



Nitrogen use efficiency and recovery in a wheat-corn rotation under tropical savannah conditions

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Abstract Stable isotopes (e.g., ^{15}N) can be used to develop best practices for fertilizer management in cereal crops under tropical conditions. The objectives of this study were to determine the N contribution from fertilizer and soil to wheat and corn grown in rotation and the residual N contribution from fertilizer applied to wheat and carried over to corn under Brazilian savannah conditions. This study was established in a no-till system on Rhodic Haplustox soil in a randomized complete block design with four replications. The N treatments applied to the wheat crops were 0, 20, 40, 60, 80 and 160 kg (urea- ^{15}N) ha $^{-1}$. The residual effects of N on the succeeding corn crop were

evaluated. During the corn phase, two additional treatments were included: a control (0 N) and a treatment with 160 kg N ha $^{-1}$. Regression analysis showed that the highest estimated wheat yield (5415 kg ha $^{-1}$) was observed with the application of 114 kg N ha $^{-1}$. Significant effects from the residual N were observed in the corn shoot biomass and straw N uptake. On average, the recovery rate of the urea N fertilizer was 34% for wheat, and the residual urea N fertilizer use in corn (when applied to wheat) was less than 5% of the amount initially applied. The effect of the residual N was not enough to meet the N demands of the succeeding corn crop. The results of this study provide producers and the scientific community with good estimates of nitrogen use efficiency for wheat and corn.

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Introduction

Brazil is a large importer of wheat (*Triticum aestivum* L.) and the world's third largest producer of corn (*Zea mays* L.) (Conab 2019). Wheat is an annual crop that has significant economic importance among cool-season cereals due to its high grain yield capacity (Fang et al. 2017; Farooq et al. 2017). In Brazil, the average wheat yield (2580 kg ha⁻¹) is low compared to high-yielding areas of Europe (e.g., Ireland, the Netherlands and the United Kingdom, with averages of 10,100, 9170, and 8580 kg ha⁻¹, respectively), Africa (e.g., Zambia, Namibia and Egypt, with averages of 7160, 6670, and 6510 kg ha⁻¹, respectively) and the USA (with an average of 3630 kg ha⁻¹) (FAO 2017; Conab 2019; USDA 2019). In the last 5 years, more than 50% of the wheat consumed in Brazil has been imported (Conab 2019).

Approximately 90% of the wheat produced in Brazil is cultivated in the southern region, suggesting that crop failure could generate a large wheat deficit in the country. For this reason, the expansion of wheat cultivation to the savannah region should be considered a food security issue. Similarly, corn is one of the most important crops for human and animal consumption (Foster et al. 2016) and ethanol production (Moreira et al. 2020). However, the mean Brazilian corn yield (5476 kg ha⁻¹) is low compared with high-yielding areas in the USA (10,840 kg ha⁻¹) and Europe (8399 kg ha⁻¹) (Eurostat 2018; USDA 2018; Conab 2019).

The Brazilian grain production system is dominated by corn and soybean rotation practices. Wheat could be integrated into rotations under savannah conditions to increase crop diversity. The low corn and wheat yields observed in Brazil are usually associated with low-fertility soils, so high fertilizer inputs are required to maximize yields. For example, as much as 15% of the total operating cost of the annual crops produced in the Brazilian savannah is spent on N side dressing application (including the fertilizer cost and field operations) (Galindo et al. 2018). It has been estimated that wheat and corn receive approximately one-third

of all the N-based fertilizers produced in the world (Ladha et al. 2016). The Brazilian savannah area comprises approximately 200 million hectares, equivalent to 23% of the country's total land area (IBGE 2019), making it the main area for potential crop expansion in Brazil.

The optimum amount of N required for optimal wheat and corn production depends on many factors, including soil type, soil organic matter, indigenous nutrient concentration in the soil, and weather conditions (Austin et al. 2019; Sanchez et al. 2019). Globally, the average N application rates for wheat and corn are approximately 52 and 80 kg ha⁻¹ year⁻¹, respectively (Ladha et al. 2016). Under tropical conditions, the maximum productivity of wheat and corn has been achieved by applying N at rates ranging between 70 and 240 kg N ha⁻¹ (Mendes et al. 2013; Munareto et al. 2019; Nunes et al. 2015; Galindo et al. 2017, 2019a, b). In recent decades, significant technological improvements in plant genetics and nutrition and soil management and machinery as well as the introduction of no-tillage practices, cover crops, and crop rotation have helped increase agricultural sustainability in Brazil. Despite this, nutrient management strategies still need improvement, particularly N management (Galindo et al. 2017, 2019a; Gil et al. 2018).

Nitrogen is a dynamic nutrient in the soil and is involved in many processes, such as ammonification, nitrification, denitrification and mineralization (Rosolem et al. 2017; Silva et al. 2017, 2018). When those processes are coupled with poor fertilizer management practices, such as the application of inadequate rates, broadcast application without incorporation, or excessively high rates applied to poorly drained soils, low nitrogen use efficiency is likely to occur (Mira et al. 2017; Cantarella et al. 2018). Poor N management has contributed to increased N losses by ammonia (NH₃) volatilization, nitrate (NO₃) leaching, and nitrous oxide (N₂O) emissions, which have both economic and environmental consequences (Martins et al. 2015, 2017; Awale and Chatterjee 2017; Snyder 2017).

Urea is the most commonly used N fertilizer worldwide, showing advantages over other common N-based fertilizers, such as high N content (45–46% N), relatively low cost per unit of N, and availability in most markets (Silva et al. 2017; Ribeiro et al. 2020). In addition, urea has high solubility and low corrosion rates, has high foliar uptake, is promptly available to

plants, and has low soil acidification rates compared to other N sources (Cantarella et al. 2018). However, it also has negative effects, such as high hygroscopicity and high potential for losses through NH_3 volatilization (Cantarella et al. 2018). Ammonia volatilization from applied fertilizers is an important issue when urea is applied to the soil surface in no-till systems and is thus not incorporated after application (Rosolem et al. 2017; Silva et al. 2017).

Under tropical conditions, urea is usually applied as a side dressing, with plant residues on the soil surface blocking or delaying soil-fertilizer contact, enhancing urease activity far above that of bare soil and favoring NH_3 volatilization (Mira et al. 2017). Losses of NH_3 are higher in no-till systems than in conventional tillage systems due to the higher activity of the urease enzyme (Liu et al. 2019). The urease enzyme is produced by soil microbes and plants (Cantarella et al. 2018), and increased plant residues on the soil surface increase its activity (Silva et al. 2017). Crop residues also reduce the contact of urea with soil particles, reducing NH_4^+ adsorption to the cation exchange sites of soil colloids and potentially increasing NH_3 losses (Silva et al. 2017; Cantarella et al. 2018; Liu et al. 2019). Up to 60% of the N from urea fertilizers applied to agricultural soils may volatilize as NH_3 (Dutta et al. 2016; Pan et al. 2016). It has been estimated that NH_3 volatilization contributes 32 Tg to the annual N loss worldwide (Beusen et al. 2008; Mandal et al. 2016). Several studies have shown that NH_3 losses in tropical and subtropical Brazilian soils typically range from 38–78% to 10–25% of the applied N, respectively (Fontoura and Bayer 2010; Viero et al. 2014, 2017; Ribeiro et al. 2020). In contrast, NO_3 leaching in Brazilian soils was estimated to range from trace amounts up to 87 kg ha⁻¹ year⁻¹, and losses from N fertilizer application usually exceed 10–15 kg ha⁻¹ year⁻¹ (Rosolem et al. 2017).

Considering that over 85% of Brazilian grain production systems are not tilled, there is an urgent need to improve N management to enhance its use efficiency and reduce losses. Studies are needed to provide a better understanding and evaluate N use efficiency and N recovery as accurately as possible. This can be achieved with stable isotope (¹⁵N) techniques. Additional studies are needed to determine the relative N contributions from fertilizer, previous plant residues, and soil and how they impact plant development and grain yield in wheat and corn in

Brazilian savannah ecosystems. This information will be valuable to farmers and will help develop the best N management practices for reducing N loss. The objectives of this study were to i) determine the fertilizer and soil N contributions to wheat and corn grown in rotation in a tropical environment and ii) determine the residual N contribution from urea applied to a wheat crop and the potential carry over to the succeeding crop (corn) under Brazilian savannah conditions.

Materials and methods

Location and management history

This study was carried out near the town of Selvíria (Savannah region), state of Mato Grosso do Sul, Brazil (20° 22' S, 51° 22' W; 335 m above sea level (a.s.l.)), (Sup. Figure 1) during the 2018 (wheat) and 2018/19 (corn) crop years. According to Köppen's classification (Alvares et al. 2013), the climate in this region is Aw (tropical with a dry winter). The soil at the experimental site was classified as a Clayey Oxisol (Rhodic Haplustox) (Soil Survey Staff 2014). The soil chemical and particle size properties were determined from soil samples collected prior to wheat planting and analyzed according to Raj et al. (2001) and Embrapa (2017), while the total N was determined by the Kjeldahl method (Otto et al. 2013) (Sup. Table 1). The experimental area has been cultivated with annual crops (cereal and legume crops) for over 30 years, with the last 14 years under no-tillage practices. Prior to planting wheat, the crop sequence was as follows: corn during the 2016/17 growing season, corn during the 2017 growing season, and cowpea [*Vigna unguiculata* (L.) Walp] during the 2017/18 growing season (Sup. Figure 2). The residue remaining following the 2017/18 cowpea crop was collected from 10 random points in the experimental area using a 0.5 m² quadrat. The residue sampled was used to characterize tissue nutrient uptake (Sup. Table 2). The maximum, average and minimum temperatures and rainfall observed during the study are presented in Sup. Figure 3.

Experimental design and treatments

The experimental design consisted of a randomized complete block with four replicates. The treatments

consisted of the application of N to wheat at six rates: 0, 20, 40, 60, 80 and 160 kg N ha⁻¹. At planting, N was applied at a rate of 20 kg ha⁻¹ in the form of urea-¹⁵N. Split applications were used for application rates of 40, 60, 80 and 160 kg N ha⁻¹: 20 kg N ha⁻¹ of a conventional urea source (nonlabeled urea) was applied at planting and 20, 40, 60 and 140 kg N ha⁻¹ of urea-¹⁵N was applied as side dressing at tillering, when the wheat was at the GS21 decimal growth stage (Zadoks et al. 1974). Split application of N fertilizer is a common practice used by farmers growing cereals in Brazil. To evaluate the residual effect of the applied N-labeled fertilizer on corn (the succeeding crop), corn planting took place in the same plots as the preceding crop (wheat). For the corn phase, two new treatments were included: i) control (total absence of N application (i.e., the negative control)) and ii) split application of 160 kg N ha⁻¹ (20 kg N ha⁻¹ of conventional urea applied at planting and 140 kg N ha⁻¹ of urea-¹⁵N applied as side dressing (i.e., the positive control)). These two treatments were used to compare the residual and in-season effects of N application (160 kg N ha⁻¹) to corn. This applied N rate was based on fertilizer recommendations for corn in the Brazilian savannah region (Galindo et al. 2019a). For wheat, the experimental plots were composed of 13.5-m planted rows spaced at a distance of 0.17 m. The useful area of the plot consisted of the eight central rows, with 0.5 m excluded from each end. The isotope solution was applied to the six central rows in an area 0.98 m long spaced at a distance of 0.17 m, totaling 1 m² per plot. For the corn, the experimental plots were composed of seven 5-m planted rows spaced 0.45 m apart. The useful area of the plot was the central four rows, with 0.5 m excluded from each end. The total row spacing of wheat (13 rows × 0.17 m = 2.21 m) was smaller than that of corn (7 rows × 0.45 m = 3.15 m). Therefore, the reference point for the wheat and corn crops was the subplot, where the isotope solution was applied. For the in-season N application (160 kg N ha⁻¹) to the corn crop, the isotope solution was applied in the two central rows, over an area 1.11 m long (with 0.45 m of space between rows), totaling 1 m² per plot. An isotopic enrichment of 5.366% excess ¹⁵N atoms was used for all the application rates. To facilitate uniform application, the N-labeled fertilizer was diluted in 200 mL of distilled water and then applied to the subplots. These subplots were carefully delimited with

wooden stakes and double sticky tape. More details concerning the experimental plots are presented in Sup. Figure 4.

Field management

Wheat

Before wheat planting, weeds were controlled by applying 2,4-D (670 g of the active ingredient [a.i.] ha⁻¹) and glyphosate (1800 g a.i. ha⁻¹). At planting, 250 kg ha⁻¹ of N-P-K 08-28-16 was applied to all the treatments based on soil analysis and the expected yield goal, except for the control and the 20 kg N ha⁻¹ (urea-¹⁵N) treatment, which received 70 and 40 kg ha⁻¹ of P₂O₅ and K₂O applied as single superphosphate and potassium chloride, respectively. Wheat genotype CD 150 was planted on 4 May 2018 at a planting rate of 70 seeds per m. Wheat emerged on 9 May 2018. Supplemental irrigation was applied using a center pivot sprinkling system at a 14 mm watering depth when necessary. The herbicide metsulfuron-methyl (3 g a.i. ha⁻¹) was applied on 29 May 2018 to control postemergence weeds. Side dressing N applications were manually performed on 9 June 2018. The N recovery for the treatment receiving 20 kg N ha⁻¹ of urea-¹⁵N at planting was used in the calculations of total N recovery in all the treatments receiving N-labeled fertilizer. Harvest was performed manually on 27 August 2018.

Corn

Before corn planting, weeds were controlled by applying 2,4-D (670 g a.i. ha⁻¹) and glyphosate (1800 g a.i. ha⁻¹). The corn hybrid DOW 2B710 PW was planted at a rate of 73,333 plants ha⁻¹ on 1 November 2018 and emerged on 6 November 2018. To assess the residual effect of N application to wheat on the succeeding crop, the corn was planted in the same plots where the wheat was planted. At planting, 250 kg ha⁻¹ of N-P-K formula 08-28-16 (equivalent to 20, 70 and 40 kg ha⁻¹ of N, P₂O₅ and K₂O, respectively) was applied to all the treatments except the control. This fertilizer application strategy is a common practice used by farmers growing cereals in this region of Brazil.

A single application of 20 kg N ha⁻¹ of urea-¹⁵N was included in the corn phase and was used in the

calculations of the total N recovery in all the treatments receiving the N-labeled fertilizer. The following recovery efficiencies were observed for the treatments that received 20 kg N ha⁻¹ of urea-¹⁵N. A total of 5.6% (representing 4.2 kg ha⁻¹) of the N taken up in the corn straw was derived from the labeled fertilizer, and the remaining 94.4% (representing 79.8 kg ha⁻¹) was derived from soil and other sources. In addition, it was estimated that the N recovery from N fertilizer application was 21.1% (4.2 kg out of 20 kg of applied N-labeled fertilizer). A total of 4.1% (representing 7.1 kg ha⁻¹) of the N taken up in the corn grain was derived from the labeled fertilizer, with the remaining 95.9% (representing 170.1 kg ha⁻¹) being derived from soil and other sources. It was estimated that the N recovery rate was 35.3% (7.1 kg out of 20 kg of applied N-labeled fertilizer). An additional treatment was added to the corn phase: the split application of 160 kg N ha⁻¹ (20 kg N ha⁻¹ of conventional urea applied at planting and 140 kg N ha⁻¹ of urea-¹⁵N applied as side dressing). All the side dressing applications were performed when the corn reached the V6 stage (six leaves completely unfolded) and took place on 6 December 2018. When necessary, the corn crop was irrigated with supplementary irrigation using a center pivot sprinkling system (same as for the wheat crop). The herbicides atrazine (1000 g ha⁻¹ of a.i.) and tembotrione (84 g ha⁻¹ of a.i.) and a vegetable oil adjuvant (720 g ha⁻¹ of a.i.) were applied to control postemergence weeds on 28 November 2018. Triflurumuron (24 g ha⁻¹ a.i.) and methomyl (215 g ha⁻¹ a.i.) were applied for pest control on 7 December 2018. The corn was manually harvested on 8 March 2019.

Data collection

We monitored the in-season leaf chlorophyll index (for wheat and corn), determined indirectly by collecting readings on 30 wheat flag leaves and 20 corn ear leaves when plants reached the flowering stage, using a portable nondestructive ClorofiLOG[®] meter, model CFL-1030 (Falker, 2008). At harvest, we recorded the plant height (from the ground level to the tip of the spike for wheat and to the apex of the tassel for corn), number of spikes per m² (wheat), and stem diameter (corn). Ten spikes of wheat and 10 ears of corn were collected to determine the number of

spikelets per spike (wheat), number of grains per spikelet (wheat), numbers of rows per ear and grains per row (corn), numbers of grains per spike and grains per ear (wheat and corn), ear length and diameter (corn), number of defective grains (by calculating the number of undeveloped spikelets per spike; wheat), hectoliter mass (corresponding to the mass of a wheat grain in a volume of 100 L, adjusted by 13% for wheat grain moisture), mass of 100 grains (using a precision scale with 0.01 g uncertainty (Marte/Shimadzu[®] model BL3200H 3200 GX), with the grain moisture content adjusted to 13% for wheat and corn), shoot biomass (determined by collecting all the wheat plants in a sampling area measuring 0.17 m² and all the corn plants in a sampling area measuring 0.45 m² (values were extrapolated to kg ha⁻¹), and grain yield (determined by harvesting the spikes and ears contained in the useful area of each wheat and corn plot, with grain moisture content adjusted to 13% (wet basis).

Nitrogen accumulation, recovery and use efficiency by wheat and corn plants

During the evaluation of ¹⁵N enrichment, we considered leaves and stems for wheat and leaves, stems, cobs and tassels (i.e., the residue) for corn (straw). The wheat plants in an area 0.50 m² and four corn plants were collected from the center row of each plot at physiological maturity. Wheat and corn grains were manually separated from the straw. The straw of both crops was cut and mixed, and grain and straw subsamples were obtained for ¹⁵N analysis. These samples were dried at 65 °C for 72 h, ground to pass through a 2-mm sieve, and weighed on a precision scale (Marte/Shimadzu[®] model AY220). The total N content and ¹⁵N (atom percentage) abundance were determined using a mass spectrometer interfaced with an N elemental analyzer (isotope ratio mass spectrometry (IRMS); HYDRA 20-20 ANCA-GLS), according to Barrie and Prosser (1996). The isotopic abundance of the applied urea-¹⁵N and the natural variation in the ¹⁵N stable isotope (0.366%) were taken into consideration during the recovery calculations for the applied labeled fertilizer.

The proportion and amount of labeled N recovered in wheat and corn, the N recovered from other sources (native biological N fixation, remaining straw, soil, rainfall and others), and the residual effect of the N

application rates on corn were calculated using the following equations:

- (a) Accumulated N in straw and grain (straw and grain N uptake): AN (kg ha⁻¹)

$$AN = (N \times DM) \div 1000 \quad (1)$$

where N is the N concentration (g kg⁻¹) in the tissue and DM is the total dry matter (kg ha⁻¹).

- (b) Proportion of N in straw and grain derived from fertilizer (Npdff, %)

The proportion of the excess ¹⁵N atoms in straw or grain and in the fertilizer were obtained using the principle of isotopic dilution:

$$\begin{aligned} \%Npdff &= (\% \text{ of } N^{15} \text{ atoms in excess in the plant} \\ &\div \% \text{ of } N^{15} \text{ atoms in excess in the fertilizer}) \times 100 \end{aligned} \quad (2)$$

where the percentage of excess ¹⁵N atoms in the plant is the percentage of ¹⁵N atoms in the plant subtracted from the natural abundance of ¹⁵N (0.366%), and the percentage of excess ¹⁵N atoms in the fertilizer is the percentage of ¹⁵N atoms in the fertilizer subtracted from the natural ¹⁵N abundance (0.366%).

- (c) Amount of N in straw and grain derived from fertilizer (ANdff, kg ha⁻¹)

$$ANdff = (\% Ndff \times AN) \div 100 \quad (3)$$

where AN is the accumulated N (straw and grain N uptake) (kg ha⁻¹).

- (d) Amount of N in straw and grain derived from soil and of the other sources (native biological N fixation, remaining straw, soil, rainfall and others) (ANdfs, kg ha⁻¹)

$$ANdfs = AN - ANdff \quad (4)$$

- (e) Proportion of N in straw and grain derived from soil and other sources (Npdfs, %)

$$\% Npdfs = (ANdfs \div AN) \times 100 \quad (5)$$

- (f) Nitrogen recovery (NRec, %) in straw and grain

N utilization refers to the N recovered from fertilizer applied in the form of urea-¹⁵N:

$$NRec = (ANdff \div ANA) \times 100 \quad (6)$$

where ANA is the amount of N applied as labeled fertilizer (kg ha⁻¹).

The total N recovery (TNRec) was calculated by using the NRec of the N in the urea-¹⁵N applied at planting added to the NRec of N in the urea-¹⁵N applied as a side dressing.

Nitrogen uptake (NUP—kg N accumulated kg N applied⁻¹), N utilization (NUT—kg grain kg N accumulated⁻¹) and N use efficiency (NUE—kg grain kg N applied⁻¹) were calculated according to Moll et al. (1982):

$$NUP = (NT \text{ at } N_f - NT \text{ at } 0 \text{ kg N ha}^{-1}) \div N_f \quad (7)$$

where NT is the total N accumulated (kg ha⁻¹) in the shoot (aerial part + grain) (shoot N uptake) and N_f is the rate of mineral fertilizer application (kg ha⁻¹).

$$NUT = (GY \text{ at } N_f - GY \text{ at } 0 \text{ kg N ha}^{-1}) \div (NT \text{ at } N_f - NT \text{ at } 0 \text{ kg N ha}^{-1}) \quad (8)$$

where GY is the grain yield (kg ha⁻¹).

$$NUE = (GY \text{ at } N_f - GY \text{ at } 0 \text{ kg N ha}^{-1}) \div N_f \quad (9)$$

Statistical analysis

All the data were initially tested for normality using a Shapiro–Wilk (1965) test and Levene's homoscedasticity test ($p < 0.05$), which showed the data to be normally distributed ($W \geq 0.90$). Regression analysis was used to discern whether there was a linear or nonlinear response to the N application rates. The data were subjected to ANOVA to compare the control treatment against the residual or in-season application of 160 kg N ha⁻¹ to corn, and mean separation was performed using a Tukey test. All the statistical tests were performed using R software (R Development Core Team 2015).

Results

Wheat response to N application rates

In wheat, significant responses to N application were observed for the leaf chlorophyll index; number of spikes per m²; plant height; number of spikelets per

Table 1 Leaf chlorophyll index, spikes per square meter, plant height, spikelets per spike, grains per spike, mass of 1000 grains, shoot biomass, grain yield, accumulated N, proportion and amount of N derived from fertilizer, soil and other sources,

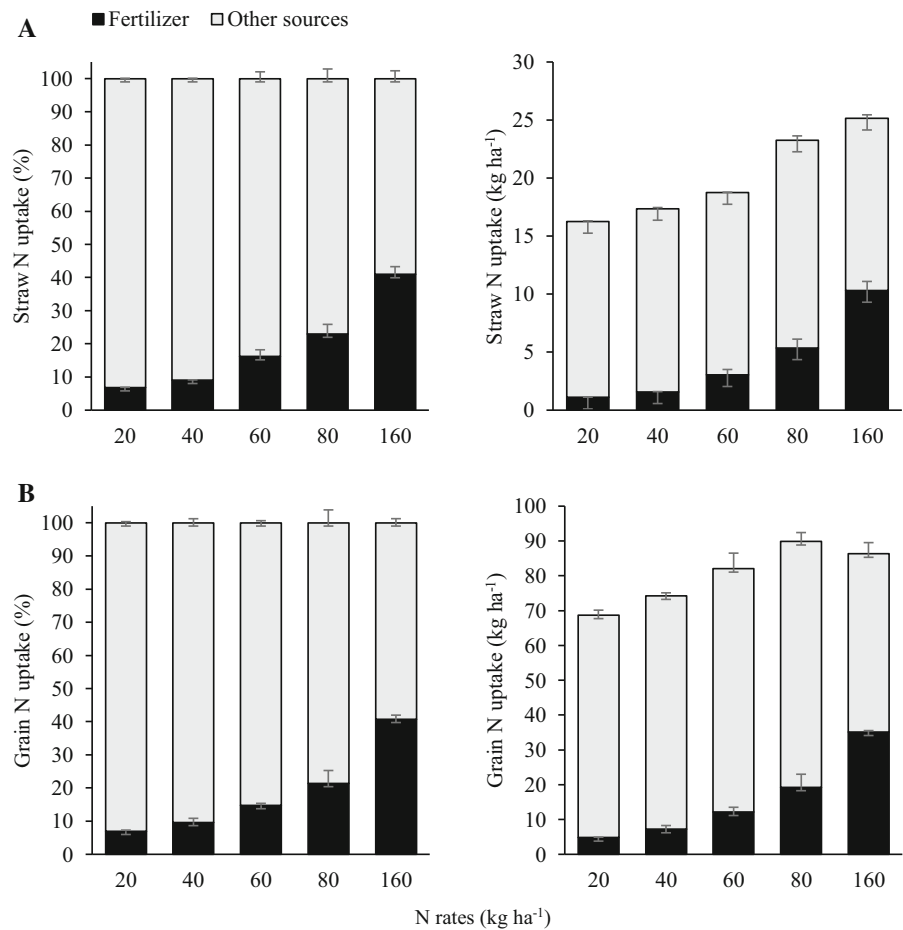
N recovery and total N recovery from N-fertilizer applied in straw and grain and N uptake as a function of N increasing rates on wheat

Source	Equation
Leaf chlorophyll index	$y = 40 + 0.11x - 0.0004x^2$ ($R^2 = 0.99^{**}$)
Spikes per square meter	$y = 424 + 0.98x$ ($R^2 = 0.87^*$)
Plant height	$y = 86 + 0.08x - 0.0004x^2$ ($R^2 = 0.80^*$)
Spikelets per spike	$y = 13.9 + 0.03x - 0.0001x^2$ ($R^2 = 0.82^{**}$)
Grains per spike	$y = 37 + 0.10x - 0.0004x^2$ ($R^2 = 0.98^{**}$)
Mass of 1000 grains	$y = 39 + 0.01x$ ($R^2 = 0.85^{**}$)
Shoot biomass	$y = 4554 + 5.29x$ ($R^2 = 0.99^{**}$)
Grain yield	$y = 4586 + 14.51x - 0.0635x^2$ ($R^2 = 0.78^{**}$)
<i>Straw</i>	
Accumulated N	$y = 15.2 + 0.07x$ ($R^2 = 0.89^{**}$)
Proportion of N derived from fertilizer	$y = 1.01 + 0.25x$ ($R^2 = 0.99^{**}$)
Amount of N derived from fertilizer	$y = -0.69 + 0.07x$ ($R^2 = 0.98^{**}$)
Proportion of N derived from soil and other sources	$y = 99 - 0.25x$ ($R^2 = 0.99^{**}$)
Amount of N derived from soil and other sources	$y = 13.4 + 0.08x - 0.0005x^2$ ($R^2 = 0.69^{**}$)
N recovery from N-fertilizer applied	$y = 4.0 + 0.10x - 0.0005x^2$ ($R^2 = 0.86^{**}$)
Total N recovery from N-fertilizer applied	$y = 4.2 + 0.07x - 0.0003x^2$ ($R^2 = 0.92^{**}$)
<i>Grain</i>	
Accumulated N	$y = 64 + 0.40x - 0.0016x^2$ ($R^2 = 0.90^{**}$)
Proportion of N derived from fertilizer	$y = 0.78 + 0.25x$ ($R^2 = 0.99^{**}$)
amount of N derived from fertilizer	$y = -0.45 + 0.22x$ ($R^2 = 0.99^{**}$)
Proportion of N derived from soil and other sources	$y = 99 - 0.25x$ ($R^2 = 0.99^{**}$)
amount of N derived from soil and other sources	$y = 57 + 0.36x - 0.0025x^2$ ($R^2 = 0.99^{**}$)
N recovery from N-fertilizer applied	$y = 22 + 0.26x - 0.0015x^2$ ($R^2 = 0.53^*$)
Total N recovery from N-fertilizer applied	$y = 21 + 0.20x - 0.0011x^2$ ($R^2 = 0.76^*$)
N uptake	$y = 0.04 + 0.007x - 0.00004x^2$ ($R^2 = 0.94^{**}$)

spike; number of grains per spike; mass of 1000 grains; shoot biomass; grain yield; straw and grain accumulated N; proportion and amount of N derived from fertilizer, soil and other sources; N recovery and total N recovery from N fertilizer applied; and N uptake (Sup. Table 3). The number of spikes per m², mass of 1000 grains, shoot biomass, accumulated N in straw, proportion and amount of N in straw and grain derived from fertilizer and proportion of N derived from soil and other sources were found to respond linearly to the N application rates (Table 1). The leaf chlorophyll index, plant height, number of spikelets per spike, number of grains per spike, grain yield, N in grains and straw derived from soil and other sources, N recovery and total N recovery from applied N

fertilizer, and grain accumulated N and N uptake were found to respond nonlinearly to the N application rates (Table 1). The highest estimated values for the leaf chlorophyll index, plant height, number of spikelets per spike and number of grains per spike were observed when the N application rates varied between 100 and 138 kg N ha⁻¹ (Table 1). The highest estimated wheat grain yield (5415 kg ha⁻¹) was observed with the application of 114 kg N ha⁻¹ (Table 1). The highest estimated N recovery in wheat straw was observed with N application rates varying between 82 and 125 kg N ha⁻¹ (Table 1). In addition, the highest estimated values for the N recoveries in wheat grains and N uptake were observed with N application rates varying between 72 and 91 kg ha⁻¹ (Table 1).

Fig. 1 Straw N uptake (a) and grain N uptake (b) on wheat derived from applied fertilizer and other sources. Error bars indicate the standard deviation of the mean (n = 4)



The application of 20, 40, 60, 80 and 160 kg N ha⁻¹ led to the uptake of 6.8, 9.0, 16.2, 23 and 41% of the total N in the wheat straw (equivalent to 1.1, 1.6, 3.0, 5.4 and 10.3 kg ha⁻¹, respectively) and 7.0, 9.7, 14.8, 21 and 41% of the total N in the wheat grains (equivalent to 4.8, 7.2, 12.1, 19.3 and 35 kg ha⁻¹, respectively) (Fig. 1). The total N recovered in the wheat shoots (straw + grains) with the application of 20, 40, 60, 80 and 160 kg N ha⁻¹ was 30, 37, 35, 38 and 32%, respectively (Sup. Table 3).

Corn responses to residual N

Significant responses to residual N were observed for the corn shoot biomass, accumulated N in the straw, proportion and amount of N derived from fertilizer and total N recovery, proportion and amount of N derived from fertilizer in the grains, proportion of N derived from soil and other sources, and N recovery and total

N recovery from the applied N fertilizer (Sup. Table 4). In most cases, a nonlinear response to N (applied to the previous crop) was observed, with the exception of shoot biomass, accumulated N in the straw and total N recovery (Table 2). The highest estimated shoot biomass, accumulated N in the straw and total N recovery were observed with the application of 104, 96 and 129 kg N ha⁻¹, respectively (Table 2). The application of 20, 40, 60, 80 and 160 kg N ha⁻¹ to wheat contributed 0.3, 0.4, 0.9, 2.0 and 5.5% of the N in the corn straw (equivalent to 0.1, 0.2, 0.7, 1.9 and 3.0 kg ha⁻¹, respectively) and 0.2, 0.4, 1.0, 2.0 and 5.5% of the N in the corn grains (equivalent to 0.3, 0.6, 1.3, 2.7 and 8.8 kg ha⁻¹, respectively) (Fig. 2). The residual N had an effect on the total N recovered from the corn shoots (straw + grain), with the recovered N accounting for 1.9, 3.0, 4.0, 6.2 and 7.6% of the total N for the application

Table 2 Shoot biomass, accumulated N in straw, proportion and amount of N derived from fertilizer, proportion of N derived from soil and other sources and total N recovery from

N-fertilizer applied in straw and grain, and N recovery from N-fertilizer applied in grain as a function of residual N increasing rates on corn

Source	Equation
Shoot biomass	$y = 5008 + 148.87x - 0.7135x^2$ ($R^2 = 0.93^{**}$)
<i>Straw</i>	
Accumulated N	$y = 24 + 1.21x - 0.0063x^2$ ($R^2 = 0.88^{**}$)
Proportion of N derived from fertilizer	$y = -1.1 + 0.04x$ ($R^2 = 0.97^{**}$)
Amount of N derived from fertilizer	$y = -0.42 + 0.02x$ ($R^2 = 0.94^*$)
Proportion of N derived from soil and other sources	$y = 101 - 0.04x$ ($R^2 = 0.97^{**}$)
Total N recovery from N-fertilizer applied	$y = -0.67 + 0.05x - 0.0002x^2$ ($R^2 = 0.86^*$)
<i>Grain</i>	
Proportion of N derived from fertilizer	$y = -1.0 + 0.04x$ ($R^2 = 0.98^{**}$)
Amount of N derived from fertilizer	$y = -1.8 + 0.06x$ ($R^2 = 0.97^{**}$)
Proportion of N derived from soil and other sources	$y = 101 - 0.04x$ ($R^2 = 0.98^{**}$)
N recovery from N-fertilizer applied	$y = 1.4 + 0.03x$ ($R^2 = 0.93^{**}$)
Total N recovery from N-fertilizer applied	$y = 0.97 + 0.03x$ ($R^2 = 0.98^{**}$)

Fig. 2 Straw N uptake (a) and grain N uptake (b) on corn derived from residual applied fertilizer and other sources. 160 IS refers to 160 kg N ha⁻¹ applied in season (corn). Error bars indicate the standard deviation of the mean (n = 4)

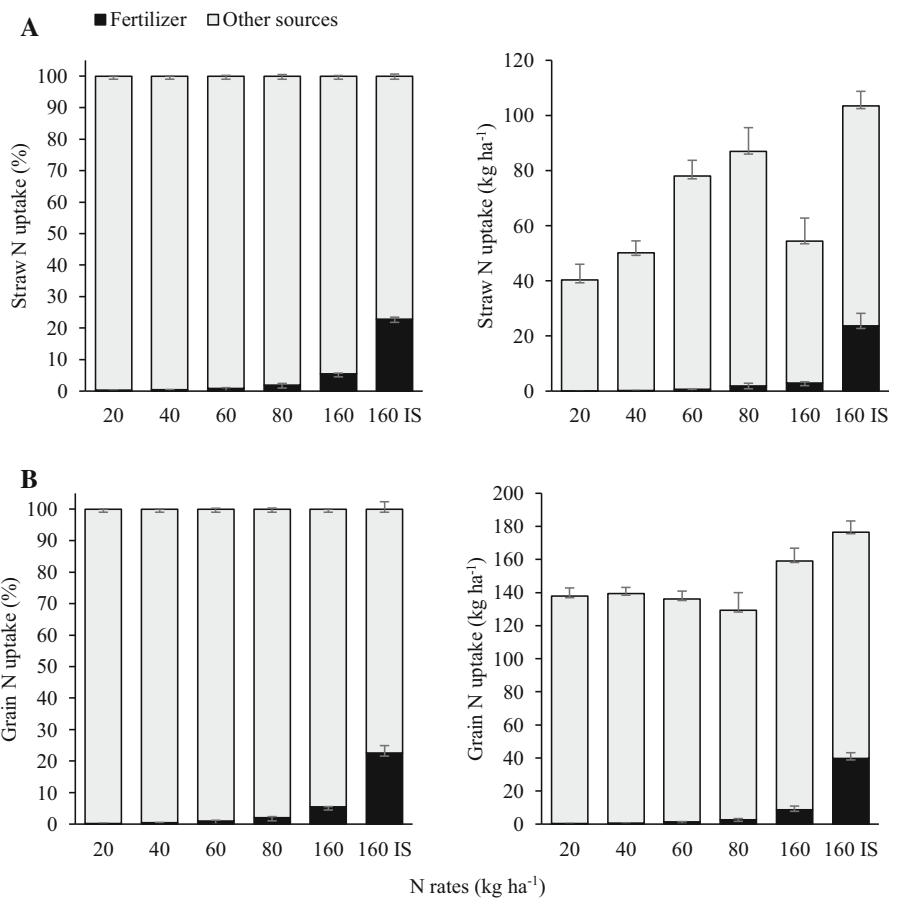


Table 3 Leaf chlorophyll index, plant height, stem diameter, rows per ear, grains per row, grains per ear, ear length, ear diameter, mass of 100 grains, shoot biomass and corn grain yield as a function of N residual, in season or in the absence of N application

Source	Leaf chlorophyll index	Plant height (m)	Stem diameter (mm)	Rows per ear	Grains per row	Grains per ear
N evaluation						
Control	59a [†]	2.4b	21b	18.5b	38a	703b
Residual (20 + 140 kg N ha ⁻¹)	63a	2.5a	23a	19.0ab	38a	731ab
In season (20 + 140 kg N ha ⁻¹)	64a	2.5a	23a	20a	40a	791a
L.S.D. (5%)	10.6	0.03	1.7	1.0	2.5	75
Source	Ear length (mm)	Ear diameter	Mass of 100 grains (g)	Shoot biomass (kg ha ⁻¹)	Grain yield (kg ha ⁻¹)	
N evaluation						
Control	200a	55a	26a	7283b	8267c	
Residual (20 + 140 kg N ha ⁻¹)	198a	56a	27a	10,508a	9492b	
In season (20 + 140 kg N ha ⁻¹)	200a	54a	28a	12,615a	10,304a	
L.S.D. (5%)	28	2.7	2.0	2581	702	

[†]Means within the column followed by different letters are significantly different (p value ≤ 0.05)

rates of 20, 40, 60, 80, and 160 kg N ha⁻¹, respectively (Sup. Table 4).

Corn response to in-season N application

For corn, the control plots showed lower plant heights, stem diameters, rows per ear, grains per ear and shoot biomass compared to the residual and in-season N application (Sup. Table 4, Table 3). The application of 160 kg N ha⁻¹ to corn resulted in greater grain yield (by 25 and 8.6%) and accumulated N in the straw (by 278 and 91%) compared with those in the control and residual N treatments, respectively (Tables 3 and 4). In addition, compared to the residual N treatment, the in-season application of 160 kg N ha⁻¹ led to a greater proportion and amount of N derived from the fertilizer in the straw and grains, N recovery and total N recovery from the applied fertilizer, and N uptake and N use efficiency (an equivalent increase, on average, of 69%) (Table 4). However, compared to the in-season N application, the treatment with residual N showed greater proportions of N derived from the soil and other sources in the straw and grains (Table 4). The in-season application of 160 kg N ha⁻¹ led to a 23% N uptake in corn straw (equivalent to 24 kg ha⁻¹) and a 23% N uptake in corn grains

(equivalent to 40 kg ha⁻¹), and the total recovered N in the shoots (straw + grain) was 47% (Fig. 2, Sup. Table 4).

Discussion

Nitrogen recovery and N use efficiency in wheat

The higher N availability resulting from N fertilizer application led to a higher leaf chlorophyll index and greater accumulated N in the straw and grains and resulted in improved shoot biomass, spikes per m², plant height, spikelets per spike, grains per spike, mass of 1000 grains and wheat grain yield. The better growth of N-fertilized plants can be attributed to N availability after application. However, the application of unnecessarily large amounts of N can result in increased losses and less utilization by cropping systems, since nutrient demand by plants is limited (Galindo et al. 2016), as verified in this study, with N uptake and grain yield showing higher responses to N application rates of 89 and 114 kg ha⁻¹, respectively, than to higher N rates.

Most of the N fertilizer applied to wheat was not used during the growing season. Our results showed

that 19% of wheat N uptake came from fertilizer and 81% was derived from native soil N and other sources (for example, organic matter), with an average N use efficiency of 7.4 kg grain kg N applied⁻¹. Similarly, Dourado Neto et al. (2010) and Karwat et al. (2017) reported 21 and 22% of wheat N uptake was derived from fertilizer, while approximately 78% of the N used by crops in rotations (wheat-rice, pasture-corn, wheat-common bean-barley, rice-wheat, and wheat-peanut, among others) was derived from other sources, including mineralization from soil organic matter.

Our results showed that, on average, the total N recovery rate was 34% (7 and 27% for the straw and grains, respectively), which was similar to the global estimate of 33% (Raun and Johnson 1999; Hirel et al. 2007; Xu et al. 2012). The greatest estimated total N recovery observed in this study was obtained with an estimated N application rates of 117 kg ha⁻¹ (for straw) and 91 kg ha⁻¹ (for grain). It has been frequently reported that only 30 to 50% of N fertilizer is taken up by grains during the growing season following fertilizer application (Duncan et al. 2018; Lollato et al. 2019). Several studies have shown that NH₃ losses from tropical and subtropical Brazilian soils typically range from 38 to 78% and 10 to 25% of applied N, respectively (Fontoura and Bayer 2010; Viero et al. 2014, 2017; Ribeiro et al. 2020). In contrast, the estimated NO₃ loss by leaching in Brazil has been reported to range from trace amounts to 87 kg ha⁻¹ year⁻¹, and losses from N fertilizer application usually exceed 10–15 kg ha⁻¹ year⁻¹ (Rosolem et al. 2017).

The recoveries of ¹⁵N fertilizer by annual crops are highly variable and affected by different management practices and biophysical factors. Although the N recoveries reported here are lower than those reported by other researchers, they are not unusual compared with studies from different climatic zones. For example, under different residue management practices, the average N use efficiency of flooded rice in experiments in California using procedures similar to those in this experiment was 37% (Eagle et al. 2001). In the Ethiopian highlands, wheat N recovery ranged between 25 and 61% (Gorfu et al. 2003); large N losses were associated with high rainfall in combination with a heavy clay soil and high soil N supply. In tropical Brazil, Martins et al. (2017) and Rocha et al. (2019) reported that corn N recovery represented only 31 to 34% of the total N applied. In the present study, a

high average air temperature (24 °C) and low rainfall amount (18 mm) were observed throughout the crop season and likely favored urease enzyme activity and NH₃ volatilization losses (Sup. Figure 3). Under tropical conditions, urea is usually applied as a side dressing, with plant residues on the soil surface blocking or delaying the contact between the soil and fertilizer granules, which enhances urease activity far above that of bare soil, favoring NH₃ volatilization (Mira et al. 2017). Losses of NH₃ are higher in no-till systems than in conventional tillage systems due to the higher activity of the urease enzyme (Liu et al. 2019). The global average losses of NH₃ from urea fertilizers are estimated to be close to 14% (range of 10–19%) (Cantarella et al. 2018), but they can reach 40% in tropical soils because of high temperatures (Cantarella et al. 2018).

Nitrogen recovery and N use efficiency in corn

Although the residual effect of the N applied to the wheat crop increased the amount of N from the fertilizer in the grain N uptake and total N recovery, most of the N taken up by the corn came from other sources (98% on average, equivalent to 198 kg ha⁻¹). Only 2%, equivalent to 3.9 kg ha⁻¹, came from the residual N that remained after fertilizer application to the wheat crop, leading to a low total N recovery (4.5%) by the corn (1.4 and 3.1% in the corn straw and grains, respectively).

In-season N application was found to provide significantly greater amounts of N for corn than residual N treatment (32%, equivalent to 67 kg ha⁻¹), with a total N recovery of 47%, 6.2 times greater than the total N recovery observed for the residual N treatment. Most of the N taken up by corn was likely derived from the soil, primarily through mineralization processes. However, N inputs from litter decomposition, biological N fixation, and wet and dry deposition cannot be neglected as additional N sources for corn (Koyama et al. 2019; Rocha et al. 2019). Studies on the use of N in soil-plant systems are abundant, but relatively few studies have reported the potential use of residual N in the successive cultivation of annual crops. Most studies using N fertilizers have evaluated fertilizer performance in only one crop cycle, ignoring the potential residual effects of fertilizers. However, the few studies that have investigated residual fertilizer effects reported residual ¹⁵N

Table 4 Accumulated N, proportion and amount of N derived from fertilizer (Npdff and ANdff), soil and other sources (Npdfs and ANpdfs), N recovery and total N recovery from N-fertilizer applied (Nrec and TNRec) on corn straw and grain, N uptake, N utilization and N use efficiency as a function of N residual, in season or in the absence of N application

Source	Accumulated N in straw (kg ha ⁻¹)	Straw Npdff (%)	Straw ANdff (kg ha ⁻¹)	Straw Npdfs (%)	Straw ANdfs (kg ha ⁻¹)	Straw NRec (%)	Straw TNRec (%)	Accumulated N in grain (kg ha ⁻¹)	
N evaluation									
Control	37b [†]	–	–	–	–	–	–	129b	
Residual (20 + 140 kg N ha ⁻¹)	54b	5.5b	3.0b	94a	51a	2.1b	1.9b	159a	
In season (20 + 140 kg N ha ⁻¹)	103a	23a	24a	77b	80a	17.0a	17.5a	177a	
L.S.D. (5%)	43	1.1	12.1	1.1	51	8.6	6.2	44	
Source	Grain Npdff (%)	Grain ANdff (kg ha ⁻¹)	Grain Npdfs (%)	Grain ANdfs (kg ha ⁻¹)	Grain NRec (%)	Grain TNRec (%)	N uptake (kg N accumulated kg N applied ⁻¹)	N utilization (kg grain kg N accumulated ⁻¹)	N use efficiency (kg grain kg N applied ⁻¹)
N evaluation									
Control	–	–	–	–	–	–	–	–	–
Residual (20 + 140 kg N ha ⁻¹)	5.5b	8.8b	95a	150a	6.3b	5.6b	0.35b	21a	7.2b
In season (20 + 140 kg N ha ⁻¹)	23a	40a	77b	137a	28a	29a	0.70a	17a	12.2a
L.S.D. (5%)	6.0	13.7	12.9	69	9.8	4.9	0.28	5.5	4.8

[†]Means within the column followed by different letters are significantly different (p value ≤ 0.05)

recovery rates of approximately 2.9–3.9% in corn (Ding et al. 2019; Rocha et al. 2019), 2.3% in sugarcane (Bastos et al. 2018) and 7.1% in rice (Silva et al. 2016).

The low recovery of residual labeled ¹⁵N in the corn plants indicates that the contribution of previously applied N fertilizer to a succeeding crop is limited or even negligible. Most of the residual N remains in the soil in its organic form due to immobilization processes and is released slowly by mineralization (Liu et al. 2015; Smith and Chalk 2018). While inorganic N fertilizer provides an immediate source of mineral N for crops, most of the N in crops is derived from N that is stored in soil from other sources (native biological N fixation, remaining straw, soil, rainfall and others). Thus, the maintenance of optimum soil organic matter levels and use of management practices

designed to maximize the synchronization between the release of N from soil sources and the time of maximum N uptake by crops will remain crucial components of sustainable agricultural practices (Dourado Neto et al. 2010; Lehmann and Kleber 2015). In a recent meta-analysis performed by Smith and Chalk (2018) with more than 100 studies on the residual value of ¹⁵N-labeled fertilizers, the authors reported that 5.4% of the initially applied N was recovered by subsequent crops. Therefore, the supply of residual N fertilizers to subsequent crops will be affected by losses through volatilization, leaching and denitrification, and the short-term balance between N immobilization and mineralization, all of which are greatly affected by local environmental conditions (Grant et al. 2016). According to Coyos et al. (2018), corn grain yield can be characterized by a linear

coefficient, with 67 kg grain ha⁻¹ produced per kg N ha⁻¹ applied. Similarly, our results showed an increase of 64 kg corn grain ha⁻¹ produced per kg N ha⁻¹ applied. This result demonstrates the need for fertilizer application to each crop to maintain optimum grain yield. The observed increase in N accumulation, N recovery in straw and grains, N uptake and N use efficiency and corn grain yield obtained with the in-season application of 20 + 140 kg N ha⁻¹ confirm the need for N fertilizer application, at least in the short term.

Conclusions

The results of our study showed that wheat responded positively to N, as indicated by the improvement in the parameters measured in the fertilized treatments compared to those in the unfertilized control. Our results also showed that the practice of applying fertilizer to wheat and expecting a substantial amount of residual N to be available for a subsequent crop (in our case, corn) might not be feasible. The results of this study showed that the majority of the residual N was stored in the soil and was not available to the corn crops during the growing season. Better N management practices are needed to improve N use efficiency by cereal crops. The overapplication of N to wheat with the expectation that residual N will be available to the subsequent crop should be avoided. Further studies aiming at increasing the use and recovery of applied N and its residuals should be performed under different climatic and edaphic conditions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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