Article

Effect of Urea-Calcium Sulfate Cocrystal Nitrogen Fertilizer on Sorghum Productivity and Soil N$_2$O Emissions

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Abstract: Urea cocrystal materials have recently emerged as high nitrogen (N) content fertilizers with low solubility capable of minimizing N loss and improving their use efficiency. However, their effects on crop productivity and N$_2$O emissions remain underexplored. A greenhouse study was designed to evaluate sorghum (*Sorghum bicolor* (L.) Moench) yield, N uptake, and N$_2$O emissions under six N treatments: C0 (without fertilizer), UR100 (urea), UC100 (CaSO$_4$·4urea cocrystal) at 150 kg N ha^{-1}, and CaSO$_4$·4urea cocrystal at 40%, 70%, and 130% of 150 kg N ha^{-1} (UC40, UC70, and UC130, respectively). The results demonstrated that UR100, UC100, and UC130 had 51.4%, 87.5%, and 91.5% greater grain yields than the control. The soil nitrate and sulfur concentration, N uptake, and use efficiency were the greatest in UC130, while UR100 had significantly greater N$_2$O loss within the first week of N application than the control and all the urea cocrystal treatments. UC130 minimized the rapid N loss in the environment as N$_2$O emissions shortly after fertilizer application. Results of this study suggest the positive role of urea cocrystal in providing a balanced N supply and increasing crop yield in a more environmentally friendly way than urea alone. It could be good alternative fertilizer to minimize N loss as N$_2$O emissions and significantly increase the N use efficiency in sorghum.

Keywords: urea; CaSO$_4$·4urea; cocrystal; nitrogen; N$_2$O; sorghum

1. Introduction

Sustainable N management has emerged as one of the critical challenges of the 21st century due to the need to minimize the influx of this important nutrient into the environment while maintaining food production needed for the growing population [1–6]. In particular, the balanced use of N fertilizers is necessary to maintain proper land use, avoid excess food production, and minimize food waste and the associated environmental impacts [7]. Losses of N from mineral fertilizers are mainly related to its instability in moist soil where urea is quickly hydrolyzed, catalyzed by the efficient dinickel urease enzyme [8–13]. As a result of the accelerated urea hydrolysis by soil enzymes in moist soil, small mobile reactive N molecules form, such as ammonium ions (NH$_4^+$) and nitrate ions (NO$_3^-$), via the nitrification process [14]. These are very mobile molecules that volatilize into the atmosphere and/or leach into the watershed with adverse environmental impacts [15]. It has been estimated that globally up to 50% of applied N is lost through leaching, runoff, or greenhouse gas emissions [16]. The N loss is also associated with significant energy pollution since ammonia (NH$_3$), a urea precursor, production consumes 3% to 5% of the total natural gas output [17] and 1% to 2% of global energy [17,18]. Significant research efforts have thus been dedicated to stabilizing urea in the environment via the design of various

chemically stabilized urea condensation polymers, such as ureaforms, that confine urea into a polymeric structure, urease enzyme inhibitors that delay urea hydrolysis, nitrification inhibitors that reduce the activity of nitrifying bacteria, and polymer- or sulfur-coated fertilizers that utilize a partially permeable coating material for controlled N release [19–21]. While using enhanced-efficiency fertilizers allows for better synchronization of N supply with crop N uptake [22], high associated costs and complex production processes remain significant obstacles in their wide use [23].

Urea-based cocrystal materials have recently emerged as high N content fertilizers [24,25] with decreased solubility [26,27] and low volatilization. These fertilizers are often produced from sustainable mineral or waste feedstock via sustainable mechanochemical routes [24,25,27–30]. In particular, $CaSO_4 \cdot 4(CO(NH_2)_2)$ (later in the text referred to as $CaSO_4 \cdot 4urea$) is typically synthesized using gypsum ($CaSO_4 \cdot 2H_2O$) and other calcium sulfate analogs [27,30–32]. The solubility of $CaSO_4 \cdot 4urea$ is almost $\times 20$ lower than that of pure urea [27]. As a result, the volatilization of $CaSO_4 \cdot 4urea$ is much lower than urea. For example, it took 85 days to achieve cumulative ~70% NH_3 emissions from $CaSO_4 \cdot 4urea$, whereas the equivalent amount of urea was volatilized only in two weeks [27]. In a field study by Barčauskaitė et al., a statistically greater maize (*Zea mays* L.) cob yield was obtained with urea + $(NH_4)_2SO_4$ than that obtained with urea only [29]. The same study also showed that the mobile sulfur content in the top 0–30 cm soil was higher when using $CaSO_4 \cdot 4urea$ than other fertilizer treatments with equal sulfur amount up to 8 weeks after the application. The concentration of the mineralized N in the soil was much higher up to 10 weeks after the application than when using other fertilizers. Recent attempts further elaborated on maize grain N uptake in a 2-year field study [33,34]. Large variation in apparent N recovery (ANR) was observed between the years, attributed to different prevalent atmospheric conditions. In particular, while there were no statistically significant differences in N uptake and ANR between the $CaSO_4 \cdot 4urea$ and comparative fertilizer treatments in 2018, significant differences in yield were observed in 2019. $CaSO_4 \cdot 4urea$ exhibited 173 kg ha^{-1} N uptake in maize grain, while ANR was only 37%. Importantly, the improvement was clearly defined when compared with the mixture of gypsum and urea containing exactly the same nutrient content of $N_{150}P_{80}K_{160}S_{42.5}Ca_{53}$ kg ha^{-1} [33]. The use of $CaSO_4 \cdot 4urea$ at 200 kg N ha^{-1} showed a very high N uptake in grains and stems of corn compared to the control and urea alone [34]. Interestingly, this high N content was not observed when $CaSO_4 \cdot 4urea$ was used at 100 kg N ha^{-1}.

Nitrous oxide (N_2O) is an important greenhouse gas with a global warming potential 300 times greater than carbon dioxide [35]. Agriculture contributes more than 60% of total anthropogenic N_2O emissions [36]. Since the contribution of synthetic N fertilizers to these emissions grew, on average, by 19% annually (0.07 to 0.68 Gt CO_2 eq yr^{-1}) from 1961 to 2010 [37], the need to develop N fertilizer which increases crop productivity and N use efficiency while reducing N loss as N_2O has been emphasized. Specifically, designing novel N-efficient materials will enhance the sustainability of agriculture and mitigate climate change, an essential step toward climate-smart soil fertility management. However, literature data are lacking on how urea cocrystals affect plant growth, reduce N_2O emissions, and related N balance. The potential of $CaSO_4 \cdot 4urea$ to address low N efficiency problems of pure urea was explored in this study using sorghum as a model crop in a greenhouse experiment.

2. Materials and Methods

2.1. Experimental Setup

The experiment was carried out in the fall of 2022 in the greenhouse of New Mexico State University Agricultural Science Center, Clovis, NM (34′35° N, 103′12° W, and elev. 1348 m). For the pot experiment, topsoil (0–15 cm) was collected from a conventionally tilled dryland field of the Agricultural Science Center. Based on the USDA soil classification system, the soil is Olton Clay loam (fine, fixed, superactive, thermic Aridic Paleustolls). Once collected from the field, the soils were homogenized, large stones, plant residues, and

macrofauna were removed, and pots were filled with 13 kg of soil. The basic soil properties and nutrient concentrations (Table 1) were analyzed during the experiment establishment using standard laboratory procedures [38].

Table 1. Basic properties of soil used in the greenhouse study.

| Soil Properties	Units	Values
Soil pH (1:1)	-	7.00
Soil organic matter (SOM)	%	1.30
Olsen P	$\mu g\,g^{-1}$	27.6
Nitrate-N	$\mu g\,g^{-1}$	40.0
Potassium	$\mu g\,g^{-1}$	517
Calcium	$\mu g\,g^{-1}$	2064
Magnesium	$\mu g\,g^{-1}$	342
Sodium	$\mu g\,g^{-1}$	13.0
Sulfate-S	$\mu g\,g^{-1}$	8.60
Zinc	$\mu g\,g^{-1}$	0.73
Iron	$\mu g\,g^{-1}$	8.30
Manganese	$\mu g\,g^{-1}$	9.91
Copper	$\mu g\,g^{-1}$	0.97
Cation exchange capacity (CEC)	$meq/100\,g^{-1}$	14.5

$CaSO_4 \cdot 4urea$ cocrystal was synthesized using the mechanochemical method using a Retsch PM 100 planetary mill equipped with a 250 mL stainless steel jar. A total of 30 g of precursors (urea: 99–100.5%, Sigma Aldrich (St. Louis, MO, USA) and gypsum: 98+%, Acros Organics (Geel, Belgium)) with a molar ratio of urea: gypsum of 4:1 was added into the jar together with fifteen 10 mm stainless steel balls and milled at 500 rpm for 1 h. The sample loading, number of balls, and milling speed were optimized via prior experiments [27] to achieve a nearly complete conversion of the precursors into the $CaSO_4 \cdot 4urea$ cocrystal. In the milling process, the crystal structure changes, whereby parent materials are combined into a new crystal structure (Figure 1). The sample obtained from the milling process was dried overnight at 50 °C to remove the water released during the mechanochemical reaction from the gypsum precursor.
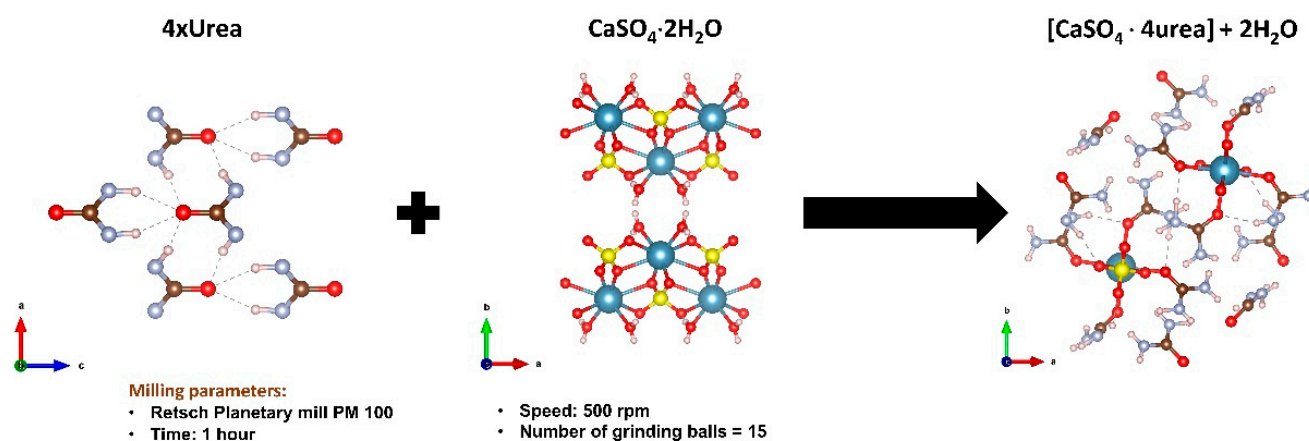
Figure 1. Mechanochemical synthesis of $CaSO_4 \cdot 4urea$ cocrystal. Crystalline unit cell fragments are shown for both reactants (urea and gypsum) and the cocrystal product.

Physicochemical characterization of the mechanochemically synthesized $CaSO_4 \cdot 4urea$ cocrystal was performed (Figure 2). The pXRD patterns were acquired using an Empyrean PANalytical B.V. diffractometer (Almelo, the Netherlands). The applied current was 40 mA, and the applied voltage was 45 kV. The X-ray mirror used was a graded, flat Bragg–Brentano HD mirror. The step size used was 0.0131 degrees. The diffraction patterns

were obtained between 10 and 50 degrees. The radiation source used was CuKα1,2 with CuKα1 wavelength 1.540598 Å and CuKα2 wavelength 1.544426 Å. The ratio of Kα1/Kα2 was 0.5. The analysis was performed with a 4 mm mask, 1/8" incident beam divergence slit, 1/2" incident beam anti-scatter slit, 7.5 mm diffracted beam anti-scatter slit, and a 0.04 mm Soller slit. The powder was placed on a glass slide and pressed into a 1 cm × 1 cm sized smooth powder film. It can be seen in Figure 2a that experimentally obtained $CaSO_4 \cdot 4$urea exhibited no peaks due to the parent compounds, e.g., $CaSO_4 \cdot 2H_2O$ or urea, rather a complex peak structure was obtained from the simulated crystal structure pattern corresponding to chemically pure $CaSO_4 \cdot 4$urea single crystal [39].

(a)   (b)
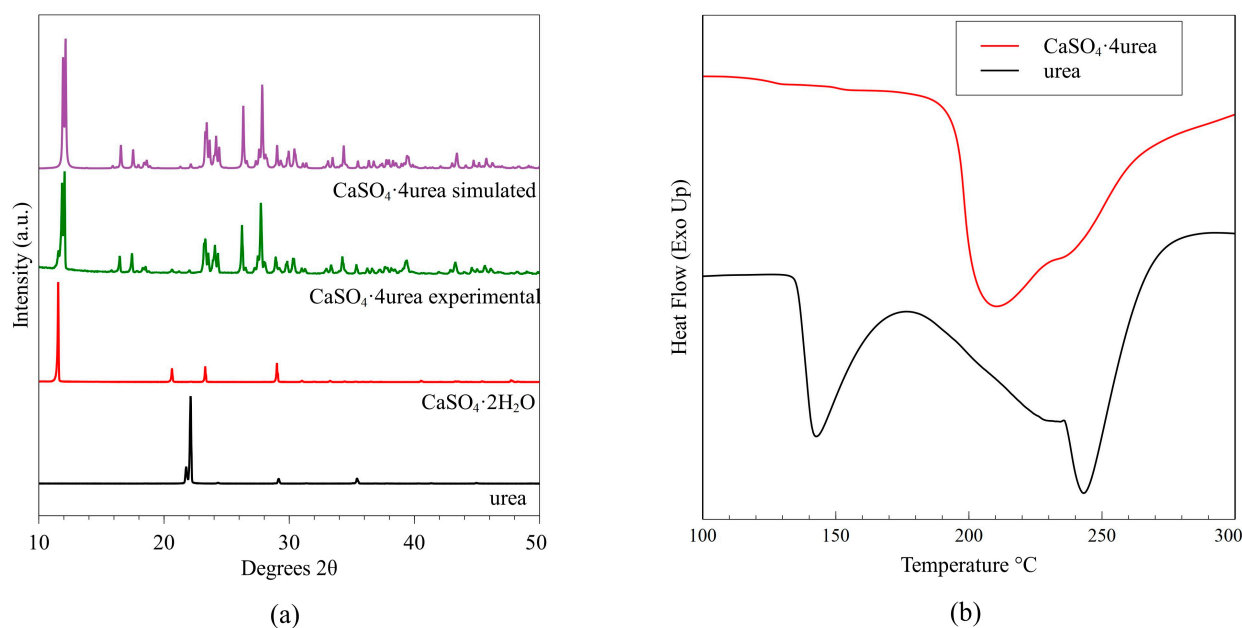
Figure 2. Physicochemical characterization of the mechanochemically synthesized $CaSO_4 \cdot 4$urea cocrystal: (**a**) XRD patterns and (**b**) DSC curves.

The thermal stability of $CaSO_4 \cdot 4$urea and urea was investigated using differential scanning calorimetry (DSC) analysis (SDT-Q600, TA Instruments, New Castle, DE, USA). During DSC measurements, a heating rate of 10 °C min^{-1} was used under an airflow of 100 mL min^{-1}. Figure 2b shows that urea exhibits an endothermal peak with an onset of 132 °C due to the melting/decomposition of urea [24,40]. Importantly, $CaSO_4 \cdot 4$urea is void of this peak, shifting to higher temperatures and overlapping with a broad endothermal peak due to the $CaSO_4$ phase transitions above 200 °C. This suggests the molecular structure of $CaSO_4 \cdot 4$urea stabilizes urea molecules more than in urea crystal and can result in lower environmental reactivity.

In the greenhouse, six treatments with three replications were tested in a randomized complete block design with eighteen pots. Treatments included a control with no N fertilizer input, urea (46% N), and $CaSO_4 \cdot 4$urea (29% N) applied at the rate recommended for irrigated sorghum and three additional $CaSO_4 \cdot 4$urea treatments equivalent to 40%, 70%, and 130% of the recommended N rate as follows:

1. Soil without N fertilizer input (C0).
2. Soil with urea equivalent to 150 kg N ha^{-1} (UR100).
3. Soil with $CaSO_4 \cdot 4$urea equivalent to 60 kg N ha^{-1} (UC40).
4. Soil with $CaSO_4 \cdot 4$urea equivalent to 105 kg N ha^{-1} (UC70).
5. Soil with $CaSO_4 \cdot 4$urea equivalent to 150 kg N ha^{-1} (UC100).
6. Soil with $CaSO_4 \cdot 4$urea equivalent to 195 kg N ha^{-1} (UC130).

Air-dried soils (13 kg) were added to 29 cm i.d. × 24 cm deep pots up to 23 cm and placed on a shallow tray, watered to bring soil moisture to ~70% of field capacity,

and four sorghum seeds (Pioneer 86 P20) were sown in each pot in the second week of August 2022. After two weeks, seedlings were thinned to two per pot, and N fertilizers were manually applied to the side of each pot, except the control, at the specified rate. No other chemical fertilizers were applied to the pots. Hand weeding removed the weeds, and uprooted weeds were allowed to decompose in the same pot. Soil moisture was measured using a HydraProbe SDI-12 (Stevens Water Monitoring Systems, Inc., Portland, OR, USA), and soil water content was maintained at ~70% of field capacity throughout the study period by regularly watering the pots. The greenhouse temperature was maintained between 18 to 35 °C by a heating and cooling system controlled by an environmental computer (Wadsworth Control System, Arvada, CO, USA). Additionally, evaporative cooling pads with water circulation maintained the moisture level and temperature inside the greenhouse.

2.2. Plant, Soil, and N$_2$O Flux Measurement and Analysis

Plant height was measured at 24 days, 44 days, and 105 days after sowing (DAS), representing seedling, boot, and maturity stages in sorghum (Figure 3). A stainless-steel ruler was used to measure the plant height from the soil level to the base of the fully expanded leaf at the seedling and boot stage. At maturity, plant height was measured from soil level to the top of the panicle without bending or stretching the plants.
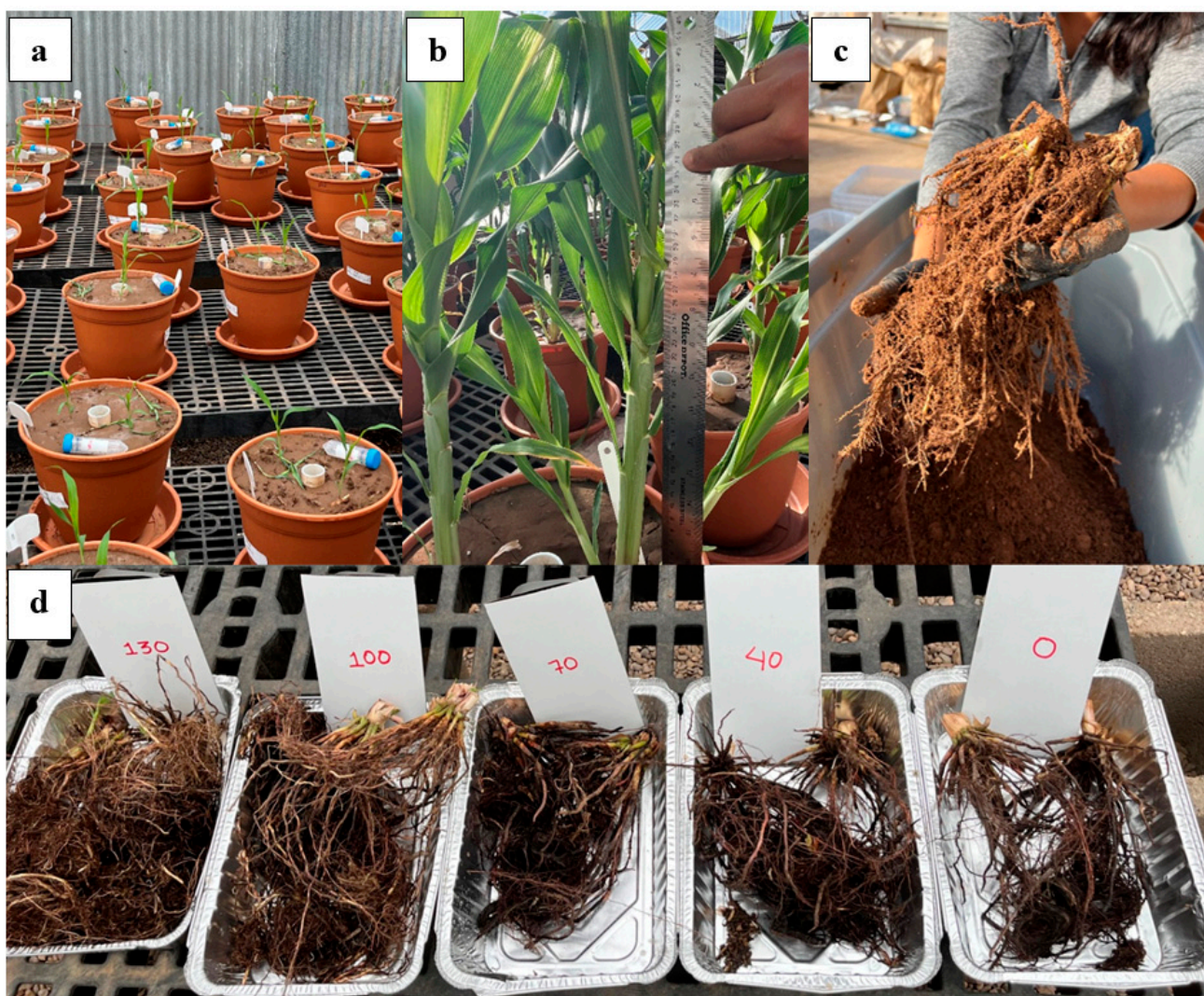
Figure 3. Urea cocrystal experimental setup (**a**), plant height measurement (**b**), root biomass separation at the end of the experiment (**c**), and root biomass at different rates of fertilizer application (**d**).

At the end of the study, grain, shoot, root, and soil samples were collected for N analysis. The whole shoot biomass was harvested by cutting shoots at the soil surface using clippers, and they were dried in an oven at 55 °C until constant weight to estimate dry matter content. Similarly, roots from bulk soil were separated by passing soil through 2 mm sieves, washing thoroughly, and oven drying at 55 °C until constant weight to estimate dry mass. Approximately 500 g root-free soil samples were taken from each pot and air-dried for two days. Oven-dried root and shoots and air-dried soil samples were sent to a commercial laboratory (Ward Laboratories, Inc. Kearney, NE, USA) for nutrient analysis. Specifically, plant samples were analyzed for biomass N content, and soil samples were analyzed for soil pH, SOM, NO_3-N, available P and K, and secondary nutrient (Ca, Mg, and S) concentration in different treatments.

The assessment of N_2O fluxes was conducted using a MIRA Pico Laser Analyzer (Aeries Technologies, Hayward, CA, USA). Polyvinyl chloride (PVC) rings, each 3 cm in diameter and 7 cm in height, were inserted 5 cm into the soil from the top at the center of each pot a day before fertilization. The rings remained in place during the entire experimental period. They were closed with PVC caps (3 cm diameter and 2 cm height) fitted with a valve for gas sampling for two minutes. After background stabilization for about a minute, aliquots of air were collected for 1 min using a sterile needle connected to a gas port in the analyzer via 3.2 mm diameter tubing. The ambient air N_2O fluxes were recorded before starting the measurement in experimental pots and later subtracted from the measurements to calculate the net gas flux in each treatment. The air samples were collected between 09:30 and 11:30 to reduce the variability in N_2O fluxes due to daily fluctuations in temperature. The gas emission rates were determined using the following equation.

$$R = \frac{Gn - G0}{Tn} \times \frac{V}{A}$$

where R is the N_2O flux rate in g m^{-2} hr^{-1}, G0 is the gas (N_2O) concentration at the time of chamber installation (T = 0), Gn is the N_2O concentration at time Tn (Tn = 60 s), A is the area of soil exposed in m^2, and V is the system volume in m^3. Cumulative N_2O emissions throughout the study period were calculated using linear interpolation of daily/weekly data and numerical integration of individual data points. Soil temperature data were collected using a HydraProbe SDI-12 (Stevens Water Monitoring Systems, Inc., Portland, OR, USA) from the top 0–5 cm depth.

2.3. Data Analysis

The data were analyzed using R version 4.1.3 using the *agricolae* package. Analysis of variance and the least significant difference test were used to evaluate the difference between the means of the three replicates under different treatments with $p < 0.05$ unless otherwise stated. The relationships between plant growth and soil properties were evaluated by using a Pearson correlation analysis. The N uptake and use efficiency (NUE) were calculated using the following formula:

$$N \; uptake = \%N \; in \; grain \; or \; biomass \times Dry \; matter \; of \; grain \; or \; biomass \left(mg \; pot^{-1} \right)$$

$$NUE \left(mg \; g^{-1} \; fertilizer \; N \right) = \frac{Total \; N \; uptake \; from \; treatment - Total \; N \; uptake \; from \; control}{Total \; applied \; N \; of \; fertilizer \; in \; the \; treatment} \times 1000$$

3. Results

3.1. Soil Properties

Changes in soil properties due to urea and urea cocrystal application measured after sorghum harvest are presented in Table 2. The highest soil NO_3-N, Mg, and SO_4-S were measured at the highest application rate of urea cocrystal (UC130); however, such differences were not evident in other soil properties. Soil NO_3-N content in UC130 was

26.9 µg g^{-1} and 19.6 µg g^{-1}, more than C0 and UR100 treatments. Similarly, SO$_4$-S was 628% and 242% greater in UC130 than in C0 and UR100. Moreover, the UC130 lowered the soil pH by 0.7 unit in calcareous Olton Clay loam soil (control: 7.8), improving the availability of macro- and micronutrients as indicated by numerically higher P, K, Ca, and Mg compared to lower rates of urea cocrystal, UR100, and the control.

Table 2. Effect of urea and urea cocrystal fertilizers on soil physical and chemical properties after sorghum harvest. Lowercase letters indicate significant differences among fertilizer treatments.

Treatments [†]	pH	SOM	Nitrate-N	Phosphorus	Potassium	Calcium	Magnesium	Sulphate-S
	-	mg g^{-1}	--µg g^{-1}--					
C0	7.77 ± 0.03	15.7 ± 0.33	4.70 ± 0.17 b	17.0 ± 0.17	488 ± 11.8	2092 ± 33.1	395 ± 17.1 b	7.20 ± 0.17 c
UC40	7.77 ± 0.03	15.3 ± 0.88	4.30 ± 0.12 b	16.6 ± 0.15	462 ± 27.0	2046 ± 15.9	356 ± 23.4 b	7.43 ± 1.27 bc
UC70	7.77 ± 0.12	15.0 ± 1.00	6.10 ± 1.73 b	14.8 ± 1.13	457 ± 18.8	2096 ± 136	405 ± 31.6 b	15.5 ± 4.18 bc
UC100	7.60 ± 0.21	16.3 ± 0.33	13.4 ± 5.02 b	16.0 ± 1.18	457 ± 14.9	2148 ± 187	383 ± 26.6 b	17.0 ± 0.83 b
UC130	7.13 ± 0.23	17.7 ± 0.33	31.6 ± 9.05 a	17.5 ± 1.99	491 ± 5.13	2352 ± 140	487 ± 38.0 a	52.4 ± 5.13 a
UR100	7.57 ± 0.15	16.3 ± 0.33	12.0 ± 4.06 b	16.3 ± 0.50	491 ± 11.3	2182 + 116	417 ± 19.1 ab	15.3 ± 2.05 bc
p-value	NS	NS	0.02	NS	NS	NS	0.05	0.001

† C0—soil without N fertilizer input; UC40—soil with CaSO$_4$·4urea at 40% N rate; UC70—soil with CaSO$_4$·4urea at 70% N rate; UC100—soil with CaSO$_4$·4urea at 100% N rate; UC130—soil with CaSO$_4$·4urea at 130% N rate; and UR100—soil with urea at 100% N rate; SOM—soil organic matter. Data are presented as mean ± standard error (*n* = 3). Means with the same lowercase letters indicate no statistical difference at $p \leq 0.05$ according to Fisher's protected least significant difference (LSD) test, NS = no significant difference.

3.2. Plant Productivity, N Uptake, and Use Efficiency

Overall, urea (UR100) and higher application rates of urea cocrystal (UC100 and UC130) positively influenced plant production by enhancing shoot and root biomass production and grain yield of sorghum compared to the control (Table 3), but there was no effect on plant height (Figure 4). UR100 had a 51.4% greater yield of sorghum grain than the control, whereas urea cocrystal application at the same N rate (UC100) had an 87.5% greater yield than the control. The grain yield increase with UC130 was 91.5% greater than the control.

Table 3. Means and standard error (*n* = 3) of grain, shoot (leaf and stem) biomass, root biomass, grain numbers, and N content in the respective plant parts in different treatments.

| Treatments [†]	Grain Yield (g Pot^{-1})	Shoot (g Pot^{-1})	Root (g Pot^{-1})	Grain Numbers	Grain N (mg g^{-1} Grain)	Shoot N (mg g^{-1} Biomass)	Root N (mg g^{-1} Biomass)	NUE (mg g^{-1} Biomass)
C0	17.7 ± 0.61 c	22.9 ± 3.33 b	13.0 ± 1.30 b	617 ± 46.0 c	22.0 ± 1.40	6.86 ± 0.60 d	8.95 ± 0.40	-
UC40	18.0 ± 0.33 bc	28.1 ± 0.77 ab	12.1 ± 0.33 b	537 ± 49.2 c	24.5 ± 2.40	9.55 ± 1.12 cd	10.65 ± 0.80	39.2 ± 6.91 c
UC70	18.6 ± 1.82 bc	29.8 ± 1.48 ab	12.9 ± 0.21 b	580 ± 42.5 c	27.3 ± 2.36	11.1 ± 0.55 bc	11.8 ± 1.64	45.5 ± 6.41 c
UC100	33.2 ± 5.83 a	34.4 ± 4.71 a	18.4 ± 5.23 b	1119 ± 190 a	27.3 ± 7.92	13.4 ± 0.92 ab	12.1 ± 0.57	82.0 ± 9.53 ab
UC130	33.9 ± 3.08 a	37.9 ± 6.06 a	31.2 ± 2.89 a	1098 ± 112 ab	28.8 ± 4.87	14.2 ± 0.89 a	12.4 ± 0.86	96.2 ± 7.98 a
UR100	26.8 ± 0.18 ab	34.8 ± 1.87 a	22.0 ± 5.67 ab	789 ± 42.0 bc	26.6 ± 5.31	11.4 ± 1.14 abc	11.5 ± 1.94	62.7 ± 12.6 bc
p-value	0.01	0.1	0.01	0.01	NS	0.01	NS	0.05

† C0—soil without N fertilizer input; UC40—soil with CaSO$_4$·4urea at 40% N rate; UC70—soil with CaSO$_4$·4urea at 70% N rate; UC100—soil with CaSO$_4$·4urea at 100% N rate; UC130—soil with CaSO$_4$·4urea at 130% N rate; and UR100—soil with urea at 100% N rate. Data are presented as mean ± standard error (*n* = 3). Means with the same lowercase letters indicate no statistical difference at $p \leq 0.05$ according to Fisher's protected least significant difference (LSD) test, NS = no significant difference.

Urea and urea cocrystal applications also improved the shoot biomass yield compared to the control, with UC130 producing the highest biomass among all the treatments. Root biomass in UC130 was 139% greater than the control. Root biomass was not statistically different between UR100 and UC100 (Table 3).

The N content in shoot biomass increased with urea and urea cocrystal application at higher application rates compared to the control (Table 3). Significantly higher (~106%) N content in shoot biomass of UC130 was measured compared to C0. Urea cocrystal treatments, UC100 and UC130, had numerically higher N content in grain, shoot, and root than urea (UR100) treatment. Moreover, the NUE of the whole plant was significantly

higher in UC130 than in UR100, and the low rate of urea cocrystal treatments (Table 3). The NUE was comparable between UR100 and UC100.
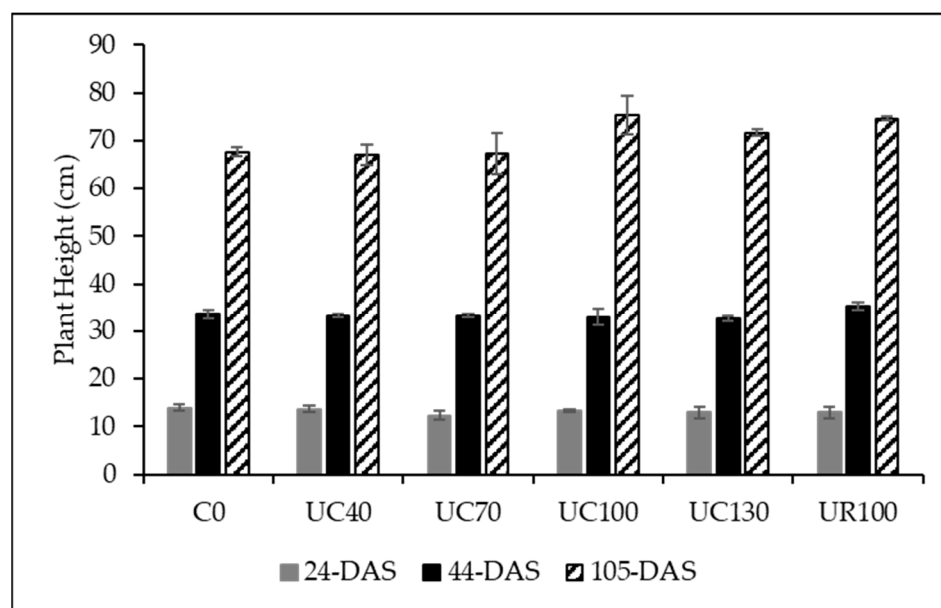
Figure 4. Plant height at 24, 44, and 105 days after seed sowing (DAS). Treatments: C0—soil without N fertilizer input, UC40—soil with $CaSO_4 \cdot 4urea$ at 40% N rate, UC70—soil with $CaSO_4 \cdot 4urea$ at 70% N rate, UC100—soil with $CaSO_4 \cdot 4urea$ at 100% N rate, UC130—soil with $CaSO_4 \cdot 4urea$ at 130% N rate, and UR100—soil with urea at 100% N rate. Data are presented as mean $\pm$ standard error ($n = 3$). A significance test was performed for each sampling date among treatments at $p \leq 0.05$ according to Fisher's protected least significant difference (LSD) test.

Correlation analysis showed soil nitrate positively influenced different yield components. We observed a significant positive relationship between grain number and soil nitrate (Table 4). The role of sulfur content in improving plant productivity was also evident from the positive correlation between sulfate-S and crop parameters.

Table 4. Correlation among soil (sulfate and nitrate), greenhouse gas (nitrous oxide), and plant (shoot, root, grain, and grain number) properties.

	Sulfate-S	N_2O-N	Shoot	Root	Grain	Grain Number
Nitrate-N	0.71 ***	0.70 ***	0.42	0.43	0.45	0.48 *
Sulfate-S		0.54 *	0.48 *	0.74 ***	0.64 **	0.58 *
N_2O-N			0.20	0.37	0.30	0.22
Shoot				0.68 **	0.51 *	0.53 *
Root					0.70 ***	0.64 ***
Grain						0.95 ***

*, **, and *** indicate significant correlation at the 0.05, 0.01, and 0.001 probability levels, respectively.

Urea and urea cocrystal fertilizers also affected N uptake in different sorghum plant parts (Figure 5). At higher N application rates of urea cocrystal, i.e., UC100 and UC130, there was a significantly greater N uptake in grain, shoot, and root compared to the control. When combined from different plant parts, the total N uptake was the highest in UC130, ~40% higher than in urea. However, N uptake in UC100 and UR100 was comparable.
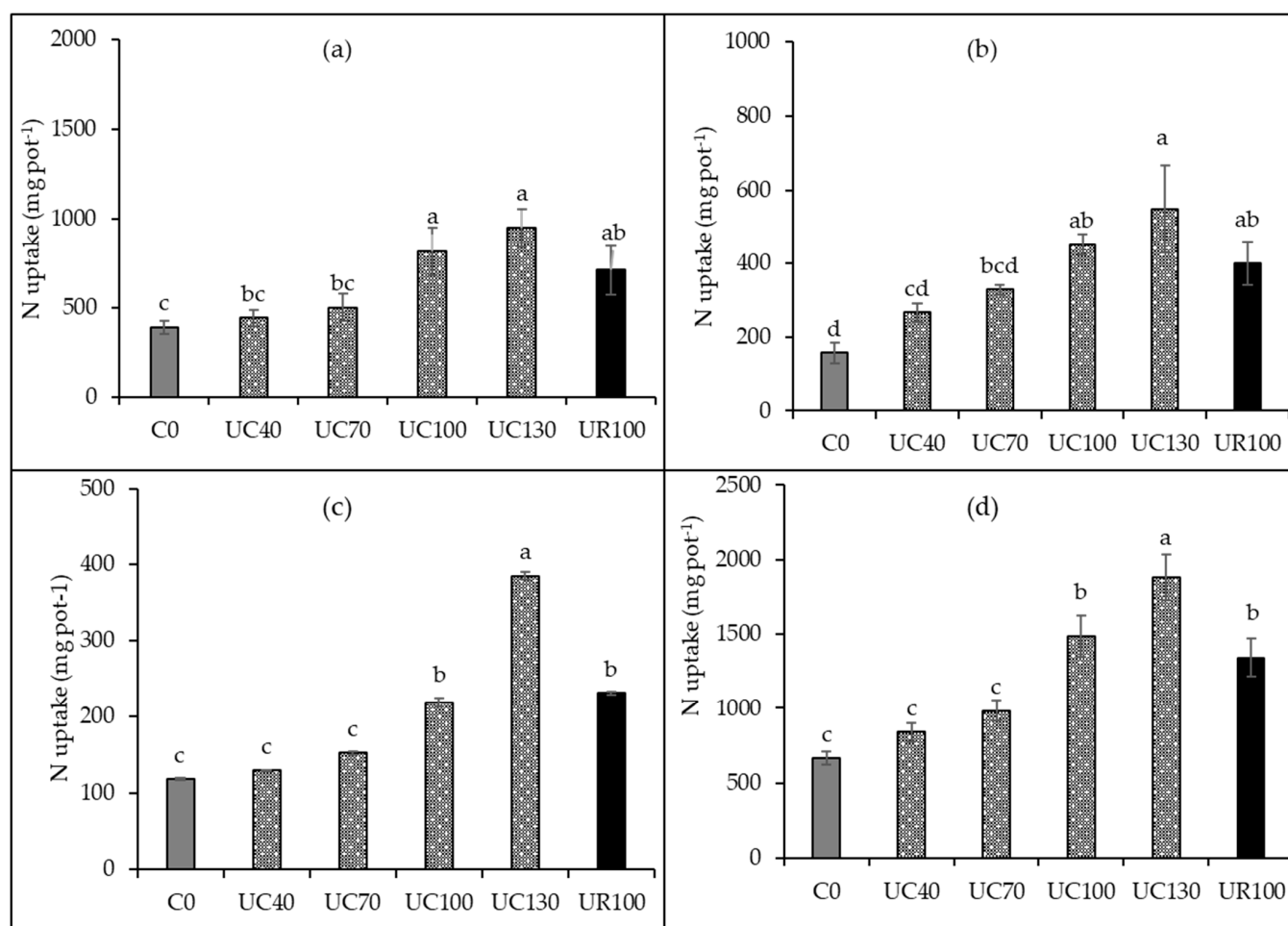
Figure 5. Effect of urea and urea cocrystals on N uptake in different sorghum plant parts: (**a**) grain, (**b**) shoot, (**c**) root, and (**d**) total in all plant parts. Error bars indicate standard errors (*n* = 3). Treatments: C0—soil without N fertilizer input, UC40—soil with CaSO$_4$·4urea at 40% N rate, UC70—soil with CaSO$_4$·4urea at 70% N rate, UC100—soil with CaSO$_4$·4urea at 100% N rate, UC130—soil with CaSO$_4$·4urea at 130% N rate, and UR100—soil with urea at 100% N rate. Different colors are used to show different fertilizer materials. Bars with the same lowercase letters indicate no statistical difference at *p* ≤ 0.01 according to Fisher's protected least significant difference (LSD) test.

3.3. Soil N$_2$O Emissions

The flux of N$_2$O emissions was significantly affected by the fertilizer treatments (Figure 6a). After fertilizer application, two different periods with high N$_2$O emissions were observed, an initial peak until day 7 in urea-applied treatment and another peak around day 28 in urea cocrystal-applied treatments. After day 28, N$_2$O emissions diminished to control levels until the end of the experiment. On the 7th day, the flux of N$_2$O emission from urea (UR100 = 0.55 ± 0.08 mg m^{-2} d^{-1}) was significantly higher (*p* < 0.01) than that of all other treatments (e.g., UC100 = 0.09 ± 0.01 mg m^{-2} d^{-1}, UC130 = 0.20 ± 0.12 mg m^{-2} d^{-1}, and control = 0.07 ± 0.02 mg m^{-2} d^{-1}). On the 28th day, N$_2$O emissions from urea cocrystal (UC130 = 0.42 ± 0.04 mg m^{-2} d^{-1}) (*p* < 0.01) treatment exceeded N$_2$O emissions from urea (UR100 = 0.16 ± 0.01 mg m^{-2} d^{-1}) and control treatments (0.08 ± 0.721 mg m^{-2} d^{-1}). However, the N$_2$O emissions from UC100 and UR100 were not significantly different, and no difference between other urea cocrystal and control treatments was observed. At the end of the experiment, the cumulative emissions from UC130 and UR100 were equivalent to 11.9 ± 2.72 mg m^{-2} d^{-1} and 11.3 ± 0.40 mg m^{-2} d^{-1}, 50.2% and 42.6%, respectively, higher than UC100 (7.95 ± 2.47 mg m^{-2} d^{-1}), and 126% and 114%, respectively, higher compared with

the control (5.29 ± 0.06 mg m^{-2} d^{-1}) (Figure 6b). The flux of N$_2$O was positively correlated with soil NO$_3$-N content (Table 4).
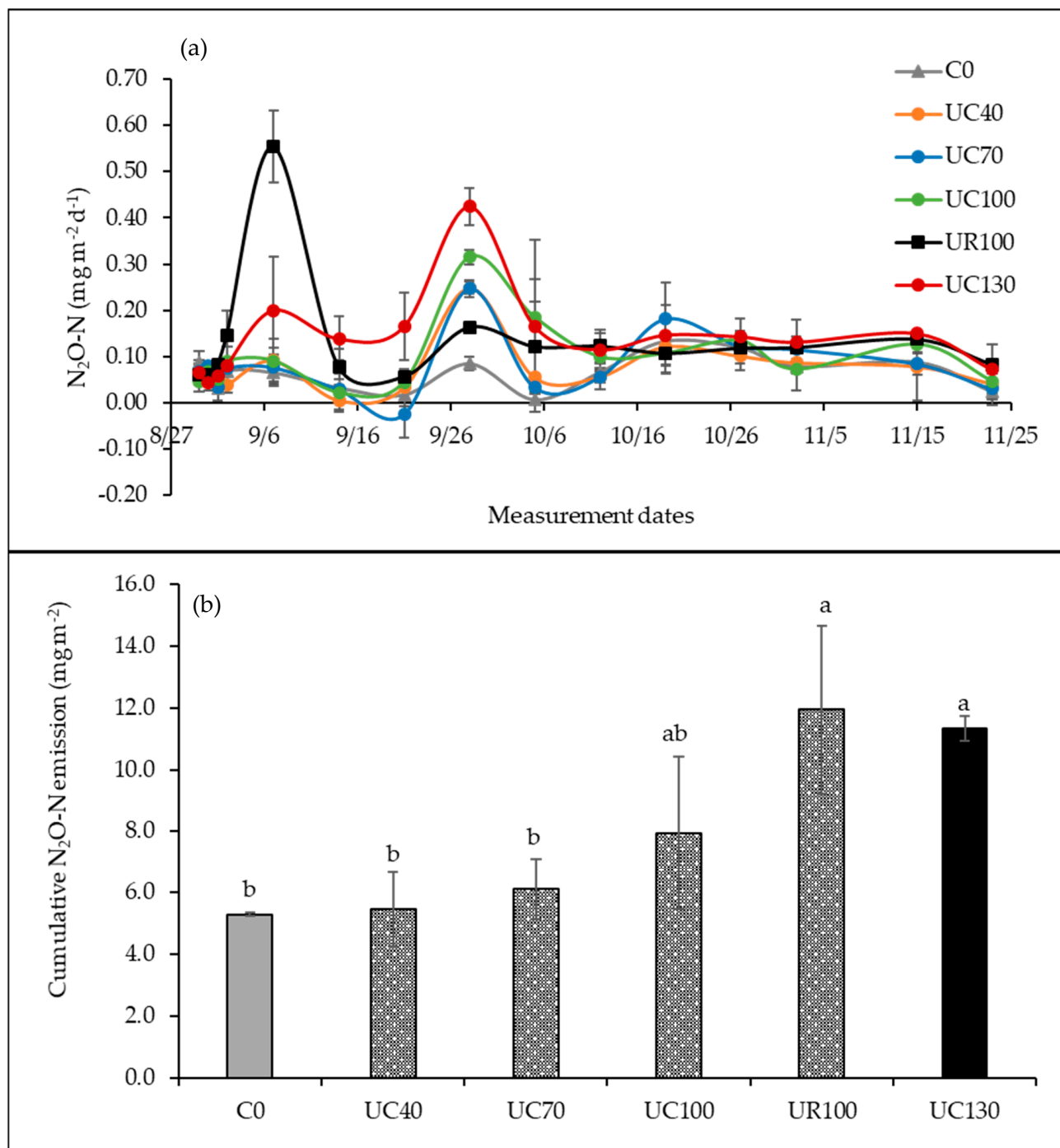
Figure 6. Daily nitrous oxide (N$_2$O) fluxes (**a**) and cumulative N$_2$O emissions over the study period for all treatments (**b**). Data are presented as mean $\pm$ standard error (n = 3). Means with the same lowercase letters indicate no statistical difference at $p \leq 0.01$ according to Fisher's protected least significant difference (LSD) test. Treatments: C0—soil without N fertilizer input, UC40—soil with CaSO$_4$·4urea at 40% N rate, UC70—soil with CaSO$_4$·4urea at 70% N rate, UC100—soil with CaSO$_4$·4urea at 100% N rate, UC130—soil with CaSO$_4$·4urea at 130% N rate, and UR100—soil with urea at 100% N rate.

4. Discussion

Nitrogen is an essential nutrient for plant growth and yield formation and the number one growth-limiting factor in modern crop production [41]. Depending on the soil, plant type, and climatic conditions, plants assimilate applied N fertilizer in two chemical forms, i.e., nitrate (NO_3^-) or ammonium (NH_4^+). Regarding the availability of both forms in soil, plants prefer to take up N as NO_3^- [42], which is the most abundant and available form in most agricultural soils [43]. Therefore, NO_3^- balance after crop harvest indicates N use efficiency and potential loss to the environment. In our study, NO_3^- after crop harvest was the highest in UC130, and this treatment also had the highest total N uptake and NUE. This was possibly due to minimum soil N loss from $CaSO_4 \cdot 4urea$. For example, measurement of N loss as N_2O revealed that N_2O attained a peak in about a month of $CaSO_4 \cdot 4urea$ application, irrespective of the fertilization rate, while it rapidly attained peak flux within a week of fertilization in UR100. It was a considerably large flux compared to control and $CaSO_4 \cdot 4urea$ treatments (Figure 6a). Urea likely loses more N due to the rapid N dissolution and oxidation when applied in the soil and hence the low soil N at harvest. Figure 2a shows multiple peaks for the $CaSO_4 \cdot 4urea$, which means it forms a unique crystal structure that makes urea less reactive to release N. This is also manifested in Figure 2b, where the 132 °C peak due to the urea sublimation is shifted, showing that fundamental properties of urea changed upon cocrystallization. In line with these findings, our previous research using Raman spectroscopy and synchrotron powder X-ray diffraction also demonstrated high ammonia (NH_3) volatilization from urea, causing 75% NH_3 emissions within two weeks. In contrast, urea cocrystals synthesized from gypsum and other calcium sulfate analogs have low solubility and reduced NH_3 volatilization [27]. Application of urea may have increased N_2O loss by enhancing nitrification and denitrification processes compared to urea cocrystal treatments, which are reduced in urea cocrystals because of reduced accessibility of N to microorganisms involved in N transformation in soils. Hydrolysis of applied ammonical fertilizer produces ammonium (NH_4^+) which is converted to oxidized N in the form of nitrite (NO_2^-) and further to nitrate (NO_3^-) via nitrification.

Studies suggest that the application of highly soluble fertilizer, e.g., urea, can temporarily create a high concentration of NH_3^+/NH_4^+, resulting in nitrite accumulation either due to inhibition of nitrite oxidation from the toxicity of high NH_3 levels in the fertilizer application zone [44] or from localized lowering of pH and production of nitric acid [45]. This change in the soil environment results in N_2O loss via nitrification and nitrifier denitrification under aerobic conditions and denitrification under anaerobic conditions [46]. In the $CaSO_4 \cdot 4urea$ treatments, the N_2O emissions were slow and only reached a peak after a month (Figure 6). In field conditions, increased crop uptake may further lower the N_2O loss because crop growth is constrained in the pots. Moreover, the cumulative N_2O emissions were not significantly different between UC130, UC100, and UR100, and NUE was the highest in UC130, despite the higher application rate in UC130. These responses of $CaSO_4 \cdot 4urea$ (UC130) suggest that it can provide a balanced N supply to the crops without increasing N loss in the environment as N_2O emissions. Therefore, $CaSO_4 \cdot 4urea$ cocrystal could be alternative N fertilizer for crop production with high NUE and in a more environmentally friendly way than urea alone.

The application of urea cocrystal fertilizers revealed many positive effects on sorghum yield components compared to urea. Grain numbers and weight are two key yield-determining components, and the grain number is the major driver of the overall yield of sorghum [47]. We found higher grain numbers in UC100 compared to control, urea (UR100), and other low N rate treatments using $CaSO_4 \cdot 4urea$ (Table 3). Furthermore, grain yield and grain N content were numerically higher in UC100 and UC130 than in UR100. It is well established in the literature that increased N inputs increase cereal grain yield up to a certain point, as N is an essential nutrient that influences yield and yield-related components such as panicle numbers and tiller numbers [48,49]. A previous study reported that an increase in N application (0, 45, and 90 kg N ha^{-1}) increased sorghum yield, and the study attributed it to an increase in grain numbers [50].

The $CaSO_4 \cdot 4$urea cocrystal fertilizer at the higher N rate (UR130) also enhanced shoot biomass, root biomass, and shoot N compared to all other treatments (Table 3). In sorghum, stem N accumulation depends on soil nutrient availability, and shoots accumulate more N under N-sufficient soils [51,52]. Accordingly, our results show that the higher the soil nitrate concentration was, the higher the stem N with the numerically highest stem N concentration in UC130 (Table 2). Grain N also followed the trend in stem N with higher N concentration in UC130, although it was not statistically different among treatments. The possible reason for no statistical difference in grain N could be related to the mechanism of grain N accumulation in sorghum which occurs primarily via the remobilization of accumulated N from vegetative parts. Grain N accumulation is a two-step process; in the first half of grain filling (i.e., within 30 days of anthesis), the panicle accumulates the majority of N due to remobilization of N accumulated in the stem and rachis, and in the latter half N translocation occurs from leaves [53]. In addition, when sufficient soil N is available in sorghum plants, it supports expressions of the stay-green trait in sorghum [53], which delays leaf senescence and lengthens the period of leaf N translocation to the grain. In our study, sorghum was harvested at 105 days. In the field condition, it is typically allowed to grow for an additional month. This could change the grain N concentration in later maturity with more N translocated to grain from other plant parts, specifically in treatments with higher soil N.

Soil NO_3-N and the total N uptake were significantly higher in UC130 than in UR100 (Figure 5d; Table 3), suggesting both high NUE and soil N conservation for the later stage of the crop or a subsequent crop. However, a higher rate of N, if not utilized by a crop at its later stage and if the subsequent crop is not planted, may lead to NO_3 leaching or NH_3 volatilization. The N uptakes corresponded with each other in UC100 and UR100, suggesting the possibility of using the same rate of $CaSO_4 \cdot 4$urea cocrystal as urea for high NUE and no or low risk of N loss. These findings are in agreement with a previous study where positive effects on corn yield components and N uptake were found with a higher N rate (200 kg N ha^{-1}) of urea cocrystals, and results were comparable between urea and urea cocrystals for 100 kg N ha^{-1} [34]. The authors suggested urea cocrystal compounds likely modified the release of N, providing a continuous supply of N through the later crop growth stage, thereby supporting an increase in crop yield. The sufficient availability of N during key developmental stages such as panicle initiation and spikelet differentiation is key to increasing grain yield as it determines the number of kernels in the sorghum [47].

The sulfur concentration in UC130 was higher than all other treatments, and there was also a strong positive correlation between sulfur and nitrate. The literature suggests that the application of a suitable sulfur source promotes the absorption of N in the soil and therefore enhances the N uptake in crops [34,54,55]. The positive correlation between sulfate and nitrate suggests the role of sulfate as an oxidant which likely facilitated NH_4^+ oxidation and enhanced N_2O production [56]. Further, in calcareous soil, as used in this study, increased sulfur concentration also helped lower the soil pH in UC130, which likely favored the release of macro- and micronutrients. In addition, the significant positive correlation between soil nitrate concentration and grain number and sulfur and several crop yield parameters (shoot, root, grain, and grain numbers) suggests that urea cocrystal positively influenced the soil properties and provided ample N supply, matching the crop N demand in different growth stages of sorghum. These findings strengthen the idea that urea cocrystal fertilizers are improved fertilizers that support sorghum grain production in an environmentally friendly way.

5. Conclusions

$CaSO_4 \cdot 4$urea cocrystal, which efficiently minimizes the high solubility of pure urea, can improve crop yield, match the crop N demand, and represent a suitable alternative fertilizer minimizing N loss into the environment from soil application. We assessed sorghum grain and biomass yield, soil properties, and N uptake from different plant parts and N_2O emissions from urea, $CaSO_4 \cdot 4$urea cocrystal, and unfertilized treatments.

Multiple positive effects of CaSO$_4$·4urea cocrystal were observed in pot experiments of sorghum, such as increased soil NO$_3^-$ and sulfur concentration resulting in increased grain yield, N uptake in various plant parts, and increased NUE in UC130 treatments. Among others, the significant retardation of N$_2$O flux was also observed, differently from pure urea, suggesting quantitatively different types of N management in soil facilitated by urea cocrystals and enhanced sustainability. Further field studies are needed to measure and quantify the long-term benefits of various urea cocrystals application on agronomic N use efficiency and greenhouse gas emissions.

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