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NITROUS OXIDE PRODUCTION IN TROPICAL SOILS UNDER DIFFERENT MOISTURE REGIMES AND N-APPLICATION RATES

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Abstract: Nitrous oxide (N₂O) is a potent trace gas responsible for approximately 6% of the current greenhouse gas (GHG) effect with 60 to 80% of total global emissions originating from the agricultural sector. Within this sector, the majority of N₂O emissions come from soils arising mainly from N fertilizer additions. In the Caribbean, no studies have been conducted to quantify the N₂O flux from our soils, although this information is crucial in gaining a better understanding of how to manage N fertilizers, such as urea, to minimize N₂O fluxes. In our region, urea represents approximately 80% of N-fertilizer used. In this laboratory study, the effects of urea-N rate and soil moisture on N₂O, CH₄ and CO₂ flux were investigated on three soils from Trinidad. The 18 treatment combinations were arranged in a completely randomized design with four replicates (72 experimental units). The equivalent of 220 g oven dried soil were placed in 1L mason jars and incubated at 50% Water Filled Pore Space (WFPS) for 7 days. Then, urea-N solutions of 0, 75 and 150 kg N/ha were applied and the soil moisture was further adjusted to 60% and 90% WFPS. Jars were then sealed and headspace air was sampled using a syringe via a rubber septum on the lid at regular intervals: three times on day 1 and then once per day for the next two weeks. Gas samples were injected into evacuated exetainers and analyzed for N₂O, CO₂ and CH₄ concentrations using a gas chromatograph equipped with a Flame Ionization Detector (FID) and an Electron Capture Detector (ECD). Increases in N-fertilizer application rate did not have a significant effect on N₂O production however it must be noted that N₂O emissions were greater as N-applicate rate increased. Soil moisture was significant to N₂O production with highest emissions under 90% WFPS compared to 60% WFPS. Soil type also had a significant effect with the greatest emission from the Nariva peaty clay soil type. The N₂O flux data presented in this paper is the first report for Trinidad soils. This study has implications for improving urea-N fertilizer use efficiency, which could enhance soil productivity while minimizing environmental pollution.

Keywords: Nitrous oxide, Nitrogen fertilizer, Fertilizer use efficiency, Water Filled Pore Space (WFPS)

INTRODUCTION

Sustainable ecosystems are necessary for people and the environment today and for future generations. Anthropogenic activities, particularly the intensive use of non-renewable natural resources has resulted in the degradation of environmental quality, adversely affecting these ecosystems (Picone et al. 2014). Global warming has been reported to be a direct consequence of such activities caused by the increase in the atmospheric concentration of greenhouse gases such as nitrous oxide (N₂O) (Picone et al. 2014). N₂O significantly contributes to global warming and mitigating N₂O is essential in combating global climate change (Chen et al. 2014).

According to the Intergovernmental Panel on Climate Change (IPCC) 2013, N₂O has a global warming potential that is 298 times higher than that of carbon dioxide (CO₂) even though it only accounts for 8% of the global anthropogenic greenhouse gas emissions (Picone et al. 2014). Once it is released into the atmosphere, the stratospheric reaction with atomic O₂ to produce nitric oxide (NO) induces the depletion of the stratospheric ozone layer (Wrage et al. 2001, Kool et al. 2010, Crutzen 1981). Over the past few decades and presently, the concentration of N₂O continues to increase at an annual rate of 0.25% which suggests there is definitely a cause for concern regarding N₂O emissions.

Most of the direct N₂O emissions arise from agricultural soils and this has contributed to the increasing atmospheric N₂O concentration in recent times (Chen et al. 2014, Kool et al. 2010, Pihlatie et al. 2004, Wrage et al. 2004, Wang et al. 2013). The IPCC second assessment report estimated that the total N₂O emission from farmland is 350×10^4 tN y⁻¹ and this quantity accounts for 61.4% of anthropogenic emissions and 23.8% of global N₂O emissions. Other authors even suggest that 80% of total anthropogenic N₂O emissions arise from agricultural activities (Yan et al. 2014).

It has therefore become imperative to understand and identify the processes producing N₂O and the key factors affecting the production rates in agricultural soils (Pihlatie et al. 2004). It is well established that the microbial processes of nitrification and denitrification represent the main sources of N₂O emissions (Firestone and Davidson, 1989). While nitrification is a two-step process which involves the oxidation of ammonium (NH₄⁺) to nitrite (NO₂⁻) and nitrate (NO₃⁻) (Garrido et al., 2002) which provides the raw material for potential N₂O formation, denitrification is the biological reduction of NO₃⁻ to nitrogen (N₂) gas by facultative heterotrophic bacteria which is the process directly responsible for the emissions (WPC, 2010). However, in a process called “nitrifier denitrification”, ammonium oxidizers containing NO₂⁻ reductase may use NO₂⁻ as an alternative electron acceptor in O₂-limiting conditions, to produce NO and N₂O emissions (Muller, 1999; Myrold, 1998)

The emissions of N₂O are greatly influenced by soil moisture content, soil temperature, mineral N (NH₄ and NO₃), organic carbon contents, placement of fertilizer and soil texture (Davidson, 1991, 1993; Smith et al., 1998, Dobbie et al., 1999, Skibia and Ball, 2002). According to Taggart et al. (2002), the interaction of soil texture, fertilization and soil moisture has a significant influence on N₂O emissions. Increasing moisture content in soils according to Simojoki and Jaakola (2000) acts as a catalyst to N₂O emissions up to 90% water-filled pore space (WFPS). The emissions of N₂O is known to be predominant in anaerobic conditions following a period of aerobic conditions and has been observed to be emitted in large bursts about 20-24 hours after rainfall (Wagner-Riddle et al., 1996).

An analysis of literature reveals that techniques for measuring N₂O generally fall into two main categories- chamber (enclosure) and micrometeorology techniques (IFA, 2001). Ryden et al. (1978) in his study concluded that the chamber technique offer the most useful approach for this measurement primarily because gases emitted by the soil into a chamber are not continually diluted with external air allowing smaller fluxes to be measured. Additionally, a properly vented chamber effectively avoids the potential for an influx of gases by mass flow from outside the

chamber by maintaining equal pressures inside and outside the enclosure (Hutchinson and Mosier 1981).

It is essential to investigate and understand how these factors as well as their interactions would affect the production of N₂O in tropical soils since no published research exist to describe N₂O production in Trinidad and by extension the Caribbean region. The main objective of this study was to investigate the influence of soil moisture, soil texture and N-application rate on N₂O production in Trinidad soils.

MATERIALS AND METHODS

Soil material and preparation

The soil types used for this trial were the A horizon of River Estate Series from the University of the West Indies field station, Mt. Hope Trinidad, Nariva Series and Arena Series from East Trinidad. River estate series is a freely drained loam soil developed from alluvium where the parent material is essentially micaceous phyllite alluvium. Nariva series has impeded drainage and a mineral topsoil of very dark gray or black humic peaty clay. Arena series is a freely drained soil where the parent material is quaternary sand.

These soils were taken from the top 0-20 cm at the respective locations and this corresponds with the depth to which they would be cultivated during land preparation. The soils were air dried, homogenized, and sieved to separate the fine-earth fraction (<2 mm) to remove visible roots and other impurities in preparation for soil testing as well as for use in the trial. Selected physical and chemical properties for each soil texture from composited samples were analyzed as listed in Table 1.

Table 1: Some physical and chemical soil properties from soil analyses before pre-incubation of air dried soils

Soil Properties	River Estate Series	Arena Series	Nariva Peaty Clay
Sand (%)	23	94	14
Silt (%)	66	6	37
Clay (%)	11	0	49
pH	5.64	4.34	4.47
Soil Organic Nitrogen (SON) (%)	0.149	0.059	0.635
Soil Organic Carbon (SOC) (%)	1.155	0.615	5.367
Ammonia (NH ₄) ⁺ (mg kg ⁻¹)	3.411	2.637	5.828
Nitrate (NO ₃ ⁻) (mg kg ⁻¹)	4.190	2.698	10.538
Bulk Density (g cm ⁻³)	1.089	1.4695	0.9328

* Methods of analysis were obtained from Soil Sampling and Methods of Analysis: Part 1 and 2 (Carter and Gregorich, 2008).

Experimental design and Treatments

In a completely randomized design, the effect of soil texture (3 soil types), soil moisture (60% and 90% WFPS) and N- application rate (0, 75 and 150kgN/ha) were investigated. WFPS is the volume of water in the soil relative to the total volume of pores (Guo et al. 2010) and was calculated as:

$$\text{WFPS} = (\text{soil gravimetric water content} \times \text{bulk density}) / [1 - (\text{bulk density} / \text{particle density})]$$

*Particle density assumed to be 2.65g/cm³

Experimental set-up

220g of each type air dried soil was moistened homogenously with distilled water to 50% WFPS and then placed in 1-L mason jars. The jars were randomly placed in an incubator and left for a 7-day aerobic incubation (jars were uncovered) at 25°C to stimulate microbial activity.

Following the 7-day aerobic pre-incubation, N-Fertilizer (urea solution) was applied uniformly to the soil surface followed by distilled water to make up the 60% and 90% WFPS. The water was added after fertilizer application to ensure even distribution of the fertilizer. After treatment application, the jars were left open to provide atmospheric air exchange between successive N₂O flux measurements. This **oxic phase** lasted approximately 7 days which is the expected time for enhanced N₂O emissions to return to ambient levels.

On day 7, the WFPS treatments in each jar was adjusted to initial levels, jars were sealed with air tight lids and subsequently flushed with N₂ for 6 minutes. The purpose of this is to replace headspace air with nitrogen until the oxygen concentration in the headspace air is less than 5% and create intensely anaerobic conditions and signal the start of the anoxic phase. An oxygen consumption pretest was conducted before the experimental set up and the results for all soil types revealed that after 6 minutes, the oxygen concentration was below 3.7%. After flushing with nitrogen, headspace air samples were taken from each of the jars and analyzed for oxygen concentration. Results revealed that the oxygen concentration in all jars were below 3.3% and N₂O flux measurements were immediately started and also lasted 7 days. The temperature inside the incubators was monitored daily to ensure that it was more or less constant (25°C).

Gas (N₂O), Soil and WFPS measurements

N₂O emissions were intensively monitored for 14 days following the application of treatments. During the first 7 days of sampling, jars were sealed only at the time of sampling (Oxic Phase) as compared to the second week where the jars remained sealed throughout the entire trial (Anoxic Phase). Immediately after applying treatments, jars were sealed and headspace air was sampled using a 20ml polypropylene syringe via a rubber septum on the lid at regular intervals: three times on day 1 and then once per day for the next two weeks. Gas samples were taken at 0 and 60 minutes during each sampling event. Gas samples were injected into evacuated 12ml exetainers (Labco, Buckinghamshire, UK) and analyzed within 2 weeks of sampling for N₂O, CO₂ and CH₄ concentrations using a gas chromatograph equipped with a Flame Ionization Detector (FID) and an Electron Capture Detector (ECD).

The increase in N₂O concentrations between 0 and 60 minutes was used to determine the N₂O emission rates following the linear model of N₂O accumulation over time. Cumulative N₂O

emission for each jar was estimated by linear interpolation between data points assuming that measured fluxes represented average daily fluxes. Fertilizer Induced Emission was calculated as the percentage of cumulative N₂O-N emissions from the applied N during a given period (Yan et al. 2014). N₂O fluxes were calculated from the change in concentration, C, inside the jars as follows and expressed as mg N₂O-N m² h⁻¹:

$$C_m = \frac{C_v \times M \times P}{R \times T}$$

C_v = Volume concentration (ppm)

GMW = Gram Molecular weight (g)

P = Pressure (mmHg)

n = Number of Moles (Unitless)

R = Gas Constant (m³ mmHg K⁻¹ mol⁻¹)

T = Temperature (K)

Gravimetric soil moisture contents were measured daily by weighing the mason jars and adjusting the WFPS to the initial levels to closely monitor the temporal variability of N₂O and WFPS. At the end of the incubation period, the soil-treatment mixture was homogenized and sub-sampled for determination of mineral N (NH₄ and NO₃⁻), pH, DOC concentration (labile C) and moisture content determination. Air temperature of the laboratory was measured using a thermometer. Wind speed, solar radiation and humidity were measured using a portable micrometeorological station.

Statistical Analyses

Repeated measures ANOVA was used to examine the main effects of Water Filled Pore Space (WFPS), soil type and fertilizer application rates on N₂O flux. Statistical analyses were conducted using GenStat Discovery Edition 4.

RESULTS AND DISCUSSION

Effect of WFPS on N₂O flux

WFPS had a significant effect on N₂O flux, cumulative N₂O emission and fertilizer induced emission as shown in Table 2. In the control jars, only the Nariva soil consistently resulted in positive emissions with higher N₂O flux values under 90%WFPS compared to 60%WFPS. All other treatment combinations produced negative or below ambient emissions (Figure 1). N₂O production here may be due to nitrification/denitrification of the resident inorganic nitrogen present in the soil prior to the start of the experiment. Ammonia and nitrate levels before the start of the experiment were in the order; *Nariva* > *River Estate* > *Arena sand*. At 60%WFPS in these control jars, Nariva showed a peak flux of 1.2 mg N/m²/h on day 8 of the trial and 4.1 mg N/m²/h on day 10 for 90%WFPS.

In all treatment combinations where 75kgN/ha was applied, N₂O flux rates were relatively low; either negative or below ambient levels (Figure 1). This indicates that for instance, where soil WFPS was 90% and soil nitrogen levels was high, N₂O flux were still low. This may be due to

the influence of other factors independent to inorganic nitrogen controlling N_2O -N losses by denitrification (Yoshinari, Hynes, and Knowles 1977) e.g. temperature and /or carbon availability. There are studies (Burton and Beauchamp 1985) that suggest that even in soils with high nitrate levels, C availability is the most limiting factor controlling denitrification. A carbon content range greater than 60 - 80 mg C kg^{-1} was even suggested to be required for denitrification (Burton and Beauchamp 1985). In this experiment, dissolved organic carbon (DOC) was measured at the end of the trial and results of the concentration are pending. This would better be able to indicate to some extent the effect of C on denitrification. On the other hand, when 150kg N/ha was applied, only the Nariva soil type produced emissions that were above ambient levels. For this soil, peak N_2O flux occurred in 48hrs (7.4 mg $\text{N}/\text{m}^2/\text{h}$) for the 90%WFPS and 72 hours for the 60%WFPS (1.9 mg $\text{N}/\text{m}^2/\text{h}$). In these treatments, peak emissions generally occurred later for the wetter treatments.

Jars fertilized with 150kg N/ha produced greater N_2O fluxes at 90% WFPS as compared to those fertilized with 75kg N/ha. More specifically, fluxes were greatest for the Nariva peaty soil (-1.5-7.4 mg $\text{N}/\text{m}^2/\text{h}$) and least for the Arena sand (-0.5-0.1 mg $\text{N}/\text{m}^2/\text{h}$). Fluxes increased in the order *Nariva*>*River Estate*>*Arena sand*. In all other treatment combinations, negative or below ambient N_2O fluxes were obtained (Figure 1). These results support the suggestion that N_2O emissions is greater in wetter soils which is in agreement with other studies (Dobbie, McTaggart, and Smith 1999, Skiba and Ball 2002, Khalil and Baggs 2005) suggesting that the emissions were predominantly due to denitrification.

During the oxic cycle for the Nariva soil 90% WFPS treatment, there was a gradual increase in N_2O flux until day 2 (peak flux) followed by a steady decline in emissions where it remained low until the end of this cycle (Figure 1). This gradual increase may have been driven by NO_3 -N availability and all other soil properties that were measured (available C and soil water content-particularly in the 90%WFPS). The decline in emission towards the end of the oxic phase could be due to low carbon availability since soil NO_3 and NH_4 levels at the end of the experiment was very high. However, this can only be concluded when DOC analysis is obtained. For the first 2 days of the anoxic cycle however, negative N_2O fluxes were obtained followed by an increase in emissions but throughout this period, all flux rates were below ambient levels (Figure 1). Again, this may be as a result of low carbon availability. Since this trend was specifically observed in the Nariva soil with 90%WFPS, denitrification is probably more influenced by the diffusion of the NO_3 , even at high concentrations to the active denitrification sites instead of its availability (Yoshinari, Hynes, and Knowles 1977). This is because, increases in WFPS generally results in more denitrification with maximum emissions occurring at WFPS values >60% (Davidson, Rogers, and Whitman 1991).

Table 2: Repeated measures ANOVA on the effect of WFPS, Soil type and N- application rate on N₂O flux, Cumulative N₂O emissions and Fertilizer derived emission factors.

Source	df	F	P
N ₂ O Flux			
WFPS	1	25.63	<.001
N- application rate	2	0.44	0.649
Soil type	2	32.33	<.001
WFPS*Soil type	2	24.63	<.001
WFPS*N application rate			
Cumulative N ₂ O Emissions			
WFPS	1	30.32	<.001
N- application rate	2	1.41	0.255
Soil type	2	37.75	<.001
WFPS*Soil type	2	27.87	<.001
WFPS*N application rate	2	2.02	0.143
Fertilizer induced emission			
WFPS	1	8.82	0.006
N- application rate	1	1.88	0.179
Soil type	2	11.54	<.001
WFPS*Soil type	2	8.86	<.001
WFPS*N application rate	1	3.36	0.076

df, degrees of freedom; *F*, F statistic; *P*, probability level.

$P < 0.05$ determines significance

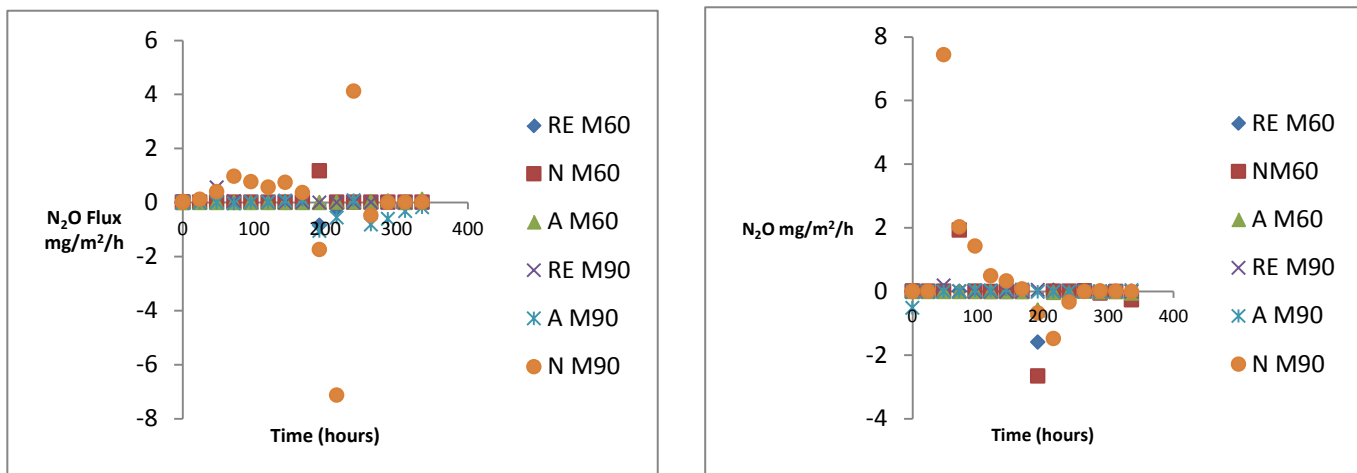


Figure 1: N₂O Flux under 0, 60 and 90%WFPS with no fertilizer addition, 75kg N/ha (left) and 150 kg N/ha (right).

Effect of N-Application Rate on N₂O flux

Increased availability of NO₃ should increase denitrification (Barnard, Leadley, and Hungate 2005) however N- application rate was not significant to the production of N₂O in this trial (Table 2). N₂O flux however increased non-linearly with increasing N application rate only in

the 90% WFPS but was highly variable to N addition in 60% WFPS treatment ranging from slightly positive to highly negative values (Figure 2). (Tiedje 1988) explains the lack of response to denitrification to fertilization because labile C may be limiting in fertile mineral soils. Negative responses to N addition may occur due to competition between heterotrophic bacteria for labile C where denitrifiers may lose (Barnard, Leadley, and Hungate 2005).

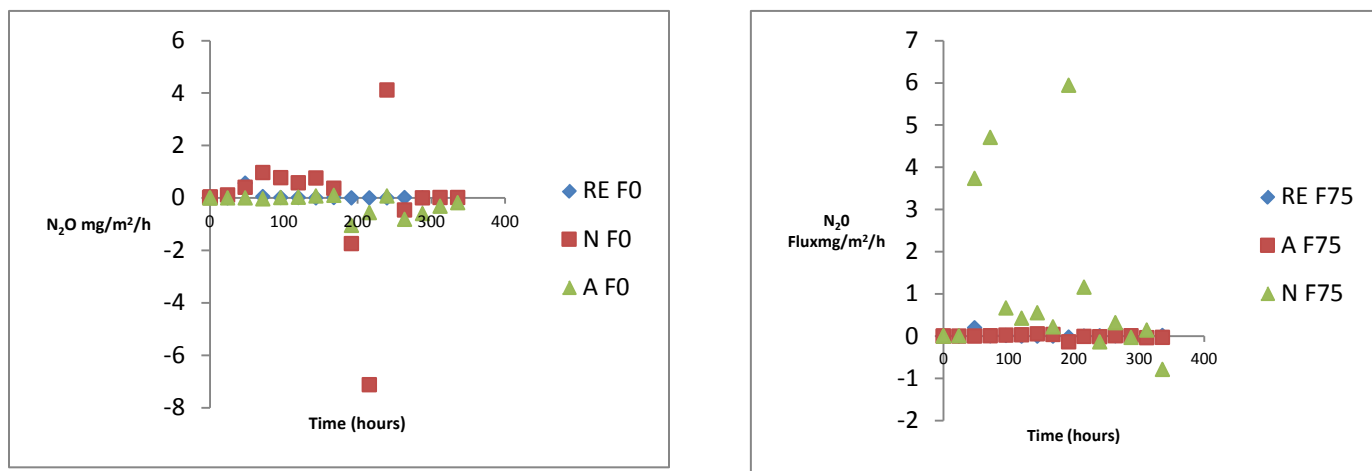
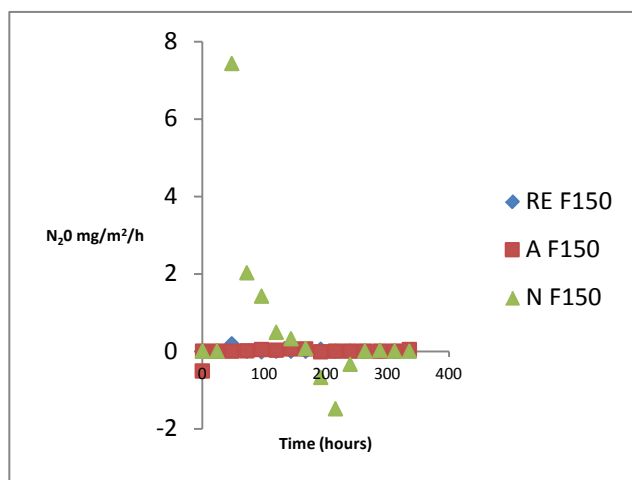


Figure 2: Effect of N-Application rate under 90%WFPS



Cumulative N₂O Losses and Soil type effect on N₂O Flux WFPS, soil type and their interaction had significant effects on cumulative N₂O losses; N –application rate did not (Table 2). This suggests that factors that determine soil aeration such as soil moisture and soil texture strongly regulates N₂O production (Pihlatie et al. 2004). Evidence of this is seen where cumulative N₂O losses was greatest in all the Nariva soil treatments at 90% WFPS followed by those under 60% WFPS (except when 75kg N/ha was applied). Arena sand had the lowest N₂O losses. Soil characterization of the Nariva soil (Table 1) revealed greater concentrations of NO₃ and NH₄ in addition to soil organic carbon compared to the other soil types, all necessary for N₂O production.

This soil also possessed the greatest clay content indicating poor aeration, again a condition favorable for denitrification (Barnard, Leadley, and Hungate 2005) . Additionally, (Chen et al. 2014) also suggests higher N₂O production in heavier textured soils possibly because they

exhibit stronger anaerobic conditions over longer periods than the lighter textured soils. This can also explain N₂O production in the unfertilized treatments due to N mineralization. Soil moisture (%WFPS) is also seen to be a strong regulator since the cumulative emissions for all soil types were generally greater in the 90% WFPS treatments.

WFPS, soil type and their interaction had significant effects on cumulative N₂O losses; N – application rate did not (Table 2). The emissions were low for all treatments with the highest being 3.2% in the Nariva treatment fertilized with 75kg N/ha at 90%WFPS followed by the other Nariva treatments that received 150kgN/ha; (0.4% under 90% WFPS and 0.3% under 60%WFPS).

CONCLUSION

This study evaluated the effect of increasing moisture content and N-fertilization on N₂O emissions in three tropical soils. Increases in N-fertilizer application rate did not have significant effects on N₂O however soil moisture did with highest emissions under 90% WFPS compared to 60%WFPS. Soil type also had a significant effect with the greatest emission from the Nariva soil possibly due to greater clay content, concentrations of NO₃ and NH₄ in addition to soil organic carbon compared to the other soil types, all necessary for N₂O production.

Peak emissions occurred at later dates in the trial for the wetter soils (90%WFPS) but only in the unfertilized jars. The jars that received the greatest concentration of N-fertilization had peak emissions occurring at later dates for the drier (60%WFPS) treatments. Soil moisture and soil type both had significant effects on cumulative N₂O losses and fertilizer derived emission. Cumulative N₂O losses was greatest in all the Nariva soil treatments at 90% WFPS followed by those under 60% WFPS (except when 75kg N/ha was applied). Arena sand had the lowest N₂O losses. Fertilizer derived emissions were low for all treatments with the highest being 3.2% in the Nariva treatment fertilized with 75kg N/ha at 90%WFPS followed by the other Nariva treatments that received 150kgN/ha (0.4% under 90% WFPS and 0.3% under 60%WFPS). Generally, this experiment concluded that N₂O emissions may be more closely related to soil properties and soil moisture than to the N application rate.

This experiment investigated some key regulating factors of N₂O production under controlled laboratory conditions. Further research is still needed to adequately understand how these factors would control N₂O production under field conditions for different crops and management practices. These studies would assist in adequately selecting agricultural practices that would ultimately reduce N₂O emissions in Trinidad.

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