Nitrogen

Article

Iodine-Enriched Urea Reduces Volatilization and Improves Nitrogen Uptake in Maize Plants

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Abstract: Urea is the primary source of nitrogen (N) used in agriculture. However, it has a high N loss potential through volatilization. Various mechanisms can be employed to reduce N volatilization losses by inhibiting urease. When added to urea, iodine (I) has high potential for this purpose. Thus, this study aimed to determine whether adding I to urea reduces volatilization losses and increases N uptake in maize plants. Maize plants were cultivated in greenhouse conditions for 36 days. Urea treatments were applied at 15 days of testing, including iodine-enriched urea, conventional urea, and no urea application. Additionally, a study concerning N volatilization from urea was conducted using the same treatments under the same environmental conditions. Iodine was incorporated and adhered to urea, at an I concentration of 0.2%, using potassium iodate (KIO_3). Under controlled conditions and over a short period of time, it was observed that the application of iodine-enriched urea increased the chlorophyll *b* content, root N accumulation, and total N accumulation in maize plants compared with conventional urea. Moreover, iodine-enriched urea reduced N losses from volatilization by 11% compared with conventional urea. The reduction in N volatilization correlated positively with the increased chlorophyll *b*, total chlorophyll, root N accumulation, and total N accumulation favored by the iodine-enriched urea treatment. Our findings demonstrated that adding I to urea is an efficient and promising strategy to reduce N losses and increase N uptake in plants.

Keywords: ammonia; chlorophyll; nitrogen losses; plant metabolism; urease

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1. Introduction

Fertilizers are essential for the adequate supply of nutrients to plants, ensuring the productivity of agricultural systems [1,2]. Among the nutrients applied through fertilizers, nitrogen (N), phosphorus, and potassium are the most widely used, with global production estimated at approximately 116.68, 48.27, and 46.64 million tons, respectively [3]. Regarding N fertilizers, less than 50% of the N applied via fertilizers in agroecosystems is absorbed by plants [1,4]. Nitrogen uptake by plants from fertilizers depends highly on environmental conditions during application, soil types, and the sources' properties [1,2,4]. Different sources are used to provide N to plants, such as organic residues, ammonium nitrate (NH_4NO_3), sulfates ($(NH_4)_2SO_4$), nitrates ($NaNO_3$, $Ca(NO_3)_2$, KNO_3), phosphates ($NH_4H_2PO_4$, $(NH_4)_2HPO_4$), aqua ammonia (NH_4OH), anhydrous ammonia ($2NH_3$), and urea ($CO\ (NH_2)_2$), with urea being the primary source used in agriculture, accounting for 56% of the current N consumption [1].

The widespread use of urea is mainly due to its lower cost than other sources and high N content (approximately 46% N) [1,2,5]. When applied to the soil, urea undergoes hydrolysis promoted by the enzyme urease, and consequently, there can be significant losses of N, as the gas ammonia (NH_3), through volatilization [1,4]. Although ammonia is not a greenhouse gas (GHG), it contributes indirectly to emissions [6,7]. Nitrogen losses from urea through volatilization can exceed 60% of the N applied [8].

Several strategies can be used to minimize N losses through volatilization, such as management practices in the field or materials that can inhibit the urease enzyme in the soil, thereby reducing its activity and consequently reducing ammonia production [9–13]. Among the compounds used as urease inhibitors, currently, only N-(n-butyl) thiophosphoric triamide (NBPT) has been used worldwide, with its market growing 16% annually over the past ten years [5,10–13]. However, several other materials and elements, especially metals, can also reduce urease activity and N volatilization from urea [1,13–15]. Among these elements, iodine (I) has shown a high capacity to reduce N losses through volatilization in urea [16].

In rare studies, it has been shown that I can reduce urease activity, primarily because it acts on specific functional groups, mainly thiol groups (-SH), oxidizing and destroying them, resulting in a drastic reduction in or complete inhibition of urease activity [17–19]. Once urease activity is reduced, N losses from urea through volatilization decrease, increasing plants' N uptake [1,5,20,21]. Additionally, in some studies, whether through the application of I to the soil along with fertilizers or isolated iodine applications to the soil, it has been shown that iodine can influence the metabolism of N, increasing its uptake by plants and also affecting the photosynthetic process in plants through different mechanisms [22–30].

Despite the potential of I to reduce urease activity and influence N metabolism, no current literature has shown that when it is incorporated into urea, it can reduce N losses through volatilization and increase N uptake by plants. This research gathers the primary information on using I to increase the efficiency of urea as a source of N in agriculture. Innovatively, the paper explores the potential of iodine-enriched urea to reduce losses due to N volatilization, a significant problem with conventional urea fertilizers. It also finds an improved N uptake and correlation with plant growth parameters. Thus, our study hypothesized that synthesizing iodine-enriched urea could produce N-enhanced efficiency fertilizers. The aims of the study were as follows: (i) to determine whether the use of iodine-enriched urea improves N accumulation in maize plants compared with conventional urea; (ii) to verify whether iodine-enriched urea reduces N volatilized as NH_3; (iii) to evaluate whether the application of iodine via urea increases chlorophyll contents in maize plants; and (iv) to examine whether the reduction in volatilization promoted by iodine-enriched urea has a positive correlation with increased chlorophyll contents and N accumulation in maize plants.

2. Materials and Methods

2.1. Synthesis of Iodine-Enriched Urea

In synthesizing iodine-enriched urea, urea (carbamide = CH_4N_2O) (reagent-grade, Synth, Diadema, São Paulo, Brazil), triethanolamine, organic dye, and an I source were used. The I source used was potassium iodate (KIO_3) (reagent-grade, Synth, Diadema, São Paulo, Brazil). Triethanolamine (surface-active agent) was incorporated and adhered I to the urea granule surface. The dye was used to ensure that all urea granules were impregnated with iodine.

The synthesis process started with KIO_3, triethanolamine, and the organic dye being initially added and homogenized in a beaker. Subsequently, urea was added to the initial mixture, and the material was homogenized again until all the urea was uniformly impregnated with the mixture and no material was retained in the beaker. The final concentration of I in the enriched urea was 0.2%, calculated from the amount of I added concerning urea's final weight. Thus, based on the molarity of I and KIO_3, the final amount of KIO_3 in urea was 0.34%. The concentration of I in urea was determined according to previous studies. After this, the samples were dried at room temperature for 24 h and stored for later use (Figure 1).

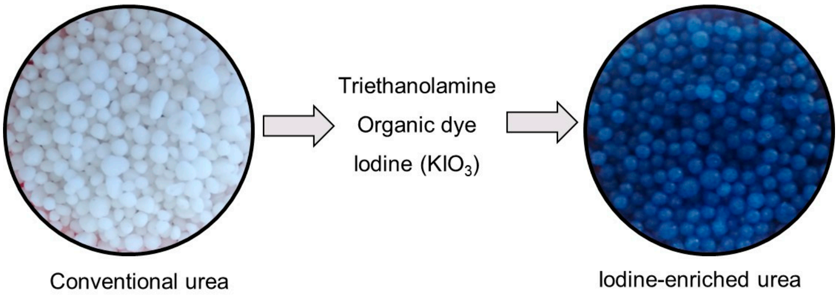


Figure 1. Conventional urea and iodine-enriched urea photos.

2.2. Maize Growing and Treatments

The study was carried out using samples of an Oxisol collected at the 0–0.20 m layer. According to the Brazilian soil classification system, the soil was classified as Latossolo Vermelho [31], which corresponds to Ferralsols [32] and Oxisols in the soil taxonomy [33]. The collected soil was dried and characterized (Table 1) and then incubated with $CaCO_3$ and $MgCO_3$ in a 3:1 molar ratio to increase the contents of Ca and Mg and to increase soil pH to ~5.5–6.0. The amount of the $CaCO_3$:$MgCO_3$ mixture used was based on the method of incubating soils with increasing doses of $CaCO_3$. The samples were then incubated for 30 days, maintaining the soil moisture at 70% of the maximum water retention capacity (MWRC). Next, the samples were dried and sieved (<4 mm). The pH values in water at a 1:2.5 (w/v) after incubation were 5.6 ± 0.03. Tropical soils under natural conditions have low availabilities of Ca and Mg and most of them have an acidic pH [34]. These conditions promote low N uptake by plants and other nutrients supplied through fertilizers [34,35]. Therefore, the correction of soil pH, as performed in our study, is essential to optimize the use of soil-added nutrients and plant yield.

Table 1. Chemical, physicochemical, and soil particle size distributions of the soil used.

Parameters	Values	Protocol
pH (1 g of soil: 2.5 mL of water)	4.5	pH determined in water
Soil organic matter (g kg^{-1})	24.9	Walkley–Black method
Total nitrogen (g kg^{-1})	2.3	Kjeldahl method
Clay (g kg^{-1})	670	Boyoucos method
Silt (g kg^{-1})	130	Boyoucos method
Sand (g kg^{-1})	200	Boyoucos method
Exchangeable calcium $^{2+}$ ($cmol_c$ kg^{-1})	0.4	1 mol L^{-1} KCl solution–soil test
Exchangeable magnesium^{2+} ($cmol_c$ kg^{-1})	0.2	1 mol L^{-1} KCl solution–soil test
Available phosphorus (mg kg^{-1})	0.4	Mehlich-1 soil test
Available potassium (mg kg^{-1})	24.8	Mehlich-1 soil test
Available zinc (mg kg^{-1})	0.2	Mehlich-1 soil test
Available iron (mg kg^{-1})	38.0	Mehlich-1 soil test
Available manganese (mg kg^{-1})	3.4	Mehlich-1 soil test
Available copper (mg kg^{-1})	1.2	Mehlich-1 soil test
Available boron (mg kg^{-1})	0.01	Hot-water extraction method
Available sulfur (mg kg^{-1})	2.9	Monocalcium phosphate diluted in acetic acid method

All methodologies are described in Teixeira et al. [36].

Pots were filled with 0.5 kg of soil, and 200 mg kg^{-1} of P and 90.4 mg kg^{-1} of N were homogeneously applied in the whole soil using $NH_4H_2PO_4$ (reagent-grade, Synth, Diadema, São Paulo, Brazil). Then, ten maize seeds (*Zea mays* L.) were sown in each pot, and the remaining planting fertilization was performed using 100, 41, 0.8, 1.33, 3.66, 0.15, 4, and 1.55 mg kg^{-1} of K, S, B, Cu, Mn, Mo, Zn, and Fe, respectively, using the following reagents: K_2SO_4, H_3BO_3, $CuSO_4.5H_2O$, $MnSO_4 \cdot H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, and $FeCl_3 \cdot 6H_2O$ (reagent-grade, Synth, Diadema, São Paulo, Brazil). Seven days after planting, thinning was performed, leaving two plants per pot until harvest.

Top-dress fertilization related to the treatments tested was performed 15 days after planting. Urea fertilization was the factor studied and three urea application conditions were tested: iodine-enriched urea, conventional urea, and no urea application. The experiment was conducted using a completely randomized design with five replicates, totaling 15 experimental units. Urea was applied superficially at a rate of 300 mg of N kg^{-1} of soil. Immediately after urea application, the pots were irrigated to incorporate the urea. Irrigation was carried out since urea is generally applied before a rain event; thus, irrigation aims to simulate the effect of incorporating urea into the soil [37].

The maize plants were grown until 36 days, reaching the V6 stage. During the maize cultivation in the greenhouse, the average daytime temperature (12 h) was maintained at 28 °C and the average nighttime temperature (12 h) at 18 °C. At 36 days, the chlorophyll *a* and *b* contents of the newly expanded leaf from each plant in each pot were determined using a portable chlorophyll meter (ClorofiLog®). The total chlorophyll content was obtained by summing the chlorophyll *a* and *b* contents. After this, the plants were harvested and divided into roots and shoots. The roots were washed with distilled water to remove any adhering soil particles. The root and shoot samples were then dried in an oven at 45 °C until constant weight was achieved, and the dry matter contents of the shoot (SDM) and root (RDM) were determined. The total dry mass was obtained by summing SDM and RDM. The Kjeldahl method determined the total N content in dried root and shoot samples [38,39].

2.3. Nitrogen Volatilization

The study used the same experimental design, type of soil (Table 1), and environmental conditions adopted for maize cultivation, evaluating three urea application conditions: iodine-enriched urea, conventional urea, and without urea application. However, although this study was conducted simultaneously, it involved different experimental units. The experiment also used a completely randomized design with five replicates, totaling 15 experimental units. The N volatilization study was conducted using mini-lysimeters. A 0.45 µm pore filter was inserted from the bottom to the upper chamber of the mini-lysimeters, which were then filled with 0.5 cm of glass wool and 0.5 kg of soil (pH-corrected soil).

On top of the soil, urea was applied at a rate of 300 mg of N kg^{-1} of soil as in the maize experiment. Immediately after urea application, the pots were irrigated to incorporate the urea, maintaining soil moisture at 70% of the maximum water retention capacity (MWRC) throughout the experimental period. A sponge (0.02 g cm^{-3} density) soaked with a phosphoric acid (75 mL L^{-1}) solution and glycerin (50 mL L^{-1}) was then inserted to capture volatilized N as ammonia ($N\text{-}NH_3$) [20,21]. The sponge with phosphoric acid and glycerin was placed about 1.5 cm above the soil. Another sponge was placed above it to protect the system and prevent contamination of the ammonia-capturing sponge.

The sponge soaked with glycerin and phosphoric acid was maintained throughout the experimental period, with sponges being replaced on the collection days. Ammonia volatilized in the sponge was collected on days 1, 2, 3, 5, 7, 10, 14, and 21 after urea application. These times were chosen based on the interval between urea top-dressing in the maize experiment and the harvest. The size of each pot limited the maximum cultivation period.

After collection, the sponges were washed with distilled water in a volumetric flask up to a final volume of 500 mL. Aliquots of 20 mL were then removed from this solution to determine the total N content through distillation, followed by titration using the Kjeldahl method [39]. In the distillation and titration procedure for N in the aliquots, 10 mL of 13 mol L^{-1} NaOH was used in each tube to promote the distillation of N as $N\text{-}NH_3$, which was captured in a boric acid solution (20 g boric acid L^{-1}) until 50 mL of distillate had accumulated in the Erlenmeyer flask. Then, the titration phase was performed using 0.07143 mol L^{-1} HCl. The cumulative volatilized N was calculated based on the total N determined in the aliquots, the total volume of the solutions, and the number of days between collections. The mini-lysimeter setup and the steps for determining the collected N are shown in Figure 2.

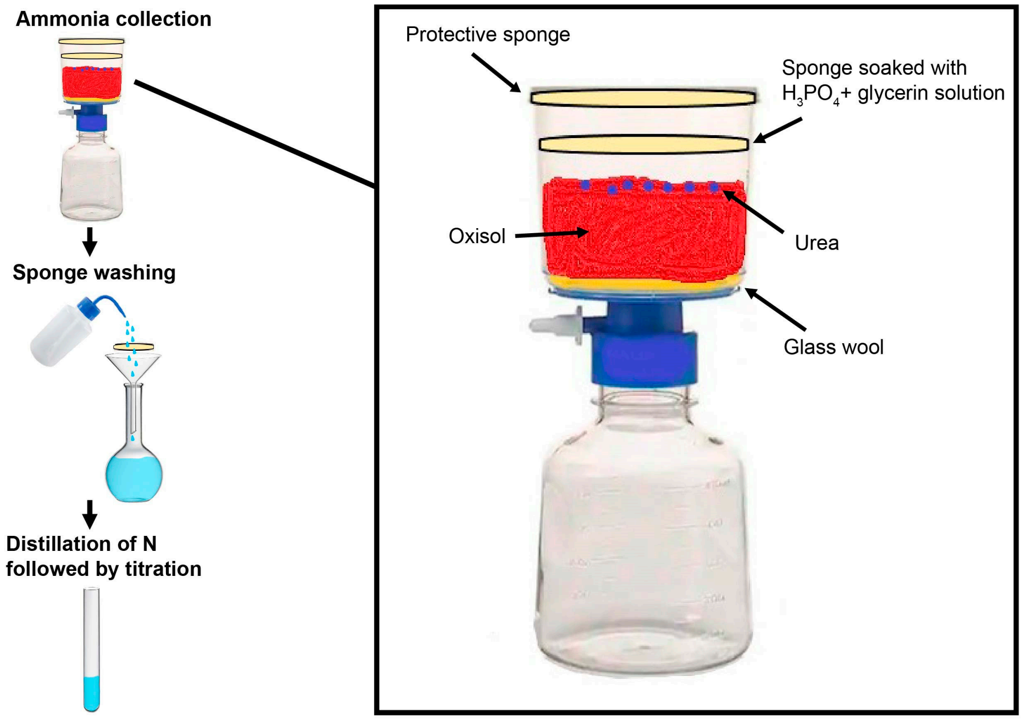


Figure 2. A scheme for the nitrogen volatilization study that was carried out.

2.4. Statistical Analysis

All statistical analyses were performed using the R software version 4.3.1 with the following packages: stats, base, tidyverse, agricolae, factoextra, FactoMineR, and corrplot [40–44]. The cumulative N-volatilization-amount dataset was adjusted to different nonlinear mathematical models to show the relationship between this variable and increasing evaluation days [45–47]. The following mathematical models were adjusted: linear model (Equation (1)), Elovich model (Equation (2)), exponential simple (Equation (3)) model, power function (Equation (4)) model, and hyperbolic model (Equation (5)).

$$\mathrm{N} = \mathrm{a} + \mathrm{b}\ x\ \mathrm{days} \tag{1}$$

$$\mathrm{N} = \mathrm{a} + \mathrm{b}\ \ln \mathrm{days} \tag{2}$$

$$\mathrm{N} = \mathrm{a}\ x\ \left(1 - \mathrm{e}^{\mathrm{b\ x\ days}}\right) \tag{3}$$

$$\mathrm{N} = \mathrm{a}\ x\ \mathrm{days}^{\mathrm{b}} \tag{4}$$

$$\mathrm{N} = \frac{\mathrm{V0} \times \mathrm{days}}{\mathrm{V0}\ x\ \mathrm{b}\ x\ \mathrm{days}} \tag{5}$$

In these equations, N is the cumulative N volatilization amount from each urea condition in the days evaluated, a is the initial value of variable used from each urea condition on one day, b is the variable rate constant for each urea condition, days are days evaluated, and V0 is the maximum value of the variable used from urea condition across the day range investigated. The best model was selected based on the highest value of the coefficient of determination (R^2), the lowest value of root-mean-squared error (RMSE) (Equation (6)), and the lowest value of the Akaike information criterion (AIC) (Equation (7)) [48]. The comparison of mathematical models fitted to each cumulative N volatilization amount over days evaluated was conducted using a 95% confidence interval created through 1000 bootstrap interactions.

$$\text{RMSE} = \sqrt{\sum_{i=1}^{n} \frac{(\text{Predicted values} - \text{Observed values})^2}{\text{Number of observations}}} \tag{6}$$

$$\text{AIC} = \text{Number of observations } x \ln\left(\frac{\text{Sum square of errors}}{\text{Number of observations}}\right) + (2\ x\,\text{number of parameters}) \tag{7}$$

Additionally, a principal component analysis (PCA) and Pearson correlation analysis were performed to determine the relation between variables analyzed in maize and volatilization studies. In the PCA and correlation analysis, data related to the lack of urea application were removed from the dataset as there was no N volatilization due to the lack of urea application. Furthermore, these analyses aimed to differentiate the effects of iodine-enriched urea versus conventional urea. The data were initially scaled in the PCA analysis, and the PCA analysis was performed.

3. Results

3.1. Maize Cultivation

When compared with plants that had received top-dressing fertilization with urea, regardless of the addition of I (iodine-enriched urea and conventional urea), the no-urea-application group showed reduced total chlorophyll (−19%) and chlorophyll *a* (−15%) contents (Figure 3a). There was no difference ($p > 0.05$) in the total chlorophyll and chlorophyll *a* contents between the application of conventional urea and iodine-enriched urea. However, for chlorophyll *b* content, fertilization with iodine-enriched urea increased the values by 10% compared with conventional urea. Both fertilization with urea had increased chlorophyll *b* contents compared with the lack of urea application, with 67% and 53% increments for iodine-enriched urea and conventional urea, respectively.

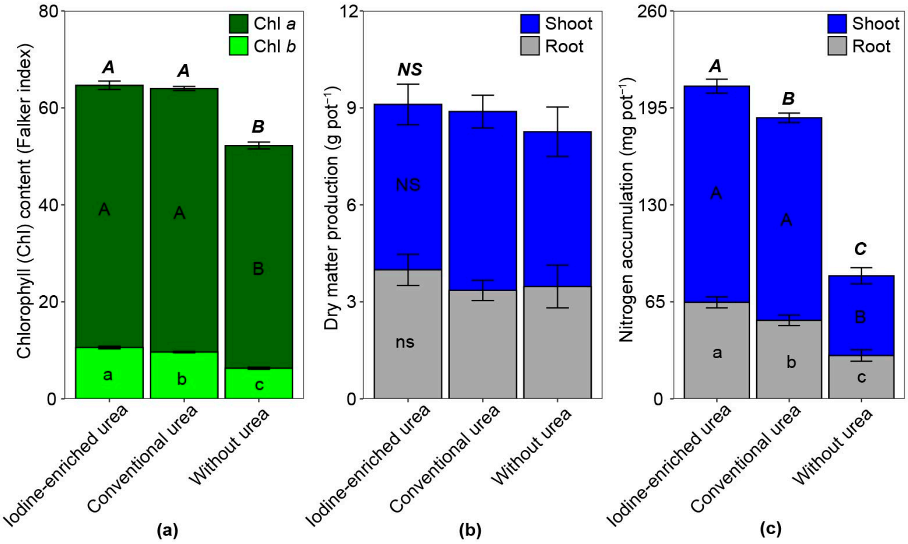


Figure 3. Chlorophyll content, dry matter production, and nitrogen accumulation in maize according to the urea fertilization. Full bar: total chlorophyll (**a**) or total biomass production (**b**) or total nitrogen accumulation (**c**). ***NS***, NS, and ns: without a statistical difference in total, shoot, and root dry matter production ($p > 0.05$). The means in Figure 3a follow the same scheme with bold–italic capitals, capital letters, and minuscule letters, not differentiating the treatments for total chlorophyll, chlorophyll *a* (Chl *a*), and chlorophyll *b* (Chl *b*) contents, respectively ($p > 0.05$). The means in Figure 3c follow the same scheme with bold–italic capitals, capital letters, and minuscule letters, not differentiating the treatments for total, shoot, and root nitrogen accumulations, respectively ($p > 0.05$).

There was no difference in maize dry matter production (total, shoot, and root) when comparing the urea application treatments with the no-urea-application treatment (Figure 3b). In the maize shoots, N accumulation did not differ for the types of urea tested ($p > 0.05$). Still, both urea treatments tested had increased N accumulation in the shoots by 162% compared with the no-urea-application treatment (Figure 3c). Nitrogen accumulation in the root and the total N accumulation in the plants had increased with urea compared with the lack of urea application. Among the types of urea evaluated, iodine-enriched urea had increased N accumulation in the roots and total N accumulation by 23% and 11%, respectively, compared with conventional urea.

3.2. Nitrogen Volatilization

The best model for N volatilization by both types of urea was the exponential model, as described in Table 2. The exponential models showed the lowest RMSE and AIC values and the highest R^2 values compared with the other models. For the treatment where urea was not applied, the models did not fit because no significant amounts of N had been volatilized.

Table 2. Coefficients of the mathematical regression models adjusted for cumulative nitrogen volatilization according to the urea fertilization treatments.

Models	**Iodine-Enriched Urea**			**Conventional Urea**			**Without Urea**		
	R^2	**RMSE**	**AIC**	**R^2**	**RMSE**	**AIC**	**R^2**	**RMSE**	**AIC**
Linear	0.48	5.07	249.32	0.55	5.16	250.76	0.19	0.23	1.75
Elovich	0.75	3.48	219.18	0.84	3.11	210.25	0.16	0.23	3.00
Exponential	0.80	3.22	213.14	0.87	2.80	201.96	0.19	0.23	1.91
Power function	0.66	4.11	232.60	0.74	3.96	229.56	0.18	19.65	229.56
Hyperbolic	0.78	3.35	216.31	0.86	2.99	207.17	0.19	0.23	1.91

R^2: regression coefficient; RMSE: root-mean-squared error; AIC: Akaike Information Criterion.

Generally, for both ureas tested, it was observed that starting from day 10, the amount of N loss through the volatilization process tended to stabilize, with the most significant N loss observed two days after urea application (Figure 4). Compared with conventional urea, it was observed that iodine-enriched urea, from day 6.5 onwards, had had a lower amount of accumulated N volatilized up to 21 days of evaluation. The final cumulative N volatilization at 21 days for conventional urea was 26.3 mg of N (17.5% of the N added), while for iodine-enriched urea, the N volatilized was 23.5 mg of N (15.7% of the N added), a reduction of 11% compared with conventional urea.

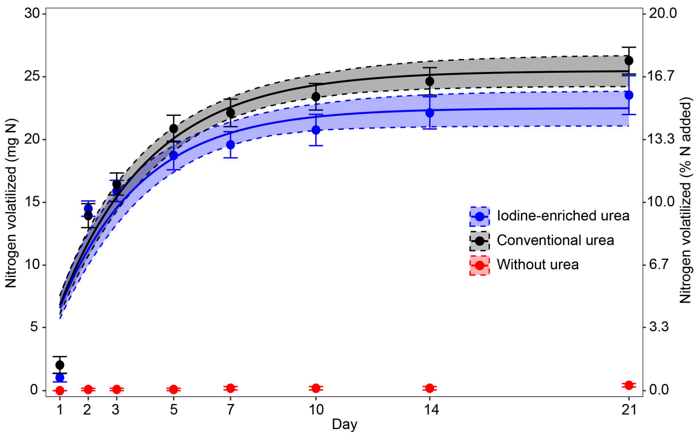
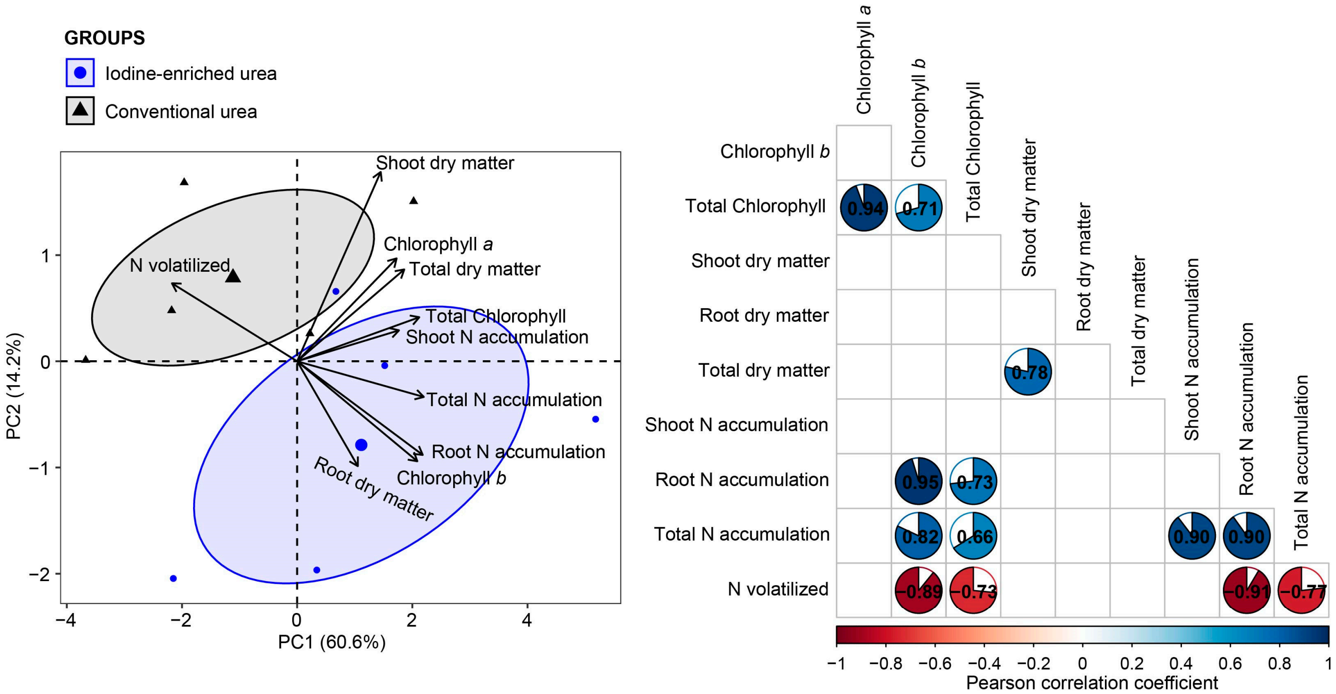


Figure 4. Cumulative nitrogen (N) volatilization according to the urea fertilization treatments. Treatments whose bootstrap-generated confidence intervals do not overlap in the figure were statistically different ($p < 0.05$). The amount of N added was 150 mg per mini-lysimeter.

3.3. Multivariate and Correlation Analysis

The chlorophyll *b* and total chlorophyll contents, root N accumulation, and total N accumulation had positive correlations (Figure 5b), favored by iodine-enriched urea application compared with conventional urea application (Figure 5a). Furthermore, the application of iodine-enriched urea showed a negative relationship with the amount of volatilized N. The amount of volatilized N exhibited a negative correlation with the chlorophyll *b* content (r: −0.89), total chlorophyll content (r: −0.73), root N accumulation (r: −0.91), and total N accumulation (r: −0.77). There was no correlation between volatilized N and chlorophyll *a* content; total, shoot, and root dry matter; or the influence of the types of urea tested.



(a) (b)

Figure 5. Principal component analysis (**a**) and Pearson correlation (**b**) for variables analyzed in the maize cultivation and N volatilization studies. Significant correlation coefficients ($p < 0.05$) are indicated by bold numbers, with positive and negative correlations distinguished by red and blue, respectively. White boxes indicate non-significance without numbers.

4. Discussion

The main source of N used in agriculture is urea (56% of all), which has significant losses due to volatilization [1]. However, we have shown that adding I to urea can reduce these losses. When hydrolyzed by the enzyme urease, urea ($CO(NH_2)_2$) forms NH_4^+ ions in the soil as follows: $CO(NH_2)_2 + 2H^+ + 2H_2O \longrightarrow 2NH_4^+ + CO_2 + OH^-$ [1,14]. Moreover, urea application raises the pH around the urea granule, and this condition promotes the conversion of ammonium (NH_4^+) to ammonia (NH_3), which is rapidly lost as a gas to the atmosphere through the volatilization process [1,13]. Urea N losses due to volatilization are influenced by several factors, such as pH, moisture, temperature, soil buffering capacity, and soil organic matter [1,12,13]. Surface-applied urea can result in volatilization losses exceeding 60% of the applied N [8]. It has been estimated that global urea N losses due to volatilization amount to USD 74.2 billion, assuming an average global urea N volatilization loss of 14% [1]. Various strategies have been adopted to reduce N losses, such as incorporating urea into the soil [9] or adding compounds inhibiting urease activity, slowing urea hydrolysis, and reducing volatilization losses [5,10,11,13].

The most common compounds applied with urea to reduce volatilization losses by decreasing urease activity include triazoles, quinones, fluorides, flavonoids, phosphorus compounds, imidazoles, Schiff base ligan metal complexes, polyphenols, hydroxamic acids, and Schiff bases [12,14]. However, an element with high potential to reduce volatilization losses is iodine, as demonstrated in our study, under controlled conditions and over a short period of time, where the application of iodine-enriched urea reduced N losses from volatilization compared with conventional urea (Figures 4 and 5).

Iodine can reduce urea volatilization since it can decrease or inactivate the urease activity [17,18], consequently lowering the conversion of urea to ammonia [13,14,18]. This condition occurs because I can act on characteristic groups in the urease molecule, such as thiol groups (-SH), oxidizing and destroying them, and it may also substitute amino ($-NH_2$) and amide (-NH-) functional groups in the urease molecule [17,18]. These structural modifications in urease directly interfere with the interactions between urease and its substrate (urea), altering and inactivating the urease [11,13,14,17,18,49]. The effect of I on urease was observed in a study conducted with *Vicia sativa* seeds, estimating the urease activity by using a medium where 1 mL of 0.1% (w/v) urea was added along with an enzyme solution (50 µL enzyme + 100 µL metal + 1.85 mL 0.2 M buffer). It was observed that the addition of I was able to reduce urease activity by 50%, an effect directly related to the thiol (-SH) group in the enzyme's active site [19].

Treatments that reduce urease activity and consequently decrease N volatilization maximize N uptake by plants, increasing the uptake of ammonium-carrying N fertilizers [1,10,12,13]. This effect was observed in our study under controlled conditions and over a short period of time, where there was a negative correlation between higher rates of N volatilization and the root N accumulation and the total N accumulation in maize plants favored by a greater efficiency of N use due to the application of iodine-enriched urea (Figures 3–5) as the apparent recovery of applied N for the iodine-enriched urea was 70%, compared with 63% for conventional urea. Additionally, applying I to plants has shown a synergistic effect on N uptake by plants, as demonstrated by several studies [26–30]. This synergistic effect of iodine application on N metabolism is poorly understood. However, in research conducted with the application of KIO_3, an increase in growth was observed in lettuce plants due to the stimulation by iodine of nitrate reductase activity and the Glutamine Synthetase/Glutamate Synthase (GS/GOGAT) cycle [25].

In a study conducted with fenugreek, the application of I increased N accumulation and the protein content [29]. In tomatoes, applying KIO_3 via soil [29] or foliar spray [46] also increased total N uptake in plants and tomato fruits. In spinach, using doses similar to our study (2 kg of I ha^{-1}, considering a depth of 0–0.20 m), the soil application of 1 and 2 mg of I dm^{-3}, equivalent to 2 and 4 kg ha^{-1} (0–0.20 m depth), increased both the N content as well as the amino acid content in spinach leaves [26]. Our study also showed an increase in N uptake with the application of iodine-enriched urea compared with no iodine application (conventional urea) (Figure 3). Similar to our study using the same amount and source of I (2 kg ha^{-1} of I supplied as KIO_3), the combined application with an N fertilizer significantly improved N utilization from mineral fertilizers for carrots [27]. In our study, this increase in N accumulation due to I application was more pronounced in the roots compared with the total accumulation, with no effect being observed in the shoot for the different urea treatments (Figure 3).

The improvement in N nutritional status due to I application can affect both N-rich cellular components, such as chlorophylls, and the photosynthetic process in plants [22–25,28,50,51]. Iodine participates in photosynthesis as a chlorophyll component and promotes the synthesis of carbohydrates and proteins [50]. This effect of iodine on chlorophyll was observed in a study with rye plants cultivated under field conditions, where the application of iodine to the soil at 2.5 and 5 kg ha^{-1} increased chlorophyll *a* and *b* contents with an increasing I concentration [28]. Evaluating coffee plants under water deficit conditions, the application of I via soil (2.5 kg ha^{-1} of KIO_3) compared with a lack of I fertilization mitigated the water deficit and also increased chlorophyll *b* (+45%) and total chlorophyll (+23%) contents, without affecting the chlorophyll *a* content. Similar to what was noted in the rye and coffee studies, applying I via urea in our study increased the chlorophyll *b* content in maize plants at doses comparable to those mentioned (Figure 3). Furthermore, in our study, this increase in chlorophyll *b* content was directly related to reduced N losses through volatilization (Figure 5). Thus, it was observed that adding iodine to urea was an effective strategy to increase the uptake of N from fertilizers. However, experiments under field conditions evaluating agronomic efficiency, feasibility, and financial return should be conducted.

5. Conclusions

This research introduces a promising and efficient strategy to enhance nitrogen (N) utilization in agriculture, addressing a key challenge of N loss through volatilization and increasing the sustainability of urea-based fertilizers. Our study, conducted under controlled conditions and over a short period of time, found a direct positive correlation between the highest values of the total accumulation of N, root accumulation of N, total chlorophyll content, and chlorophyll *b* and a negative correlation with the accumulated iodine-enriched urea favoring lower N losses. Compared with conventional urea, iodine-enriched urea reduces N losses through volatilization, increasing N accumulation in maize plants. Additionally, the iodine contained in urea could modify plant metabolism, leading to an increased chlorophyll content. Therefore, coating urea with iodine is an effective strategy for creating fertilizers that improve N uptake. However, the full potential of this technology can only be realized through further field studies. These studies should evaluate N uptake by plants under these conditions, as well as the financial returns of such technology, and are crucial for advancing this research.

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