Soil nitrous oxide emissions from sugarcane field affected by nitrogen fertilizer rate and inhibitors of urea hydrolysis and nitrification process

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Research Article

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Abstract

Reducing the use of nitrogen (N) fertilizer and inhibitors of urea hydrolysis and the nitrification process (UIs and NIs, respectively) could be the key to the successful mitigation of soil nitrous oxide (N_2O) emissions, such information is limited for non-Annex I countries. This study focused on sugarcane (*Saccharum officinarum* L.) and bare soil fields to evaluate the growth potential of the fertilizer rate with UIs, NIs and their combinations (UINIs) on soil N_2O emissions. The N fertilizer rates applied were based on standard farmer practice (F) and soil analysis (S). The synthetic UI and NI used in this study were N-(n-butyl) thiophosphoric triamide (NBPT) and dimethylpyrazole phosphate (DMPP), the UIs and NIs from plants were garlic (*Allium sativum* L.) and ground neem seed (*Azadirachta indica* A. Juss. var. siamensis Valeton), and praxelis (*Praxelis clematidea* (Griseb.) R.M. King & H. Rob). The results showed that compared to F, reducing the N fertilizer rate and adding NIs in the sugarcane field decreased soil N_2O emissions by up to 19.1%, followed by UINIs and UIs (17.0 and 15.9%, respectively), while there was 18.9% N_2O reduction with praxelis. When applying the dressing, all inhibitors seem to have potential to inhibit microbial activity but there was an inconsistent significant difference on mitigated cumulative N_2O. The use of inhibitors decreased soil N_2O emission factor (EF) values in the sugarcane plots by 1.1–1.6%, compared to 1.8% for S treatment. Notably, F had a lower soil N_2O EF (0.6%), possibly due to the relatively high N fertilizer rate applied in this study. The findings suggested that reducing N fertilizer use and applying UIs, NIs and UINIs had great potential to minimize greenhouse gas (GHG) emissions from the soil. These results regarding the use of soil N_2O EFs in a sugarcane field could provide supporting data for the national GHG inventory and be adopted for mitigation measures in Thailand.

1 Introduction

Nitrogen (N) plays an important role in tropical agricultural systems due to its low availability in soil and often being insufficient for crop use. Synthetic N fertilizer has been applied in the field to improve crop productivity; however, sometimes it has been over applied based on the crop's requirements [1]. Excessive application of N fertilizer, especially urea, has increased environmental problems, such as ammonia (NH_3) volatilization, nitrous oxide (N_2O) emission through the nitrification, and potentially losses to groundwater from nitrate (NO_3^-) leaching. Anas, Liao, Verma, Sarwar, Mahmood, Chen, Li, Zeng, Liu, Li [2] reported over 50% of N fertilizer added was unable to be used by many crops and up to 80% was not utilized by sugarcane crops.

Sugarcane (*Saccharum officinarum* L.) is an important cash crops in the world and one of Thailand's important crops. For nearly a decade, Thailand has been the second largest sugarcane exporter in the world, 11 million tons, behind Brazil (28.2 million tons) and the fourth largest sugar producer behind Brazil, India and the European Union [3]. However, growing sugarcane is one of the largest contributors of greenhouse gas (GHG) emissions in the world especially N_2O emissions. Globally, N_2O emissions from soils was the main source of GHG emissions in seven of the ten regions. The emission trends in the agricultural sector in developing regions show N_2O emission from soil (mainly associated with N fertilizer and manure applied to soil) as contributing the greatest source (about 38%) of total non-carbon dioxide (CO_2) emissions and this was predicted to continue to increase. Due to amount of N fertilizer use and animal manure production increased agricultural N_2O emissions are projected to increase up to 60% by 2030 [4]. However, the investigation of the soil N_2O emission factor (EF) from croplands is limited and country-specific N_2O EF values remain under often preliminary investigation particularly for non-Annex I countries.

Many researchers have studied ways to improve nitrogen use efficiency (NUE) by enhancing fertilizer management practices, along with increasing productivity and farmer income, while protecting the environment. The concept of best practice management followed by increased fertilizer efficiency and reduced nutrient losses from the soil could achieve economic, social and environmental goals [5]. However, an optimum fertilizer rate and the correct fertilizer type might not be sufficient to produce an agronomic profit; consequently, other technologies are required to contribute to efficiency gains.

The addition of urease inhibitors (UIs) and nitrification inhibitors (NIs), together with N fertilizer has been developed to reduce N losses from soils [6]. UIs can increase the urea contents in soil by delaying the hydrolysis of the urea and reducing NH_3 volatilization so that the urea remains in the soil. Many compounds have been investigated to delay urea hydrolysis through different reactions with the urease enzyme, such as forming a complex with the Ni atom at the active site, reacting with the sulfhydryl (mercapto) groups of urease and binding to the active site of urease as competitive inhibitors [7]. However, the most commonly used UI is N-(n-butyl) thiophosphoric triamide (NBPT). Many commercial NIs have been considered, such as dimethylpyrazole phosphate (DMPP) and dicyandiamide (DCD), which depress the activity of ammonia-oxidizing bacteria (AOB) in the nitrification process, inhibiting ammonia monooxygenase (AMO) that converts NH_4^+ to hydroxylamine (NH_2OH). In addition, NIs can block N_2O emission by keeping the NH_4^+ in the soil and helping plants to absorb more minerals from the soil [8].

However, it is very important to prevent NH_3 volatilization, N_2O emission and NO_3^- leaching. [9] reported that DMPP had the potential to increase productivity and mitigate N_2O emissions in the maize and wheat seasons by up to 55% and 47%, respectively; however, the efficiency was decreased when DMPP blended with NBPT in both the maize and wheat seasons. On the other hand, the use of UIs significantly reduced volatilization, whereas adding NIs could increase organic matter and retain soil NH_4^+ (Gioacchini, Nastri, Marzadori, Giovannini, Vittori Antisari, Gessa [10], [11]. The addition of UIs with NIs with them working in different ways could enhance NUE by slightly transforming urea to NH_3 while retaining NH_4^+ in the soil and this may help to decrease N_2O emission and increase productivity.

Several inhibitors have been investigated to improve environmental profit; however, there have been concerns about side-effects and sustainability and profitability. Therefore, natural inhibitors would provide the chance to enhance the efficiency of N fertilizer use while being environmental friendly with a reasonable cost [12]. Some parts of plants have been tested for their performance as UIs, such as thiosulfinate, an organosulfur compound from garlic [13]. Yellow and white onion, cabbage and brussels sprouts showed urease inhibitory activity [14], while. Various compounds containing fatty acids, phenylpropanoids, flavonoids, quinines, diterpenoids and isothiocyanates have been reported as NIs, such as neem, mint, tea and karanja [15–17].

Praxelis (*Praxelis clematidea* (Griseb.) R.M. King & H. Rob) is an invasive weed in wild agricultural land in Thailand [18] that contains phenylpropanoid, flavonoids and other compounds [19] that have been evaluated for antibacterial and antifungal activities [20]. New biological nitrification inhibitors (BNIs) are required that can reduce the excessive N emissions into the air and water and that are eco-friendly and achieve higher crop yields with lower costs.

Although many researchers have tested for urease and nitrification inhibition from natural sources, there have been few studies on soil N_2O emissions as affected by chemical and natural UIs and NIs using field experiments, especially in Thailand. Therefore, the objective of this study was to investigate: 1) the potential of applying the right rate of fertilizer application (based on soil and leaf analysis) using synthetic inhibitors (NBPT and DMPP), BNIs (garlic, neem and praxelis) and their combinations on soil N_2O emissions; and 2) the relationship between the N fertilizer rate applied and the soil N contents with soil N_2O emission in sugarcane and an unplanted field in Thailand. In addition, soil N_2O EFs were investigated and developed. We assumed that the application of reducing the N fertilizer rate and adding UIs with NIs could mitigate soil N_2O emission in sugarcane filed and bare soil plot should reveal the same correlation with the sugarcane plot and be more pronounced.

2 Materials And Methods

2.1 Field experiment

The study site was located at the Lopburi Research Station, Faculty of Agriculture, Kasetsart University, Khok Charoen district, Lopburi province, Thailand (15°21' N, 100°55' E). The patterns of rainfall and air temperature of this research station are shown in Fig. 1. Characteristics of the soil in this study were: sand 29%, silt 24% and clay 47% (clayey soil); pH 6.7; organic C 2.93%; P 87 mg kg^{-1}; and K 394 mg kg^{-1}. The sugarcane plots and the unplanted plots were arranged in a randomized complete block design with 4 and 3 replications, respectively. Both the individual planted and unplanted plot sizes were 28 m^2 (4 rows per plot, 7 meters per row) with 1.5 × 0.5 m row by plant spacing. The sugarcane plot and the unplanted plot had inputs of basal and dressing fertilizers at the same rates to compare the influence of plants on soil N_2O emission patterns. The treatments were C (no N fertilizer application), F (N fertilizer with commonly applied rate by farmers), S (N fertilizer based on soil and leaf analysis according to Department of Agriculture, Thailand recommended) and 7 inhibitor treatments (DM/NB (DMPP/NBPT), DM/DM (DMPP/DMPP), DM/NBDM (DMPP/NBPT + DMPP), NE/GL (neem/garlic), NE/NE (neem/neem), NE/GLNE (neem/garlic + neem) and PR/PR (praxelis/praxelis)), detailed in Table 1.

Table 1
Details of fertilizers and inhibitors used in this study

Treatments	**Fertilizer rate (kg ha^{-1})**				**N fertilizer rate (kg N ha^{-1})**		**Inhibitors used**			**Inhibitors rate (% of N fertilizer) ***		
	Basal		**Dressing**		**Basal**	**Dressing**	**Basal**	**Dressing**		**Basal**	**Dressing**	
	16-20-0	**16-16-8**	**46-0-0**	**0-0-60**			**NI**	**UI**	**NI**	**NI**	**UI**	**NI**
C	-	-	-	-	-	-	-	-	-	-	-	-
F	312.5	-	312.5	125	50	143.75	-	-	-	-	-	-
S	-	118.75	43.75	50	19	20.125	-	-	-	-	-	-
DM/NB	-	118.75	43.75	50	19	20.125	DMPP	NBPT	-	5	5	-
DM/DM	-	118.75	43.75	50	19	20.125	DMPP	-	DMPP	5	-	5
DM/NBDM	-	118.75	43.75	50	19	20.125	DMPP	NBPT	DMPP	5	2.5	2.5
NE/GL	-	118.75	43.75	50	19	20.125	neem	garlic	-	20	10	-
NE/NE	-	118.75	43.75	50	19	20.125	neem	-	neem	20	-	20
NE/GLNE	-	118.75	43.75	50	19	20.125	neem	garlic	neem	20	5	10
PR/PR	-	118.75	43.75	50	19	20.125	praxelis	-	praxelis	200	-	200
Note: * Inhibitor rate used in this study was calculated with dry weight.												

[Table 1 about here]

The sugarcane plots used Khon Kaen 3 as a check variety. Healthy sugarcane plants were selected and each stem was split with 3 to 4 joints per piece and placed horizontally into the furrows and the basal fertilizer was added when the sprouts first emerged before covering with soil.

For the basal application, the fertilizer used for F was ammonium phosphate at 312.5 kg ha^{-1} or 50 kg N ha^{-1} (16-20-0; N-P-K) and for S at 118.75 kg ha^{-1} or 19 kg N ha^{-1}(16-16-8; N-P-K). All inhibitor treatments received the same rate and formula of fertilizer as S along with the NIs. The DM/NB, DM/DM and DM/NBDM treatments were added with DMPP at 5% of the N fertilizer amount. The NE/GL, NE/NE and NE/GLNE treatments were added with ground neem seed (*Azadirachta indica* A. Juss. var. siamensis Valeton) at 20% of the N fertilizer amount and the PR/PR treatment was added with praxelis at 200% of the N fertilizer amount.

After 2 months of basal application, urea fertilizer was added as the dressing application. F was added at the rate of 312.5 kg N ha^{-1} or 143.75 kg N ha^{-1}. S and all inhibitor treatments were added at a rate of 43.75 kg ha^{-1} or 20.125 kg N ha^{-1}. The 0-0-60 fertilizer was added at rates of 125 kg ha^{-1} for F and 50 kg ha^{-1} for S and the rest. NBPT and DMPP were added in the DM/NB and DM/DM treatments at the rate of 5% of the N fertilizer amount. NBPT and DMPP were added in the DM/NBDM treatment at the rate of 2.5% of the N fertilizer amount. The NE/GL, NE/NE and PR/PR treatments were added with garlic (*Allium sativum* L.), ground neem seed and praxelis at rates of 10%, 20% and 200%, respectively, of the N fertilizer amount. Garlic and neem were added at rates of 5 and 10% of the N fertilizer amount in the NE/GLNE treatment. Watering was applied as necessary and monitoring was carried out for pests and diseases until maturation.

[Figure 1 about here]

2.2 Field measurements

A gas sample was taken 2–3 times per week for the first month after fertilizer application and then once every 2 months until maturation (22 November 2019 to 29 August 2020). Each chamber was used in each plot and covered the fertilizer band. The individual enclosed static chamber consisted of two parts: the chamber body was equipped with a small fan and thermometer (diameter 30 cm and height 10 cm) and a polyvinyl chloride collar (diameter 30 cm and 10 cm height) installed as a collar into the soils at 5 cm depth. Gas samples were taken from the chamber headspace at 5, 10 and 20 min after chamber closure. The flux of N_2O was analyzed using an Agilent 6890 gas chromatograph (GC) with a micro electron capture detector (μECD) according to [21]. The GC used a valve temperature of 60°C, an oven temperature of 60°C and a methanizer temperature of 375°C. N_2 was used as the carrier gas. N_2O was analyzed using the μECD at 350°C and measured based on a calibration N_2O standard concentration of 1 ppmv.

The N_2O flux was calculated using the following Eq. (1) [22] :

$$\mathrm{F} = \rho \times \mathrm{V}/\mathrm{A} \times \varDelta\mathrm{C}/\varDelta\mathrm{t} \times 273/(\mathrm{T}+273)$$

1

where F is the N_2O flux (μg m^{-2} h^{-1}), ρ is the density of N_2O (kg m^{-3}), V is the volume of the headspace in the chamber (m^3), A is the base area of the chamber (m^2), ΔC/Δt is the average rate of change of concentration with time (ppmv h^{-1}) and T is the temperature (°C) in the chamber.

Then, 30 days after fertilization (DAF) the seasonal cumulative N_2O emissions were calculated as follows Eq. (2) [23]:

$$\mathrm{Cumulative N_2O emission} = \sum_{i=1}^{\mathrm{n}} 1/2 \times (\mathrm{F_i} + \mathrm{F_{i+1}}) \times (\mathrm{T_{i+1}} - \mathrm{T_i}) \times 24$$

2

where F is the N_2O flux (μg m^{-2} h^{-1}), i is the i^{th} measurement in the term (T_{i+1} - T_i), which is the time between two adjacent days of measurements and n is the total number of measurements. The N_2O emission factor (%) of N fertilizer with and without inhibitor treatment to the soil were calculated using the following Eq. (3):

$$\mathrm{N_2OEF} = \left(\left(\mathrm{N_2O_F} - \mathrm{N_2O_C}\right)/\mathrm{Napplied}\right) \times 100$$

3

where N_2O_F is the cumulative N_2O emissions in the N fertilizer with and without inhibitor treatment (kg N ha^{-1}), N_2O_C is the cumulative N_2O emissions from the control treatment (kg N ha^{-1}) and N applied is the total N fertilizer applied rate (kg N ha^{-1}).

N_2O emission reduction was calculated using the following Eq. (4) [24]:

$$\mathrm{N_2Oreduction}\,(\%) = \left(\left(\mathrm{A}-\mathrm{B}\right)/\left(\mathrm{A}-\mathrm{C}\,\right)\right) \times 100$$

4

where A is the cumulative N_2O emission in the only-N fertilizer treatment (F, S), B is the cumulative N_2O emission in the inhibitor treatments and C is the cumulative N_2O emission in the C treatment.

2.3 Statistical analysis

Analysis of variance (ANOVA) was determined to evaluate the effects of the UI and NI applications and times on soil N_2O emissions and cumulative N_2O emissions. The statistical model used was a repeated measure. The multiple comparison test used was Duncan's multiple range test with $P < 0.05$ or 0.1 as the significance levels.

3 Results And Discussion

3.1 Soil N_2O fluxes

Soil N_2O fluxes from the bare soil and sugarcane plots showed a pattern related to the fertilizer application (Fig. 2). Soil N_2O fluxes increased rapidly for about one month after the basal and dressing N fertilizer applications, and then sharply decreased thereafter. We found a higher soil N_2O emission trend in all treatments in unplanted plots than in the sugarcane plots because, in the planted area, some of the inorganic N in the form of NH_4^+-N may have been taken up by plants, resulting in lower levels of emitted soil N_2O through the decrease in N substrate in the nitrification process. The presence of mineral N may have contributed to soil N_2O production from the nitrification process because in the first step of nitrification, soil N_2O is produced during the oxidation of NH_2OH to nitrite (NO_2^-) via the enzyme hydroxylamine oxidoreductase (HAO) in addition to NO_2^- being oxidized to NO_3^- via nitrite reductase (NIR) and nitric oxide reductase (NOR) [25, 26].

In Fig. 2, after basal application, the F treatment had the highest soil N_2O flux after one month of fertilization in the unplanted and planted plots, while the S and the inhibitor treatments had significantly ($P < 0.05$) lower soil N_2O flux. The highest soil N_2O rate occurred in the F treatment (193.75 kg N ha^{-1}), perhaps due to the excess N input to the soil not being consumed by plants [27]. In this study, applying the right rate as in the S treatment (at 39.125 kg N ha^{-1}) may have potentially reduced soil N_2O emission and N losses from the soil [5, 28]. The NIs used (DMPP, neem and praxelis) in this study reduced the soil N_2O flux by suppressing or inhibiting the AMO enzyme that converts NH_4^+-N to NH_2OH in the first step of nitrification. The commercial NI (DMPP) seemed to have a lower soil N_2O flux than the BNIs (neem and praxelis) (but not consistently significant) in both planted and unplanted plots. The efficiency of NIs was more pronounced for about 14 DAF and slightly decreased after that. The results agreed with the concept that applying NIs could delay the NH_4^+-N oxidation process, which could help increase the opportunity for uptake of any available mineral N, clearly reducing soil N_2O emissions and decreasing NO_3^- leaching [29–31].

[Figure 2 about here]

After applying urea as the dressing, the F treatment had the highest soil N_2O flux, followed by S with and without the inhibitors applied in both planted and unplanted plots ($P < 0.05$), as shown in Fig. 2. The synthetic inhibitors (NBPT and DMPP) provided lower levels of soil N_2O flux than the inhibitors from plants (garlic, neem and praxelis) for about 30 DAF in both plots. In contrast the UIs (NBPT and garlic) produced lower levels of soil N_2O flux than the NIs (DMPP, neem and praxelis) at 7 DAF and then the levels were higher after that. The increase in soil N_2O fluxes at 7 DAF in the DM/NB and NE/GL treatments could be explained by the inhibitory effect of UIs by: slowing down soil urease activity in which urea is hydrolyzed to NH_4^+ as competitive inhibitors; and reacting with the sulfhydryl group of urease and have a complex form with one of the Ni atoms as an active site [7, 32].

The effects of the UIs and NIs used in the basal and dressing fertilizer applications strongly supported mitigation in the nitrification process due to the applied UIs and NIs with different fertilizer types (urea or ammonium) and expressed efficiency in different ways. Application of the NIs with ammonium fertilizer in the 1st fertilizer application showed the potential of the commercial NI (DMPP) was greater than for the BNIs (neem and praxelis) for a short period after application (3 DAF), but there was no difference in effects among these inhibitors after that perhaps because of the form of fertilizer used in the basal application, for which the NH_4^+ contents were better absorbed via the negative surface charge in the fine textures. There was clearly stability in the ammonium fertilizer and NIs used with no significant effect on retardation of NH_4^+ transformation in the soil; subsequently the efficiency of inhibitors used was not observed.

Nevertheless, we found the use of NIs with ammonium-base fertilizer could help retard soil N_2O emission more than using urea. The urea was unstable once applied to the soil, as the transformation process usually started immediately, which hydrolyzed to NH_3 within days and generated soil acidity through nitrification and a proportion of NH_3 was initially lost in the form of soil N_2O. Some studies reported the fertilizer type significantly affected soil N_2O emission, with ammonium-based types having the lowest soil N_2O emission compared to the rest [33]. However, climatic conditions, soil texture, timing of application and management practices probably caused different interactions among these factors in the different experiments.

The effects of the UIs and NIs on soil N_2O emission were evident in the 2nd fertilizer application due to the different performance levels according to the different pathways after applying urea in the field. The UIs was active during the urea hydrolysis process, while the NIs was effective during the nitrification process. After the long measurement period there was significantly higher soil N_2O emissions in the UI treatments than for the NIs and UINIs. This confirmed that the application of UIs helped to decrease urea hydrolysis and reduce NH_4^+ concentrations, which significantly slowed the release of NH_4^+ and improved N uptake by plants. However, the UIs were not effective in the nitrification process, resulting in greater potential for soil N_2O emission. However, adding NIs delayed the transformation of NH_4^+ through nitrification by suppressing nitrifiers that oxidize NH_3 to NH_2OH in the first step of the nitrification process and provide NH_4^+ available for plants before losses via soil N_2O from the next step of the nitrification process [34].

3.2 Cumulative soil N_2O emission

The cumulative soil N_2O emission throughout the cropping season (Table 2) showed the lower value (not consistently significant) in the S treatment with and without inhibitors than for the F treatment, with reductions of up to 21.33% in the unplanted plots and 19.08% in the sugarcane plots. In addition, all inhibitors produced lower (but not significant) cumulative soil N_2O emissions than the S treatment by up to 8.65 and 7.35% for unplanted and planted plots, respectively. However, the effect of the N rate with inhibitors was significant only in the unplanted plots ($P < 0.05$) perhaps because the unplanted plot had a higher emission level than all fertilizer plots due to the lack of plants resulting in less absorption of some mineral N from the soil, with the effectiveness of inhibitors being to minimize N losses from soil being more clearly observed than in the planted plot. This results agreed with McSwiney, Robertson [35], reported that increasing N fertilizer rate leaded to higher N_2O emissions with a nonlinear response. Plant presence on N addition area strongly affected N_2O emissions, which seriously reduced N_2O emissions compared to bare soil [36]. In this study, both synthetic and natural NIs achieved lower cumulative soil N_2O emission levels than the UIs and UINIs, except for GLNE in the unplanted plot (Table 2). Praxelis (PR/PR), DMPP (DM/DM) and the combination of garlic and neem (NE/GLNE) in the unplanted plots had significant lower levels of cumulative soil N_2O emission than the F treatment by 21.33, 20.70 and 20.50%, respectively. For the sugarcane plot, the neem (NE/NE) and praxelis (PR/PR) had the highest inhibitory effect (but not significant) by 19.08 and 18.85%, respectively, compared to the F treatment.

Table 2
Cumulative N_2O emissions following treatment using N fertilizer with UIs and NIs in field experiment

Treatment	Unplanted plot			Sugarcane plot		
	Cumulative N_2O (kg/ha)	% Reduction		Cumulative N_2O (kg/ha)	% Reduction	
		Compared to F	Compared to S		Compared to F	Compared to S
C	2.63 ± 0.38 c*			3.10 ± 0.53 a		
F	4.83 ± 0.35 a			4.35 ± 0.51 a		
S	4.16 ± 0.30 ab	13.9		3.81 ± 0.78 a	12.4	
DM/NB	4.12 ± 0.26 ab	14.7	1.0	3.69 ± 0.30 a	15.2	3.2
DM/DM	3.83 ± 0.25 b	20.7	7.9	3.59 ± 0.35 a	17.5	5.8
DM/NEDM	4.12 ± 0.80 ab	14.7	1.0	3.74 ± 0.23 a	14.0	1.8
NE/GL	4.05 ± 0.37 ab	16.2	2.6	3.66 ± 0.47 a	15.9	3.9
NE/NE	4.01 ± 0.11 ab	17.0	3.7	3.52 ± 0.33 a	19.1	7.6
NE/GLNE	3.84 ± 0.56 b	20.5	7.7	3.61 ± 0.69 a	17.0	5.2
PR/PR	3.80 ± 0.56 b	21.3	8.6	3.53 ± 0.27 a	18.8	7.4
F-test	0.0018			NS		

Note: * Means followed by the same lowercase letter in the same column are not *significantly different* according to Duncan's multiple range test at $P < 0.05$. NS = not significant.

[Table 2 about here]

To gain better understanding of the effect of the N rate and inhibitors applied, we focused on cumulative soil N_2O emission during 30 DAF for each treatment in this study (Fig. 3). With both basal and dressing fertilizer applications, the cumulative soil N_2O emissions at 30 DAF clearly revealed the potential of reducing the N rate (S treatment) with and without the inhibitors to mitigate soil N_2O emission compared to the F treatment ($P < 0.05$). At the N rate for the S treatment, the application of UIs and NIs had a significant inhibitory effect on cumulative soil N_2O emission during 30 DAF ($P < 0.05$), with more emphasis in the dressing fertilization and in the unplanted plot. However, there were no significant differences among the inhibitor treatments.

After dressing fertilization in unplanted plot, the DM/NB, NE/GLNE and PR/PR treatments had significantly lower levels of cumulative soil N_2O emission at 30 DAF ($P < 0.0001$) than the S treatment, which was similar to the observation for the cumulative soil N_2O emission. With the dressing fertilization in the planted plot, the inhibitory effect of UIs and NIs was only significantly lower ($P < 0.0001$) for the levels of cumulative soil N_2O emission in the DM/NB (up to 30 DAF) and the DM/DM (up to 21 DAF) than for the S treatment.

Notably, in this study, the dressing application with urea produced a greater level of 30-day cumulative soil N_2O emission than the basal application with ammonium sulfate for both planted and unplanted plots (Fig. 3). This was supported by [33] who reported that ammonium-based fertilizers had the lowest levels of soil N_2O emission. Based on this observation, the inhibitory effect of NIs on cumulative soil N_2O emission was brief (3–7 DAF) for the unplanted plot when the ammonium fertilizer (basal fertilization) was applied in this study (Fig. 3). However, this inhibitory effect was not significant in the planted plot (Table 2).

The findings regarding the levels of cumulative soil N_2O emission during 30 DAF and for the whole cropping season in this study suggested that UIs and NIs were effective in reducing soil N_2O emissions, even with the very small amount of N fertilizer used in this study (39.125 kg N ha^{-1}). However, the inhibitory effect only significantly reduced soil N_2O emissions for about one month after the fertilizer was applied. This may suggest that split application of inhibitors may enhance soil N_2O mitigation from applying N fertilizer. Notably, this study revealed that praxelis seems to be a good candidate for mitigating soil N_2O emission levels for both a short period (one month) after fertilizer application or throughout the cropping season.

[Figure 3 about here]

3.3 Soil N_2O EF

Table 3 shows the soil N_2O EF in this study and other sugarcane fields and croplands in the tropics. In the sugarcane plot in this study, the soil N_2O EF values were in the range 0.65–1.82%. The S treatment had the highest value (1.82%) followed by the treatment with only-N fertilizer, while all inhibitors achieved lower EF values in the range 1.07–1.62%. The NI applications (DM/DM, NE/NE and PR/PR) had lower EF values than for the UIs applications. Interestingly, the soil N_2O EF was not the highest (0.65%) when the highest N rate was applied (F treatment), In contrast, the S treatment with and without inhibitors seemed to produce a greater value.

Table 3
N_2O emission factors (EF) of unplanted plot and sugarcane plot

Country	Crop	Details	N form	N fertilizer rate (kg N ha^{-1})	inhibitor	EF (%)		Reference
						Planted	Unplanted	
Thailand	Sugarcane	F	AS + urea	193.75	-	0.65	1.14	This study
		S	AS + urea	39.12	-	1.82	3.92	
		DM/NB	AS + urea	39.12	NBPT + DMPP	1.49	3.80	
		DM/DM	AS + urea	39.12	DMPP	1.25	3.07	
		DM/NBDM	AS + urea	39.12	NBPT + DMPP	1.62	3.80	
		NE/GL	AS + urea	39.12	Garlic + neem	1.43	3.63	
		NE/NE	AS + urea	39.12	Neem	1.07	3.53	
		NE/GLNE	AS + urea	39.12	Garlic + neem	1.31	3.10	
		PR/PR	AS + urea	39.12	Praxelis	1.08	3.01	
Brazil	Sugarcane	0 crop residued*	AS	120	-	0.12		Gonzaga, Carvalho, Oliveira, Soares, Cantarella [39]
		5 crop residued	AS	120	-	0.52		
		10 crop residued	AS	120	-	1.44		
		15 crop residued	AS	120	-	0.91		
		0 crop residued + NI	AS	120	DCD	0.00		
		5 crop residued + NI	AS	120	DCD	0.25		
		10 crop residued + NI	AS	120	DCD	0.69		
		15 crop residued + NI	AS	120	DCD	0.49		

Global	Sugarcane	Meta-analysis	114		1.21	Yang, Deng, Wang, Zhang, Shi, Chen, Lakshmanan, Zhang [38]
Tropics		Meta-analysis	115		1.20	
Sub-tropics		Meta-analysis	108		1.24	
Global		Synthetic fertilizer (SN)	118		1.09	
Global		Organic amendment (OA)	48.7		1.19	
Global		SN + OA	153		2.47	
Global		EEF	116	DCD, DMPP and polymer-coated urea	0.419	

Note: * Sugarcane crop residues rates (Mg ha^{-1}) on dry basis incorporated with N fertilizer. EEF is enhanced efficiency fertilizer by adding e.g. DCD, DMPP and polymer-coated urea.

The large variation in soil N_2O EFs in Table 3 were primarily due to variations in the climate, soil fertility and practice management, especially the N input. The soil N_2O EFs confirmed that excessive N application potentially affects soil microorganisms that contribute to direct soil N_2O emissions. The soil texture also had a strong impact on soil N_2O emissions, the Organic matter is an available source for microorganisms that could affect the emission of soil N_2O. But NH_4^+ availability for nitrification could limited due to higher cation exchange capacity from fine textured [37] in a result of variation in soil N_2O EFs found in this study. However, even the F treatment clearly showed that the highest soil N_2O rate where the N input exceeded the crop's needs had the lowest EF. This was possibly due to calculating the EF by dividing the total N fertilizer at the highly applied rate. A proportion of N might have been lost into the environment through NH_3 volatilization or NO_3^- leaching into the groundwater and increasing the N rate, which was the lowest for soil N_2O EF in the S treatment.

[Table 3 about here]

Both the only-fertilizer and inhibitor treatments produced soil N_2O EF values rarely higher than the mean global values for tropical and sub-tropical soil of N_2O EF from sugarcane of 1.21, 1.20 and 1.24%, respectively [38] and the EF from a sugarcane field in Brazil was reportedly in the range 0.00-0.44% [39], as shown in Table 3. According to the IPCC defaults, soil N_2O EF of synthetic N fertilizers are 1% (0.1–1.8%) for all regions and 1.6% (1.3–1.9%) for wet climates [40]. In this study, the soil N_2O EF value for the S (without inhibitors) treatment seemed to be in the upper level of this EF range, possibly because a much lower rate of N fertilizer was applied (39.12 kg N ha^{-1}) and practice management. After the inhibitors had been applied, most of the soil N_2O EFs were lower than or close to the IPCC default for wet climates. These observations suggested that the inhibitors used in this study could effectively retard soil N_2O emissions in sugarcane fields in Thailand. These finding on EFs could be used as supportive data for the Thailand national GHG inventory in the future.

In this study, soil N_2O EFs in the unplanted plots were provided as a database for further meta-analysis (Table 3). As mentioned earlier, more N fertilizer was available in the unplanted plots, so the soil N_2O EFs were higher (1.14–3.92%) than for the planted plots in this study.

3.4 Relationships of N factors and soil N_2O emissions

Figure 4 showed the positive relationships of soil N_2O emissions and N fertilizer rate. The soil N_2O emissions from planted and unplanted plots revealed that adding more N fertilizer could drive higher N_2O emissions. This effect of N fertilizer rate was more pronounced in the unplanted plot than the planted plot. As was explained earlier, the planted plots had lower levels of soil N_2O emissions with the N rate applied compared to the unplanted plots. The N fertilizer rate added in the soil contributed to soil N_2O emissions due to the availability of inorganic N sources in the soil having a positive effect on soil microbial activity. For aerobic conditions, N in the form of NH_4^+-N is oxidized into NO_3^--N and then both of the mineral N forms are subsequently taken up by plants or microbes. This result clearly suggests that plants have a major impact on soil N_2O emissions, due to the uptake of N on their part, so subsequently, the soil N_2O emissions decreased.

[Figure 4 about here]

We investigated the relationship between soil N_2O emission and soil N to determine which soil N form would be a good driver of N_2O emission that could be adopted into the relevant simulation model of nutrient biogeochemistry. Figures 5 and 6 showed the positive relationships of soil N_2O emissions and the various fates of N in the soil (total N, NH_4^+ and NO_3^-) in topsoil and subsoil from planted and unplanted plots. The soil N_2O emissions from both plots revealed that adding more N fertilizer increased soil N and subsequently induced higher soil N_2O emissions, as shown in the steeper slope in F compared to S. Application of UIs and NIs also gave a positive response of soil N_2O flux with soil N, but seemed to have flatter slope compared to F and S. These effects of N fertilizer rate and UIs and NIs were more pronounced in the unplanted plot than the planted plot. The unplanted plots showed a more positive response of soil N_2O emissions to total N and mineral N in the soil than the planted plots. N_2O emissions from unplanted plots, which occurs under aerobic conditions the through nitrification process, was related to the total N, NH_4^+-N and NO_3^--N contents in both the topsoil and subsoil. However, when planted with a crop, the correlation between soil N_2O emission and N concentration in the soil was less pronounced.

[Figure 5 about here]

The different relationships between N in the soil and N emissions from different land uses implied that plants had a primary effect on N_2O emissions. Some of the NH_4^+-N in the soil was taken up by plants before it could undergo nitrification and loss via N_2O emissions to the atmosphere [41]. On the other hand, the results showed that NO_3^--N in the soil had the same pattern of a weak correlation with N_2O emissions in both unplanted and planted plots. Even higher NO_3^--N remaining in the soil did not change the pattern of N_2O production, indicating that the NO_3^--N content was not the key to N_2O emission from the nitrification process [42].

[Figure 6 about here]

4 Conclusions

The outcomes from this study supported the application of N fertilizer use to agricultural field crops having a powerful effect on soil N_2O emissions, due to the positive correlation with mineral N in the form of NH_4^+-N in the soil. The use of an optimum N rate based on soil and leaf analysis could be an alternative option to mitigate GHG emissions, especially soil N_2O emission. Furthermore, applying UIs, NIs and UINIs along with an appropriate N application could greatly improve %N_2O emission reductions, when applied together with urea. These UI, NIs and UINIs play a role in retarding urea hydrolysis and the nitrification pathway as a result of the decrease in N_2O EF values. This study showed that the N contents in the soil had an important effect on the N_2O emissions and effects of N fertilizer rate and inhibitors were also observed on soil N availability and subsequently soil N_2O emissions. Furthermore, the application of UIs and NIs could be an additional option for farmers to help delaying transformation of N in the soil, providing the available form of N to plants, improving crop production and subsequently, mitigating N_2O emission. Notably, applying natural NIs, such as praxelis, seemed to have potential in delaying N transformation, resulting in lower N_2O emission that was closely correlated to other inhibitors used in this study that should be considered in more detail regarding chemical compounds that affect microbial activity in the nitrification process.

Declarations

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Phatchariya Welutung, Patthra Pengthamkeerati and Boonlue Kachenchart. The first draft of the manuscript was written by Phatchariya Welutung and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

1. Dheri GS, Lal R, Verma S. Effects of nitrogen fertilizers on soil air concentration of N_2O and corn growth in a greenhouse study. J Crop Improv 2015;29(1):95-105.
2. Anas M, Liao F, Verma KK, Sarwar MA, Mahmood A, Chen ZL, et al. Fate of nitrogen in agriculture and environment: agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. Biol Res 2020;53(1):47.
3. USDA. Sugar: world markets and trade, https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads; 2022.
4. Smith P, Bustamante M, Ahammad H, Clark H, Dong H, Elsiddig EA, et al. Agriculture, Forestry and Other Land Use (AFOLU). *Climate Change 2014 Mitigation of Climate Change. Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press; 2014:811–922.
5. Johnston AM, Bruulsema TW. 4R nutrient stewardship for improved nutrient use efficiency. Procedia Eng 2014;83:365-70.
6. Drury CF, Yang X, Reynolds WD, Calder W, Oloya TO, Woodley AL. Combining urease and nitrification inhibitors with incorporation reduces ammonia and nitrous oxide emissions and increases corn yields. J Environ Qual 2017;46(5):939-49.
7. Folina A, Tataridas A, Mavroeidis A, Kousta A, Katsenios N, Efthimiadou A, et al. Evaluation of various nitrogen indices in N-fertilizers with inhibitors in field crops: A review. Agron 2021;11(3).
8. Stein LY, Arp DJ. Loss of ammonia monooxygenase activity in nitrosomonas europaea upon exposure to nitrite. Appl Environ Microbiol 1998;64(10):4098-102.

9. Zhao Z, Wu D, Bol R, Shi Y, Guo Y, Meng F, et al. Nitrification inhibitor's effect on mitigating N_2O emissions was weakened by urease inhibitor in calcareous soils. Atmos Environ 2017;166:142-50.
10. Gioacchini P, Nastri A, Marzadori C, Giovannini C, Vittori Antisari L, Gessa C. Influence of urease and nitrification inhibitors on N losses from soils fertilized with urea. Biol Fertil Soils 2002;36(2):129-35.
11. Cantarella H, Otto R, Soares JR, Silva AGB. Agronomic efficiency of NBPT as a urease inhibitor: A review. J Adv Res 2018;13:19-27.
12. Arora K, Srivastava A. Nitrogen losses due to nitrification: plant based remedial prospects. Int J of Bioassays 2013;2:984-91.
13. Mathialagan R, Mansor N, Al-Khateeb B, Mohamad MH, Shamsuddin MR. Evaluation of allicin as soil urease inhibitor. Procedia Eng 2017;184:449-59.
14. Modolo LV, de Souza AX, Horta LP, Araujo DP, de Fatima A. An overview on the potential of natural products as ureases inhibitors: A review. J Adv Res 2015;6(1):35-44.
15. Pengthamkeerati P, Modtad A. Nitrification inhibitor, fertilizer rate, and temperature effects on nitrous oxide emission and nitrogen transformation in loamy sand soil. Commun Soil Sci Plant Anal 2016;47:1-8.
16. Kiran U, Patra DD. Medicinal and aromatic plant materials as nitrification inhibitors for augmenting yield and nitrogen uptake of Japanese mint (*Mentha arvensis* L. Var. Piperascens). Bioresour Technol 2003;86(3):267-76.
17. Sahrawat K, Parmar D, Mukerjees S. Note on the nitrification inhibitor in the seeds, bark and leaves of Pongamia glabra Vent. Indian J Agric Sci 1974;44:415-8.
18. Thepphakhun T, Intanon S. Total phenolics, flavonoids, antioxidant activity, and allelopathic potential of praxelis. Journal of Current Science and Technology 2020;10:59-65.
19. Yang JN, Zhou XQ, Nong XH, Cao J, Hui Y, Wen M, et al. Phytochemical investigation of the flowers of *Praxelis clematidea* (Griseb.) R.M. King & H. Rob. Nat Prod Res 2020;35:3504–8.
20. Nguyen CC, Nguyen TQC, Kanaori K, Binh TD, Vang LV, Kamei K. Isolation and identification of antibacterial and antifungal compounds from *Praxelis clematidea* R. M. King & H. Robinson as an effective potential treatment against rice pathogens. Agron 2021;11(11):2366-76.
21. Maneepitak S, Ullah H, Datta A, Shrestha RP, Shrestha S, Kachenchart B. Effects of water and rice straw management practices on water savings and greenhouse gas emissions from a double-rice paddy field in the Central Plain of Thailand. Eur J Agron 2019;107:18-29.
22. Singh J, Kunhikrishnan A, Bolan NS, Saggar S. Impact of urease inhibitor on ammonia and nitrous oxide emissions from temperate pasture soil cores receiving urea fertilizer and cattle urine. Sci Total Environ 2013;465:56-63.
23. Cai Y, Ding W, Luo J. Nitrous oxide emissions from Chinese maize–wheat rotation systems: A 3-year field measurement. Atmos Environ 2013;65:112-22.
24. Dong D, Kou Y, Yang W, Chen G, Xu H. Effects of urease and nitrification inhibitors on nitrous oxide emissions and nitrifying/denitrifying microbial communities in a rainfed maize soil: A 6-year field observation. Soil Tillage Res 2018;180:82-90.
25. Degaspari IAM, Soares JR, Montezano ZF, Del Grosso SJ, Vitti AC, Rossetto R, et al. Nitrogen sources and application rates affect emissions of N_2O and NH_3 in sugarcane. Nutr Cycling Agroecosyst 2020;116(3):329-44.
26. Norton J, Ouyang Y. Controls and adaptive management of nitrification in agricultural soils. Front Microbiol 2019;10:1-18.
27. Snyder CS, Bruulsema TW, Jensen TL, Fixen PE. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agric Ecosyst Environ 2009;133(3-4):247-66.
28. Fixen PE. A brief account of the genesis of 4R nutrient stewardship. Agronomy Journal 2020;112(5):4511-8.
29. Wang H, Köbke S, Dittert K. Use of urease and nitrification inhibitors to reduce gaseous nitrogen emissions from fertilizers containing ammonium nitrate and urea. Glob Ecol Conserv 2020;22:e00933.

30. Weiske A, Benckiser G, Herbert T, Ottow J. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biol Fertil Soils 2001;34(2):109-17.
31. Hooper AB, Vannelli T, Bergmann DJ, Arciero DM. Enzymology of the oxidation of ammonia to nitrite by bacteria. Antonie Van Leeuwenhoek 1997;71(1-2):59-67.
32. Zaman M, Zaman S, Adhinarayanan C, Nguyen ML, Nawaz S, Dawar KM. Effects of urease and nitrification inhibitors on the efficient use of urea for pastoral systems. Soil Sci Plant Nutr 2013;59(4):649-59.
33. Bouwman AF, Boumans LJM, Batjes NH. Emissions of N_2O and NO from fertilized fields: Summary of available measurement data. Glob Biogeochem Cycles 2002;16(4):6-1-6-13.
34. Signor D, Cerri CE. Nitrous oxide emissions in agricultural soils: A review. Pesqui Agropecu Trop 2013;43:322-38.
35. McSwiney CP, Robertson GP. Nonlinear response of N_2O flux to incremental fertilizer addition in a continuous maize (*Zea mays* L.) cropping system. Glob Change Biol 2005;11(10):1712-9.
36. Bowatte S, Hoogendoorn CJ, Newton PCD, Liu Y, Brock SC, Theobald PW. Grassland plant species and cultivar effects on nitrous oxide emissions after urine application. Geoderma 2018;323:74-82.
37. Jarecki MK, Parkin TB, Chan ASK, Hatfield JL, Jones R. Greenhouse gas emissions from two soils receiving nitrogen fertilizer and swine manure slurry. J Environ Qual 2008;37(4):1432-8.
38. Yang L, Deng Y, Wang X, Zhang W, Shi X, Chen X, et al. Global direct nitrous oxide emissions from the bioenergy crop sugarcane (*Saccharum* spp. inter-specific hybrids). Sci Total Environ 2021;752:141795.
39. Gonzaga LC, Carvalho JLN, Oliveira BGd, Soares JR, Cantarella H. Crop residue removal and nitrification inhibitor application as strategies to mitigate N_2O emissions in sugarcane fields. Biomass and Bioenergy 2018;119:206-16.
40. IPCC. 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. In: Calvo Buendia E, Tanabe, K., Kranjc, A., Baasansuren, J., Fukuda, M., Ngarize S., Osako, A., Pyrozhenko, Y.,, Shermanau PaF, S., eds. Switzerland; 2019.
41. Laungani R, Knops JMH. Microbial immobilization drives nitrogen cycling differences among plant species. Oikos 2012;121(11):1840-8.
42. Liu S, Wang JJ, Tian Z, Wang X, Harrison S. Ammonia and greenhouse gas emissions from a subtropical wheat field under different nitrogen fertilization strategies. J Environ Sci (China) 2017;57:196-210.

Figures

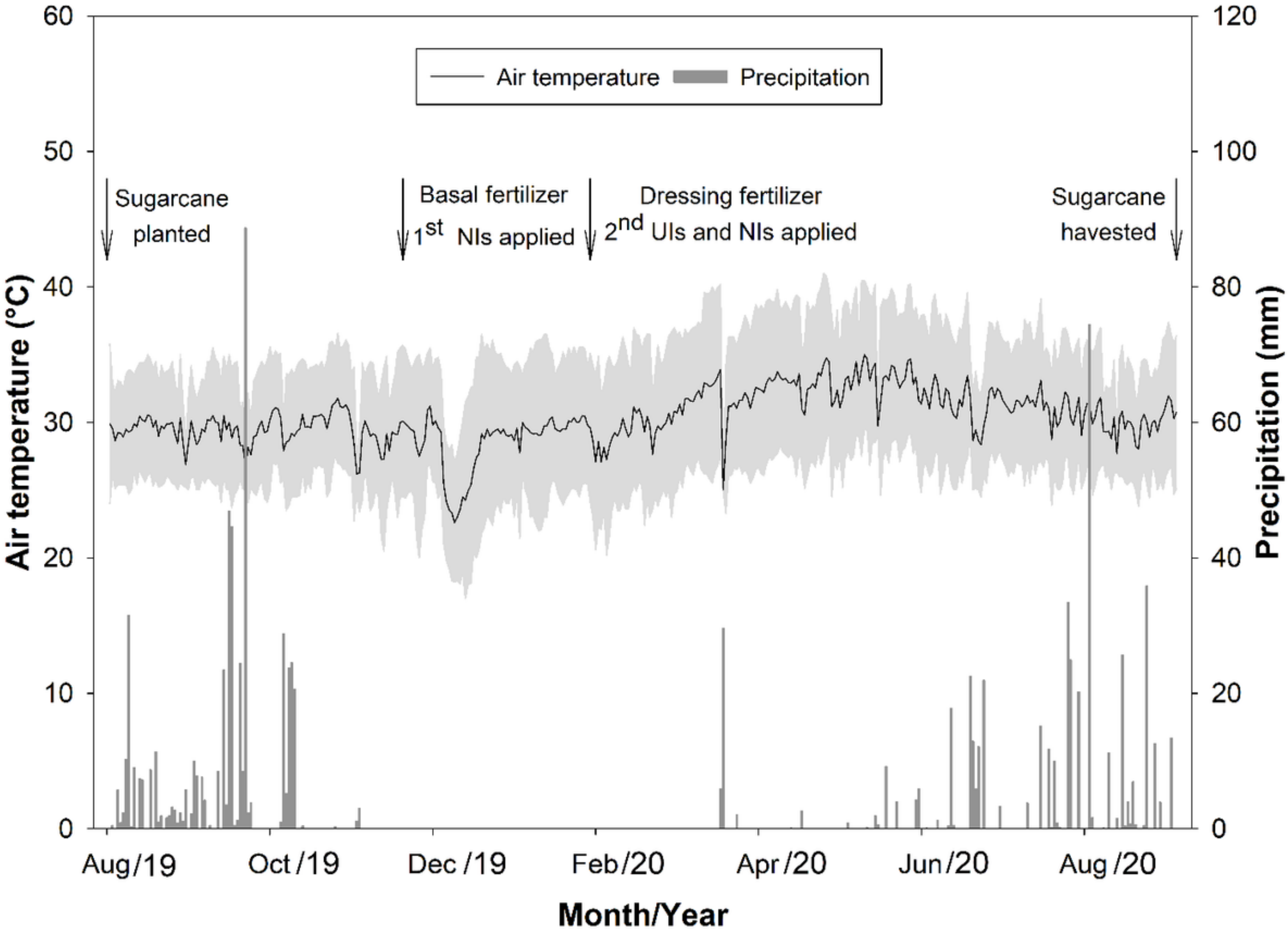

Figure 1

Daily air temperature (line) and precipitation (bars) during cropping season at Lopburi Research Station, Faculty of Agriculture, Kasetsart University, Khok Charoen district, Lopburi province, Thailand. Arrows indicate times of planting, fertilizer and inhibitors applications and harvesting events for cropping season.

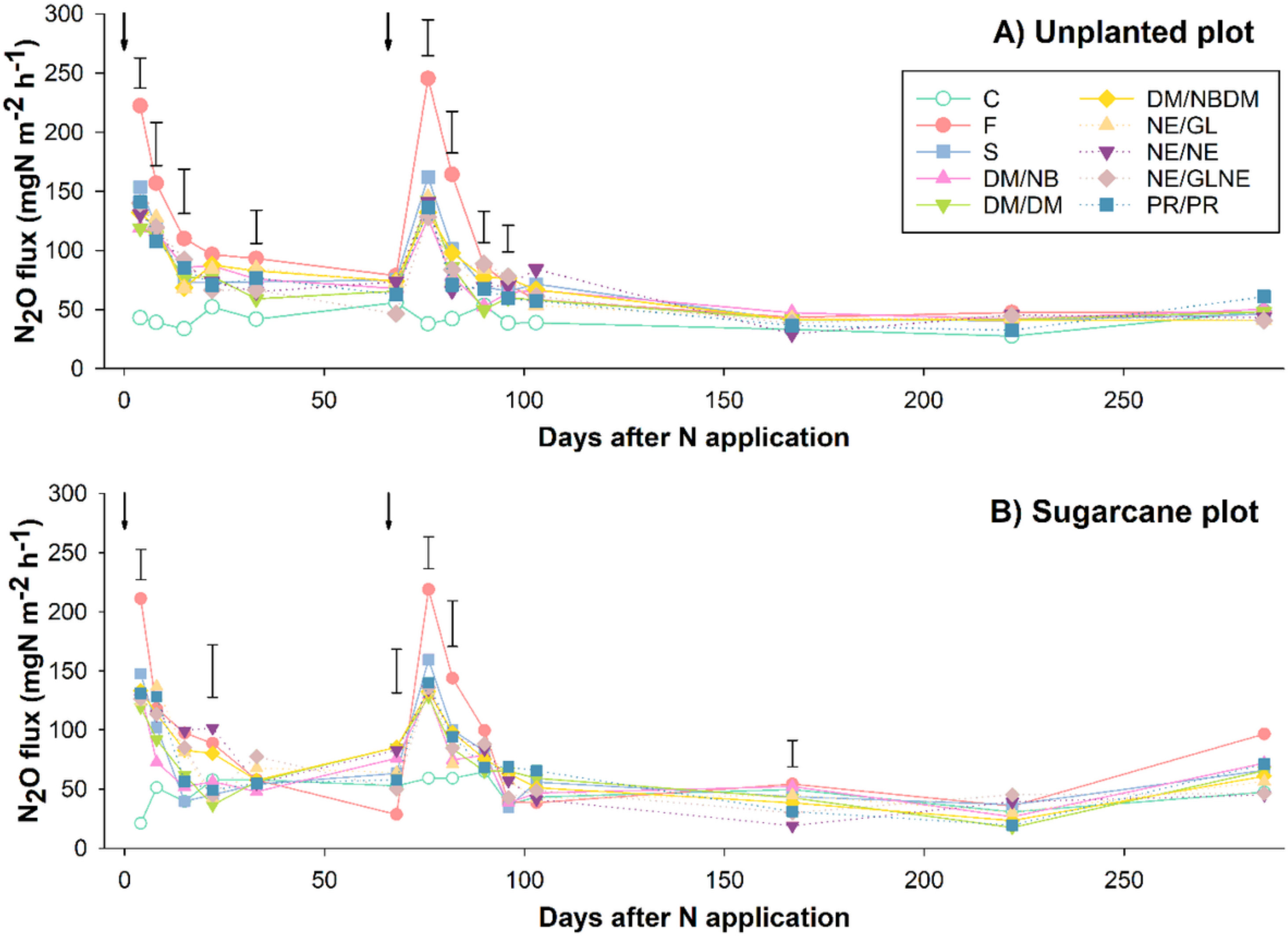

Figure 2

Soil N_2O fluxes on treatment of N fertilizer with UIs and NIs compared to treatment with only N fertilizer in field experiment. Bars indicate significant differences (P<0.05) according to Duncan's multiple range test. Line arrows indicated timing of fertilizer application.

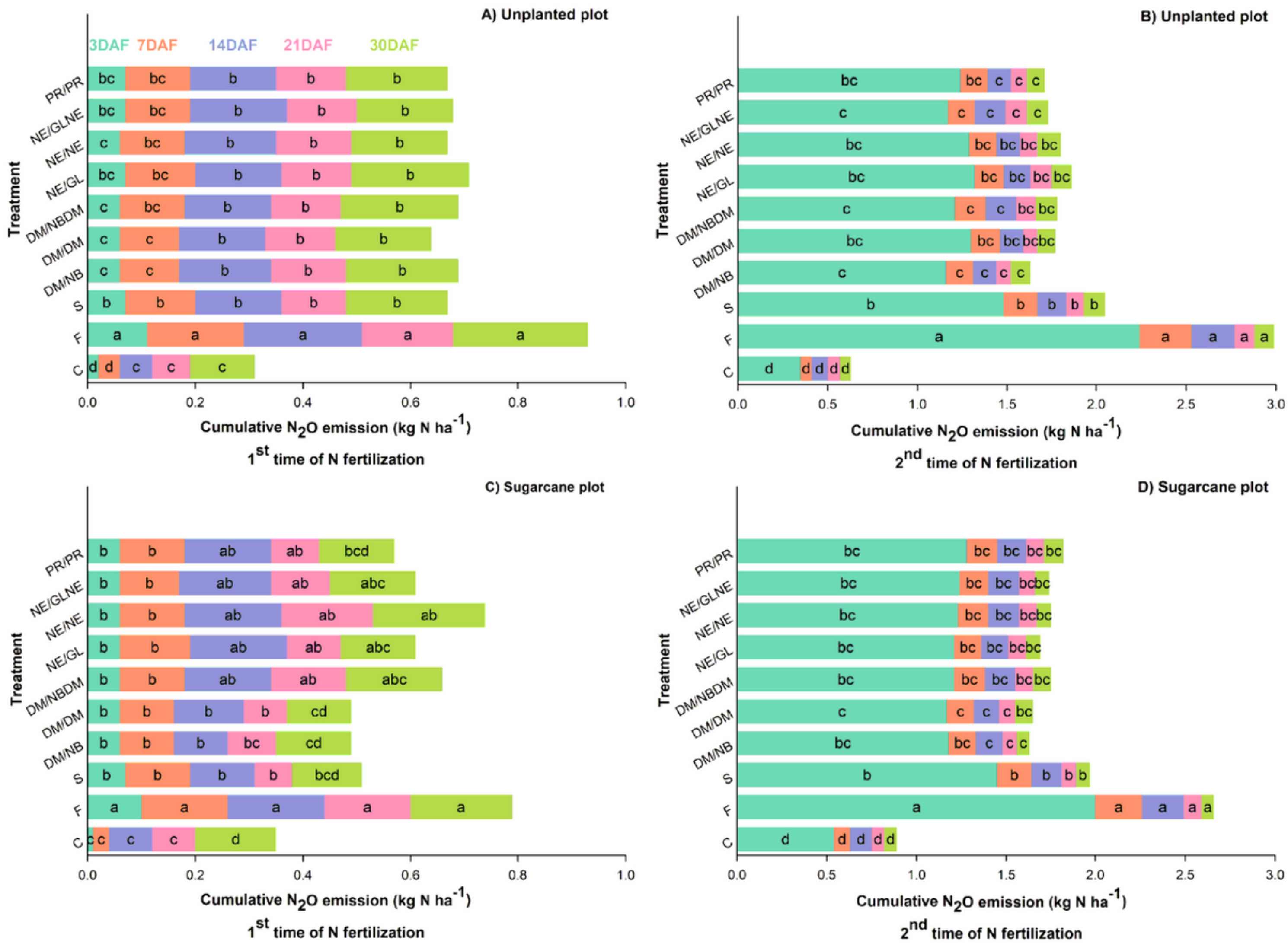

Figure 3

Cumulative of N_2O emissions on treatment of N fertilizer with UIs and NIs compared to treatment with only N fertilizer in field experiment on (A) 1st time, (B) 2nd time of N fertilization on sugarcane plot and (C) 1st time, (D) 2nd time of N fertilization on unplanted plot. %Reduction of cumulative N_2O emission calculated at 30 DAF compared to F treatment.

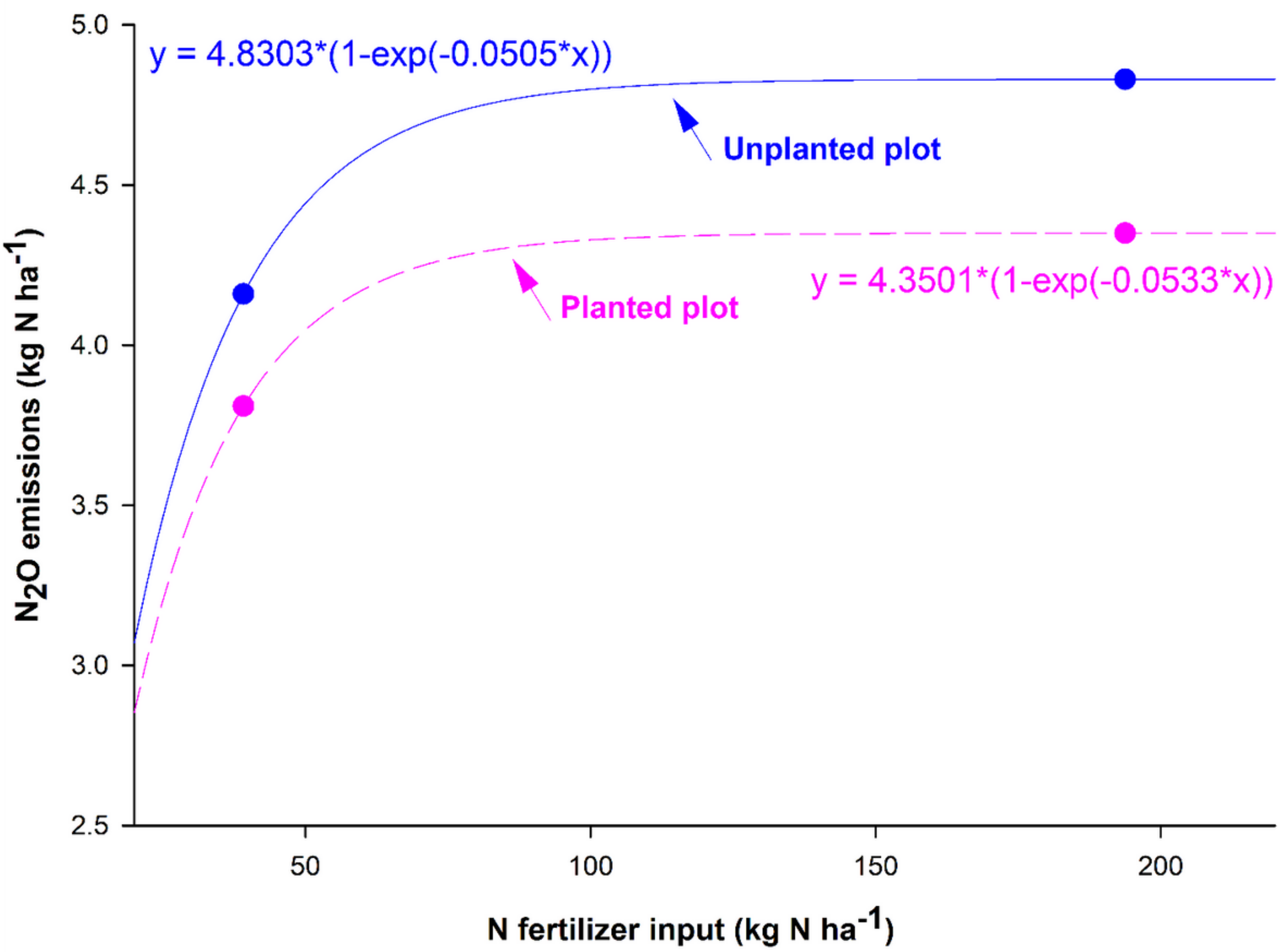

Figure 4

Relationship between N fertilizer input and N_2O emissions from planted plot and unplanted plot.

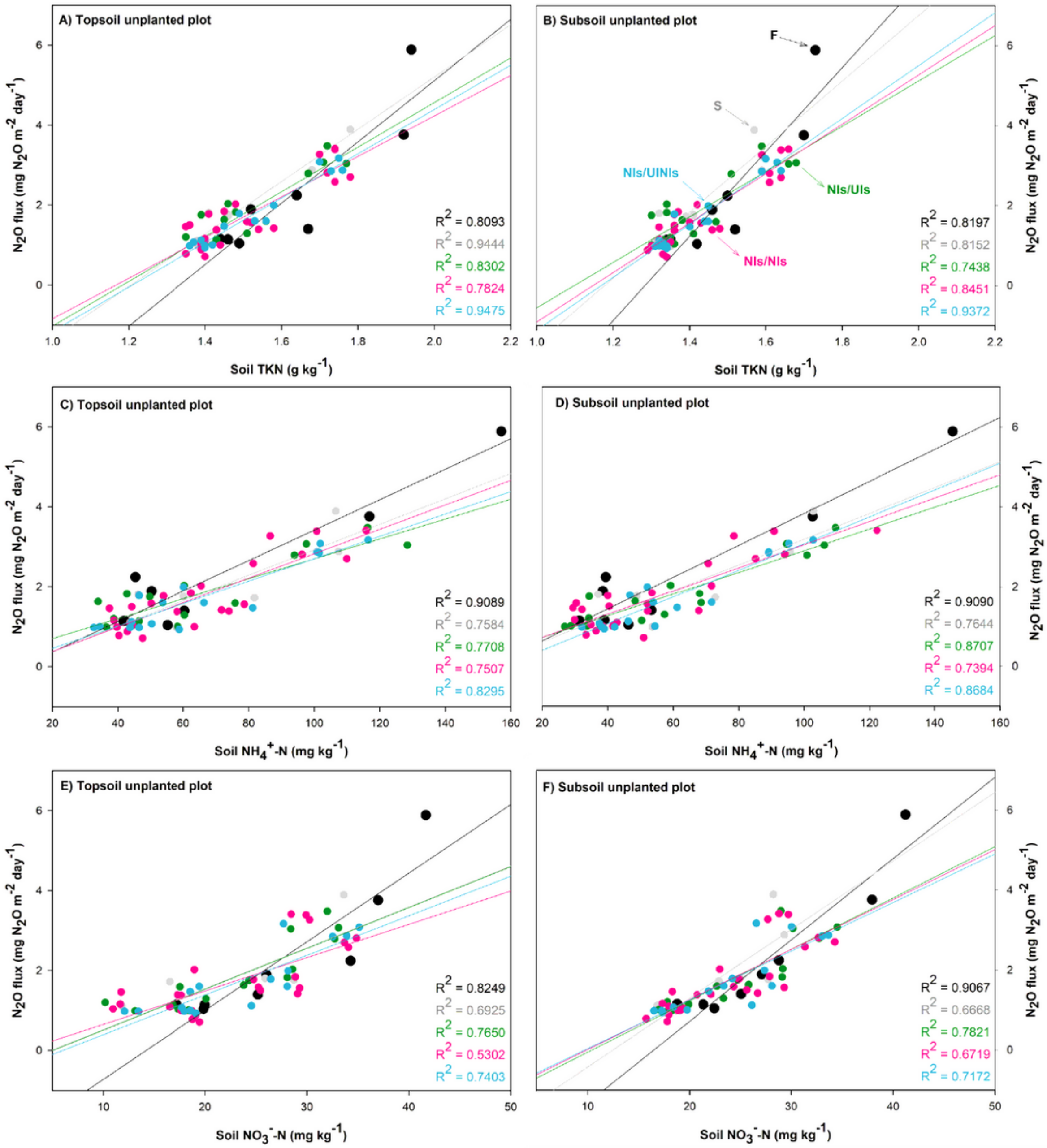

Figure 5

Relationship between various soil N and N_2O fluxes on treatment of N fertilizer with and without UIs and NIs in field experiment in topsoil and subsoil of unplanted plot.

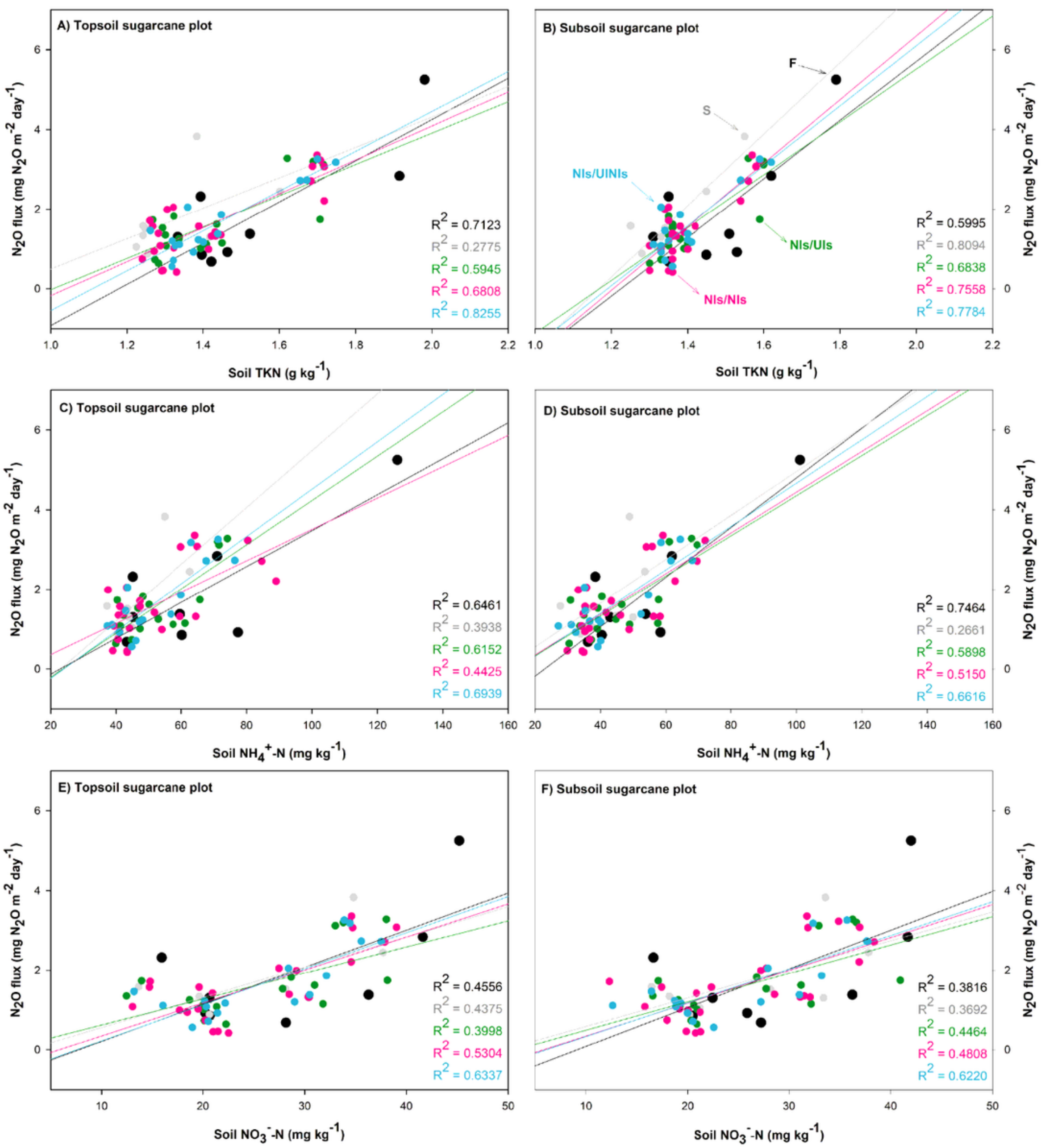

Figure 6

Relationship between various soil N and N_2O fluxes on treatment of N fertilizer with and without UIs and NIs in field experiment in topsoil and subsoil of sugarcane plot.