [R₇ phenological stage; (Fehr and Caviness, 1977); March 2020], before leaf senescence, six soybean 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 were carried out. Plants from each main plot (maize stover and grains, ruzigrass, and soybean stover and grains) were subjected to the same procedure above to assess the natural ¹⁵N abundance. All milled plant tissue was used to determine the total N concentration and ¹⁵N measurements.

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

2.7. ¹⁵N calculations

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

$$Ndff \text{ (kg ha}^{-1}\text{)} = (a/b) \times TN \quad (3)$$

$$Ndfs \text{ (kg ha}^{-1}\text{)} = TN - Ndff \quad (4)$$

$$^{15}\text{N recovery (\%)} = (Ndff/NFR) \times 100 \quad (5)$$

$$^{15}\text{N unrecovered}_{FS} \text{ (\%)} = 100 - ^{15}\text{N recovery}_{TFS} \quad (6)$$

$$^{15}\text{N remaining (\%)} = ^{15}\text{N recovery}_{TFS} - ^{15}\text{N recovery}_{maize \text{ grain}} \quad (7)$$

$$^{15}\text{N unrecovered}_{SS} \text{ (\%)} = ^{15}\text{N remaining} - ^{15}\text{N recovery}_{SS} \quad (8)$$

where Ndff is the N derived from fertilizer; *a* and *b* are the ¹⁵N enrichment (atom % ¹⁵N excess) in the plant (maize/ruzigrass/soybean) fractions (stover and grain) or soil and in the substrate (fertilizer), respectively (a natural abundance of 0.368 atom % ¹⁵N was considered in the calculations); *TN* represents the N accumulation (kg ha⁻¹) in the plant fractions or soil; Ndffs is the N derived from other sources (BNF

and/or soil); ¹⁵N recovery is the percentage of fertilizer N recovery; *NFR* is the N fertilizer rate applied (kg ha⁻¹); ¹⁵N unrecovered_{FS} and ¹⁵N unrecovered_{SS} are the percentages of N fertilizer unaccounted for (i.e., potential losses) after maize + ruzigrass (¹⁵N-labeled fertilizer applied in the maize + ruzigrass season) and after soybean (¹⁵N-labeled fertilizer residual in the second season), respectively; ¹⁵N recovery_{TFS} is the total N recovery (%; sum of maize stover and grain, ruzigrass, and soil) in the maize + ruzigrass season; ¹⁵N remaining is the amount of ¹⁵N available in the system before soybean was grown; ¹⁵N recovery_{maize grain} is the amount of ¹⁵N exported in maize grain; ¹⁵N recovery_{TFS} is the total N recovery (%; sum of soybean stover and grain and soil) in the second season.

Isotopic analysis was performed at the Stable Isotopes Center at São Paulo State University – UNESP, Brazil. Dry and homogenized soil samples were weighed to a mass of 30–35 mg in 5 × 8 mm tin capsules (PN 24006400, Thermo Scientific, Germany). The capsules were analyzed in a continuous flow isotope ratio mass spectrometry system (CF-IRMS) in which an IRMS (Delta V, Thermo Scientific, Germany) was coupled to an elemental analyzer (Flash EA, Thermo Scientific, Germany) through a gas interface (ConFlo IV, Thermo Scientific, Germany). The IRMS determined the isotopic ratio of N (¹⁵N/¹⁴N) expressed as the relative difference in the isotopic ratio (δ15N) in ‰ according to the following equation (Coplen, 2011):

$$\delta^{15}\text{N} = \frac{R(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{R(^{15}\text{N}/^{14}\text{N})_{\text{Air}}} - 1 \quad (9)$$

The results were normalized via two-point anchoring (Paul et al., 2007) using the IAEA-N-1 and IAEA-311 standards. The CF-IRMS standard uncertainty for δ15N is ± 0.15‰ and ± 56.64‰ for IAEA-N-1 and IAEA-311, respectively.

2.8. Statistical analysis

Our dataset were tested for normality [Anderson–Darling test; (Nelson, 1998)] and homoscedasticity [Levene's test; (Levene, 1960)]. Subsequently, the means were subjected to analysis of individual variance (one-way ANOVA) by the F test (*p* ≤ 0.05). Lime rates were included as fixed effects. Blocks, growing seasons and depth (soil variables) were considered as random factors. When significant, means were compared using the modified t test [Fisher's protected least significant difference (LSD), at *p* ≤ 0.05].

3. Results

3.1. Soil chemical analysis

The lasting effects of surface liming at 48 months after the last lime reapplication (2016) maintained soil pH ≥ 5.0 to a depth of 20 cm in 2 RLR-amended soil, whereas at lower lime rates, soil pH ≥ 5.0 occurred only to a depth of 10 cm (Table 2). In addition, soil managed with 2 RLR presented the highest soil pH values to a depth of 100 cm, ranging from 6 (0–5 cm) to 4.06 (80–100 cm), whereas the pH range with depth was 4.08 (0–5 cm) to 3.75 (80–100 cm) in the control treatment.

Over time, the application of 2 RLR also increased CEC to a depth of 20 cm, whereas BS was higher in all soil layers to a depth of 100 cm, with a range of 72.8% (0–5 cm) to 6.55% (80–100 cm). By contrast, in the control treatment, BS ranged from 15.3% (0–5 cm) to 0.63% (80–100 cm). In general, the higher the lime rate, the higher the BS in all soil layers.

Even in deep layers, the lasting effects of the two highest lime rates (1 RLR and 2 RLR) reduced the Al³⁺ concentration. Down to a depth of 10 cm, the C stock was highest in 2 RLR-amended soil (20.3 Mg ha⁻¹), but at deeper soil layers, the C stock did not differ between 1 RLR and 2 RLR. Interestingly, the N stock down to a depth of 20 cm was highest at 2 RLR (4.05 Mg ha⁻¹), followed by 1 RLR (3.53 Mg ha⁻¹); however, at

Table 2

Soil attributes according to the lime rate [control (no liming), half the recommended lime rate ($\frac{1}{2}$ RLR), recommended lime rate (1 RLR) and twice the recommended lime rate (2 RLR)] in seven stratified soil layers to a depth of 100 cm.

Soil depth (cm)	Lime rates	pH (CaCl ₂)	C stock (Mg ha ⁻¹)	N stock (Mg ha ⁻¹)	BS %	CEC (mmol _c kg ⁻¹)	Al ³⁺
0–5	Control	4.08 d [†]	4.88c	0.84c	15.3 d	92 b	4.45 a
	$\frac{1}{2}$ RLR	5.32c	6.80 b	0.92c	40.9c	106 ab	1.16 b
	1 RLR	5.66 b	7.92 b	1.09 b	59.0 b	108 ab	0.15c
	2 RLR	6.00 a	10.8 a	1.37 a	72.8 a	126 a	0.16c
5–10	Control	3.68c	4.10 d	0.72 b	13.0 d	99c	6.68 a
	$\frac{1}{2}$ RLR	5.01 bc	5.71c	0.82 ab	36.7c	110 b	1.34 b
	1 RLR	5.25 b	6.81 b	0.86 a	55.6 b	108 b	0.26c
	2 RLR	5.40 a	9.50 a	0.94 a	70.4 a	125 a	0.24c
10–20	Control	4.01c	10.4 b	1.09c	6.78 d	103 a	14.9 a
	$\frac{1}{2}$ RLR	4.66 bc	12.4 ab	1.39 b	22.3c	99 a	2.65 b
	1 RLR	4.83 ab	13.6 a	1.58 ab	38.1 b	100 a	1.42c
	2 RLR	5.01 a	14.1 a	1.74 a	51.6 a	104 a	1.06c
20–40	Control	3.98c	22.8 b	2.34 a	4.82 d	124 b	15.5 a
	$\frac{1}{2}$ RLR	4.11bc	26.1 a	2.40 a	15.9c	120 b	14.3 b
	1 RLR	4.30 ab	27.2 a	2.53 a	25.8 b	146 a	3.87c
	2 RLR	4.53 a	28.6 a	2.49 a	41.5 a	143 a	2.85c
40–60	Control	3.83 b	20.6 b	2.47 b	2.16 d	164 a	16.7 a
	$\frac{1}{2}$ RLR	4.00 ab	22.7 a	2.91 a	7.14c	160 a	14.1 b
	1 RLR	4.03 ab	23.7 a	2.30 b	11.1 b	162 a	10.3c
	2 RLR	4.14 a	24.3 a	2.31 b	17.5 a	157 a	9.40c
60–80	Control	3.76c	20.7 a	2.22 a	0.89 d	186 a	20.5 a
	$\frac{1}{2}$ RLR	3.91 bc	21.4 a	2.61 a	3.24c	186 a	17.2 ab
	1 RLR	4.02 ab	22.3 a	2.28 a	5.33 b	189 a	14.7 bc
	2 RLR	4.13 a	22.7 a	1.59 b	9.15 a	187 a	11.3c
80–100	Control	3.75 b	17.6 b	1.93 ab	0.63 d	181 a	20.9 a
	$\frac{1}{2}$ RLR	3.84 ab	18.8 ab	2.14 a	2.20c	187 a	18.2 a
	1 RLR	3.97 a	19.5 a	1.86 b	3.65 b	181 a	14.1 b
	2 RLR	4.06 a	19.7 a	1.37c	6.55 a	184 a	11.7 b

[†] Different lowercase letters for each soil layer indicate significant differences between treatments by Fisher's protected LSD test at $p \leq 0.05$.

depths greater than 40 cm, the N stock was highest at $\frac{1}{2}$ RLR (7.66 Mg ha⁻¹) and in the control (6.62 Mg ha⁻¹), followed by 1 RLR and 2 RLR (average = 5.86 Mg ha⁻¹). At a depth of 100 cm, the total C stock increased with increasing lime rate as follows: 2 RLR (130 Mg ha⁻¹), 1 RLR (121 Mg ha⁻¹), $\frac{1}{2}$ RLR (114 Mg ha⁻¹), and control (101 Mg ha⁻¹) (Fig. S1A). By contrast, the total N stock at 100 cm was highest at $\frac{1}{2}$ RLR (13.2 Mg ha⁻¹) and lowest in the control (11.6 Mg ha⁻¹) (Fig. S1B).

3.2. Total dry matter production and grain yield

Surface-applied lime resulted in pronounced differences in total

aboveground dry matter production during the first (maize + ruzigrass) and second (soybean) growing seasons (Fig. 2; Table S1). During the maize + ruzigrass season, soil managed with 2 RLR produced 20.57 Mg ha⁻¹ of aboveground dry matter (stover = 6.24 Mg ha⁻¹; grain = 6.25 Mg ha⁻¹; ruzigrass = 8.08 Mg ha⁻¹), an increase of ~75% compared with the control treatment (total dry matter = 11.76 Mg ha⁻¹; stover = 4.11 Mg ha⁻¹; grain = 2.98 Mg ha⁻¹; ruzigrass = 4.67 Mg ha⁻¹) (Fig. 2A). Moreover, applying 2 RLR increased total dry matter by ~53.3% and 13.3% compared with $\frac{1}{2}$ RLR and 1 RLR, respectively. Similar results were obtained for soybean (Fig. 2C). Specifically, the control treatment produced ~4.35 Mg ha⁻¹ of aboveground dry matter (stover = 2.4 Mg ha⁻¹; grain = 1.95 Mg ha⁻¹), ~28.9%, 59.5% and 80% less than $\frac{1}{2}$ RLR (stover = 2.95 Mg ha⁻¹; grain = 2.6 Mg ha⁻¹), 1 RLR (stover = 3.35 Mg ha⁻¹; grain = 3.59 Mg ha⁻¹), and 2 RLR (stover = 3.61 Mg ha⁻¹; grain = 4.23 Mg ha⁻¹). Considering the grain yield (grains at 13% of humidity), the highest lime rate increased the grain yield of maize from 3.43 (control) to 7.18 Mg ha⁻¹ (2 RLR), and soybean by 2.24 (control) to 4.86 Mg ha⁻¹ (2 RLR) (Fig. 2B and D).

3.3. Nitrogen use efficiency during the maize + ruzigrass season

The rates of N use by maize and ruzigrass were also positively influenced by liming (Fig. 3; Table S2). The N usage index of maize and ruzigrass were higher at 2 RLR (maize = 1.0 kg DM kg⁻¹ N_{ac}; ruzigrass = 0.4 kg DM kg⁻¹ N_{ac}) and 1 RLR (maize = 0.91 kg DM kg⁻¹ N_{ac}; ruzigrass = 0.35 kg DM kg⁻¹ N_{ac}) than in the other treatments (average maize = 0.71 kg DM kg⁻¹ N_{ac}; average ruzigrass = 0.22 kg DM kg⁻¹ N_{ac}) (Fig. 3A, B). The N uptake efficiency of maize and ruzigrass also increased with increasing lime rate (Fig. 3C, D). The N uptake efficiency of maize and ruzigrass plants was highest at 2 RLR (maize = 1.55 kg N_{ac} kg⁻¹ N_{ap}; ruzigrass = 1.62 kg N_{ac} kg⁻¹ N_{ap}), followed by 1 RLR (maize = 1.35 kg N_{ac} kg⁻¹ N_{ap}; ruzigrass = 1.45 kg N_{ac} kg⁻¹ N_{ap}), $\frac{1}{2}$ RLR (maize = 0.94 kg N_{ac} kg⁻¹ N_{ap}; ruzigrass = 1.11 kg N_{ac} kg⁻¹ N_{ap}), and the control (maize = 0.73 kg N_{ac} kg⁻¹ N_{ap}; ruzigrass = 1.07 kg N_{ac} kg⁻¹ N_{ap}).

3.4. Fate of ¹⁵N fertilizer in the soil-plant system

¹⁵N fertilizer recovery by the crops was positively affected by the lime rate (Fig. 4). Here, the values presented as percentages are equivalent to the values in kg ha⁻¹, as the applied dose of N was 100 kg ha⁻¹. During the first season (maize + ruzigrass), ¹⁵N recovery was highest at 2 RLR (stover = 8.2%; grain = 29.6%; ruzigrass = 30.1%), followed by 1 RLR (stover = 7.2%; grain = 26.4%; ruzigrass = 25.9%), $\frac{1}{2}$ RLR (stover = 6.1%; grain = 16.1%; ruzigrass = 24.5%), and the control (stover = 5.9%; grain = 10.3%; ruzigrass = 22.9%) (Fig. 4A). The treatments had the strongest effect on grain ¹⁵N recovery. Considering all treatments, approximately 75% of the ¹⁵N from fertilizer found in maize shoots was exported by the grains. The ¹⁵N found in maize grains (fraction exported by harvest) was 187%, 156% and 56% higher at 2 RLR (29.6%) than in the control, $\frac{1}{2}$ RLR and 1 RLR, respectively. Soil ¹⁵N retention (to a depth of 100 cm) was higher at $\frac{1}{2}$ RLR and 1 RLR (average = 21.9%) than in the control and 2 RLR (average = 18%). Importantly, unrecovered ¹⁵N was highest in the control treatment (43.1%), followed by $\frac{1}{2}$ RLR (32.2%), 1 RLR (17.8%) and 2 RLR (13.9%); even though ¹⁵N export was highest at 1 RLR and 2 RLR, the amount of ¹⁵N remaining at the end of the first season was ~7% higher in these treatments (average = 52.6%) than in the control and at $\frac{1}{2}$ RLR (average = 49.2%) (Fig. 4B). Interestingly, most of the total N found in the shoots of maize and ruzigrass came from the soil (76–80%) rather than N fertilizer (20–24%) (Fig. 5A and B). Regardless of which fraction (N fertilizer or soil) the N is sourced from, 2 RLR provided the highest N accumulation in maize and ruzigrass plants.

Unlike maize, the recovery of ¹⁵N fertilizer by soybean was low (Fig. 4C). On average, soybean stover and grains recovered 2.1% of the ¹⁵N applied to the system. Interestingly, ¹⁵N recovery by soybean was highest in the control (stover = 0.82%; grain = 2.11%), which, although

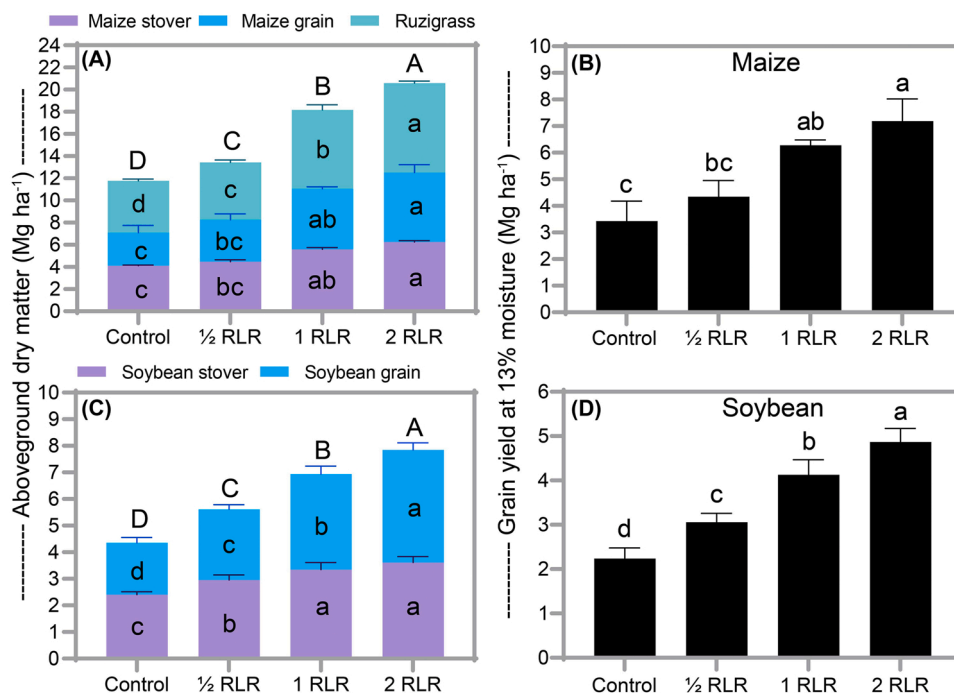


Fig. 2. Aboveground (stover + grain) dry matter yield in the (A) first (maize + ruzigrass) and (B) second (soybean) growing seasons in response to lime rate [control (no liming), half the recommended lime rate (1/2 RLR), recommended lime rate (1 RLR) and twice the recommended lime rate (2 RLR)]. Different 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 ($n = 4$).

low, was on average ~58% higher than in the other treatments. The amount of ^{15}N retained in the soil (down to 100 cm depth) did not differ among the treatments with liming (average = 37.36%) and was ~13.5% higher in these treatments than in the control treatment (32.9%). Additionally, the amount of unrecovered ^{15}N fertilizer during the soybean season did not differ between treatments (average = 14.3%). After the soybean harvest, the amount of ^{15}N remaining in the control treatment (33.7%) was lower than the average for the liming treatments (37.9%) (Fig. 4D). Considering the entire agricultural year (first + second seasons), total unrecovered ^{15}N was lowest in the treatments that received liming (Fig. 4E). Unrecovered ^{15}N was highest in the control treatment (53.9%), whereas ^{15}N loss was lowest in 2 RLR (30.3%), followed by 1 RLR (34.5%) and 1/2 RLR (45.4%).

The N accumulation in the soybean shoots (stover + grain) showed that ~98% of the total N came from other sources (BNF + soil), whereas only ~2% came from the N fertilizer (Fig. 5C). Soybean plants established in acid soil (control treatment) were more dependent on soil N (even if in low proportion) than in more fertile soils. On the other hand, N derived from other sources increased by 50% using 1/2 RLR, 92% on 1 RLR and by 125% on 2 RLR treatments.

3.5. ^{15}N fertilizer retention in the soil profile

^{15}N retention in the soil profile after the first and second seasons varied with the liming rate (Fig. 6). Long-term application of higher lime rates (1 RLR and 2 RLR) increased ^{15}N retention in the uppermost soil layers (0–20 cm; average = 12.9%), but in layers below 60 cm, ^{15}N fertilizer retention was highest at 1/2 RLR (5%) and lowest at 2 RLR (2.1%) (Fig. 6A). After the soybean harvest, ^{15}N fertilizer retention was highest in the 0–5 cm layer (average = 9.67%), and ^{15}N fertilizer retention in this layer decreased in the order 2 RLR (11.71%) > 1 RLR (11.15%) > 1/2 RLR (8.74%) > control (7.08%) (Fig. 6B). At a depth of 5–20 cm, ^{15}N fertilizer retention was higher at 1 RLR and 2 RLR (9.5%) than the average of 1/2 RLR and the control (5.6%). Interestingly, in the deepest layer (80–100 cm), ^{15}N fertilizer retention was highest at 1/2 RLR (5.74%), followed by the control (4.95%), 2 RLR (2.47%), and 1 RLR

(1.79%).

4. Discussion

4.1. Soil profile fertility and biomass production

Surface application of lime without soil disturbance is a viable long-term practice to reduce subsoil acidity and increase soil profile fertility in tropical agricultural systems managed under no-till, but the magnitude of the effect varies depending on the rate of lime application. Here, we determined the long-term impact on subsoil fertility of four applications (2002, 2004, 2010 and 2016) of lime rates over 17 years. Even 36 months after the last lime reapplication, the highest lime rate (2 RLR) was associated with $\text{pH} > 5.0$ to a depth of 20 cm, the soil layer where the concentration of crop roots is generally greatest (Rellán-Álvarez et al., 2016; Rosolem et al., 2017). Compared with 1 RLR, 2 RLR also increased soil pH to a depth of 80 cm. Increased pH directly impacts the concentration of free Al^{3+} in the soil layers. According to Bossolani et al. (2022), high lime rates reduce Al^{3+} and boost the growth and distribution of the root system in the soil profile, leading to increased acquisition of water and nutrients from the soil. Al^{3+} toxicity is one of the main factors negatively affecting root growth (Parker et al., 1988; Reis et al., 2018). The increase in soil pH [which induced deprotonation of acidic groups and increased negative charges (increase in CEC)] (Limousin and Tessier, 2007) combined with the supply of Ca^{2+} and Mg^{2+} by sedimentary dolomitic lime (calcium and magnesium carbonates) (Bossolani et al., 2020b) contributed to greater retention of Ca^{2+} and Mg^{2+} and, consequently, to increased BS along the soil profile. Notably, there was a strong effect of the applied lime rates on BS variations at soil layers below 20 cm.

As a consequence of the increase in soil fertility at higher lime application rates, aboveground biomass (straw and grain) production by maize, ruzigrass and soybean was increased. Numerous studies have reported increased biomass and grain production in lime-amended soils (Joris et al., 2016; Carmeis Filho et al., 2017a; Bossolani et al., 2018; Anderson et al., 2020; Crusciol et al., 2022a), primarily due to improved

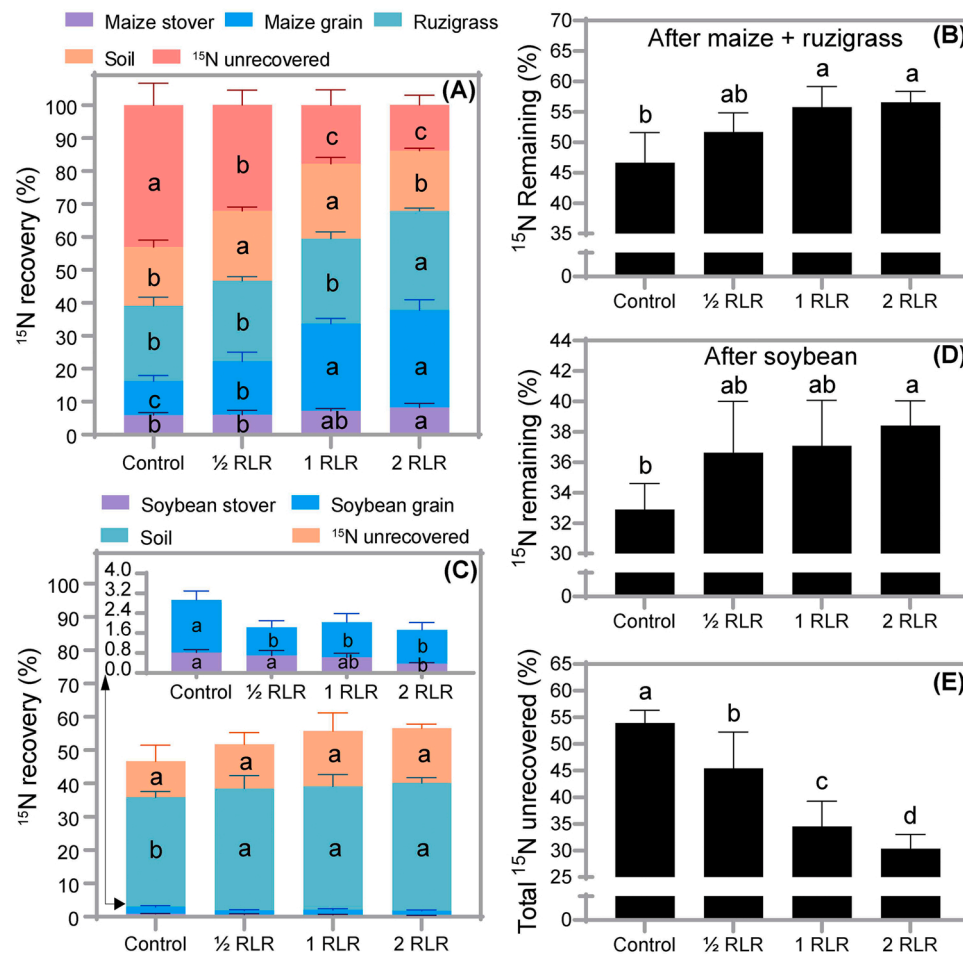


Fig. 3. Usage index (A = maize; B = ruzigrass) and uptake efficiency (C = maize; D = ruzigrass) of nitrogen in response to lime rate [control (no liming), half the recommended lime rate (1/2 RLR), recommended lime rate (1 RLR) and twice the recommended lime rate (2 RLR)]. Different lowercase or capital 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 ($n = 4$).

soil profile fertility, lower Al^{3+} levels and increased root growth (greater exploitation of soil resources). Interestingly, we also observed an increase in the N use index of maize and ruzigrass (N usage index and N uptake efficiency) in the present study, which, together with the increased availability of nutrients in the soil profile, may have positively impacted plant growth.

Here, our results showed that high lime rates are essential for building up fertility in the soil profile over the years. Tropical regions are characterized by low rainfall during autumn/winter cultivation (maize season) and recurrent dry spells during spring/summer cultivation (soybean season) (Cunningham, 2020); therefore, improving the production environment is essential for maize and soybean to reach high grain yields and production stability (Carmeis Filho et al., 2017a; Bossolani et al., 2021b, 2022a).

In our study, we observed high input of organic residues into soil managed with higher lime rates. C input from biomass and its relationship with SOC stocks are important indicators of the influence of lime on C sequestration (Briedis et al., 2012; Carmeis Filho et al., 2017b; Inagaki et al., 2017) and soil quality (Inagaki et al., 2016; Bossolani et al., 2021a). Crop residues are the main source of SOM (Briedis et al., 2012). Both the quantity and quality of the crop biomass (crop rotation; Table S1) that accumulated between 2002 and 2019 impacted the accumulation of C in the soil profile, as evidenced by the increase in the C stock with increasing lime rate.

The N stock is influenced by many factors (Song et al., 2018). For example, after mineralization by soil microbes, SOM becomes a potential source of mineral N for crops (Bertol et al., 2022). Interestingly,

increasing pH by liming helps increase the rate of mineralization of SOM (Carmeis Filho et al., 2017b; Holland et al., 2018), resulting in greater N release to the soil. However, SOM mineralization alone does not explain the reduction in the N stock in the soil profile in lime-amended soil; nutrient export also plays a direct role. The higher the grain yield, the higher the export of nutrients, including N (Jones et al., 2013). In general, maize export (nutrient removal from area by crop harvest) approximately 15–18 kg N Mg⁻¹ of grain produced (Bender et al., 2013), whereas soybean, which are largely benefited from BNF, exports ~45–60 kg N Mg⁻¹ of grain produced (Bender et al., 2015; Esper Neto et al., 2021). Nutrient export may explain the higher N stock in the soil profile at 1/2 RLR (which had the lowest grain yield with the exception of the control) compared with 1 RLR and 2 RLR. Nutrient export can directly influence the soil N stock. The N stock was low in the control treatment due to the lower biomass C input by the crops over the years and the lower N fertilizer use efficiency due to low absorption and the high rate of denitrification in acidic soils (Jones et al., 2013), which caused a large part of the N be lost to the environment (leaching and/or denitrification) (Zhou et al., 2021; Tamagno et al., 2022).

4.2. ¹⁵N fate in the soil-plant system

Increasing soil fertility by applying lime altered ¹⁵N fate in the soil-plant system. Maize and ruzigrass plants grown in lime-amended soils, particularly when 2 RLR was applied, presented higher ¹⁵N fertilizer recovery in the aboveground biomass (stover of maize and ruzigrass, and maize grain). The N fertilizer recovered by crops is exported

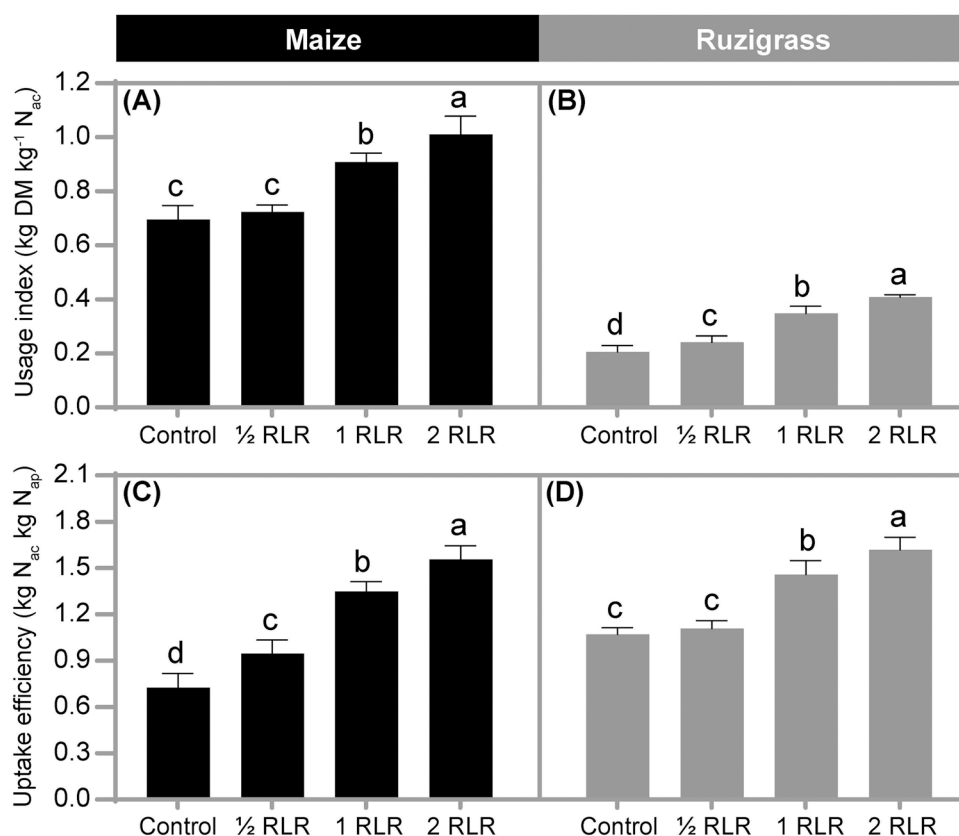


Fig. 4. (A, C) ^{15}N recovery in each compartment (plant, soil or unrecovered) in the (A) first (maize + ruzigrass) and (C) second (soybean) growing seasons; (B, D) ^{15}N fertilizer remaining after the (B) first and (D) second growing seasons; (E) total ^{15}N unrecovered (first + second growing seasons) in response to lime rate [control (no liming), half the recommended lime rate (1/2 RLR), recommended lime rate (1 RLR) and twice the recommended lime rate (2 RLR)]. 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 ($n = 4$).

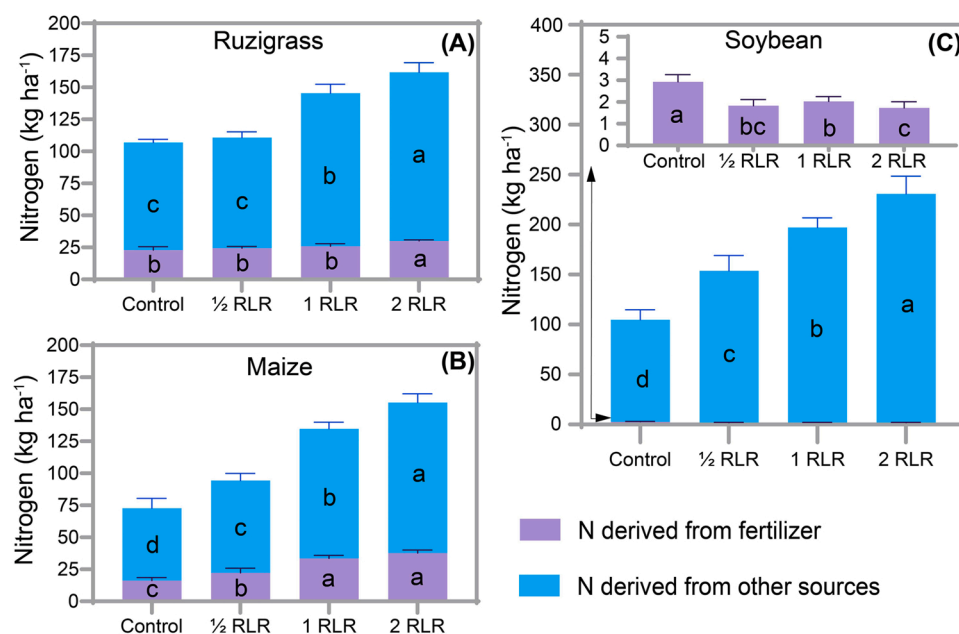


Fig. 5. Unlabeled nitrogen derived from fertilizer and from other sources (BNF and/or soil) in ruzigrass (A), maize (B) and soybean (C) shoots in response to lime rate [control (no liming), half the recommended lime rate (1/2 RLR), recommended lime rate (1 RLR) and twice the recommended lime rate (2 RLR)]. 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 ($n = 4$).

in the grain harvest or returned to the system by nutrient cycling from crop residues (Chen et al., 2014), as supported by our results for ^{15}N remaining in the system. To increase N fertilizer use efficiency in agricultural systems, most of the applied fertilizer must be absorbed and assimilated by the crops and converted into biomass (Fageria and Moreira, 2011). The combination of soil acidity correction and soil fertility improvement increases soil exploitation by plant roots (Bossolani et al., 2022a), leading to greater fertilizer absorption capacity

(Fageria and Nascente, 2014). In our study, the N fertilizer efficiency was one of the main factors for maize to achieve higher grain yield. Interestingly, Bossolani et al. (2022) reported that the number of grains per plant and the 100-grains weight were the main production components affected by higher lime rates, which directly impacted the final maize grain yield. Ammonium-based fertilizers undergo nitrification (conversion of NH_4^+ to NO_3^-), which is enhanced in acidity-corrected soils (Karaivazoglou et al., 2007). Although nitrification is more likely to

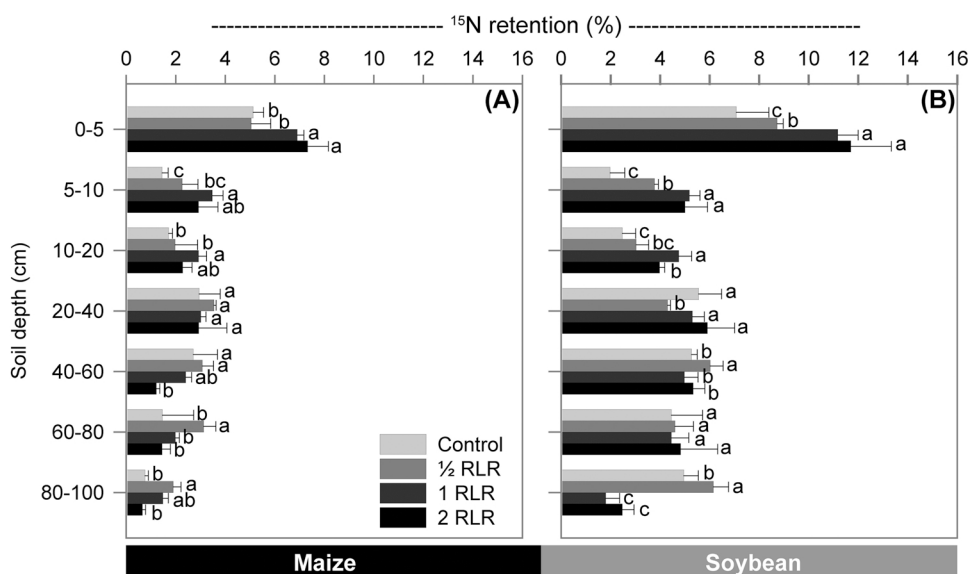


Fig. 6. ^{15}N retention in seven stratified soil layers to a depth of 100 cm after the (A) first (maize + ruzigrass) and (B) second (soybean) growing seasons in response to lime rate [control (no liming), half the recommended lime rate ($\frac{1}{2}$ RLR), recommended lime rate (1 RLR) and twice the recommended lime rate (2 RLR)]. 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 ($n = 4$).

increase in soils that are managed with high lime rates (Holland et al., 2018; Bossolani et al., 2020a), increasing the tendency for N losses through leaching and denitrification (Tamagno et al., 2022), such soils are also more fertile and boost crop root growth (Rellán-Álvarez et al., 2016). Under these conditions, most of the N present in the soil (NH_4^+ or NO_3^-) is quickly absorbed by the plants, reducing their propensity to lose N to the environment (Caires et al., 2016; Rosolem et al., 2017). The results of the analysis of unrecovered ^{15}N fertilizer during the maize + ruzigrass season are consistent with these effects. Soils managed with 2 RLR and, to a lesser extent, 1 RLR presented the lowest potential N losses, whereas unrecovered ^{15}N was highest in the control (no amendments applied) and at $\frac{1}{2}$ RLR.

Nitrogen crop demand can be met by supplying inorganic N (fertilizers) and/or through N mineralization from SOM (SOM-N) (Chen et al., 2014). Based on these assumptions, it is important to emphasize that the increase in biomass production of maize and ruzigrass plants was not totally dependent on the N derived from the fertilizer. Most of the N accumulated in stover and grains was derived from the soil, especially from the SOM-N fraction (Dourado-Neto et al., 2010). These authors confirmed that N from the net N mineralization of SOM was the dominant source of N in the crops. In this way, N fertilizer functions as a readily available source for plant uptake, while SOM-derived N is released throughout the crop cycle (Mwfulirwa et al., 2017). Therefore, long-term management with lime is a important tool that increases the crop N use efficiency from mineral N fertilizers (Crusciol et al., 2022a), and increases the increment of SOM in the system by increasing the production of biomass by the roots and shoots of cultivated plants (Bossolani et al., 2022a; b), as supported by our results of SOC contents. As a consequence, SOM will become a natural reservoir of N and other elements in the soil, in addition to several other benefits linked to soil quality (Nunes et al., 2018).

Crop rotation of maize (intercropped or not with forage grasses) and soybean is the most common model in Brazil (Cecon et al., 2013). Here, we showed that managing soil with 2 RLR led to higher production of biomass (stover and grain) and greater recovery of ^{15}N fertilizer by crops during the maize + ruzigrass season (and consequently, less unrecovered ^{15}N); by contrast, ^{15}N recovery by soybean was highest in the control and at $\frac{1}{2}$ RLR. There are two main explanations for the opposing results for these crops. First, because ^{15}N fertilizer recovery was higher during the first season (part exported by grain, part retained in crop residues), a smaller amount of fertilizer remained in the soil to be absorbed by soybean in succession. Nitrogen fertilizer recovery by the successor crop is usually low (Smith and Chalk, 2018), especially when

it comes to a leguminous plant with high BNF efficiency, such as soybean. Second, most of the N present in soybean plant tissues ($\sim 90\%$) comes from BNF (Freitas et al., 2022), which is improved by liming (Alves et al., 2021). Our results also showed that only 2% of the total N in soybean shoots was derived from fertilizer, whereas 98% was derived from other sources (presumed to come predominantly from BNF, and to a lesser extent from SOM mineralization). Liming increases soybean root growth (Bossolani et al., 2021b) and, in turn, the area available for nodulation by *Bradyrhizobium* sp. (Alves et al., 2021). The higher availability of nutrients in soils managed with lime [e.g., P, Ca, Mg, (Costa et al., 2018)] also increases the efficiency of nodules in converting atmospheric N_2 into ureides. The combination of these effects may have reduced the dependence of soybean on fertilizer-derived ^{15}N . Several studies have shown that the abundance (Andrade et al., 2002) and activity (Zhalnina et al., 2013) of Rhizobia species are lower in highly acidic soil, leading to lower BNF capacity of soybean.

Interestingly, after the soybean harvest, ^{15}N fertilizer remaining was highest in soil amended with 2 RLR, followed by 1 RLR, $\frac{1}{2}$ RLR, and the control. This pattern is consistent with the pattern of unrecovered ^{15}N fertilizer during the agricultural year (maize + ruzigrass/soybean seasons). When ^{15}N recovery by crops is greater, fertilizer loss to the environment is reduced. ^{15}N fertilizer losses were $\sim 71\%$ higher in the control (highest loss, 54%) than at 2 RLR (lowest loss, 31%).

4.3. Stratified ^{15}N retention in the soil profile

In addition to the amount of ^{15}N fertilizer remaining in the soil (down to a depth of 100 cm), its distribution in the soil profile is an important factor in better understanding the dynamics of N in soil managed with liming. According to our results, as the lime rate and, consequently, soil fertility increased, maize and ruzigrass, both highly N-demanding cereals (Omara et al., 2019), became more able to recover high amounts of ^{15}N fertilizer (based on data from ^{15}N recovery) and store them in aboveground crop residues. After nutrient cycling, this ^{15}N is available in the uppermost soil layers, as supported by our ^{15}N retention results. In addition, due to the improvement in the soil profile (i.e., higher availability of nutrients and lower levels of Al^{3+}), root development can also increase (Rosolem et al., 2017), enabling the absorption of high amounts of N (mostly as NO_3^-) (Yu et al., 2015) and reducing the N concentration in intermediate soil layers (5–20 cm). Beginning with the 20–40 cm layer (in which ^{15}N retention did not differ significantly among the treatments), the amount of ^{15}N fertilizer in deeper layers tended to be the highest in soil managed with $\frac{1}{2}$ RLR. The

same trend was observed after soybean cultivation in the control and $\frac{1}{2}$ RLR treatments. These results have an important implication for tropical agricultural systems: applying low lime rates may increase N leaching (probably as NO_3^-). The surface application of lime at low rates (e.g., $\frac{1}{2}$ RLR) increases soil pH in the surface layers (where N fertilizer is applied and the release of N forms is greatest after nutrient cycling) and, consequently, soil nitrification rates (Beekman et al., 2018; Holland et al., 2018) in the same manner as higher lime rates. Liming has been shown to potentiate soil N transformation by microorganisms (Bossolani et al., 2020a), thereby regulating the rates of conversion of NH_4^+ to NO_3^- by nitrification. However, in the present study, soil fertility at depths below 10 cm was lower when $\frac{1}{2}$ RLR was applied. As a consequence, the NO_3^- generated by nitrification in the uppermost soil layers was absorbed in smaller amounts than in soils corrected with higher lime rates, given that root growth and distribution are better in the fertile soils, as observed by Bossolani et al. (2022) in a study of the root development of soybean and maize in the same experimental area.

During crop development, as long as there are no limitations on root development and/or water restriction, the NO_3^- generated by nitrification is taken up by crops; conversely, when N uptake is low, there is a risk of NO_3^- leaching (Holland et al., 2018). Plants established in soils corrected with 1 RLR and, in particular, 2 RLR produced higher amounts of biomass (grain and/or straw) and recovered greater amounts of ^{15}N fertilizer than plants grown in less fertile soils (control and $\frac{1}{2}$ RLR). This indicates that a higher amount of ^{15}N fertilizer was lost to the environment (supported by unrecovered ^{15}N data) and that part of the N fertilizer may have been leached (high amounts of ^{15}N in deep layers) and/or denitrified (Zhou et al., 2021). Although the supply of N is greater in less fertile soils due to lower crop uptake, the remaining N is liable to loss by leaching and denitrification, processes that are harmful to the environment and indicate low sustainability of the agricultural system. Despite the strong evidence validating the hypotheses raised here, more studies must be carried out to better understand the dynamics of N in soils treated with low lime rates.

5. Conclusions

Lime application to the soil surface under a no-till system ameliorated subsoil acidity by increasing soil pH, base saturation and reducing Al^{3+} toxicity. The effects were proportional to the lime rates used (2 RLR > 1 RLR > $\frac{1}{2}$ RLR > control). In addition, higher lime rates increased C stock on soil profile, even at 1 m depth. Maize intercropped with ruzigrass and soybean grown in fertile soils by the application of high lime rates presented higher aboveground biomass production (grains and/or straw), which led to an increase in the recovery of ^{15}N -labeled fertilizer, mainly by maize and by ruzigrass. Soybean recovered a small amount of ^{15}N , regardless of treatment, but larger amounts were found in plants established in acidic soils (control and $\frac{1}{2}$ RLR). The final balance of potential ^{15}N -fertilizer losses (total ^{15}N unrecovered) showed that the higher the recovery of ^{15}N -fertilizer, the smaller the amount that can be lost to the environment. Interestingly, this study showed that large amounts of ^{15}N -fertilizer were found in deep soil layers when $\frac{1}{2}$ RLR was applied, indicating that low lime rates, or even its absence, can increase losses (leaching) of unabsorbed N-fertilizer by the crops, resulting in low sustainability of agricultural systems.

CRediT authorship contribution statement

João William Bossolani: Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft. **Carlos Alexandre Costa Crusciol:** Project administration, Funding acquisition, Supervision. **Eduardo Mariano:** Formal analysis, Visualization, Writing – review & editing. **Luiz Gustavo Moretti:** Investigation, Validation, Writing – review & editing. **José Roberto Portugal:** Investigation, Validation, Writing – review & editing. **Mariley Fonseca:** Investigation, Validation, Writing – review & editing. **Letusa Momesso:** Visualization,

Writing – review & editing. **Andressa Selestina Dalla Còrt:** Visualization, Writing – review & editing. **Vladimir Eliodoro Costa:** Isotopic analysis, Visualization, Writing – review & editing. **Heitor Cantarella:** Visualization, Validation, Writing – review & editing.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2023.108971.

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