Nitrous Oxide Emissions from a Sugarcane Field with Green Manure and Chemical Fertilizer Applications in Okinawa, Japan

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Abstract

The objective of this study was to evaluate N_2O emissions during the first three months after different nitrogen (N) source treatments involving green manure and chemical fertilizer in a sugarcane field in Okinawa, Japan. Four treatment plots were established: 100 and 300 kg N ha^{-1} chemical fertilizer (100N and 300N plots, respectively); green manure (*Crotalaria juncea*, 150 kg N ha^{-1}, C:N=20; GM plot); and the control (0N plot). We regularly measured N_2O flux and soil inorganic N (NH_4^+-N and NO_3^--N), and also recorded environmental data. There were no significant differences in the fluctuation pattern and peaks of N_2O emissions between the 100N and GM plots, and total N_2O emissions were comparable, though the applied N amount was higher in the GM plot. Little rainfall in the early period should limit microbial activity in the 100N plot, though soil NH_4^+-N and NO_3^--N contents were higher in the 100N plot than in the GM plot. Total N_2O emissions in the 300N plot were 1.5-times higher than in the 100N and GM plots. Collectively, the results suggest that the amount of applied N more strongly affected N_2O emission than the difference in N substrate quality.

Discipline: Agricultural Environment
Additional key words: nitrous oxide, green manure, chemical fertilizer, sugarcane

Introduction

Global warming is a serious problem for human well-being. Nitrous oxide (N_2O) is an important greenhouse gas that contributes to global climate change. The concentration of atmospheric N_2O has increased at a rate of 0.2%-0.3% per year since 1750 (WMO Greenhouse Gas Bulletin 2016, IPCC 2006). Agricultural activities are the main source of N_2O (Mosier & Kroeze 2000). Given that the largest N_2O sources are from terrestrial landscapes at subtropical latitudes (Stehfest & Bouwman 2006), it is necessary to evaluate N_2O emissions at subtropical latitudes. The emission of N_2O from soil is generally a result of denitrification and nitrification processes (Bremner 1997, Stevens & Laughlin 1998). The application of such materials as chemical fertilizers or organic matter containing nitrogen (N) to cropland soil

stimulates N_2O emission (Davidson 1991, Conrad 1996). The predominant controlling factors for N_2O emission are the quantity and quality of the N substrate (Velthof et al. 2002, Van Groenigen et al. 2004), such as the form of applied N (i.e., various mineral N fertilizers, slurry, others) or the carbon-to-nitrogen (C:N) ratio of organic residue. In addition, soil environmental factors (e.g., soil temperature, soil moisture content) also affect N_2O emission from soil through their influence on microbial activity (Conrad 1996, Bouwman 1998).

In Japan's southernmost prefecture of Okinawa, sugarcane (*Saccharum* L.) fields constitute approximately one-third of all croplands, thus making sugarcane an important commercial crop in the area (Ministry of Agriculture, Forestry and Fisheries of Japan 2016), especially on outlying islands such as Kita-Daito Island (Yoshida et al. 2016, Yoshida et al. 2017). To improve

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sugarcane productivity, substantial quantities of chemical N fertilizer are widely applied (generally 200-300 kg N ha⁻¹); consequently, high N_2O emissions from sugarcane cropland in Okinawa have been reported (Bellido et al. 2015). Previous research conducted on sugarcane fields in Brazil and Australia has shown that 1.0%-6.7% (Allen et al. 2010), 1.1 ± 0.8% (Carmo et al. 2013), 0.5 ± 0.3% (Neto et al. 2016), and 0.7%-0.8% (Soares et al. 2015) of input N (chemical fertilizer) are emitted as N_2O, and most N_2O flushes were mainly observed during the initial 3-6 month cropping period after fertilization. In addition, the peaks of N_2O emissions from agricultural fields were generally observed within 1-2 weeks after chemical fertilizer application (Liu et al. 2005, Schils et al. 2008). Our study area of Okinawa is typically affected by several typhoons during the sugarcane planting season, resulting in heavy rainfall within a few days (Sakihama et al. 2008) and substantial fluctuation in soil moisture content. Generally, N_2O emissions strongly increased immediately after precipitation (Breuer et al. 2000); therefore, it is necessary to monitor and evaluate N_2O emissions in the sugarcane fields of Okinawa, especially for the early sugarcane cultivation period (e.g., 1-3 months after planting and N fertilization treatment) and the typhoon season.

Given the critical depletion of soil organic matter in the Okinawa area, the incorporation of green manure (GM) in sugarcane cropland to improve and maintain soil fertility has recently been recommended (Miyamaru et al. 2008), although not widely practiced in most of the Okinawa Islands. The incorporation of GM may also reduce total N_2O emissions during sugarcane cultivation by replacing a portion of chemical N fertilizer as an alternative N source, because the N supply from GM decomposition is slower than that from chemical N fertilizer. In fact, the incorporation of GM reduced total N_2O emissions by 10%-50% compared with those following chemical fertilizer application in the sugarcane cropland of Brazil (Bordonal et al. 2012) and Australia (Cajas et al. 2016). Moreover, the incorporation of GM in the absence of fertilizer decreases N_2O emissions compared with those after the application of chemical N fertilizer, such as in a maize cropland in southeastern England (Sarkodie-Addo et al. 2003). However, the relationship between N_2O emissions and GM application

in Okinawa's sub-humid tropical area remains unclear. Thus, the objective of the present study was to evaluate and compare N_2O emissions and soil N condition during the early 3-month cultivation period, in response to GM and chemical fertilizer application in a sugarcane cropland of Okinawa. We hypothesized that organic N application can decrease total N_2O emissions as compared with inorganic N application. In this study, we used *Crotalaria juncea* as GM, due to its increasing popularity in this area, high plant biomass, and easy cultivation (Mambu et al. 2018).

Materials and methods

1. Description of the study site

We conducted a field experiment from August to November 2017 (90 days in total) in a sugarcane field on Kita-Daito Island, Okinawa, Japan (25°56′N, 131°45′E). Based on data covering a recent 10-year period (2008-2017, Japan Meteorological Agency), the mean annual air temperature of the study area is 23.8°C and the mean total annual precipitation is ca. 1,500 mm. The current study was conducted during the typhoon season, which is also the typical cultivation season of summer-planted sugarcane in the study district. The experimental field has a clayey acidic soil. Table 1 lists the physicochemical characteristics of the soil surface layer at a depth of 0 cm-15 cm (Mambu et al. 2018). Widely distributed on Kita-Daito Island, the soil is classified as an inceptisol (Soil Survey Staff 2014) known locally as *Daito-mahji* (Tokashiki 1993, Onaga & Yoshinaga 1988, Miyamaru 2013).

2. Experimental design

The experimental plots were laid out in a randomized block design with four treatments:
(1) control plot (no N); hereafter '0N'
(2) plot treated with chemical fertilizer (ammonium sulfate) (100 kg N ha⁻¹); hereafter '100N'
(3) plot treated with chemical fertilizer (300 kg N ha⁻¹); hereafter '300N'
(4) plot incorporating green manure (*Crotalaria juncea*) (ca. 150 kg N ha⁻¹); hereafter 'GM'.

We established 16 study plots (4 treatments × 4 replications = 16 plots). The area of each plot was 12 m²

Table 1. Physicochemical characteristics of the soil surface layer (0 cm-15 cm depth) in sugarcane cropland on Kita-Daito Island, Okinawa, Japan

pH (H₂O)	TC	TN	Sand	Silt	Clay	CEC	Bulk density
	(%)	(%)	(%)	(%)	(%)	(cmolc kg⁻¹)	(g cm⁻³)
5.4	1.9	0.2	1.4	8.3	90.3	22.1	1.0

(3 m × 4 m), except for the GM plots, which measured 18 m² (3 m × 6 m) in order to minimize the ridge effect of GM application.

Before starting the experiment, we cultivated *Crotalaria juncea* in each plot from 9 May to 31 July 2017. On 6 August 2017 (day 0 of the experiment), we removed the entire aboveground plant biomass from each plot, except in the GM plot, where the aboveground plant biomass was incorporated into the soil (at a depth of 0 cm-15 cm) using a rotary tiller. GM residues were mechanically chopped (into lengths of 5 cm-10 cm) before being incorporated into the soil of the GM plot. We determined the amount of aboveground biomass and its N content, and then calculated the quantity of applied N in the GM plot (i.e., 150 kg N ha^{-1} for a C:N ratio of 21.6), which was within the normal range applied in the study district and for the plant species (170 kg N ha^{-1}: Zougmoré et al. 2010, 130-170 kg N ha^{-1}: Mambu et al. 2018). Although the belowground biomass of GM (e.g., roots) was equally incorporated into the entire treatment plots, its effect should be quite limited due to the small amount of N (ca. 5-10 kg N ha^{-1}) based on our previous measurement. The aforementioned amounts of chemical N fertilizer (ammonium sulfate) were broadcast on the 100N and 300N plots on 6 August 2017. The amount of chemical N fertilizer reflected the conventional application amounts recommended for the Okinawa area. N$_2$O emission in the 300N plot was evaluated in response to the conventional and recommended rate of N fertilizer application. In the study area, the recommended application is 100 kg N ha^{-1} only to the ridges (which comprise ca. one-third of the total plot area) and thus was extrapolated to 300 kg N ha^{-1} in the case of broadcast application. For homogeneity in N$_2$O measurement from the entire treatment plots, we conducted broadcast application rather than ridge fertilization. N$_2$O emission in the 100N plot was evaluated in response to broadcasting one-third of the recommended rate of N fertilizer application.

Along with establishing the plots, we also applied phosphorus (P) and potassium (K) as chemical fertilizer to all plots. The application rates of P (as P$_2$O$_5$) and K (as K$_2$O) were 36 kg P ha^{-1} and 36 kg K ha^{-1}, respectively. On 13 August (day 8), we conducted mechanical deep-plow tilling (to a depth of ca. 50 cm) to enhance drainage performance, followed by rotary tilling (to a depth of ca. 15 cm) on 23 August (day 18). Sugarcane 'KN00-114' setts were planted mechanically on 25 August (day 20). On 16 September (day 42), sugarcane setts were manually replanted in blank spaces. These practices reflect conventional management in this area. No pesticides were applied during the experiment.

3. Measurements of soil environmental characteristics

Air temperature at the study site and soil temperature in two plots (GM and 100N) at a depth of 5 cm were continuously measured using time-domain temperature probes (108 Temperature Probe; Campbell Scientific, Inc., Logan, UT, USA). The volumetric water content in the surface soil at a depth of 0 cm-10 cm in two plots (GM and 100N) was continuously measured with time-domain reflectometer probes (CS616 Water Content Reflectometer; Campbell Scientific, Inc.). Volumetric soil water content was converted to water-filled pore space (WFPS) by using bulk density to calculate available soil porosity. Rainfall was measured with a tipping bucket rain gauge (TE525; Campbell Scientific, Inc.) at the study site. The climatic data on air temperature, soil temperature, soil moisture, and rainfall were recorded using a CR1000 Datalogger (Campbell Scientific, Inc.) at 30-min. intervals.

4. Soil sampling and analysis

Undisturbed soil core samples were collected at the study site from a soil depth of 0 cm-5 cm with five replications, using a 100-cm^3 (5-cm height) core sampler. All core samples were used for measuring bulk density (Grossman & Reinsch 2002). During the experimental period, surface soil samples at a depth of 0 cm-10 cm were collected on 11 dates (7, 17, 22 and 28 August, 3, 11, 20 and 29 September, 9 and 23 October, and 3 November). Each soil sample was collected with five replications, which were located within 20 cm of a sugarcane plant randomly selected inside the plot to avoid an edge effect. The five subsamples were mixed to form a composite soil sample, per replication. In addition, soil samples at a depth of 10 cm-30 cm were collected on 11 September, 9 October, and 3 November. After passing through a 4-mm sieve, the fresh soil samples were stored in a refrigerator at 4°C until the following analyses was conducted. To calculate the soil water content, 10 g of the soil samples were thoroughly dried at 105°C for more than 24 h and then weighed. Soil samples were also used to measure the soil inorganic N (SIN) content, namely ammonium-N (NH$_4^+$-N) and nitrate-N (NO$_3^-$-N). Inorganic N in the fresh soil samples was promptly extracted (within 1-2 days) from 6 g of soil with 30 mL of 2 M KCl for 30 min. The suspension was centrifuged and filtered through filter paper (Advantec No. 5C; Advantec, Tokyo, Japan). Several drops of 1 mg kg^{-1} copper (II) bromide solution were added to the extract for inhibiting microbial activity, and then the extract was stored in a refrigerator at 4°C. NH$_4^+$-N in the extract was analyzed using the modified indophenol blue method (Rhine et al. 1998) with a spectrophotometer (UV-1200; Shimadzu Co., Ltd, Kyoto,

Japan). NO_3^--N in the extract was analyzed by flow injection analysis using a flow-through visible spectrophotometer (S/3250; Soma-Kogaku Co., Ltd., Tokyo, Japan) connected to a double-plunger pump (PE-230; Aqua-lab Co., Ltd., Tokyo, Japan) equipped with a copperized cadmium column to reduce nitrate to nitrite in the sample solutions.

5. Measurement of N_2O flux

Nitrous oxide flux was measured using the closed static-chamber technique. Gas samples were collected once every one or two days during the first two months after the start of the experiment, and thereafter once or twice per week during one month (for a total of 43 sampling times during the experiment). In each plot, a chamber (15 cm in diameter, 15 cm in height) was inserted into the soil to a depth of 5 cm, and was placed within 10 cm of a sugarcane ridge at least one day before measurement to settle down. Gas samples of the chamber headspace were collected with a plastic 50 mL syringe, and injected into 10 mL pre-evacuated vials. We collected gas samples at three fixed time intervals (0, 15, and 30 min. after the chamber had been closed). We began collecting the gas samples at 10:00. Each gas sample was analyzed using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) with a column (Porapak Q 50/80) and an electron capture detector at 300°C. The rate of increase in N_2O concentration was calculated by linear regression analysis. The minimum detectable concentration of N_2O was 0.007 ppmv, and the measured N_2O concentration was mostly 0.2-1.5 ppmv. Cumulative N_2O emissions during the experimental period were calculated using the trapezoid rule to interpolate emission between the sampling points.

To calculate the N_2O emission factor (EF) due to the application of chemical fertilizer and GM, we used the methodology described in *Guidelines for National Inventories of Greenhouse Gases* (IPCC 2006) as follows:

$$EF \ (\%) = (E_{input} - E_{control}) \ / \ N_{applied} \times 100 \qquad \text{(Eq. 1)}$$

where E_{input} and $E_{control}$ are the cumulative emissions of the input N plots (100N, 300N, and GM) and control plot (0N), respectively (kg N_2O-N ha^{-1}), and $N_{applied}$ is the amount of input N (kg N ha^{-1}).

Many studies have found that the effect of chemical N application on N_2O emission predominantly occurs within 30-45 days after treatment (Smith et al. 1997, Akiyama et al. 2000, Hyde et al. 2006). Therefore, we focused on the first 45 days after treatment and divided the experimental period into the early period (comprising the non-planted and plowed period of 21 days from 6 to 26 August), the middle period (of 24 days immediately after planting from 27 August to 19 September), and the latter period (of 45 days from 20 September to 3 November) to assess the effect of N application on N_2O flux in each period of cultivation.

6. Statistical analysis

All statistical analyses were performed with SYSTAT 12.5 (Systat Software, San Jose, CA, USA). All data are expressed on a dry weight basis, and $P < 0.05$ was considered significant. One-way analysis of variance (ANOVA) was used to compare the cumulative N_2O emissions and EF followed by Tukey's test. Repeated-measure ANOVA was used to assess the effects of treatment and seasonal variation on N_2O emission and SIN (NH_4^+-N and NO_3^--N) content. All variables were tested for normality of the distribution and transformed by reciprocal or reciprocal square root, when necessary to minimize the variation.

Results

1. Environmental factors

Figure 1 (a) shows the fluctuation in rainfall and WFPS during the experimental period. Cumulative rainfall during the experimental period totaled 214 mm (22 mm in the early period, 61 mm in the middle period, and 131 mm in the latter period), which was relatively lower than the annual average. In particular, cumulative rainfall in the early period (22 mm) was lowest in this experiment. There were five heavy rainfall events (> 10 mm within 2-4 hours in a day) during the experimental period (two in the middle period; three in the latter period). In the experimental period, fewer typhoons struck the Okinawa area than the annual average, although a typhoon in mid-October brought heavy rainfall. WFPS increased after each distinct rainfall event throughout the experimental period. Before the rotary tilling was conducted, no distinct difference in daily averaged WFPS was observed between the 100N and GM plots, and WFPS was maintained at about 20% in all plots. However, after the rotary tilling, WFPS in the GM plot was maintained higher than that in the 100N plot, and fluctuated between 26% and 58% in the GM plot and between 25% and 51% in the 100N plot.

No difference was observed in daily averaged soil temperature throughout the experimental period between the GM and 100N plots (100N: 30.0°C; GM: 30.0°C). Daily averaged soil temperature was highest at 37.8°C on 18 August and thereafter declined to 24.1°C on 1 November.
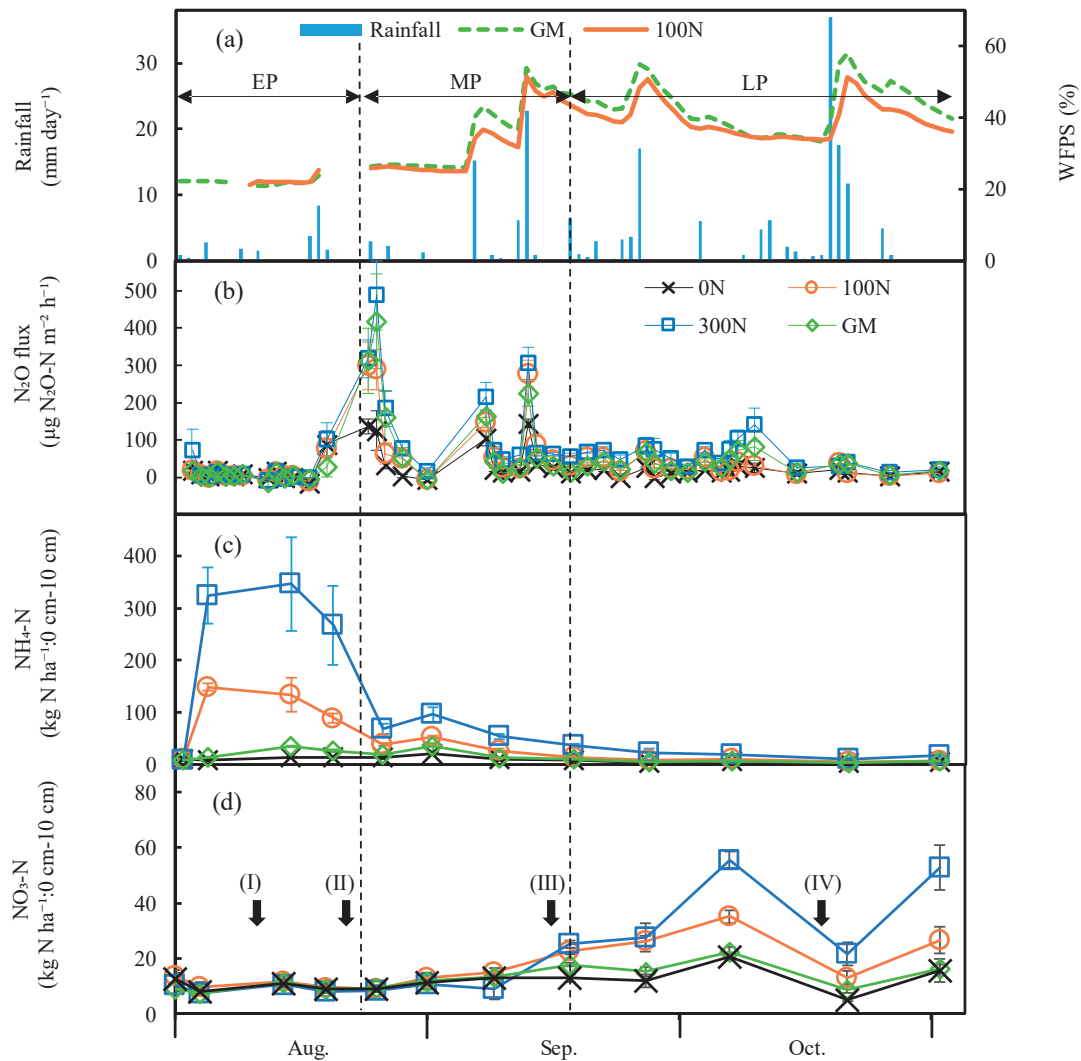
Fig. 1. Fluctuation in (a) rainfall and water-filled pore space (WFPS), (b) nitrous oxide (N2O) flux, (c) soil ammonium-nitrogen (NH$_4$-N) content (0 cm-10 cm depth), and (d) soil nitrate-nitrogen (NO$_3$-N) content (0 cm-10 cm depth) during the experimental period in sugarcane cropland on Kita-Daito Island, Okinawa, Japan.

Error bars indicate the standard deviation.

0N   = the control
100N = 100 kg N ha-one chemical fertilizer
300N = 300 kg N ha-one chemical fertilizer
GM   = 150 kg N ha-one provision of green manure
EP   = early period (from 6 August to 26 August)
MP   = middle period (from 27 August to 19 September)
LP   = latter period (from 20 September to 3 November)
(I)   = plow tilling on 13 August
(II) = rotary tilling and seed planting on 23-26 August
(III) = replanting seed on 16 September
(IV) = typhoon during 20-22 October

2. N$_2$O flux

There were no clear differences in the temporal change and timing of peaks in N$_2$O emissions among the treatments in the early period (Fig. 1 (b)). After the planting of sugarcane in the middle and latter periods, clear differences were observed in N$_2$O flux among the treatments, and N application (i.e., 100N, 300N, GM) significantly increased N$_2$O emission (Table 2). There were three peaks of N$_2$O emission in the experimental period (on 28 August, 10 September, and 15 September). The highest N$_2$O fluxes were observed on 28 August with peak values of 124, 288, 488, and 418 µg N$_2$O-N m^{-2} h^{-1}

Table 2. Results of repeated-measure ANOVA for the effects of treatment and seasonal variation on nitrous oxide (N2O) emission, and contents of ammonium-nitrogen (NH4-N) and nitrate-nitrogen (NO3-N) under different nitrogen-source treatments

			Overall			Early period[1]			Middle period[2]			Latter period[3]		
			N₂O	NH₄-N	NO₃-N	N₂O	NH₄-N	NO₃-N	N₂O	NH₄-N	NO₃-N	N₂O	NH₄-N	NO₃-N
			(n=43)	(n=11)	(n=11)	(n=13)	(n=3)	(n=3)	(n=10)	(n=3)	(n=3)	(n=20)	(n=5)	(n=5)
(a) 100N×GM	Treatment (T)	F	0.2	200.7	10.1	2.2	82.1	0.3	0.3	31.0	0.1	0.3	9.5	54.0
		P	NS[4]	***	*	NS	***	NS	NS	**	NS	NS	*	***
	Seasonal	F	22.4	31.1	30.0	7.0	2.0	26.3	20.4	25.6	12.0	4.4	15.9	15.2
	Variation (S)	P	***	***	***	***	NS	***	***	***	**	***	***	***
	T×S	F	0.9	17.8	3.9	1.4	3.1	3.4	0.9	0.4	0.6	1.3	1.3	1.3
		P	NS	***	***	NS	NS	NS	NS	NS	NS	NS	NS	NS
(b) 100N×0N	Treatment (T)	F	10.1	285.2	17.9	0.2	105.4	0.2	11.1	94.1	0.7	5.8	16.1	97.3
		P	*	***	**	NS	***	NS	*	***	NS	*	**	***
	Seasonal	F	26.6	26.5	20.4	12.2	2.0	9.0	28.6	50.9	25.0	4.6	20.4	12.4
	Variation (S)	P	***	***	***	***	NS	**	***	***	***	***	***	***
	T×S	F	3.6	22.5	4.6	0.1	2.7	0.3	3.6	7.4	1.1	1.3	1.7	0.5
		P	***	***	***	NS	NS	NS	**	**	NS	NS	NS	NS
(c) 100N×300N	Treatment (T)	F	2.7	18.3	3.2	0.0	11.6	0.8	2.6	21.7	2.3	2.3	19.3	12.6
		P	NS	*	NS	NS	**	NS	NS	**	NS	NS	**	**
	Seasonal	F	27.7	22.1	46.6	7.8	1.1	13.1	27.1	13.0	3.2	3.5	10.4	20.5
	Variation (S)	P	***	***	***	***	NS	***	***	***	NS	***	***	***
	T×S	F	1.0	4.3	7.3	0.1	1.0	0.7	0.9	0.7	1.6	1.0	2.5	4.7
		P	NS	***	***	NS	NS	NS	NS	NS	NS	NS	NS	**

(a) 100N (100 kg N ha-one chemical fertilizer) and GM (150 kg N ha-one provision of green manure), (b) 100N and 0N (the control), (c) 100N and 300N (300 kg N ha-one chemical fertilizer) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)
[1] from 6 August to 26 September
[2] from 27 August to 19 September
[3] from 20 September to 3 November
[4] Not significant

for the 0N, 100N, 300N, and GM plots, respectively. After 20 September (the latter period), no clear peaks of N₂O emission were observed until 3 November, although rainfall events occurred continuously and WFPS was maintained at a relatively high percentage. However, the average N₂O fluxes for the 100N and GM plots in the latter period (100N: 28.0 µg N₂O-N m⁻² h⁻¹; GM: 34.6 µg N₂O-N m⁻² h⁻¹) were distinctly higher than those in the early period (100N: 8.7 µg N₂O-N m⁻² h⁻¹; GM: 4.7 µg N₂O-N m⁻² h⁻¹).

Cumulative N₂O emissions over the 90 days differed among the treatment plots (Fig. 2). Cumulative N₂O emissions in the 300N plot (1.53 kg N ha⁻¹) were significantly higher than in all the other plots. No significant differences were observed in cumulative N₂O emissions between the 100N (0.95 kg N ha⁻¹) and GM plots (1.05 kg N ha⁻¹). Cumulative N₂O emissions in the 0N plot (0.54 kg N ha⁻¹) were significantly lower than those in the 300N and GM plots. About 70% of the cumulative N₂O emissions in the N-input plots was recorded in the middle period. The N₂O EF in the 100N, 300N, and GM plots was 0.41%, 0.33%, and 0.34%,
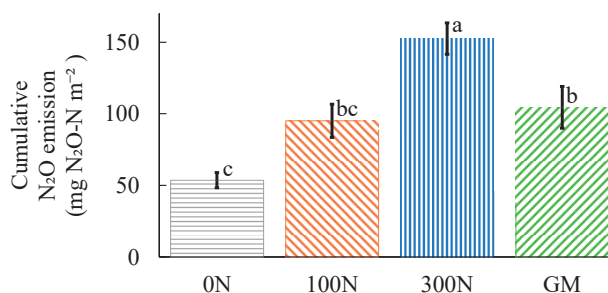
Fig. 2. Cumulative nitrous oxide (N₂O) emissions during the experimental period under different nitrogen-source treatments
0N = the control
100N = 100 kg N ha-one chemical fertilizer
300N = 300 kg N ha-one chemical fertilizer
GM = 150 kg N ha-one provision of green manure in sugarcane cropland on Kita-Daito Island, Okinawa, Japan.
Different letters above the bars indicate a significant difference ($P < 0.05$).
Error bars indicate the standard deviation.

respectively, and there was no significant difference among the treatments.

3. Dynamics of soil inorganic nitrogen

(1) Soil NH$_4^+$-N content

Soil NH$_4^+$-N content in the 100N and 300N plots increased markedly two days after N input, and was maintained at a high level until 12 days after the application (on 17 August; Fig. 1 (c)). The NH$_4^+$-N content in the 100N and 300N plots decreased from 22 to 28 August, and gradually decreased until the end of the experiment. The NH$_4^+$-N content in the GM plot increased 12 days after GM incorporation, and gradually decreased thereafter until the end of the experiment. The amount of NH$_4^+$-N in the 300N plot was maintained at a higher level than in the other treatment plots during the experimental period.

Figure 3 shows the soil NH$_4^+$-N content at depths of 0 cm-10 cm and 10 cm-30 cm in each treatment. The soil NH$_4^+$-N content of chemical N fertilizer plots (100N and 300N) decreased from 7 August at a depth of 0 cm-10 cm (100N: 148 kg N ha^{-1}; 300N: 324 kg N ha^{-1}) to 11 September at a depth of 0 cm-30 cm (100N: 47 kg N ha^{-1}; 300N: 138 kg N ha^{-1}). In contrast, the soil NH$_4^+$-N content of the GM plot did not decrease during this period.

(2) Soil NO$_3^-$-N content

The NO$_3^-$-N content in the 100N, 300N, and GM plots increased from 37 days (11 September) to 65 days after treatment (9 October; Fig. 1 (d)). The highest NO$_3^-$-N content in all of the N-input plots was observed 65 days after treatment (9 October) at a depth of 0 cm-10 cm (100N: 35 kg NO$_3^-$-N ha^{-1}; 300N: 55 kg NO$_3^-$-N ha^{-1}; GM: 22 kg NO$_3^-$-N ha^{-1}). The NO$_3^-$-N content decreased in the 100N and 300N plots from 9 to 23 October when the typhoon struck (on 20-22 October). The amount of NO$_3^-$-N was highest in the 300N plot, followed by the 100N, GM, and 0N plots in the latter period, whereas no distinct differences were observed among treatments in the early and middle periods.

Figure 3 shows the soil NO$_3^-$-N stock at depths of 0 cm-10 cm and 10 cm-30 cm in all plots. In the latter period, the soil NO$_3^-$-N stock was predominantly distributed in the deeper layer (at a depth of 10 cm-30 cm) in all treatment plots. The soil NO$_3^-$-N stock at a depth of 0 cm-30 cm in the 100N and 300N plots increased from 11 September (100N: 76 kg N ha^{-1}; 300N: 76 kg N ha^{-1}) to 9 October at a depth of 0-30 cm (100N: 97 kg N ha^{-1}; 300N: 143 kg N ha^{-1}), whereas little increase was observed from 9 October to 3 November at a depth of 0 cm-30 cm (100N: 100 kg N ha^{-1}; 300N: 161 kg N ha^{-1}). The soil NO$_3^-$-N stock at a depth of 0 cm-30 cm in the GM plot barely changed from 11 September (at 54 kg N ha^{-1}) to 3 November (at 55 kg N ha^{-1}).

4. Effect of N application on N$_2$O flux and soil inorganic nitrogen

Table 2 presents the results of the repeated-measure ANOVA. No significant differences in N$_2$O emissions were detected between the 100N and GM treatments, although significant differences in NH$_4^+$-N and NO$_3^-$-N contents were observed between 100N and GM for the overall experimental period (Table 2 (a)). No significant differences in N$_2$O emissions between 100N and GM treatments were observed in the early, middle, and latter periods, whereas significant differences were observed in NH$_4^+$-N content in the early, middle, and latter periods, and in NO$_3^-$-N content in the latter period.

There were significant differences in N$_2$O emissions between the 100N and 0N treatments for the overall experimental period and the middle period (Table 2 (b)). Significant differences were also observed in NH$_4^+$-N content for the overall experimental period, the early, middle, and latter periods, and in NO$_3^-$-N content for the overall experimental period and the latter period.

There were no significant differences in N$_2$O emissions between the 100N and 300N treatments for the overall experimental period and the early, middle, and latter periods. However, significant differences were observed in soil NH$_4^+$-N content for the overall experimental period, the early, middle, and latter periods,
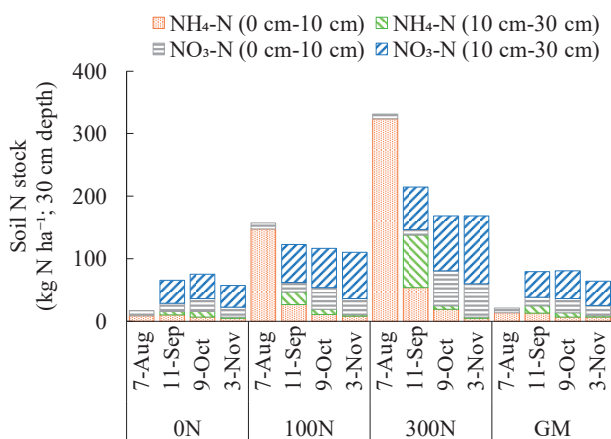
Fig. 3. Temporal variation in soil ammonium-nitrogen (NH$_4$-N) and nitrate-nitrogen (NO$_3$-N) stock (at depths of 0 cm-10 cm and 10 cm-30 cm) during the experimental period under different nitrogen-source treatments.
0N = the control
100N = 100 kg N ha-one chemical fertilizer
300N = 300 kg N ha-one chemical fertilizer
GM = ca. 150 kg N ha-one provision of green manure in sugarcane cropland on Kita-Daito Island, Okinawa, Japan

and in soil NO_3^--N content in the latter period (Table 2 (c)).

Discussion

The temporal pattern and peaks of N_2O flux were not significantly different between the 100N and GM plots, resulting in similar cumulative N_2O emissions and N_2O EF (Fig. 1 (b), Fig. 2, Table 2 (a)), even though the amount of applied N in the GM plot (150 kg N ha⁻¹) was 1.5 times higher than that of the 100N plot (100 kg N ha⁻¹). In addition, SIN (NH_4^+-N and NO_3^--N) in the 100N plots was maintained at a higher content than in the GM plots; therefore, N_2O emissions should be theoretically larger in the 100N plot (Jones et al. 2007), though that was not the case in the present experiment. These results suggest no clear effect of the applied N quality, such as chemical fertilizer and GM, on N_2O emissions at this experimental site and during its periods. Meng et al. (2005) reported that no significant differences were detected between the N_2O flux patterns of mineral fertilizer and organic manure in a wheat cropland in China. Jin et al. (2010) also reported that high peaks of N_2O fluxes were observed in both the chemical fertilizer and manure plots within several weeks after N application in a reed canary grassland in Japan. However, a significant effect of N substrate quality on N_2O emission, such as N_2O EF, has often been reported. For example, Neto et al. (2016) reported that the N_2O EF of chemical N fertilizer (ammonium nitrate; 0.44%) was higher than that of organic N (sugarcane filter cake; 0.10%-0.17%) in a sugarcane cropland in Brazil. Alluvione et al. (2010) observed that the N_2O EF of GM (hairy vetch; 1.34%) was significantly lower than that of urea (3.42%) in a corn field in Italy. The reasons for the non-significant differences in N_2O emission and EF between the different N treatments, such as 100N and GM, in the present study remain unclear. We consider two possible reasons to be 1) low rainfall received in the early period, causing low N_2O emission in the 100N plot, and 2) favorable conditions for N-mineralization and nitrification caused by an adequate C:N ratio of the GM substrate, possibly stimulating the soil microbes for N_2O emission in the GM plot. As we only observed the first three months of the sugarcane cropping period that generally lasts 1-1.5 year, N_2O emissions through the cultivation period must also be evaluated.

With regard to the first reason, little rainfall was received in the early period (22 mm), which resulted in low N_2O emissions from all plots in this period. The effect of N application on N_2O emission should particularly be observed in this early period, as many studies have found that most N_2O emission peaks occurred 1-2 weeks after chemical fertilizer application in croplands (Liu et al. 2005, Schils et al. 2008). Weier (1999) also observed distinct N_2O emission peaks immediately after heavy rainfall with chemical fertilizer application (ammonium sulfate, urea) in a sugarcane field of Australia. However, in the present study, WFPS was maintained at around 20% in the early period, which represented unfavorable conditions for microbial activity, especially for denitrification (Linn and Doran 1984). As there were no clear N_2O emission peaks in the early period in the 300N plots, which has higher soil NH_4^+-N content, low WFPS should limit microbial activity in all the plots. Based on the SIN content of the subsoil on 11 September, NH_4^+-N in the topsoil in the 100N and 300N plots moved downward in the soil profile to the subsoil, possibly due to the rotary tilling on 23 August. These factors may have contributed not only to lower-than-expected N_2O emissions in the 100N and 300N plots, but also to lower N_2O EF in the 100N (0.41%) and 300N plots (0.33%) than those observed in major sugarcane production countries such as Brazil (Carmo et al. 2013, Soares et al. 2015) and Australia (Denmead et al. 2010, Cajas et al. 2016).

Concerning the second reason, high temperature and the decomposability of GM will also promote rapid N mineralization of GM, resulting in high N_2O emissions. Average soil temperature throughout the experimental period was ca. 30°C, which is sufficient to promote rapid decomposition of applied GM in tropical regions (Cobo et al. 2002). The C:N ratio of GM (*Crotalaria juncea*) in the present experiment was ca. 20, and thus rapid N mineralization could be expected after the incorporation of GM (Vigil and Kissel 1991, Janssen 1996, Fernandes et al. 1997). Based on our field CO_2 flux analysis in the same field but for different objective research, we estimated that 30%-50% of applied GM was decomposed after the first four months, indicating that 45-75 kg N ha⁻¹ should be theoretically mineralized from GM (*unpublished data*). We also observed higher WFPS in the GM plots in the middle and latter periods, rather than in the early period, thus indicating the stimulated nitrification and denitrification of microbes. After the rotary tilling on 23 August, WFPS in the GM plots was maintained at a higher percentage compared with that of the 100N plots after rainfall. Phiri et al. (2003) observed that GM (*Sesbania sesban*) application enhanced soil water storage through enhanced water infiltration into the soil in Zambia. Previous studies of sugarcane cropland have reported that soil moisture content is positively correlated with N_2O emission (esp. from 20% to 50% of the WFPS range; Neto et al. 2016, Soares et al. 2015,

Allen et al. 2010). Thus, we suggest that low C:N GM incorporation caused the higher WFPS, along with immediate decomposition and subsequent N-mineralization, which possibly resulted in higher N$_2$O emission in the GM plot. Although not evaluated in this study, the effect of sugarcane N uptake on SIN dynamics and N$_2$O emission through the crop growth period to achieve an efficient N cycle should be evaluated in future experiments.

Nitrification and denitrification are microbially mediated processes involving NH$_4^+$-N oxidation and NO$_3^-$-N reduction, respectively. In addition, N$_2$O production during nitrification has been measured in soils with WFPS of 60% or less, whereas denitrification rates rapidly increase when WFPS exceeds 60% (Linn & Doran 1984, Abbasi & Adams 2000). During the middle period, three clear N$_2$O flux peaks coincided with the period in which soil NH$_4^+$-N content decreased and WFPS increased. In the present study, it is thus likely that N$_2$O emission in the middle period was mostly derived from nitrification, rather than denitrification. However, in the latter period, SIN certainly existed in the substrates for nitrification and denitrification in the 100N and 300N plots under a higher WFPS (54%). Therefore, N$_2$O emission during the latter period might be derived both from nitrification and denitrification, though N$_2$O emission in the latter period was lower than that in the middle period. Regardless of treatment, nitrification is probably the main cause of N$_2$O emission in the present experiment, as 70% of the total N$_2$O emissions in the N-input plots was recorded in the middle period. Bateman and Baggs (2005) observed that (1) nitrification was the main process of N$_2$O emission, accounting for 68%-81% of emitted N$_2$O, and that (2) daily fluxes of N$_2$O were positively correlated with available NH$_4^+$-N by an incubation experiment under different WFPS conditions between 35% and 60%. Similarly, Bellido et al. (2015) also observed a distinct N$_2$O emission peak immediately after ammonium sulfide fertilizer input in a sugarcane field in Okinawa, and indicated that most N$_2$O emissions are derived from nitrification.

Considering that the 300N, 100N, and 0N plots shared the same N quality, but differed in N quantity, significant differences were observed in N$_2$O emissions, and the larger amount of SIN resulted in higher N$_2$O fluxes and higher cumulative N$_2$O emissions. The present results are consistent with previous findings that the rate of N$_2$O emission increases with elevation in the amount of soil available N (Smith et al. 1997, Skiba & Smith 2000, Sehy et al. 2003). To achieve climate smart agriculture with sufficient crop yields, further study is needed to evaluate sugarcane yields under various N management practices throughout the entire 1-1.5 year cultivation period.

Conclusion

We evaluated N$_2$O emissions under different N application treatments to sugarcane cropland during the first three months after planting and N fertilization. There were no clear differences in N$_2$O flux and its emission factor between the GM and 100N plots, indicating that GM application, which had a larger amount of N (150 kg N ha^{-1}), did not cause larger N$_2$O emission than that in the 100N plot (100 kg N ha^{-1}). We also observed a distinct increase in cumulative N$_2$O emissions in the 300N plot compared with the 100N and 0N plots, suggesting that the amount of applied N clearly affected N$_2$O emission as a result of differences in SIN content. Given that GM application is expected to improve soil fertility conditions, such as soil organic carbon stock, water permeability, and other biological properties, the incorporation of GM should be an effective management practice in sugarcane croplands in Okinawa, not only for climate change mitigation but also for long-term sustainable land management.

Acknowledgments

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