

Nitrogen fertilization and of N₂O emission in corn production in the Comarca Lagunera

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Abstract

An experiment was established to quantify the level of N₂O emissions in the soil in response to the application of increasing doses of nitrogen fertilization for corn production. The treatments were as follows: 1) control, without application of nitrogen (N); 2) application of 200 kg ha⁻¹ of N; 3) application of 400 kg ha⁻¹ of N. An experimental design in random blocks with three repetitions was used. The corresponding treatments with forage corn were established in July 2018. To carry out the measurements of N₂O emissions, two PVC static chambers were placed in each experimental unit: one at the ridge (B), where the fertilizer was applied, and another at the bottom of the furrow (F). Periodic measurements were carried out and at the same time soil samples from 0 to 15 cm deep were collected to measure moisture content and inorganic N (N-NO₃⁻ + N-NH₄⁺) and soil temperature. The content of inorganic N of the soil fluctuated on average from 28 to 59 mg kg⁻¹ of soil and the flux of N₂O emissions fluctuated from 22 to 60 g ha⁻¹ day⁻¹. Both were closely related to the increasing doses of nitrogen fertilization, both at the ridge and at the bottom of the furrow, the R² fluctuated between 0.81 and 0.99. The trend was adjusted to an exponential model. Dry forage and grain production reached 17.5 and 9.6 t ha⁻¹, respectively, with the highest nitrogen fertilization dose. There were no significant differences ($p < 0.05$) between 200 and 400 units of N ha⁻¹, which indicated a low utilization of N by the crop and a higher emission of N₂O with the latter.

Keywords: greenhouse gas emission, fertilization, soil nitrogen.

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Introduction

The Comarca Lagunera is an area comprising five municipalities of the state of Coahuila and 10 of Durango. It is the most important region in milk production in the country, so the demand for fodder for livestock feeding is important (Yescas *et al.*, 2015). In 2020, around 68 000 ha of forage maize (*Zea mays* L.) and a similar area for grain production were established (SIAP, 2020), of which 64% were grown with gravity irrigation and 36% with pumping of deep well. Due to the low rainfall, which is 225 mm per year, irrigation is required for cultivation, so the production systems that prevail are generally intensive and technified.

The atmospheric concentration of nitrous oxide (N₂O) in the pre-industrial period was 269 parts per billion (ppb); however, in 2021 the atmospheric concentration of this gas is estimated at 334 ppb (NOAA, 2021). Approximately 70% of the world's annual anthropogenic emissions are derived from agricultural production (Signor *et al.*, 2013). According to González-Estrada and Camacho-Amador (2017), in Mexico the N₂O emissions produced by the applications of nitrogenous chemical fertilizers in agriculture represent around 25 000 Gg annually.

The use of agricultural inputs is also excessive, especially the use of nitrogenous fertilizers, due to the low content of organic reserves that agricultural soils frequently contain (Cueto *et al.*, 2006). However, the irrational use of fertilizers causes serious environmental pollution problems, mainly nitrate leaching (N-NO₃⁻) and NO_x emissions, especially nitrous oxide (N₂O) (Saynes-Santillán *et al.*, 2016). The first contributes to the pollution of aquifers, by leaching, and the second, considered as a powerful greenhouse gas, which contributes to a greater extent to the phenomenon of global climate change.

Another important factor that adds to the problem of contamination in the dairy basin of the Comarca Lagunera is the high production of bovine manure. According to Figueroa-Viramontes *et al.* (2009) and Figueroa-Viramontes *et al.* (2015), in the Comarca Lagunera, about one million t year⁻¹ are produced, on a dry basis, while the excreted N is estimated at 44 154 t year⁻¹. According to the authors, the regional balance expressed as N incorporated and the N requirement for the production of forages is 187 kg ha⁻¹.

This indicates that the incorporation of this byproduct into the farmland, in theory, should require the application of a moderate dose of nitrogenous chemical fertilizer to achieve maximum yields; however, the irrational use of this input in the region is widespread, so the environmental impact caused by the excess of N, which is supplied in agricultural production systems, is serious. Acevedo-Peralta (2017) mention that up to 150 t ha⁻¹ of fresh manure, without prior treatment, are applied to agricultural soils in the region for corn production.

In a study carried out in the Comarca Lagunera, N¹⁵ was used, finding that forage corn recovers between 30 and 50% of the N applied as fertilizer (Cueto *et al.*, 2013). Another study conducted in the region with Sudan grass (*Sorghum sudanense* (Piper) Stapf) showed similar results (Quiroga-Garza *et al.*, 2010). According to Figueroa-Viramontes *et al.* (2015), the efficiency of the use of N by the crop, from the chemical fertilizer, in the production of forage corn in the region is 40%, meanwhile the N that is applied from the bovine manure incorporated into the soil barely reaches

15%. Saynes *et al.* (2016) point out that agricultural activities are the third leading cause of generation of greenhouse gas emissions, with a contribution of 12% to national emissions, most of them generated by enteric fermentation, manure management and the use of fertilizers in production areas.

The losses of N in the form of N₂O emissions in the production systems of forage corn have not been quantified in the Comarca Lagunera, so it is hypothesized that they contribute significantly to the greenhouse gas emissions emitted by the agricultural sector. The objective of this study was to evaluate the flux of this gas (N₂O) in response to increasing doses of nitrogen fertilization applied to forage corn, under the conditions of the Comarca Lagunera.

Materials and methods

An experiment under irrigation conditions was conducted in the 2018 summer cycle at the Campo Experimental La Laguna (CELALA), located in Matamoros, Coahuila, Mexico. The Experimental Field is in the southwest of the state, at coordinates 103° 13' 4" west longitude and 25° 31' 41" north latitude, at an altitude of 1 110 masl. The average annual temperature is 22.6 °C, while the average annual rainfall is 258 mm (Villa-Castorena, 2005).

Physicochemical characteristics of the soil

The soil was clay loamy, with 47% sand, 21.8% silt and 31.3% clay. The field capacity was estimated at 26.5% and the permanent wilting point at 13.1%, with a usable moisture retention capacity of 13.1% (Pedroza-Sandoval *et al.*, 2015). The pH was alkaline (8.4), with an electrical conductivity of 0.52 dS m⁻¹ and had a very low content of organic matter (1.1%). The content of extractable P and inorganic N (N-NO₃+N-NH₄) were about 13 mg kg⁻¹ of soil.

Treatments and experimental design

Three treatments were evaluated: (1) without nitrogen fertilization; (2) 200 units of N ha⁻¹; and (3) 400 units of N ha⁻¹ (Table 1). Farmers in the Comarca Lagunera frequently apply 400 or more units of N ha⁻¹ for corn production. In the evaluated treatments, the N₂O emissions from these increasing doses of nitrogen fertilization were studied. An experimental design in completely random blocks with three repetitions was used. Statistical analysis was performed using the software: Statistical Analysis System (SAS) version 9.4 (SAS institute, 2008). The comparison of means was made using Tukey's test, $\alpha=0.05$.

Table 1. Evaluated treatments.

Treatment	Units of N (kg N ha ⁻¹)	Urea (kg ha ⁻¹)
1	0	0
2	200	435
3	400	970

All treatments received 90 kg ha⁻¹ of P₂O₅ in the form of phosphoric acid (52% P₂O₅).

Agronomic management

During the 2017 winter cycle, unfertilized forage oats were established at the experimental site, with the aim of homogenizing the edaphic conditions of the soil. Subsequently, tillage work was performed and the leveling of the terrain was carried out with Laser Plane[®] equipment. The terrain was fallowed with vertical plow and then two passes of cross harrow were performed, finally the furrows were made with a separation between them of 0.76.

Sowing and fertilization

The sowing was carried out in dry soil on July 12, 2018, with a Gaspardo pneumatic precision planter supreme model. The variety of white corn ‘Cimarrón’ of Asgrow[®] was used. The seed was treated one day before sowing with 500 ml of Thiamethoxam (350 g L⁻¹ of ia.) per 100 kg of seed, to prevent the attack of soil pests. The sowing density was 105 000 seeds per hectare (8 seeds per linear meter).

One hundred percent of the phosphorus and 50% of the dose of N corresponding to each treatment were applied at the time of sowing and immediately afterwards the establishment irrigation was applied. At 34 days after sowing (DDS), a cultural practice and ridging were carried out with a moldboard plow in order to break the ‘crust’ of the soil, eliminate weeds and apply the second nitrogen fertilization (50% remaining), to later apply the first supplemental irrigation (August 15).

Irrigation

Irrigation was carried out by gated pipe supplied by a well. The total volume of irrigation was estimated at 0.84 m. An over-irrigation to favor the emergence of the seed and four supplemental irrigations during the growth cycle were applied as shown in Table 2. The number of irrigations was carried out based on the water needs of the crop and the low retention of moisture of the sandy soil (13.1%).

Table 2. Frequency of irrigation, periodicity and irrigation volume.

Type of irrigation	Date	Days after sowing	Irrigation volume applied (cm)	Accumulated irrigation volume (cm)
Sowing irrigation	July 12	0	18	18
Over-irrigation	July 23	10	09	27
1° supplement	August 15	34	18	45
2° supplement	September 9	59	14	59
3° supplement	September 25	75	12	71
4° supplement	October 8	88	13	84

Pest and weed control

Pest monitoring in the crop was carried out periodically, especially during the phenological stages of emergence, flowering and grain filling. To control the fall armyworm (*Spodoptera frugiperda*), permethrin (0.4% GR) granulates were directly applied to the bud (‘salereado’), when the crop was

15 DDS, subsequently, three applications of chlorpyrifos-ethyl 44.5% CE were made at 25, 33 and 65 DDS. At 60 DDS, a mixture of imidacloprid (19.6%) + betacyflutrim (8.4%) was applied in doses of 500 ml ha⁻¹ for the control of corn rootworm (*Diabrotica ssp*) and corn earworm (*Heliothis zea*). For the control of red spider, two applications of Abamectin (18 g ia. L⁻¹) were made at a dose of 1 L ha⁻¹ at 8 and 32 DDS.

Weed control was mainly performed manually, however, two herbicide applications were made: The first at 34 DDS in doses of 1 L ha⁻¹ of dimethylamine (2,4 D 49.4% S) in 300 L of water for broadleaf weeds, the second at 51 DDS, Nicosulfuron (4.21%) for narrow-leaf weeds, especially Johnson grass (*Sorghum halepense*).

Meteorological and soil variables

Temperature and precipitation data were recorded in the automated meteorological station of the Experimental Field throughout the crop cycle, the dynamics of inorganic N (N-NO₃⁻ + N-NH₄⁺) in the soil from 0 to 15 cm depth was also recorded, as well as the gravimetric moisture. Soil samples were collected around the PVC chambers during each gas sampling.

Measurement of nitrous oxide emission

Two PVC static flux chambers (Matson *et al.*, 1996) of six inches in diameter and volume of 3 216.9 ml were permanently placed in each experimental plot during the cultivation cycle. These were placed in the central furrow of each plot: one at the top of the ridge (B) and one at the bottom of the furrow, to evaluate the level of N₂O emissions in both conditions of the farmland (F) (Figure 1). Each chamber had a lid to close it hermetically when taking gas samples, which were performed once or twice a week.



Figure 1. Placement of the static flux chambers at the ridge (B) and at the bottom (F) of the furrow with the necessary material for gas sampling.

On each date, gas samples were collected at 0, 10, 20 and 30 min, using syringes of 20 cm³, which were introduced through a septum strategically located in the lids of the chambers. Before taking the first sample (time zero), the needle was inserted through the septum of the chamber and by

pumping the syringe, the air from the internal atmosphere of the chamber was mixed. After this, the samples were taken at the specified times. The samples were stored in sealed amber glass vials (14 ml) previously depressurized.

The gas samples were analyzed in a gas chromatograph (Shimadzu Model GC-2014 Greenhouse gas Analyzer). The chromatograph is equipped with an electron capture detector (ECD) that has a radiation source of ^{63}Ni and operates at a temperature of 325 °C. For the calibration of the chromatograph, a certified standard (Scotty Analyzed Gases, USA) with a concentration of 1 ppm of N_2O was used. For internal quality control, a standard with a concentration of 0.54 ppm of N_2O was used.

All gas samplings were performed at 10:00 am. Before each sampling, the ambient temperature and a blank sample were taken, which was collected from the environment outside the chambers. The evaluation of N_2O emissions was calculated according to Millar *et al.* (2018), using the following equation: $\text{N}_2\text{O} = (\alpha \times V \times \text{WA} \times 60) / (A \times \text{MVcorr})$. Where, α is the slope of the concentration line vs time obtained in each sampling and it is measured in (ppmv min^{-1}), V is the volume of the upper space of the chamber (L), WA is the mass of N in N_2O (28), 60 is the conversion from hours to minutes, A is the surface area of the ground covered by the chamber (m^2) and MVcorr is the standard molecular volume corrected by temperature and pressure of the locality.

The fluxes per hour and per m^2 were converted to daily fluxes per hectare ($\text{g N}_2\text{O-N ha}^{-1} \text{d}^{-1}$). The average fluxes between one measurement date and another, by treatment, were obtained. The quantity of N_2O accumulated was calculated from the following equation: $C_B = C_A + \left(\frac{D_A + D_B}{2}\right) * (B - A)$. Where: C_A = accumulated gas emission on day A prior to day B, D_A = daily gas flux on day A, D_B = daily gas flux on day B, A/B = day number.

Soil moisture and measurement of inorganic N

At the same time as N_2O emission measurements were made in the field, soil samples (0-15 cm) were collected and placed in aluminum canisters previously weighed and labeled, both at the ridge and at the bottom of the furrow on 24 occasions. The gravimetric moisture of the soil was estimated, for which the samples were first weighed wet at the sampling site, then they were dried in an oven for 24 h at 105 °C, and then the dry weight was recorded. Before drying the soil samples, a wet subsample of 9 g was extracted and 90 ml of KCl 1M was added and stirred (1 min).

Subsequently, it was left to rest for 24 hours at room temperature and stirred again (1 min), it rested (60 min), then it was filtered (Whatman® glass microfiber 1 μm GF/B). An aliquot (20 ml) was transferred to a polypropylene cup and subsequently evaluated for inorganic N content ($\text{N-NO}_3 + \text{N-H}_4$) by steam and Kjeldahl distillation (Jackson, 1976).

Results and discussion

The temperature and precipitation conditions that prevailed throughout the corn cultivation cycle are shown in Figure 2. The volume of rainfall during 2018 was 282.2 mm, 30 mm above the average monthly rainfall. From July 12 to October 11, the period that comprised the crop cycle, the average

diurnal temperatures were warm and fluctuated between 20 and 25 °C, while the distribution of rainfall was irregular and was only 136.4 mm, 48.3% of the total annual precipitation. The strongest event occurred on September 14, with 48.8 mm of rainfall (Figure 2). On the other hand, the average monthly temperatures, during the same period, fluctuated between 25.1 and 27.5 °C, with August being the warmest month.

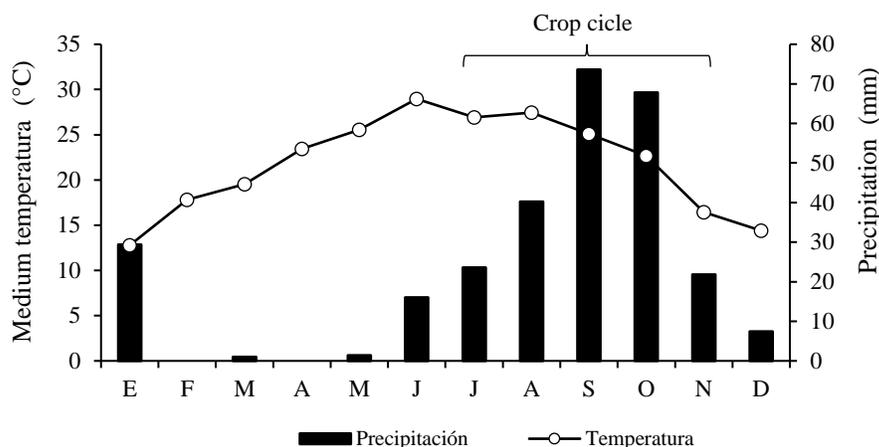


Figure 2. Ombrothermic diagram of the Experimental Field La Laguna, Matamoros, Coahuila, 2018.

Content of inorganic N in the soil

The average content of inorganic N of the soil ($\text{N-NO}_3^- + \text{N-H}_4^+$) was closely related to the applied doses of nitrogenous fertilizer, both at the ridge and at the bottom of the furrow. The trend was adjusted to an exponential statistical model, obtaining a correlation coefficient (R^2) that fluctuated between 0.81 and 0.99 (Figure 3). The average content of inorganic N in the soil, which can be considered as a base, in the treatment where no fertilizer was applied, was 28 mg kg^{-1} of N. With the application of $200 \text{ kg of N ha}^{-1}$, the availability of this element increased about 60%, with respect to the previous treatment, and about double when $400 \text{ kg of N ha}^{-1}$ were applied.

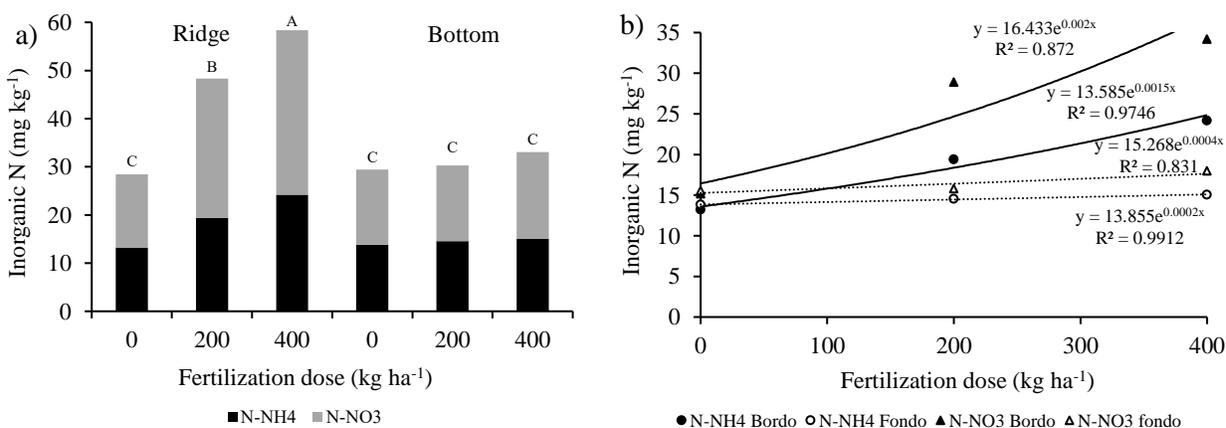


Figure 3. Average content of inorganic N. a) N-NO_3 and N-NH_4 at the ridge and at the bottom of the furrow; b) relationship between the content of N-NO_3 and N-NH_4 and dose of nitrogen fertilization at the ridge and at the bottom of the furrow.

However, at the bottom of the furrow the increase in N was more tenuous, barely more noticeable in the treatment with application of 400 kg of N ha⁻¹ and there were no significant differences between treatments ($p < 0.05$) for this sampling site.

In general terms it was observed that, of the total inorganic N, the fraction in the form of N-NO₃ was on average 60% higher in the treatments with application of 200 and 400 kg ha⁻¹ of fertilizer, and the rest corresponded to the ammoniacal form (N-NH₄). A high nitrate content in the soil when not synchronized with crop demand is usually associated with an increased nitrogen loss by leaching and emission into the atmosphere. The nitrate concentrations found in this study coincide with those reported by Millar *et al.* (2018).

Daily N₂O emissions

The average results of the flux of N₂O from the soil, in the corn production system, showed that there were significant differences ($p < 0.05$) due to the effect of the treatments. The flux of this greenhouse gas increased as a function of nitrogen fertilization doses, and the trend fitted an exponential model, both at the ridge and at the bottom of the furrow (Figure 4a). It was observed that the flux of N₂O in the treatment where no fertilizer was applied, in both sites, was similar ($p < 0.05$), with 22 g ha⁻¹ day⁻¹. In contrast, with the application of 400 units of N at the ridge, this was more than triple, while, at the bottom of the furrow, this difference was more than double; evidently there was migration of the N applied as fertilizer from the ridge towards the bottom of the furrow.

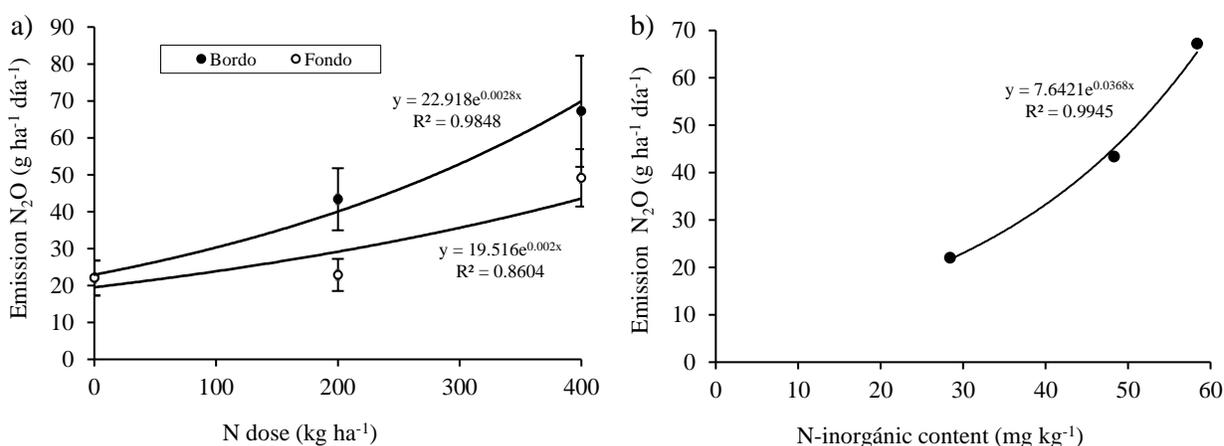


Figure 4. a) relationship between the average N₂O emission and the fertilization dose at the ridge and at the bottom of the furrow; b) relationship between average N₂O emission and average content of inorganic N of the soil (N-NO₃⁻+N-NH₄⁺).

The average N₂O emission corresponding to the ridge, where the fertilizer was placed, was closely related ($R^2 = 0.99$) to the content of inorganic N of the soil. These results coincide with that reported by Millar *et al.* (2018) in that the application doses of nitrogen fertilizers, in this case of 400 units ha⁻¹ or more, at least double the emissions of N₂O, increase production costs and reduce the efficiency in the use of N by plants, which can be 40% or less (Peña-Cabriales *et al.*, 2001).

The N_2O emissions reported in various investigations are highly variable, even in the case of the same corn crop. No doubt this is due to the great variability of environments in which it is grown, the equally diverse doses and fertilization technology used, and the methods used in measuring emissions. The results of this research coincide with what was reported by Millar *et al.* (2018) and Halvorson *et al.* (2010); however, the maximum level of nitrogen fertilization that these researchers evaluated was 280 kg ha^{-1} . Perhaps for this reason, in this research a higher emission of N_2O ($270 \text{ g ha}^{-1} \text{ day}^{-1}$) was obtained at the ridge with the application of 400 kg of N ha^{-1} (Figure 4b). On the other hand, Alluvione *et al.* (2014) report a flux of N_2O of up to $800 \text{ g ha}^{-1} \text{ day}^{-1}$ with the application of only 130 kg ha^{-1} of urea.

The dynamics of N_2O flux throughout the cultivation cycle, in this evaluation, show that it increased rapidly after the application of the N fertilizer and irrigation events ($> 60\%$) (Figure 5). With the soil saturated with moisture, the potential of N_2 emission is favored, rather than N_2O as the final product of denitrification (Bouwman, 1998; Robertson and Groffman, 2015). Denitrification is the reduction of the nitrate of the soil to gases NO , N_2O and N_2 . A wide variety of bacteria, mostly heterotrophic, can denitrify, so they use NO_3 instead of oxygen (O_2) as a terminal electron acceptor during respiration.

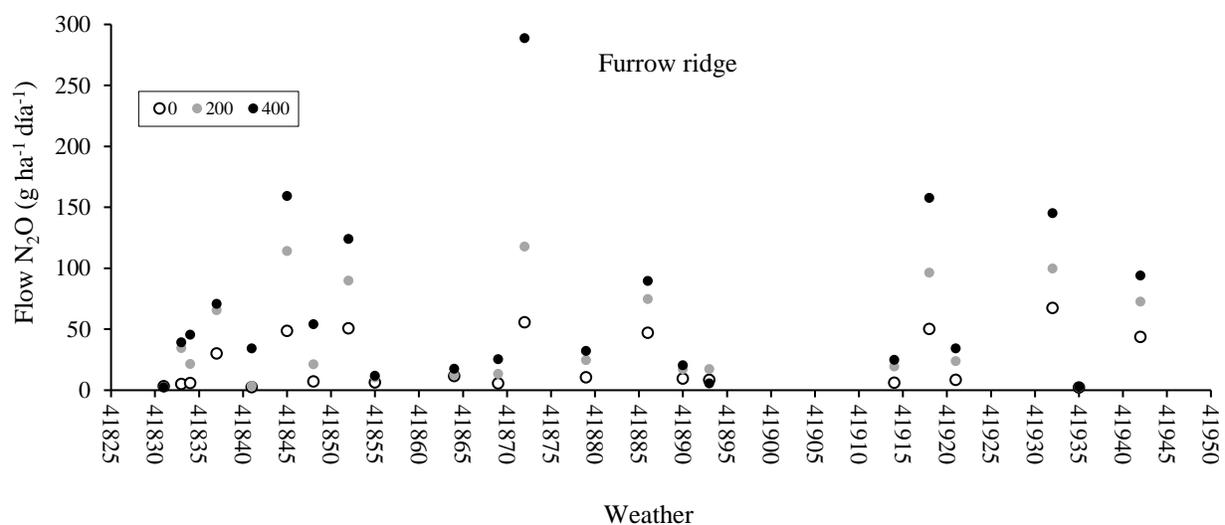


Figure 5. N_2O emissions of the soil in response to the application of increasing doses of nitrogen in forage corn in the Comarca Lagunera.

Because nitrate is a less efficient electron acceptor than O_2 , most denitrifiers undertake denitrification only when O_2 is not available, which occurs when drainage in the soil is insufficient when it is saturated with water (Robertson and Groffman, 2015).

After applying the second fertilization the phenomenon occurred again; however, a few weeks before the end of the cycle, other peaks of $100 \text{ g ha}^{-1} \text{ day}^{-1}$ are observed at the ridge (Figure 6). The high inherent spatial variability of N_2O emissions associated with large N inputs in agricultural production systems has been reported by Parkin (1987); Riley *et al.*, (2001).

The weighted daily emissions increased according to the level of fertilization and then decreased rapidly to the flux level at the bottom of the furrow, where the fertilizer was not applied, that is, to 50 g ha⁻¹ day⁻¹ or less (Figure 6), due, on the one hand, to the desiccation of the soil, which limits the microbial activity, and on the other hand, to the fact that it was already consumed by the plants or lost by the different routes of the N cycle.

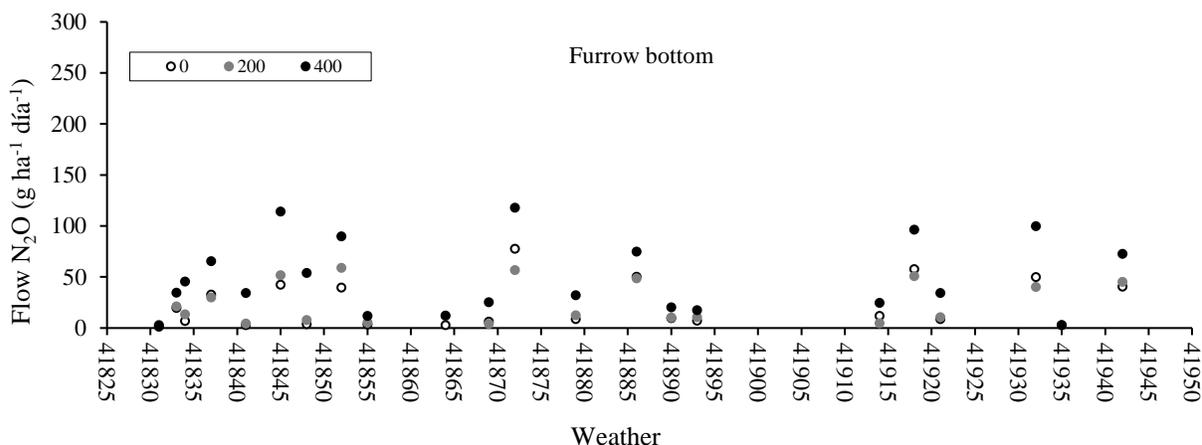


Figure 6. N₂O emissions of the soil in response to the application of increasing doses of nitrogen in forage corn in the Comarca Lagunera.

Accumulated N₂O emission

The N₂O emission accumulated during the culture cycle was exponentially related to the fertilization doses at the ridge (R²= 0.98) and at the bottom of the furrow (R²= 0.75) (Figure 7a). This behavior coincides with what is reported by Hoben *et al.* (2011) and Millar *et al.* (2018), who also reported the same trend in N₂O emissions. However, the highest level of N₂O emission that was reached in this study: 7 000 g ha⁻¹ of N₂O at the ridge and 4 600 g ha⁻¹ of N₂O at the bottom of the furrow, compared to the cited studies, can be attributed to the higher dose of N applied, to the high temperature (maximums up to 45 °C) and the humidity available during the crop cycle (six irrigations were applied).

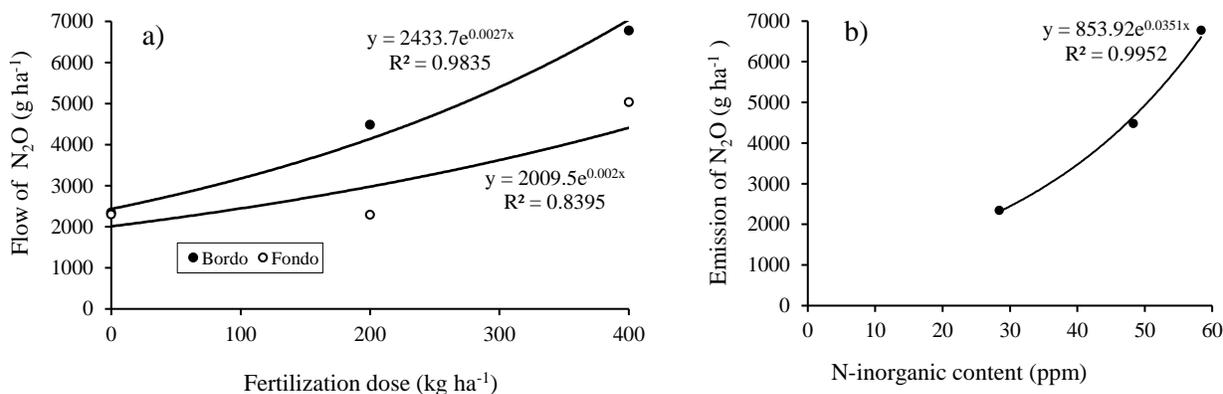


Figure 7. a) relationship between accumulated flux of N₂O and dose of nitrogen fertilization; b) relationship between accumulated N₂O emission and content of inorganic N of the soil.

There was also a high correlation at the level of concentrations of inorganic N in the soil and N₂O emissions ($R^2= 0.99$) (Figure 7b), but not necessarily greater use of N by the crop. These data coincide with what was published by Saynes *et al.* (2016), where they suggest that the GHG inventory in Mexico for the case of agriculture is underestimating N₂O emissions, since the emissions measured in the field were 5.3 kg N₂O-N ha⁻¹, which were not significantly different from 4.7 kg N₂O-N ha⁻¹ estimated by the Nitrogen Index ($p < 0.7$).

Crop yield

Forage and grain production increased as a function of N dose (Table 3). There were significant differences ($p < 0.05$) between the treatments without application of N (1) and the application of 200 and 400 kg of N (2 and 3), about double; however, between 200 and 400 kg of N (2 and 3), there were no significant differences ($p < 0.05$). These results suggest that 400 kg ha⁻¹ of N is an excessive dose, which was clearly manifested in the level of N₂O emissions, which increased exponentially with the increased application of N. According to Castellanos *et al.* (2005), the nutritional demand for N of the corn crop is 22.5 kg N per tonne of grain produced on one hectare.

Table 3. Yield of forage and grain in the evaluation test of N₂O emission. 2014 summer cycle. Matamoros, Coahuila.

Treatment	Fertilization	Fresh forage	Dry forage	Grain yield
	(kg ha ⁻¹)		(t ha ⁻¹)	
1	0	24.3 b*	8 b	3.1 b
2	200	50.6 a	16.7 a	8.4 a
3	400	53 a	17.5 a	9.6 a

*= Equal letters are statistically similar ($\alpha = 0.05$).

According to the highest level of grain production in this study (9.6 t ha⁻¹), the internal requirement of the crop was just over 200 kg ha⁻¹, so 400 units of N ha⁻¹ implied higher emission of N₂O. The soil, with 47% sand, necessarily favors the flow of water and nutrients to the interior of the profile, so the loss of the N that was applied in the fertilizer can be greater than in soils with less sand. Based on the above, it is necessary to implement agronomic management practices to increase the rate of use of N by the crop in the region. A more fractioned application of nitrogen fertilization or use of slow-release fertilizers could be alternatives to reduce N₂O emissions to the environment.

Conclusions

N₂O emissions increased exponentially as a function of N application doses and were closely related to the content of inorganic N of the soil. The concentration of inorganic N in the soil significantly increased in response to the inorganic N present in the soil before sowing and to the doses of N applied as fertilizer. In turn, N₂O emissions increased exponentially and with high correlation with the content of inorganic N of the soil. Dry forage and grain production reached 17.5 and 9.6 t ha⁻¹, respectively, with the highest nitrogen fertilization dose. There were no significant differences ($p < 0.05$) between 200 and 400 units of N ha⁻¹; which indicated a low utilization of N by the crop and a higher emission of N₂O with the latter.

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