

Efficiency of ^{15}N -fertilizer uptake in coffee plants: short-term comparison of foliar, root, and combined pathways

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Editor: Samuel Pereira de Carvalho

Received in September 25, 2025 and approved in March 11, 2026

ABSTRACT

Despite technological advances, agriculture still faces challenges to sustainably feed a growing global population, including the low efficiency of nitrogen (N) absorption from fertilizers. Fertigation can improve this efficiency and may be applied via foliar, soil, or a combination of both approaches, even in perennial crops such as coffee. The aim of this short-term study was to compare N uptake efficiency by evaluating different urea application methods (foliar, soil, or ‘foliar plus soil’) and two amounts of N using isotopic methodology. Based on total N content and ^{15}N abundance in leaves, we calculated the percentage and amount of N derived from the fertilizer, as well as N uptake efficiency at 24, 48, and 72 hours after application. Rapid N uptake occurred, with the combined foliar plus soil at 2.3 g N per plant showing the highest efficiency. These findings suggest that combining foliar and soil applications can optimize N use efficiency in coffee cultivation.

Key words: *Coffea arabica*; isotope; urea.

1 INTRODUCTION

One major challenge in making agriculture more sustainable is overcoming the intensive use of fertilizers (FAO, 2022). Improving nitrogen (N) uptake efficiency is critical, as N is an essential nutrient for plant growth and often overapplied (Kirkby, 2023). Excess reactive N from fertilizers can pollute the environment and reflect agriculture’s dependence on synthetic N inputs (Congreves et al., 2021; FAO, 2022). However, in some regions, limited nitrogen availability still reduces crop productivity.

Fertigation is a promising strategy to improve fertilizer use efficiency. By applying water and nutrients simultaneously, it reduces labor demands, minimizes environmental losses, and allows the total fertilizer rate to be applied in smaller fractions. This approach aligns nutrient supply with the plant’s growth stages, thus improving nutrient uptake and optimizing fertilization (Bruno et al., 2011; Sun et al., 2023).

Although global and Brazilian estimates of N use in coffee cultivation are lacking (IFA, 2024), studies indicate that Brazilian coffee plantations achieve high yields despite low N recovery from fertilizer (Bruno et al., 2011; Fenilli et al., 2008). Given coffee’s high N demand, economic importance (ICO, 2023), and the large area under cultivation worldwide (FAO, 2024), improving the sustainability of coffee production is essential. In this context, this study aimed to compare N uptake efficiency in fertigated coffee plants by applying ^{15}N -labeled urea via foliar, root, and combined ‘foliar plus root’ in a short-term experiment.

2 MATERIAL AND METHODS

The experiment was conducted in a commercial coffee plantation in Barreiras, Bahia, Brazil (12°09’10” S and 44°59’24” W), at 452 meters above sea level. The soil is classified as a Red-Yellow Latosol according to the Brazilian Soil Classification System, corresponding to an Oxisol in the USDA Soil Taxonomy (Santos et al., 2018). It contains 75 % sand, 3 % silt, and 22 % clay.

Before the experiment, soil chemical analysis was performed at a depth of 0-0.6 m using composite samples collected from an adjacent area. The soil fertility characteristics and respective methodologies were: pH (CaCl_2 0.01 mol L⁻¹) = 4; organic matter (colorimetry) = 20 g dm⁻³; phosphorus (ion exchange resin) = 53 mg dm⁻³; sulfur (turbidimetry) = 30 mg dm⁻³; potassium (ion exchange resin) = 1 mmol_c dm⁻³; calcium (ion exchange resin) = 11 mmol_c dm⁻³; magnesium (ion exchange resin) = 5 mmol_c dm⁻³; exchangeable aluminum (titrimetric method 1 mol L⁻¹) = 7 mmol_c dm⁻³; potential acidity (pH SMP) = 32 mmol_c dm⁻³; sum of bases = 17 mmol_c dm⁻³; cation exchange capacity = 49 mmol_c dm⁻³; base saturation = 30 % (Raij et al., 2001).

Three-year-old plants of *Coffea arabica*, cv. Catuaí Vermelho (IAC 144) in full production were used in this experiment. They were planted at a spacing of 1.8 x 0.5 m (11,111 coffee plants per hectare), in a circular arrangement, under a central pivot system for fertigation with LEPA sprinklers, on a flat land area (slope of less than 1 %).

The experiment was conducted from July 20th to 23rd, 2009, shortly after the coffee harvest, during the resting/

senescence stage (Camargo & Camargo, 2001). A randomized block design was used in a 3x3 factorial arrangement, with nine treatments and four replicates, totaling 36 plots. The factors were the application method (foliar, soil, or a combination of foliar plus soil) and the amount of N (0, 2.3, and 4.6 g N per plant), with the zero-N treatment serving as the control. Each plot consisted of three coffee plants arranged in a row, and a border with five plants on each side of the plot. According to the treatment, plants received either a solution of urea labeled with ^{15}N (1.4 %) or water (control), and only the central plant in each plot was used for analyses.

The ^{15}N -labeled urea was diluted in water to match an irrigation depth of 4 mm. The N amounts applied were based on the farm's standard fertilization rate of 600 kg ha⁻¹ year⁻¹ (equivalent to 54 g N per plant per year). To simulate monthly or biweekly fractions of the total rate, this rate was divided by 12 (4.6 g N per plant) and by 24 (2.3 g N per plant), respectively. Therefore, this study reports N application as amount per plant rather than area-based rates. Control plants received only water.

The treatments were applied in the early morning (between 8:00 and 9:00 AM) using a watering can. For the foliar application, a plastic sheet covered the soil beneath and around the canopy to prevent ^{15}N -urea from reaching the soil and being absorbed by the roots. For the soil application, care was taken to avoid contact between the solution and the leaves. In the combined 'foliar plus soil' treatment, the solution was applied above the plant canopy, ensuring the leaves were wetted before the solution was drained onto the soil, simulating a typical low energy precision application (LEPA) system.

Leaves were sampled at 24, 48, and 72 hours after ^{15}N -urea application to determine total N and ^{15}N abundance. From each central plant, nine leaves were collected (three from the upper third, three from the middle third, and three from the lower third), forming a composite sample for each replicate. The leaves were immediately washed with running water, air-dried at room temperature, stored in paper bags, and dried at 60 °C until constant weight. Leaf dry mass was determined using a coffee plant in an area adjacent to the experimental plots.

For the determination of N and ^{15}N concentrations, dry leaf samples were ground and homogenized to take representative sub-samples for analysis in an automated continuous flow isotope ratio mass spectrometer (model ANCA-SL, Europa Scientific) (Barrie & Prosser, 1996; Bruno et al., 2011). The total N content in the leaves (QN) was calculated from the values of leaf dry mass and N concentration.

The following calculations (Bruno et al., 2011; Reichardt & Timm, 2020) were made for each collection time (24, 48, and 72 hours) of each treatment:

Percentage of nitrogen derived from fertilizer in the leaves, Ndff (%):

$$\text{Ndff} = (\text{Ab}_{\text{lea}} - \text{Ab}_{\text{nat}}) / (\text{Ab}_{\text{fer}} - \text{Ab}_{\text{nat}}) \times 100 \quad (1)$$

Where Ab_{lea} is the abundance of ^{15}N in the leaves; Ab_{nat} is the natural abundance of ^{15}N (0.3660 %); and Ab_{fer} is the fertilizer abundance of ^{15}N (1.4 %).

Quantity of N derived from fertilizer in the leaves, QNdff (g plant⁻¹):

$$\text{QNdff} = (\text{Ndff} \times \text{QN}) / 100 \quad (2)$$

Where QN is the total N content in the leaves (g plant⁻¹)

N uptake efficiency, Efficiency (%):

$$\text{Efficiency} = (\text{QNdff} / \text{U}) \times 100 \quad (3)$$

Where U is the amount of N applied as ^{15}N -urea to each plant (g plant⁻¹)

QN data were first tested for normality using the Shapiro-Wilk test. As the data followed a normal distribution, they were analyzed by analysis of variance (ANOVA), followed by the Tukey test at a 5 % significance level, using Sisvar 5.8 software. In contrast, Ndff, QNdff, and N uptake efficiency did not meet normality assumptions. Therefore, they were analyzed using the nonparametric factorial method aligned rank transform (ART). ART begins with a preprocessing step that "aligns" the data for each main effect or interaction before ranking. In case of tied values, average ranks were used. To facilitate the alignment and ranking processes, the authors developed the open-source program ARTool (2024), which handles all main effects and interactions. To avoid inflating Type I error rates when performing multifactor contrast tests, the ART-C procedure was adopted, along with its extended computer application. Repeated measures nonparametric ANOVA was also used to compare means of Ndff, QNdff, and Efficiency across sampling times using ARTool as described above. Statistical significance was set at $p = 0.05$. When $p < 0.05$, Tukey's HSD post-hoc test was performed to detect differences for each main effect and interaction. All ANOVAs were performed using Statistica software (Version 14.0).

3 RESULTS

The shoot dry matter of a representative coffee plant from our sampling area was 2,037 g, distributed as follows: stem (880 g), leaves (733 g), and branches (424 g). The average QN in the leaves across all treatments was 20 g per plant (i.e., 20 g of N in 733 g of leaves), corresponding to 27 g N kg⁻¹ of leaf dry matter (CV = 6.8 %). There was no interaction effect

between application method and the amount of N applied on QN at any sampling time. After 24 hours, the average QN was $19.7 \text{ g plant}^{-1}$ (CV = 5.1 %). No significant differences were observed among application methods; however, N application amount had a significant effect. The control treatment (without N fertilization) showed significantly higher QN ($20.8 \text{ g plant}^{-1}$) than plants that received 2.3 g ($19.4 \text{ g plant}^{-1}$) and 4.6 g of N ($19.0 \text{ g plant}^{-1}$). At 48 and 72 hours, neither application method nor N amount applied affected QN, with average values of $19.1 \text{ g plant}^{-1}$ (CV = 7.0 %) and 20 g plant^{-1} (CV = 6.9 %), respectively.

Figure 1 shows the evolution of Ndff, QNdff, and Efficiency from 24 to 72 hours after ^{15}N -urea application, regardless of application method and N amount. The results indicate that coffee plants continued to absorb the applied N over 72 hours after the fertilizer application. Ndff increased significantly between 24 and 48 hours (Figure 1 A). In contrast, both QNdff (Figure 1 B) and Efficiency (Figure 1 C) increased progressively at each time point.

The evolution of Ndff, QNdff, and Efficiency is shown in Figure 2 according to the application method of ^{15}N -urea, regardless of the N amount. At 24 hours, foliar and ‘foliar plus soil’ applications resulted in significantly higher Ndff and QNdff than soil application (Figure 2 A and B). By 48 hours, all application methods differed significantly, with the ‘foliar plus soil’ application showing the highest values. At 72 hours, the ‘foliar plus soil’ continued to have the highest Ndff and QNdff values, although differences with the soil application were no longer statistically significant. Foliar application alone led to faster initial uptake than soil application, but this advantage diminished over time.

The ‘foliar plus soil’ application was the most efficient, with significantly higher N uptake efficiency compared to the other methods at all time points (Figure 2 C). Soil application showed the lowest efficiency, except at 72 hours, when it became statistically equivalent to foliar application.

Figure 3 shows the progression of Ndff, QNdff, and Efficiency as a function of the amount of N applied, independent of the application method. The highest amount of N (4.6 g of N per plant) resulted in significantly higher values of Ndff and QNdff throughout the evaluation period, except at 24 hours, when it did not differ from the 2.3 g of N (Figure 3 A and 3 B). In contrast, the highest Efficiency occurred with the lowest N amount at all time points (Figure 3 C).

Figure 4 A-C shows Ndff values considering the interaction of each application method (upper case letters) and N amount. At 2.3 g of N per plant, the ‘foliar plus soil’ application resulted in the highest Ndff values, except at 24 hours when it was similar to foliar application alone. At 4.6 g of N per plant, foliar and ‘foliar plus soil’ applications showed statistically similar Ndff values up to 48 hours. By 72 hours, the application method no longer influenced Ndff at either N amount.

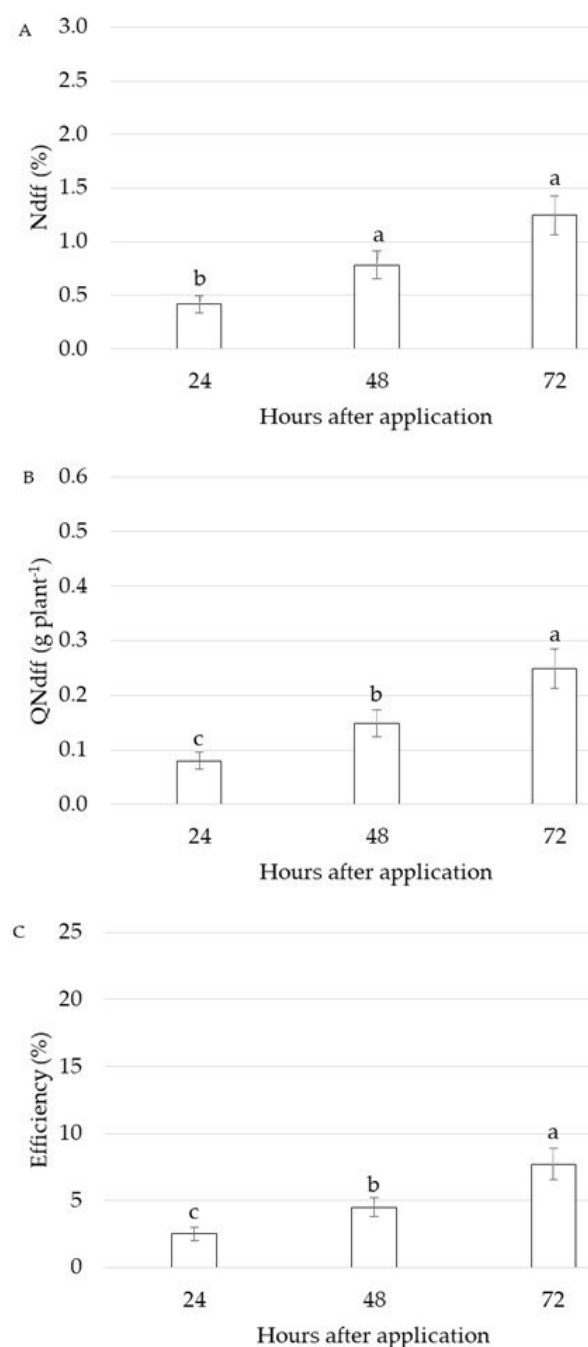


Figure 1: Percentage of N derived from fertilizer (Ndff, %) (A), the quantity of N derived from fertilizer (QNdff, g plant⁻¹) (B), and N uptake efficiency (Efficiency, %) (C) in coffee plant leaves at 24, 48, and 72 hours after ^{15}N -urea application, regardless of the application method and the amount of N applied as ^{15}N -urea. Different letters indicate significant differences ($p < 0.05$). Error bars represent standard errors.

QNdff followed the same pattern as Ndff, except at 24 hours for the 2.3 g of N treatment, where foliar and soil applications were statistically similar (Figure 4 D-F).

At 2.3 g of N, the ‘foliar plus soil’ application showed the highest Efficiency at all time points (Figure 4 G-I). However,

by 72 hours, it was not significantly different from that of the soil application. At 4.6 g of N, Efficiency was lower for the soil application up to 48 hours, while foliar and 'foliar plus soil' applications did not differ. After 72 hours, no significant difference was observed among the three application methods.

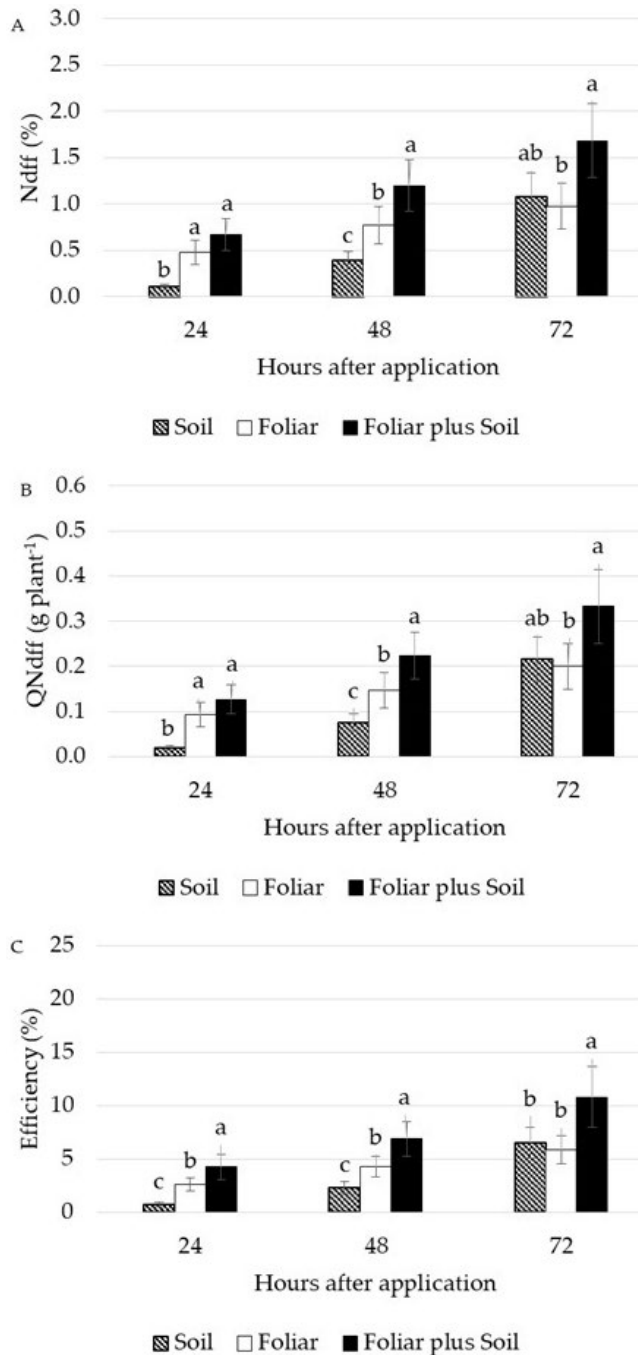


Figure 2: Percentage of N derived from fertilizer (Ndff, %) (A), the quantity of N derived from fertilizer (QNdff, g plant⁻¹) (B), and N uptake efficiency (Efficiency, %) (C) in coffee plant leaves at 24, 48, and 72 hours after ¹⁵N-urea application, according to the application method and regardless of the amount of N. Different letters indicate significant differences ($p < 0.05$). Error bars represent standard errors.

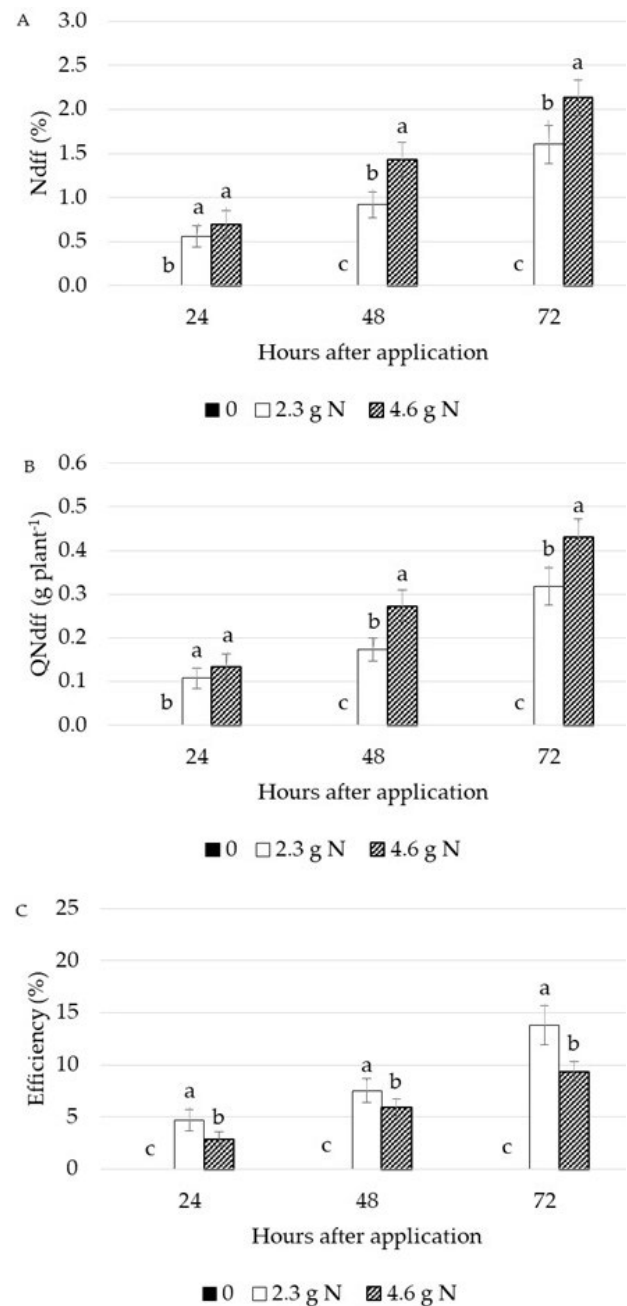


Figure 3: Percentage of N derived from fertilizer (Ndff, %) (A), the quantity of N derived from fertilizer (QNdff, g per plant) (B), and N uptake efficiency (Efficiency, %) (C), in coffee plant leaves at 24, 48, and 72 hours after ¹⁵N-urea application, according to the applied N amount and regardless of the application method. Different letters indicate significant differences ($p < 0.05$). Error bars represent standard errors.

When analyzing the effect of N amount within each application method on Ndff, QNdff, and Efficiency (Figure 4 - lower case letters), no significant differences were found between the two N amounts (2.3 g and 4.6 g N) for soil application at any time point, only between fertilized and unfertilized plants. The only exception was

at 72 hours, when QNdff was significantly higher with 4.6 g of N (Figure 4 F).

For the foliar application, significant differences in Ndff, QNdff, and Efficiency were observed at 24 hours only between fertilized and unfertilized plants (Figure 4 A, D, and G). At 48 hours, plants that received 4.6 g of N via foliar application showed significantly higher Ndff and QNdff compared to those that received 2.3 g, and both fertilized treatments had significantly higher values than the control (Figure 4 B and E). By 72 hours, Ndff and QNdff remained significantly higher for the 4.6 g of N treatment. However, QNdff in the plants that received 2.3 g of N was no longer different from the unfertilized plants (Figure 4 C and F). Despite these differences in Ndff and QNdff, Efficiency was not significantly affected by N amount at any time point (Figure 4 G-I).

For the ‘foliar plus soil’ application, significant differences in Ndff and QNdff were observed only

between fertilized and unfertilized plants at all time points (Figure 4 A-F).

In terms of Efficiency, coffee plants that received 2.3 g of N via the ‘foliar plus soil’ application showed significantly higher values than those receiving 4.6 g of N at all time points. For both foliar and soil applications, significant differences were observed only between fertilized and unfertilized plants. Efficiency for the ‘foliar plus soil’ application ranged from approximately 4 % (24 hours after applying 4.6 g N) to just over 21 % (72 hours after applying 2.3 g N) (Figure 4 G-I, lower case letters). When comparing each N amount, the 2.3 g of N consistently showed the highest Efficiency with the ‘foliar plus soil’ application at all time points (Figure 4G-I, upper case letters). For the 4.6 g of N, foliar and ‘foliar plus soil’ applications were equally efficient and outperformed soil application at 24 and 48 hours. At 72 hours, no differences were observed among application methods.

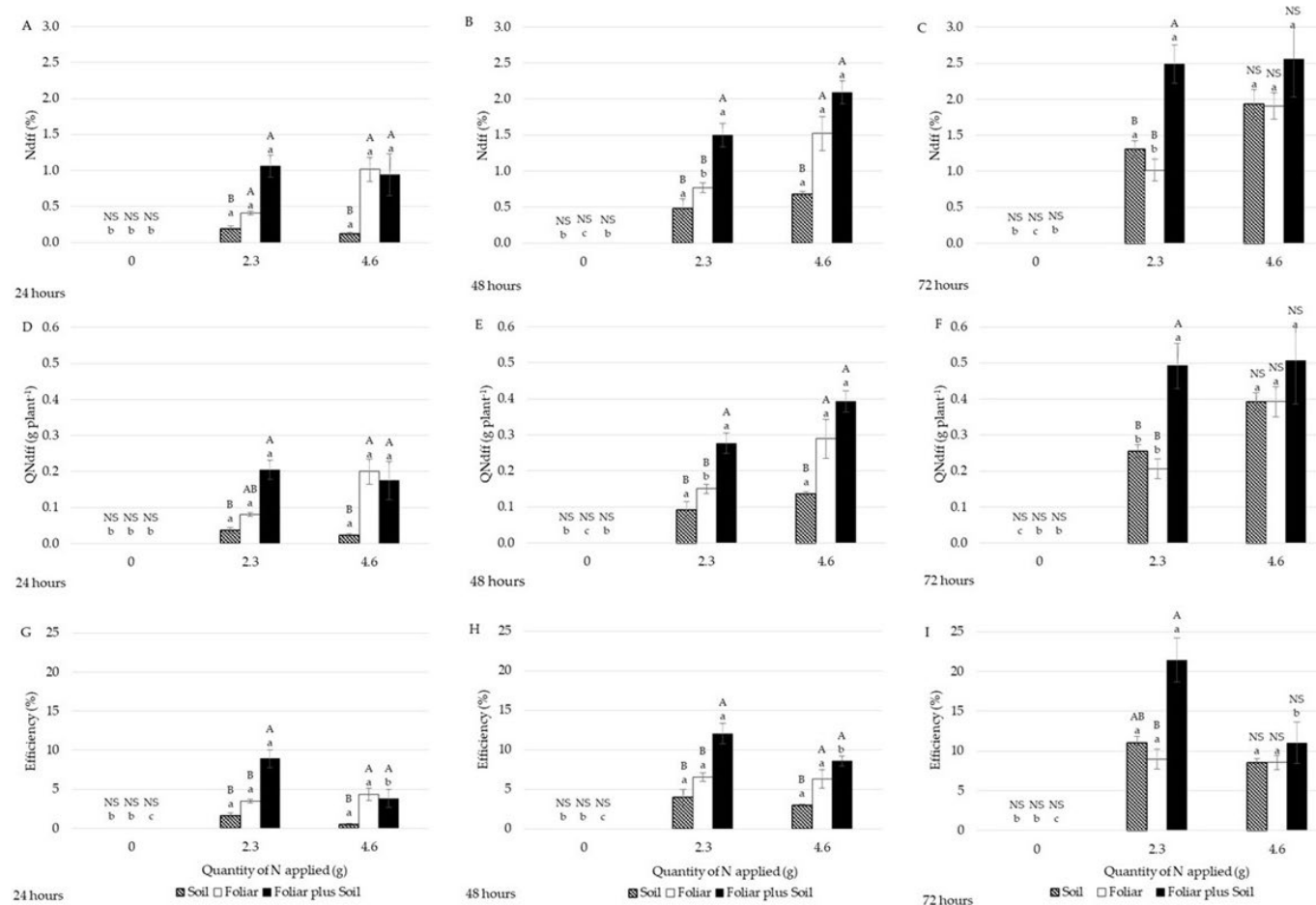


Figure 4: Average values of percentage of N derived from fertilizer (Ndff, %) (A, B, C), quantity of N derived from fertilizer (QNdff, g plant⁻¹) (D, E, F), and N uptake efficiency (Efficiency, %) (G, H, I) in coffee leaves. The analysis considers the interaction between N application methods (soil, foliar, and ‘foliar plus soil’) and N amounts (0, 2.3, and 4.6 g of N plant⁻¹), measured at 24, 48, and 72 hours after ^{15}N -urea application. Lower case letters indicate comparison among N amounts within the same application method ($p < 0.05$). Upper case letters indicate comparison among application methods for the same N amount applied ($p < 0.05$). Error bars represent standard errors.

4 DISCUSSION

Shoot dry matter observed in this study aligns with that reported for 3-year-old *C. arabica* plants of the same cultivar in Southeast Brazil, which received soil-applied N from planting and reached 2,222 g per plant (Fenilli et al., 2007). The average leaf N concentration fell within the adequate range for *C. arabica* under conventional fertilization (26-32 g N kg⁻¹; SBCS/NEPAR, 2017), indicating that the plants were nutritionally balanced at the time of the experiment.

The unexpectedly higher QN values observed in the control treatment at 24 hours are likely not due to actual N accumulation, as this period is too short for significant changes in QN to occur.

The temporal dynamics of Ndff (Figure 1 A) suggest that most of the fertilizer-derived N was absorbed within the first 24 to 48 hours, as no significant difference was observed between 48 and 72 hours. In contrast, QNdff continued to increase over the entire study period (Figure 1 B), likely reflecting not only N from the fertilizer but also N already present in the soil or remobilized from other plant tissues. Although QNdff and Efficiency (Figures 1 B and C) were low overall, both nearly doubled between 24 and 48 hours and continued to rise up to 72 hours, indicating active N uptake. This response occurred even though the plants were in the resting/senescence phase of branch development, suggesting that nutrient-demanding processes such as new leaf formation were occurring (Camargo & Camargo, 2001). Since less than 1.5 % of Ndff has been absorbed by the end of the experiment, a longer evaluation period is needed to fully capture the dynamics of N uptake (Figure 1).

The observed variations in Ndff, QNdff, and Efficiency across application methods (Figure 2) indicate that this factor significantly influences short-term N uptake in coffee plants. The ‘foliar plus soil’ treatment resulted in the highest values for all parameters over the 72 hours, although some values were not significantly different from those of the soil application. This pattern suggests a complementary effect between foliar and root uptake pathways.

Similar patterns have been reported in other crops. For instance, in cotton grown in both saline and non-saline soils, plants receiving ‘foliar plus soil’ fertilization exhibited significantly higher ¹⁵N concentrations within 72 hours compared to those fertilized only via soil, regardless of salinity conditions (Luo et al., 2015). The authors attributed this effect to a more balanced N distribution among roots, stems, and leaves after 28 days. In our study, the rapid uptake through leaves was likely complemented by continued root absorption. This is supported by significantly higher Ndff and QNdff values for foliar compared to soil application at 24 and 48 hours, with values becoming similar at 72 hours (Figure

2 A, B). Similarly, grapevine seedlings treated with foliar N application nearly double Ndff in leaves compared to those receiving soil application (Sun et al., 2017).

The ‘foliar plus soil’ application showed the highest Efficiency at all time points (Figure 2 C). Although foliar application initially outperformed soil application in Efficiency, this advantage decreased over time, and both treatments became statistically similar by 72 hours. This contrasts with findings in oak trees, where foliar N application maintained higher efficiency for up to three weeks (Da Ros et al., 2023), highlighting that N uptake dynamics through different pathways can vary substantially among species. Given that coffee is a cultivated species frequently managed with frequent fertilization - especially through fertigation - longer evaluation periods beyond 72 hours are needed to better understand the relative contribution of foliar and root uptake pathways, and to determine how long the combined ‘foliar plus soil’ method remains the most efficient.

The evolution of Ndff, QNdff, and Efficiency in response to the amount of N applied (Figure 3) is consistent with year-long results from fertigated coffee plants receiving biweekly N via the LEPA system, which is equivalent to our ‘foliar plus soil’ treatment (Bruno et al., 2011). In that study, leaf QNdff increased significantly with higher N rates, peaking between 237 and 265 days after application (during the grain filling stage). As in our experiment, Efficiency was highest at the lowest applied rate. These authors reported Efficiency values ranging from 20 % to 50 % for 200 kg ha⁻¹, and from 10 % to 30 % for 800 kg ha⁻¹. These values were higher than those observed in our study, likely due to the longer evaluation period. In our experiment, Efficiency increased from 4.7 % at 24 hours to 13.8 % at 72 hours with 2.3 g N, and from 2.9 % to 9.4 % with 4.6 g N (Figure 3 C). This nearly threefold increase in Efficiency over a short period underscores the importance of frequent, short-term evaluations to better understand N uptake.

Regarding Ndff and QNdff (Figure 4 A-F), our results on the interaction between N application methods and amounts differ from previous studies. For example, an 11-day field trial in corn showed higher QNdff-equivalent values with foliar application compared to soil application (Roberts et al., 2018). In contrast, we found no significant difference between foliar and soil applications at 2.3 gN in any of the evaluation times. At 4.6 g N, foliar application resulted in higher values than soil application during the first 48 hours, but this difference disappeared by 72 hours. These observations suggest that the effect of application method - and thus the contribution of foliar and root uptake pathways - on QNdff varies with N amount and across species.

The Efficiency results (Figure 4 G-I) were similar to those observed in corn by Roberts et al. (2018), where soil application showed lower Efficiency than foliar application

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after 11 days. In that study, the low Efficiency of soil-applied N was attributed to the absence of rain or irrigation, which limited urea incorporation into the soil. In our study, however, N-urea was applied in solution and incorporated through fertigation, yet soil application still proved less efficient, especially within the first 48 hours, and particularly at the higher amount of N. This may be due to the short evaluation period and the exclusive focus on leaf sampling. N absorbed by roots as nitrate or ammonium can be either assimilated locally or transported via xylem to the leaves (Tegeder & Masclaux-Daubresse, 2018). As Ndff in leaves already indicated root uptake within 24 hours, longer evaluation periods including root sampling would help clarify N uptake efficiency through different uptake pathways.

To our knowledge, this is the first study to compare different urea application pathways in coffee plants and show that the combined ‘foliar plus soil’ approach enhances short time N uptake efficiency. Despite the short evaluation period, the findings highlight the potential of integrated application methods to enhance N uptake and use in fertigated coffee systems.

5 CONCLUSIONS

Ndff increased significantly between 24 and 48 hours, indicating rapid absorption of N derived from fertilizer. The combined ‘foliar plus soil’ application of 2.3 g of N achieved the highest Efficiency, suggesting complementary uptake pathways. These findings demonstrate that simultaneous urea application through both leaf and soil enhances short-term N uptake Efficiency in coffee plants.

6 AUTHORS' CONTRIBUTION

Conceptual Idea: Bruno, I.P.; Dourado-Neto, D.; Reichardt, K.; Methodology design: Bruno, I.P.; Dourado-Neto, D.; Reichardt, K.; Data collection: Bruno, I.P.; Data analysis and interpretation: Bruno, I.P.; Araujo, A.G.; Dourado-Neto, D.; Reichardt, K., and Writing and editing: Bruno, I.P.; Araujo, A.G.

7 ACKNOWLEDGMENTS

The authors would like to thank ‘Fazenda Morena’ and Agronomist Wesley Moreira for their assistance with the fieldwork, and the ‘Center for Nuclear Energy in Agriculture - University of São Paulo’ for their support with the ^{15}N analyses.

8 DATA AVAILABILITY STATEMENT

Data available upon request to authors.

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