

Article

Selected Carbon and Nitrogen Compounds in a Maize Agroecosystem under the Use of Nitrogen Mineral Fertilizer, Farmyard Manure, Urease, and Nitrification Inhibitors

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Abstract: Carbon and nitrogen compounds in agroecosystems have attracted much attention in recent years due to their key roles in crop production and their impacts on environment quality and/or climate change. Since fertilization profoundly disrupted the C and N cycles, several mitigation and/or adaptation strategies, including the application of farmyard manure (FYM) and/or urease and nitrification inhibitors (UI and NI), have been developed. The aim of this study was to evaluate the contents of soil organic carbon and its fractions, the total and mineral forms of nitrogen, as well as CO₂ and N₂O emissions under mineral and organic fertilization with and without urease and nitrification inhibitors in a maize agroecosystem. A two-year field study was carried out on Cambisols (silt) in Poland. The experiment scheme included nine treatments: C (the control without fertilization), UAN (Urea Ammonium Nitrate), UAN+UI, UAN+NI, UAN+UI+NI, FYM with N mineral fertilizer base, FYM with N mineral fertilizer base+UI, FYM with N mineral fertilizer base+NI, and FYM with N mineral fertilizer base+UI+NI. It was found that treatments fertilized with cattle FYM were higher sinks and sources of C and N compounds in comparison to the UAN plots. The organic carbon, humic and humin acid, and total nitrogen concentrations, in contrast to ammonium and nitrate nitrogen, were not affected by the inhibitors added. Nitrification and urease inhibitors were effective in decreasing N₂O emissions only in treatments that were exclusively applied with UAN and had no significant influence on CO₂ emissions.

Keywords: UAN; FYM; urease inhibitor; nitrification inhibitor; CO₂ emission; N₂O emission; organic carbon; mineral nitrogen; humus fractions



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1. Introduction

Soil organic carbon (SOC) and nitrogen (N) in agricultural ecosystems have achieved tremendous attention over the past few decades due to their crucial roles in soil health maintenance, the efficiency of crop productivity, environmental quality, and/or climate change mitigation and adaptation [1].

Soil organic matter (SOM), which is critical to diverse soil functions and ecosystem services, represents the largest organic carbon reservoir in terrestrial ecosystems, which can accumulate 5–15% of the annual global C emissions mainly in humic substances (HS), which are classified according to their solubility into humins (insoluble at all pH conditions), humic acids (soluble in pH > 2), and fulvic acids (soluble at all pH values) [1,2]. Even small changes in SOC stocks may have a substantial effect on the atmospheric CO₂ concentrations [3]. It was reported that 25–29% of anthropogenic CO₂ emissions originate from agricultural soils. SOM also determines the amount of nitrogen in agroecosystems. Its

microbiological decomposition results in the formation of mineral nitrogen—ammonium ($\text{NH}_4\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$), which are available to plants but also easily subjected to losses via leaching, surface runoff, and volatilization [4,5]. These bring threats to the environmental quality, including the eutrophication and acidification of land and water ecosystems, ozone depletion, the formation of particulate matter, smog and acid rains, biodiversity loss, health problems, and effects on global warming [6,7]. Soil mineral forms of nitrogen can also be consumed by microorganisms and immobilized into organic forms. Approximately 833 kg of nitrogen is needed to sequester 10,000 kg of carbon in humus, assuming a C:N ratio of 12:1 [8].

The close connection of soil C and N cycling, driven mainly by the degradation and fixation of C and N, as well as nitrification and denitrification, means that even slight changes in their pools can have serious production and environmental consequences [9]. Agricultural practices, such as fertilization, have profoundly disrupted the C and N cycles [10]. The application of mineral and organic fertilizers into soil may lead to significant short-term changes in the organic matter cycling and acceleration of SOM mineralization or the immobilization of C and N. According to some authors, N mineral fertilizers initially intensify SOM mineralization; however, in the long-term period, due to increasing yields and crop residues, which can enhance carbon sequestration, they positively affect the SOC content. Some researchers, however, believe that one-sided mineral fertilization leads not only to the acceleration of mineralization processes and CO_2 emission, but also to the deterioration of humus quality [1,11]. Other studies indicate that mineral fertilizers in doses covering the fertilization needs of crops only prevent a decrease in the humus level [12].

Nitrogen fertilizer use aiming to replace microbially mediated N mineralization and supply this nutrient directly to crops [13] increases the content of total nitrogen and its mineral forms. This is caused by mineral N not being absorbed by the plants and the larger biomass of crop residues left in the field after harvesting. In contrast, organic fertilization providing SOC and nutrients such as N in agroecosystems can create a more tightly coupled cycle of C and N [13]. A meta-analysis based on 101 studies with a total of 592 treatments showed that the use of farmyard-(FYM), cattle-, and pig manure caused the highest SOM increases of 50%, 32%, and 41%, respectively [14]. If manure inputs were combined with N mineral fertilizers, the SOC accumulation was even greater. There are also studies showing no or adverse effects on SOM [15]. Therefore, according to some authors [14,15], there is a need for further research under local management and environmental conditions concerning the magnitude of change in SOC stocks as the result of manure application.

Organic inputs alter the soil organic carbon (SOC) dynamics affecting the chemical structure of soil organic matter. The identification of organic carbon sequestration in humic substances (HS) (humins and humic and fulvic acids) is crucial for understanding SOC stabilization and carbon cycling [16]. It is commonly believed that FYM fertilization increases the contents of humic acids and humins. However, some authors indicated the high susceptibility of the OM of soils treated with manure to oxidation, which may favor mineralization, leading to N losses.

Nitrogen and carbon inputs affect the emissions of CO_2 and N_2O [17]. Agroecosystems treated with mineral and organic fertilizers, however, have the potential to be not only sources but also sinks of CO_2 [18,19]. Nitrogen-containing fertilizers also boost the production of the most powerful long-lived greenhouse gas— N_2O —by providing a substrate for microbial denitrification, nitrification, and nitrifier denitrification processes, i.e., $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ [18,20,21]. A direct emission factor for N_2O from N inputs (mineral and organic) applied to soils is 0.01 kg $\text{N}_2\text{O-N/kg N}$ input [22]. Several mitigation strategies have been developed to decrease the N emissions in agroecosystems, including the application of urease and/or nitrification inhibitors (UI and NI) [20,21,23,24]. NIs delay the bacterial oxidation of $\text{NH}_4\text{-N}$ by depressing the activities of nitrifiers in soil, whereas UIs slow down the hydrolysis of urea to $\text{NH}_4\text{-N}$ by preventing its binding to the enzyme urease [23,25]. UI and NI temporarily retard the microbiological transformations of nitrogen-based fertilizers,

improving the synchronization of N bioavailability with plant uptake and mitigating N losses [25].

Previous studies indicated inconsistent or no impacts of inhibitors on CO₂ and N₂O emissions under maize cropping [20,21]. Recent research has combined UI with NI to increase the effectiveness in the reduction of N₂O emissions [21]. In Zaman's and Nguyen's experiment [26], the DIs (1:7 ratio w/w of UI and NI) were more effective in minimizing N losses. Chen et al. [27], after synthesizing data from 26 meta-analyses and reviews, stated that future research should focus on double inhibitors (DIs). There is also a lack of studies comprehensively examining the impacts of UI, NI, and/or DI inhibitors under organic and mineral fertilization on the quantity and quality of soil C and N compounds as well as CO₂ and N₂O emissions. We hypothesized that there were such relationships. Thus, the aim of this study was to evaluate the contents of soil organic carbon and its fractions, total and mineral forms of nitrogen, as well as CO₂ and N₂O emissions under mineral and organic fertilization with and without UI, NI, and DI.

2. Materials and Methods

2.1. Site Description and Experimental Design

A two-year field study was carried out in the 2020–2021 period in a randomized complete block design with three replicates at a farm situated in Piława Górna, Poland (50°66'74.73" N, 16°76'47.16" E) on Cambisols (silt) with a soil pH of 6.5, an SOC content of 10 g C kg⁻¹, a total nitrogen (TN) content of 1.11 g N kg⁻¹, and a CEC of 11.28 cm(+) kg⁻¹. The test plant was maize, specifically the SY Talisman variety (FAO 220–230). The annual precipitation was 589.0 mm and 603 mm in the first and second years of the experiment, respectively. Average monthly temperatures in January and July were −1.2 °C and 19.4 °C and −1 °C and 20.0 °C, respectively. Each treatment plot had dimensions of 13.5 m in width × 13.5 m in length. The experiment scheme included nine treatments (Table 1).

Table 1. Experimental treatments.

Treatment	Mineral N Fertilization	Organic Fertilization	Inhibitor
C	–	–	–
UAN	UAN (150 kg N ha ⁻¹)	–	–
UAN+UI	UAN (150 kg N ha ⁻¹)	–	NBPT *
UAN+NI	UAN (150 kg N ha ⁻¹)	–	DMPP *
UAN+UI+NI	UAN (150 kg N ha ⁻¹)	–	NBPT+DMPP *
FYM	UAN (150 kg N ha ⁻¹)	Cattle FYM (129 kg N ha ⁻¹)	–
FYM+UI	UAN (150 kg N ha ⁻¹)	Cattle FYM (129 kg N ha ⁻¹)	NBPT **
FYM+NI	UAN (150 kg N ha ⁻¹)	Cattle FYM (129 kg N ha ⁻¹)	DMPP **
FYM+UI+NI	UAN (150 kg N ha ⁻¹)	Cattle FYM (129 kg N ha ⁻¹)	NBPT+DMPP **

Notes: C—the control without fertilization; UAN—urea ammonium nitrate; FYM—farmyard manure; UI—urease inhibitor; NI—nitrification inhibitor; NBPT—*N*-(*n*-butyl) thiophosphoric triamide; DMPP—3,4-dimethylpyrazole phosphate; *—the inhibitor applied with the mineral fertilizer; **—the inhibitor applied with the organic fertilizer.

2.2. Soil Sampling and Analysis

Soil samples (25 per plot) were collected before the beginning of the experiment and at the end of each growing season. Soil samples were air-dried, homogenized, and sieved (2 mm sieve-mesh). The soil samples for NH₄-N and NO₃-N analysis were placed in polyethylene bags and stored in the refrigerator. In air-dried soil samples, the following parameters were determined: soil organic carbon by sulfochromic oxidation with titration of excess K₂Cr₂O₇ with FeSO₄(NH₄)₂SO₄·6H₂O, total nitrogen according to the Kjeldahl method, and particle size distribution using the laser diffraction method (LDM) based on the light intensity distribution pattern of the scattered light emitted from that particle group [28]. The isolation of HA, FA, and H from soil samples were carried out according to the Schnitzer method [29].

Mineral nitrogen content was determined using Skalar SAN plus Segmented Flow Analyzer (Skalar Analytic B.V., De Breda, The Netherlands) after extraction with 1% of

K_2SO_4 [28]. The data are presented in this paper in the form of the means of the two years of studies.

2.3. CO_2 and N_2O Measurements

Soil CO_2 and N_2O emissions were measured (30 measurements per year) in situ using a portable FTIR analyzer model, GT5000 Terra (Gasetm Technologies Oy, Vantaa, Finland), equipped with the device chamber. The soil CO_2 and N_2O emissions were measured at randomly selected locations in each experimental plot between 11 am and 1 pm to eliminate the diurnal variability. The results were extrapolated to 24 h and 1 ha. The data were presented in the paper in the form of the means of the two years of studies.

2.4. Statistical Analysis

The statistical analysis of the results was performed using Statistica 13.3. A one-way analysis of variance (ANOVA) and Tukey's mean separation were used to determine the statistical significance at $p < 0.05$. Pearson's linear correlation coefficient was calculated with a significance level of $p < 0.05$.

3. Results and Discussion

Mineral and organic fertilization, as well as the use of nitrification and urease inhibitors, caused quantitative and qualitative changes in the contents of carbon and nitrogen compounds in the maize agroecosystem. It is worth noting that, in the experiment conducted, the largest statistically significant differences were observed in the nitrogen pool under the conditions of mineral fertilization and the application of urease and nitrification inhibitors. The weaker effects of UI and NI under organic fertilization may have indicated the occurrence of their adsorption, faster degradation, and effectiveness reduction at higher SOC contents [25].

3.1. Soil Organic Carbon and Total Nitrogen in the Soil

Significantly higher contents of SOC (11.28 g kg^{-1}) and TN (1.40 g kg^{-1}) were found in the soil applied with farmyard manure combined with mineral fertilizer compared to the unfertilized control (Figures 1 and 2), suggesting that organic and mineral fertilization had a beneficial effect on these parameters. Several authors also reported that the SOC levels increase in treatments with farmyard manure [17,30,31] and/or with N inorganic fertilizers [30,32]. This was linked to the amount of additional C and N applied with fertilizers and/or improvements in the crop yields and higher C and N input via rhizodeposition and plant residues [30,33]. In the present study, urease and nitrification inhibitors did not have a significant effect on the SOC and TN concentrations (Figures 1 and 2). The SOC/TN ratios were low (below 10; Figure 3).

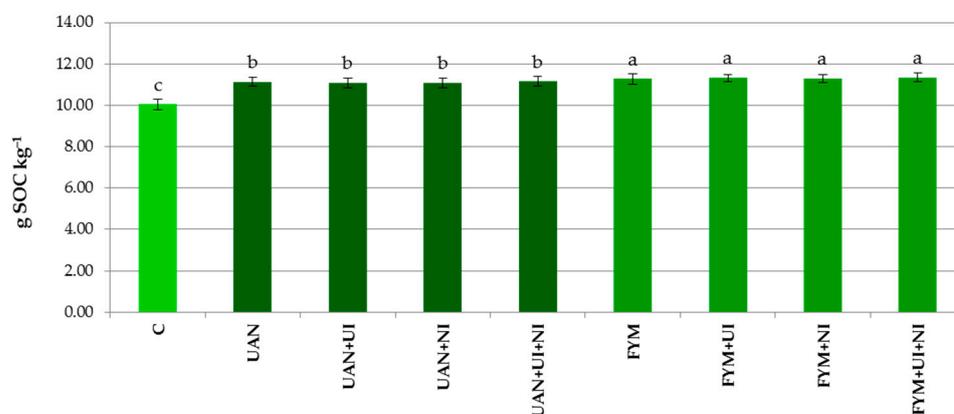


Figure 1. Soil organic carbon (SOC) content. The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

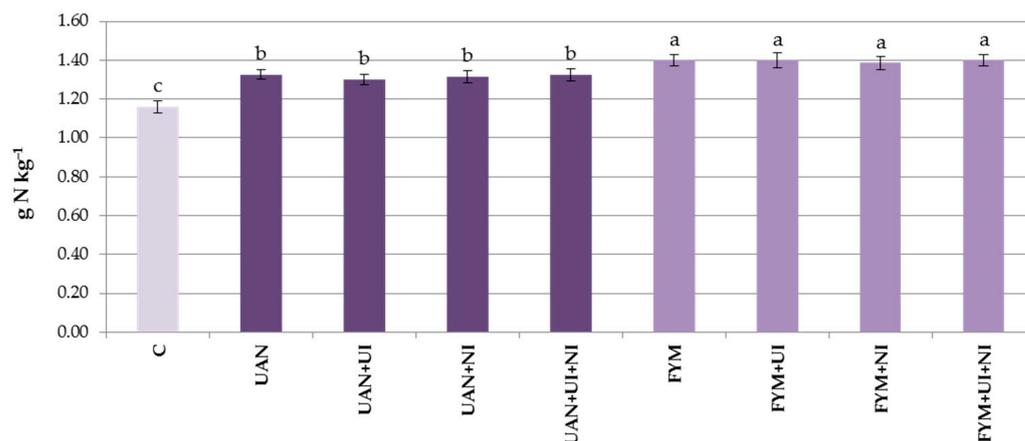


Figure 2. Total nitrogen (TN) content. The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

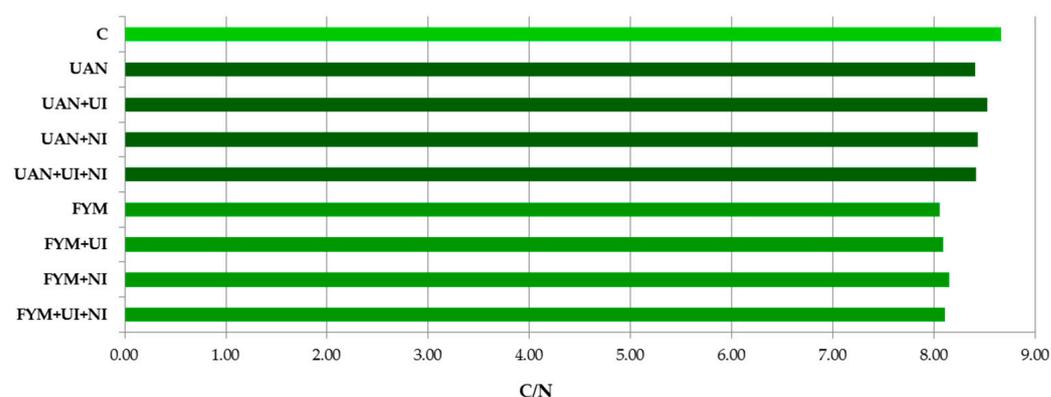


Figure 3. SOC/TN ratios. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

3.2. Humus Fractions in the Soil

In the present study, the SOM content fluctuations were accompanied by changes in the fractional composition of the humus. Our results showed significant quantitative differences between the major components of the soil organic matter, i.e., humic substances (HS). The HS fractions in the soils tested were dominated by C-HA (2.21–2.62 g kg⁻¹) and C-H (5.42–6.22 g kg⁻¹), which is consistent with the previous research [34]. Organic and mineral-amended plots registered higher C-FA, C-HA, and C-H contents compared to the control treatment (Figures 4–6). However, significant differences in impact between organic and mineral fertilization were found only in the case of the C-HA and C-H concentrations. According to some authors [34,35], OM application into soil favored HA formation mainly from FAs during the inception phase of the humification process.

The ratios between C-HA and C-FA expressed as a humification index (HI), indicating the intensity of humification [34,36] were higher in the soils of the FYM treatments (1.10–1.12) than in the plots with UAN (1.0–1.04) (Figure 7). According to some authors [35], HI values > 1 showed longer residence periods for the humic acid fraction in soils. In the present study, urease and nitrification inhibitors did not have significant impacts on the HS fractions (Figures 4–6).

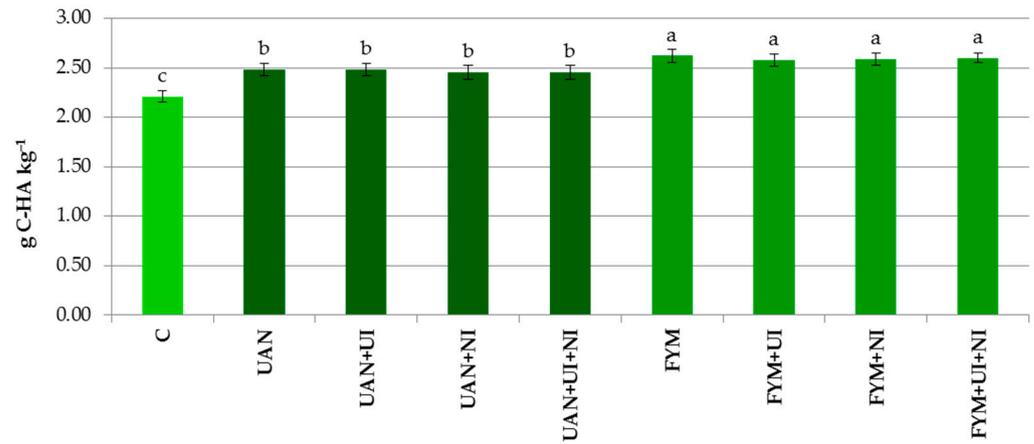


Figure 4. Content of humic acids (C-HA). The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

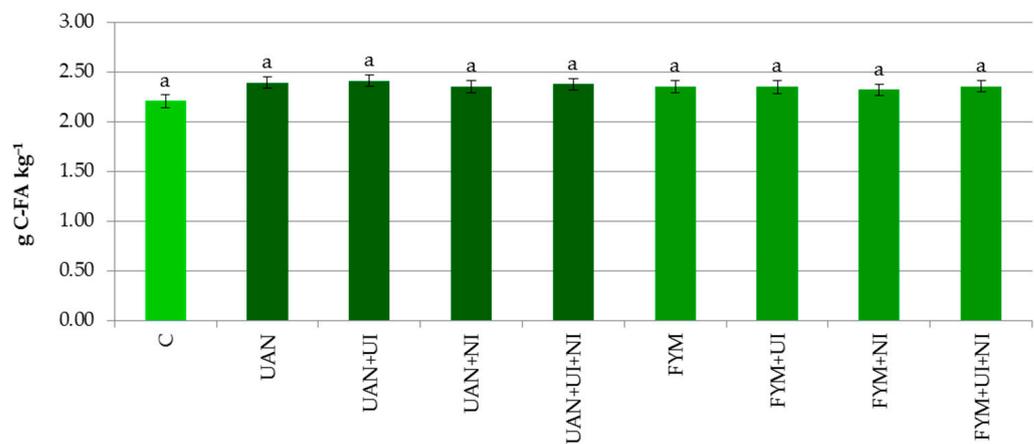


Figure 5. Content of fulvic acids (C-FA). The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

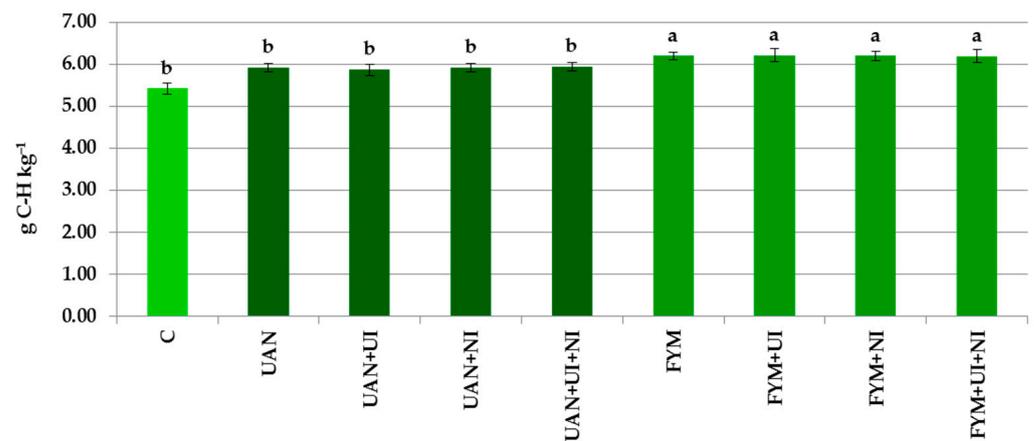


Figure 6. Content of humins (C-H). The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

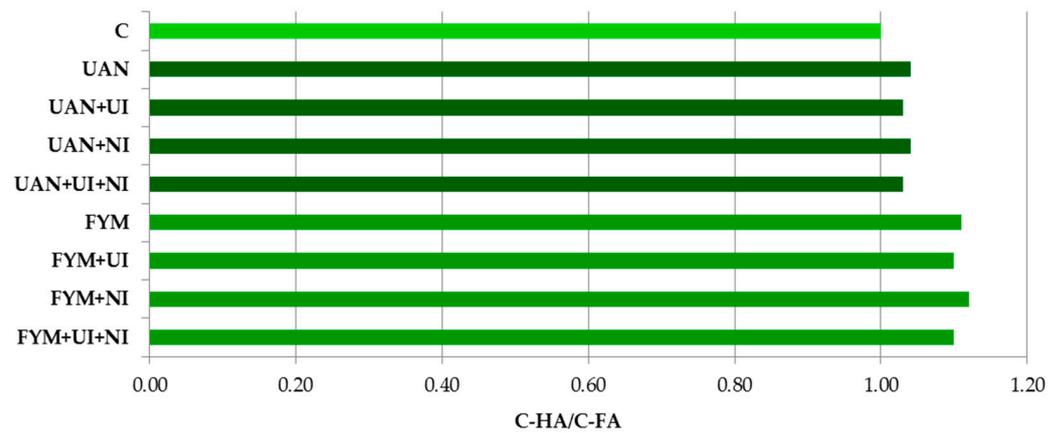


Figure 7. Humification index (HI). C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

3.3. Mineral Nitrogen in the Soil

The transformations of nitrogen in the soil are mainly determined by mineralization, immobilization, oxidation, and reduction. Two of the key compounds in these processes are ammonium and nitrate ions.

In our research, the FYM treatment had the highest $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ contents, respectively, which were $7.70\text{--}8.01 \text{ mg kg}^{-1}$ and $3.15\text{--}3.24 \text{ mg kg}^{-1}$ (Figures 8 and 9). Several authors also noticed that a combination of organic and inorganic fertilizers plays a significant role in the improvement of N bioavailability in soils [37–39]. The mineral nitrogen concentrations rose considerably under FYM application with an increasing N rate to $\geq 80 \text{ kg N}\cdot\text{ha}^{-1}$ in 0–15 cm and to $120 \text{ kg N}\cdot\text{ha}^{-1}$ in 15–60 cm depths [40].

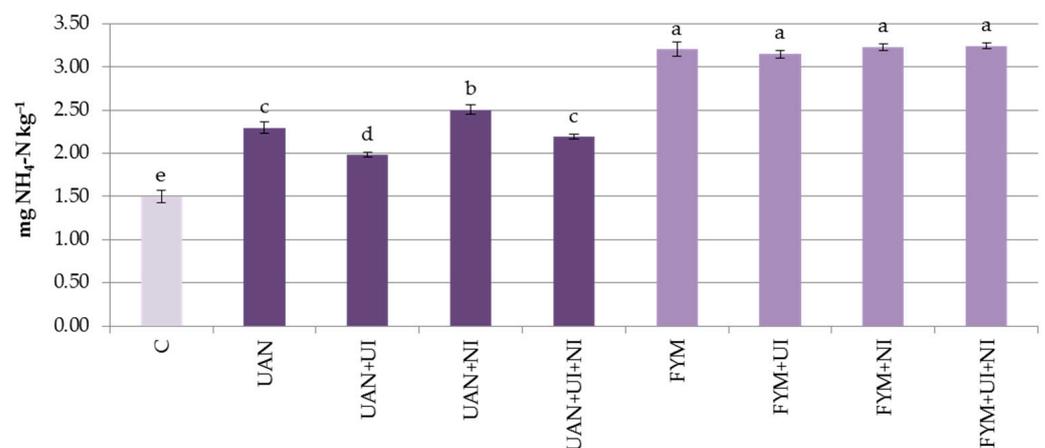


Figure 8. Ammonium content ($\text{NH}_4\text{-N}$) in the soil. The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

In the present study, the inhibitor addition (NBPT, DMPP, DI) decreased the soil $\text{NO}_3\text{-N}$ content compared to the soil with applied FYM (Figure 9). However, this reduction was not statistically significant. There were also no significant effects of inhibitor use with farmyard manure on the $\text{NH}_4\text{-N}$ concentrations, although the treatments with nitrification and double inhibitors had numerically greater contents than the FYM and FYM+UI treatments.

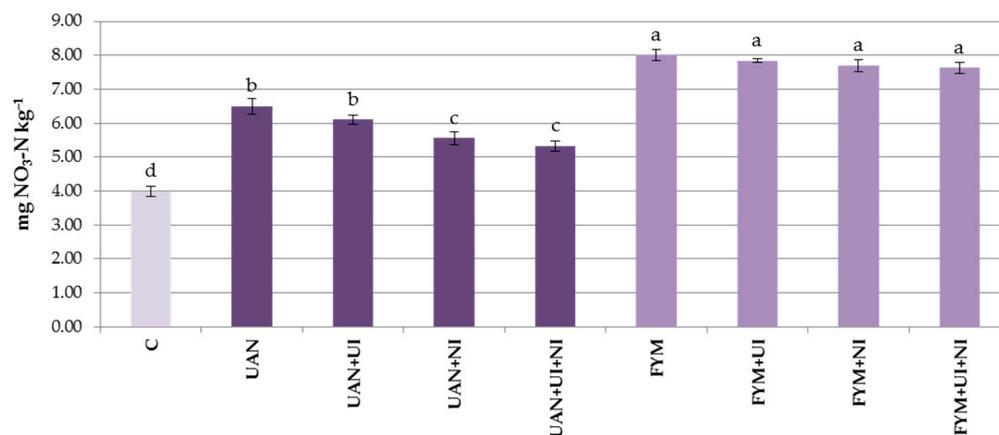


Figure 9. Nitrate content (NO₃-N) in the soil. The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

The addition of DMPP with the UAN fertilizer effectively decreased the nitrification rates in the soil, raised the content of NH₄-N by 8.70%, and lowered the NO₃-N concentration by 14.46% (Figures 8 and 9), which is consistent with the results of other studies [7,24,41]. DMPP extends the residence time of NH₄-N in soils due to the deactivation of the enzyme responsible for the first step of nitrification, i.e., the oxidation of NH₄⁺ to NH₂OH via indiscriminate binding and suppressing ammonium monooxygenase activity [23–25,41–43]. Abalos et al. [44] noticed that NI applications could diminish denitrification-induced N losses by decreasing the soil NO₃-N contents for denitrification, which allows the available nitrogen to be retained in the soil and become absorbed by the plants for a longer time.

In the present study, the application of NBPT with mineral fertilizer led to a decrease in the contents of both mineral forms of nitrogen (Figures 8 and 9). N-(n-butyl)thiophosphoric triamide is quickly converted to its more effective O analog, N-(n-butyl)phosphoric triamide, which forms a tridentate ligand with the urease enzyme to slow down the catalytic hydrolysis of urea to NH₄⁺ [25,45]. As a result of these processes, NH₄⁺ is gradually produced, which enables its more efficient uptake by plants and, at the same time, reducing the extent of nitrification and the potential loss of NO₃-N [25,42].

In previous studies [43], mineral fertilization with NI and UI inhibited not only nitrification but also urea hydrolysis, which resulted in significant decreases in the NO₃-N and NH₄-N concentrations, which was observed in the present experiment in the case of the latter (Figures 8 and 9).

3.4. CO₂ and N₂O Emissions

In the conducted experiment, changes were observed not only in the pool of soil carbon and nitrogen but also in the emissions of their gaseous compounds, i.e., CO₂ and N₂O.

The carbon dioxide emissions ranged between 100 and 125 kg CO₂ ha⁻¹ d⁻¹ (Figure 10). The control treatment showed the lowest CO₂ losses, and the application of UAN did not induce significant changes in the CO₂ emissions in comparison to the unfertilized treatment, which was consistent with other authors' research [46,47]. The addition of DMPP and NBPT with the UAN had no significant impact on CO₂ efflux (Figure 10).

Previous studies showed contradictory effects of UI and NI on CO₂ fluxes. Zhang et al. [48] noticed a decrease in CO₂ emissions following the use of DMPP with mineral fertilizers in field experiments. Other authors reported that the application of NBPT together with nitrification inhibitors in wheat fields mitigated soil CO₂ release by blocking soil carbon mineralization. Huéfrano et al. [46] and Wang et al. [49] observed that CO₂ emissions were not affected by inhibitors. Zhang et al. [48] reported that CO₂ efflux from

a clay loam grassland was unaffected by DMPP addition. The discrepancy observed in the inhibitory impact on the soil CO₂ emission may partially lie in the differences in soil physicochemical properties (pH, Eh, and clay content) [48].

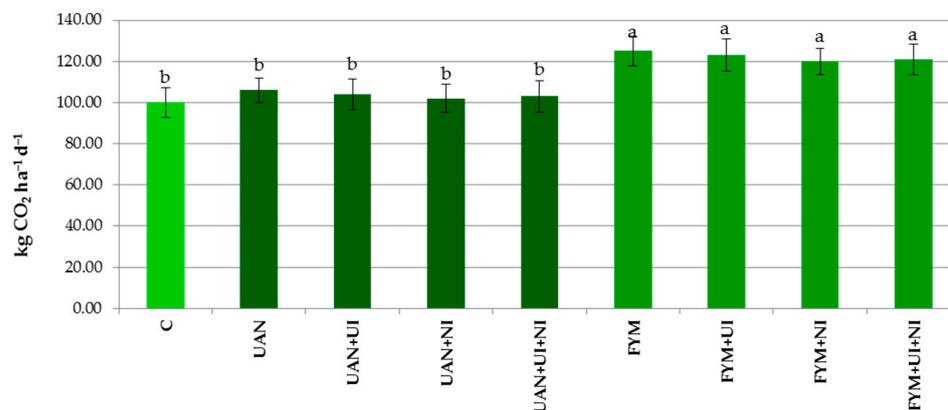


Figure 10. CO₂ emission in a maize agroecosystem. The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

Since CO₂ emission is closely linked with the decomposition of organic matter [48,50], the cattle manure treatments significantly stimulated the soil CO₂ emissions by 17.92% and 25%, respectively, in comparison to the UAN and C plots (Figure 10). Sistani et al. [47] also stated that animal manure applied in farmland may be beneficial to soil health. However, it can also increase the production of GHG. Several authors reported [17,30] that carbon dioxide emissions are greater from soils treated with manure compared to inorganic fertilizer, which was attributed to a greater OM availability for microbial respiration in soil. According to previous studies, the soil C/N ratio plays a key role in controlling CO₂ emissions [17], which is consistent with our study. The low SOC/TN ratios in our experiment imply that a prevailing decomposition of OM resulted in significantly higher CO₂ emissions in the treatments characterized by lower values of this parameter (FYM, FYM+UI, FYM+NI, and FYM+UI+NI treatments; Figures 3 and 10; $r = -0.963$). Abdalla et al. [30] reported that organic fertilization enhances microbial growth and activities, resulting in accelerated SOM mineralization and a priming effect. It should be underlined that, in the present study, despite the greater SOM mineralization in the FYM treatments, the final SOC level was still significantly higher in comparison to the mineral-fertilized plots (Figure 1). Significant correlations were noticed between CO₂ emissions and concentrations of SOC ($r = 0.622$), TN ($r = 0.794$), C-HA ($r = 0.833$), C-H ($r = 0.844$), NH₄-N ($r = 0.921$), and NO₃-N ($r = 0.932$) (Table 2). Other authors reported earlier that the availability of N and SOM quality are the key factors to C mineralization in soils [17,34].

Table 2. Correlation coefficients between determined soils’ parameters.

Parameter	SOC	TN	C-HA	C-FA	C-H	NH ₄ -N	NO ₃ -N	CO ₂	N ₂ O
SOC		0.930 ***	0.935 ***	0.750 ***	0.914 ***	0.790 ***	0.818 ***	0.622 ***	0.786 ***
TN	0.930 ***		0.948 ***	0.529 ***	0.942 ***	0.913 ***	0.908 ***	0.794 ***	0.884 ***
C-HA	0.935 ***	0.948 ***		0.585 ***	0.965 ***	0.904 ***	0.947 ***	0.833 ***	0.931 ***
C-FA	0.750 ***	0.529 ***	0.585 ***		ns	ns	ns	ns	ns
C-H	0.914 ***	0.942 ***	0.965 ***	ns		0.930 ***	0.918 ***	0.844 ***	0.907 ***
NH ₄ -N	0.790 ***	0.913 ***	0.904 ***	ns	0.930 ***		0.929 ***	0.921 ***	0.924 ***
NO ₃ -N	0.818 ***	0.908 ***	0.947 ***	ns	0.918 ***	0.929 ***		0.932 ***	0.987 ***
CO ₂	0.622 ***	0.794 ***	0.833 ***	ns	0.84 ***4	0.921 ***	0.932 ***		0.936 ***
N ₂ O	0.786 ***	0.884 ***	0.931 ***	ns	0.907 ***	0.924 ***	0.987 ***	0.936 ***	

Notes: *** $p < 0.001$; ns—not significant.

Changes in N₂O emissions significantly depended on the fertilization used. The highest flux rate (5.68 g ha⁻¹ day⁻¹) was observed for the FYM treatment, while the lowest one was found in the control treatment (Figure 11). This was consistent with other studies where organic fertilization increased N₂O emissions [38,51,52], and this might be explained by two crucial mechanisms. Firstly, the total nitrogen input was higher in the FYM treatments. Several researchers reported that the amount of N₂O emissions rose strongly when the N rate was higher than 90 kg N ha⁻¹–200 kg N ha⁻¹ [20]. According to some authors [45], the N₂O emission flux increased exponentially with a nitrogen rate of 0–225 kg N ha⁻¹. A positive correlation was also observed between N application rates and N-N₂O emissions [45,51]. Secondly, FYM provides a source of readily available C, which could stimulate nitrifying and denitrifying bacteria and N₂O production in soils [40,51,52]. The growth of active microorganisms and O₂ consumption in soil pores may result in the formation of a micro-anaerobic environment, favoring denitrification and N-N₂O production [51]. Cai et al. [45] stated that, under conditions with limited O₂, nitrous oxide may be produced via nitrifier denitrification or nitrification-coupled denitrification, and under highly anaerobic conditions, its production is dominated by denitrification.

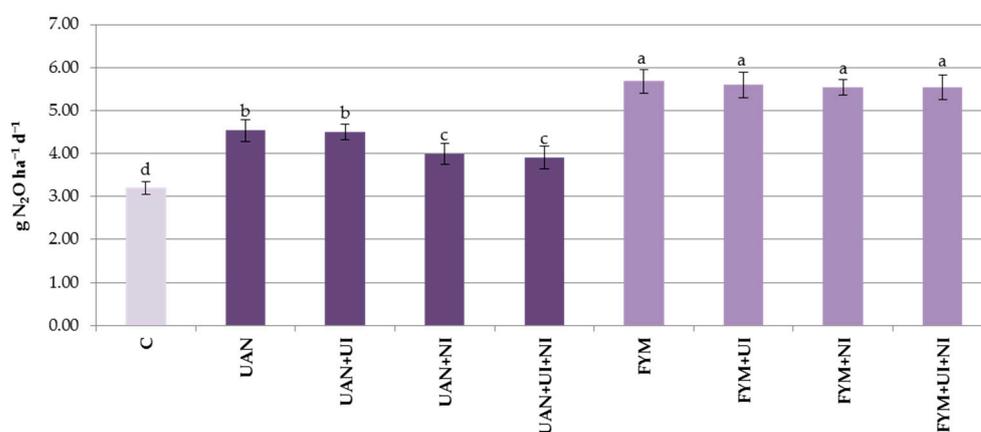


Figure 11. N₂O emissions in a maize agroecosystem. The same letter means not significantly different. C—control without fertilization; UAN—urea ammonium nitrate; UI—urease inhibitor; NI—nitrification inhibitor; FYM—farmyard manure with N mineral fertilizer base.

In the present study, the application of urease, nitrification, and double inhibitors with organic fertilizer did not significantly affect the N₂O emissions, which were within the range of 5.54–5.59 g ha⁻¹ day⁻¹ (Figure 11). Other studies also did not report an impact of UI, NI, and DI added to manure regarding the reduction in nitrous oxide emissions [53], which was due to the gradual release of mineral forms of nitrogen from FYM and the absorption or increasing decomposition of inhibitors in the presence of higher organic C concentrations [53,54].

Compared with the UAN treatment, the use of DMPP and NBPT+DMPP significantly decreased the N₂O emissions by 11.89% and 13.88%, respectively (Figure 11). This was in line with studies by other authors [20], who noticed that the application of nitrification inhibitors reduced the soil N₂O emissions by 1.8–61.0%. Cui et al. [7] and Ma et al. [41] reported that DMPP with urea-based fertilizers significantly inhibited the potential of the nitrification rate since it is characterized by low mobility, slow biodegradation, and persistence in the soil environment as well as interactions with ammonium monooxygenase to suppress the first rate-limiting step of soil nitrification.

The results of the current research indicate that the addition of NBPT to the mineral fertilizer decreased the value of the N₂O emissions, but the reduction was not significant. Urease inhibitors, such as NBPT, influence the conversion of amide nitrogen to ammonium nitrogen during urea hydrolysis. Hence, they could retard the N-NH₄ supply, which decreases the substrate availability for both nitrification and subsequent denitrification, i.e., the main processes of N₂O production [20,43]. According to the data in the literature [25,42],

although NBPT may delay urea hydrolysis for 3–15 days, depending on environmental factors, its influence on N_2O emissions is highly variable, ranging from no effect to reduced or even increased emissions of nitrous oxide. The relatively low N_2O reductions under the inhibitor used in the conducted experiment could be the result of the high contents of clay and silt in the soil tested. According to some authors, NBPT and DMPP applications were more effective in depleting N_2O emissions in coarse than in fine soil due to the larger adsorption of inhibitors by clays in the latter [25,41].

There were positive correlations between the emission of N_2O and concentrations of SOC ($r = 0.786$), TN ($r = 0.884$), C-HA ($r = 0.931$), C-H ($r = 0.907$), NH_4-N ($r = 0.924$), and NO_3-N ($r = 0.987$) (Table 2), which is consistent with other studies [17,53,55]. The high correlation between N_2O emission and NH_4-N concentration in the tested soil confirms that nitrification can be an important source of N_2O [32,45]. The soil mineral N content is regarded as one of the key drivers of N_2O emissions since ammonium and nitrate serve as sources for nitrification and denitrification, respectively, which are processes that produce nitrous oxide [49,55]. Some authors [53] reported that the NO_3^- intensity explained 80–90% of the variability in N_2O emissions. In the present experiment, more than 97.48% and 85.35% of the variability in N_2O emissions may be explained by the regression equation, in which the explanatory variables are NO_3-N and NH_4-N , respectively (Figures 12 and 13). According to some authors [55], NH_4-N enhances soil N_2O emission only at a lower soil moisture.

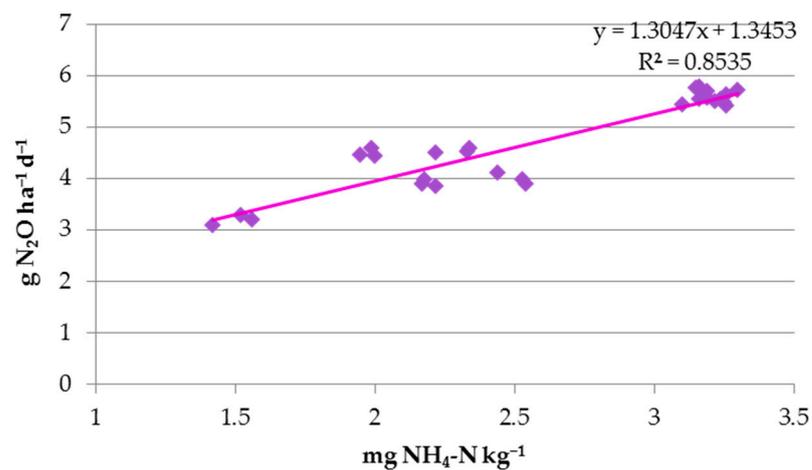


Figure 12. The relationship between N_2O emissions and NH_4-N contents in the soil.

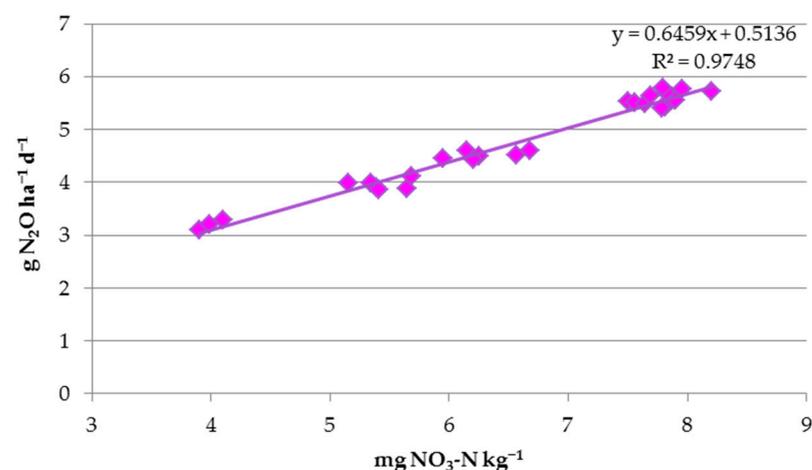


Figure 13. The relationship between N_2O emissions and NO_3-N contents in the soil.

It should be kept in mind that agronomic treatments impact GHG efflux differently, and relationships between CO₂ and N₂O emissions and soil properties are not universal [17].

4. Conclusions

The treatments that included fertilization with farmyard manure were higher sinks and sources of carbon and nitrogen compounds compared to the UAN plots. Despite the significant increases in CO₂ and N₂O emissions in organic fertilized soils, the final levels of soil organic carbon, humic acids, humins, total nitrogen, ammonium, and nitrate nitrogen were still significantly higher in comparison to the mineral-fertilized ones. Nitrification and double inhibitors were effective in decreasing N₂O emissions only in treatments that exclusively applied UAN and had no impact on the CO₂ efflux. In the present study, the soil organic carbon, humic acid, humin, and total nitrogen concentrations, in contrast to the NH₄-N and NO₃-N contents, were not affected by the inhibitors added. The analyzed soil parameters were positively correlated with CO₂ and N₂O emissions, indicating their contributions to the processes of soil respiration, nitrification, and denitrification. Further, longer-lasting studies on quantitative and qualitative changes in the contents of carbon and nitrogen compounds in agroecosystems under mineral and organic fertilization, as well as the use of nitrification and urease inhibitors, are recommended under different climate and soil conditions.

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