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SHORT COMMUNICATION

Soil N_2O emissions and $N_2O/(N_2O+N_2)$ ratio as affected by different fertilization practices and soil moisture

E. Ciarlo · M. Conti · N. Bartoloni · G. Rubio

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Abstract The objective of this work was to evaluate the effect of the chemical nature and application frequency of N fertilizers at different moisture contents on soil N_2O emissions and $N_2O/(N_2O+N_2)$ ratio. The research was based on five fertilization treatments: unfertilized control, a single application of 80 kg ha^{-1} N-urea, five split applications of 16 kg ha^{-1} N-urea, a single application of 80 kg ha^{-1} N–KNO_3, five split applications of 16 kg ha^{-1} N–KNO_3. Cumulative N_2O emissions for 22 days were unaffected by fertilization treatments at 32% water-filled pore space (WFPS). At 100% and 120% WFPS, cumulative N_2O emissions were highest from soil fertilized with KNO_3. The split application of N fertilizers decreased N_2O emissions compared to a single initial application only when KNO_3 was applied to a saturated soil, at 100% WFPS. Emissions of N_2O were very low after the application of urea, similar to those found at unfertilized soil. Average $N_2O/(N_2O+N_2)$ ratio values were significantly affected by moisture levels ($p=0.015$), being the lowest at 120% WFPS. The $N_2O/(N_2O+N_2)$ ratio averaged 0.2 in unfertilized soil and 0.5 in fertilized soil, although these differences were not statistically significant.

E. Ciarlo (✉) · M. Conti
Cátedra de Edafología, Facultad de Agronomía de la Universidad de Buenos Aires (FAUBA),
Av. San Martín 4453,
1417 Buenos Aires, Argentina
e-mail: ciarlo@agro.uba.ar

N. Bartoloni
Cátedra de Métodos Cuantitativos Aplicados, FAUBA,
Buenos Aires, Argentina

G. Rubio
Cátedra de Fertilidad y Uso de Fertilizantes, FAUBA,
Buenos Aires, Argentina

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Introduction

Nitrogen fertilization is one of the main factors controlling N_2O emissions (Dittert et al. 2005; Stehfest and Bouwman 2006). Although the effect of the chemical nature of N fertilizers on denitrification and N_2O emissions is still subject of study (Eichner 1990; Stehfest and Bouwman 2006), it has been observed that nitrate (NO_3^-)-based fertilizers may lead to high N losses from predominantly anaerobic soils (Scheer et al. 2008), whereas the application of ammonium sulfate to aerobic soils can increase N_2O emissions up to 25.7 times (Trujillo-Tapia et al. 2008). The influence of the chemical nature of N fertilizers on the $N_2O/(N_2O+N_2)$ ratio is poorly understood. Weier et al. (1993) found that NO_3^- application caused a decrease in N_2O reduction to dinitrogen (N_2). In sandy clay loam soils, Estavillo et al. (2002) found that the application of calcium ammonium nitrate (CAN) increased $N_2O/(N_2O+N_2)$ ratio with respect to unfertilized soils. On the contrary, Ellis et al. (1998) concluded that N_2 represented an important portion of the final denitrification products after ammonium nitrate application, although the emissions of both gases could not be compared due to different measurement time scales.

The use of NH_4^+ (urea, ammonium mono-, or diphosphate) and NO_3^- (potassium nitrate)-based N fertilizers has significantly increased during the last years in the Pampean region, in coincidence with the increase in annual precipitation, which has raised the groundwater levels; as a consequence, some areas of the region can remain saturated during part of the year (Tanco and Kruse 2001). Under flooding conditions, the presence of a superficial water layer may promote the consumption of secondary electron acceptors as N_2O (Ciarlo et al. 2007), resulting in low $N_2O/(N_2O+N_2)$ ratio values.

The objective of this work was to evaluate the effect of the chemical nature and application frequency of N fertilizers at different moisture contents on soil N_2O emissions and $N_2O/(N_2O+N_2)$ ratio values. We tested the following hypotheses: (1) split N fertilizer applications will decrease N_2O emissions due to a reduction in the average soil $N–NO_3^-$ contents, specially with nitrate-based fertilizers and (2) the $N_2O/(N_2O+N_2)$ ratio will not be affected at oversaturation by application frequency or by the chemical nature of fertilizers because at this moisture condition, both NO_3^- and N_2O are completely reduced to N_2.

Materials and methods

Soil sampling and handling

The research was conducted with undisturbed soil cores (0–10 cm) taken from a sandy loam Typic Hapludoll located at an agricultural field at Buenos Aires Province (35°22′38.9″ S, 60°03′48.5″ O). The soil had the following properties: $N–NO_3^-$ 19.9 mg kg^{-1}; $N–NH_4^+$ 3.6 mg kg^{-1}; total organic C 8.6 g kg^{-1}; total N (Nt) 0.81 g kg^{-1}; pH H_2O 1:2.5 6.01; 75%, 10%, and 15% of sand, silt and clay, respectively; bulk density 1.102 g cm^{-3} and water-filled pore space (WFPS) at field capacity 32.11%.

Soil treatments and incubation

The experiment was conducted under laboratory conditions where temperature ranged between 18°C and 22°C. Treatments were randomly assigned to the soil cores in a completely randomized factorial design with two factors (fertilization and moisture) and with three replicates. Five fertilization treatments were carried out: unfertilized control, CK; application of 16.1 mg N-urea per soil core as a single dose at the beginning of the incubation and equivalent to a rate of 80 kg N ha^{-1}, UF; application of 16.1 mg N-urea per soil core split in five applications during the incubation period, US; application of 16.1 mg $N–KNO_3$ per soil core as a single dose at the beginning of the incubation and equivalent to a rate of 80 kg N ha^{-1}, NF; application of 16.1 mg $N–KNO_3$ per soil core split in five applications during of the incubation period, NS. The US and NS treatments involved five applications each of 16 kg N ha^{-1} at 0, 2, 5, 10, and 20 days. Potassium nitrate was chosen because it does not contain NH_4^+ and urea because it easily hydrolyzes to NH_4^+ at a wide range of soil conditions and it is the most used N fertilizer at the Pampean Region. The required N amount at 1-cm depth was injected in at least ten points of the soil cylinder with a disposable syringe; the aqueous solutions had concentrations of 1 g urea per milliliter and 0.25 g KNO_3 per milliliter, respectively.

Before fertilization, soil moisture value was adjusted by adding distilled water to soil cores. The following moisture values were reached: 32% WFPS, field capacity; 100% WFPS, saturated; and 120% WFPS, oversaturated with about 2 cm overlying surface water layer. Water-filled porosity space was calculated by considering the measured soil bulk density data (arithmetic means of five samples) and using a particle density of 2.65 g cm^{-3}. Soil moisture was maintained constant by adding water lost by evaporation.

Measurements

Denitrification emissions (N_2O+N_2) were measured by the acetylene blockage technique (Yoshinari et al. 1977). Each replication included a pair of intact soil cores, one incubated with acetylene and the other one without acetylene, and this allowed us to calculate N_2 emissions and thus the $N_2O/(N_2O+N_2)$ ratio; these measurements were performed on the same soil cores throughout. Ten percent of the headspace air was replaced with a syringe by an equal amount of acetylene at the beginning of each measurement in the acetylene-treated soil cores. Three additional jars without soil were used as blanks. Both N_2O and N_2 emissions accumulated within the jars headspaces for 24 h before being determined; jars were left open between measurements. Triplicate gas samples (2 ml) were immediately analyzed by the Gaseous Chromatograph Agilent 6890 (Palo Alto, CA, USA) equipped with ECD detector and a capillary column Carboplot; helium (He) gas was used as a carrier; the oven, injector, and detector temperatures were of 100°C, 100°C, and 250°C, respectively. Nitrogen emissions were measured at 1, 6, 8, 14, and 22 days. Daily emissions were expressed in micrograms N per kilogram per day. Cumulative emissions over the incubation period were obtained by integrating daily fluxes.

In addition to the two soil cores for gaseous emissions measurements, at each measurement day, three additional soil cores were used for chemical analysis. The pH was measured with a soil/water ratio of 1:2.5 (Thomas 1996). Nitrate-N was determined by extracting fresh soil (20 g) with 100 ml 0.25% $CuSO_4$ + 0.01 M H_3BO_3 solution; the soil mixture was filtered and the $N–NO_3^-$ content determined colorimetrically by the hydrazine reduction method (Carole and Scarigelli 1971).

Statistical analysis and calculations

Statistical Analysis System SAS package was used for data analysis (SAS Inc. Institute 1995). Daily and cumulative emissions and $N_2O/(N_2O+N_2)$ ratio were log-transformed to obtain their normality due to the high data skewness. Simple and multiple regression analysis between the measured soil variables were performed with PROC REG procedure of SAS. Daily and cumulative emissions and average $N_2O/(N_2O+N_2)$ ratio values were evaluated through conventional two-way analysis of variance with PROC GLM procedure of SAS, with means separation by Duncan test when *F* statistic was significant.

Results

Nitrate contents under split applications usually increased with time, but they were lower than with single-dose applications ($p<0.0001$), with the exception of urea application at 100% WFPS. Mean soil pH value of urea-treated soil was 5.48, and it was significantly lower ($p=0.01$) than the mean pH value of the unfertilized soil and the soil receiving split applied KNO_3 (5.65), probably due to the well-known acidifying effect of ammonia oxidation. The mean pH of soils fertilized with a single dose ok KNO_3 was 5.6.

Cumulative emissions of N_2O+N_2 generally increased by increasing moisture content ($p<0.0001$), except in the urea-treated soil, and they were highest when nitrate fertilizer was applied ($p<0.0001$; Fig. 1). Cumulative N_2O emissions were affected by moisture ($p<0.0001$) and fertilization treatments ($p<0.0001$) and by their interaction ($p=0.0002$). Cumulative N_2O emissions were unaffected by

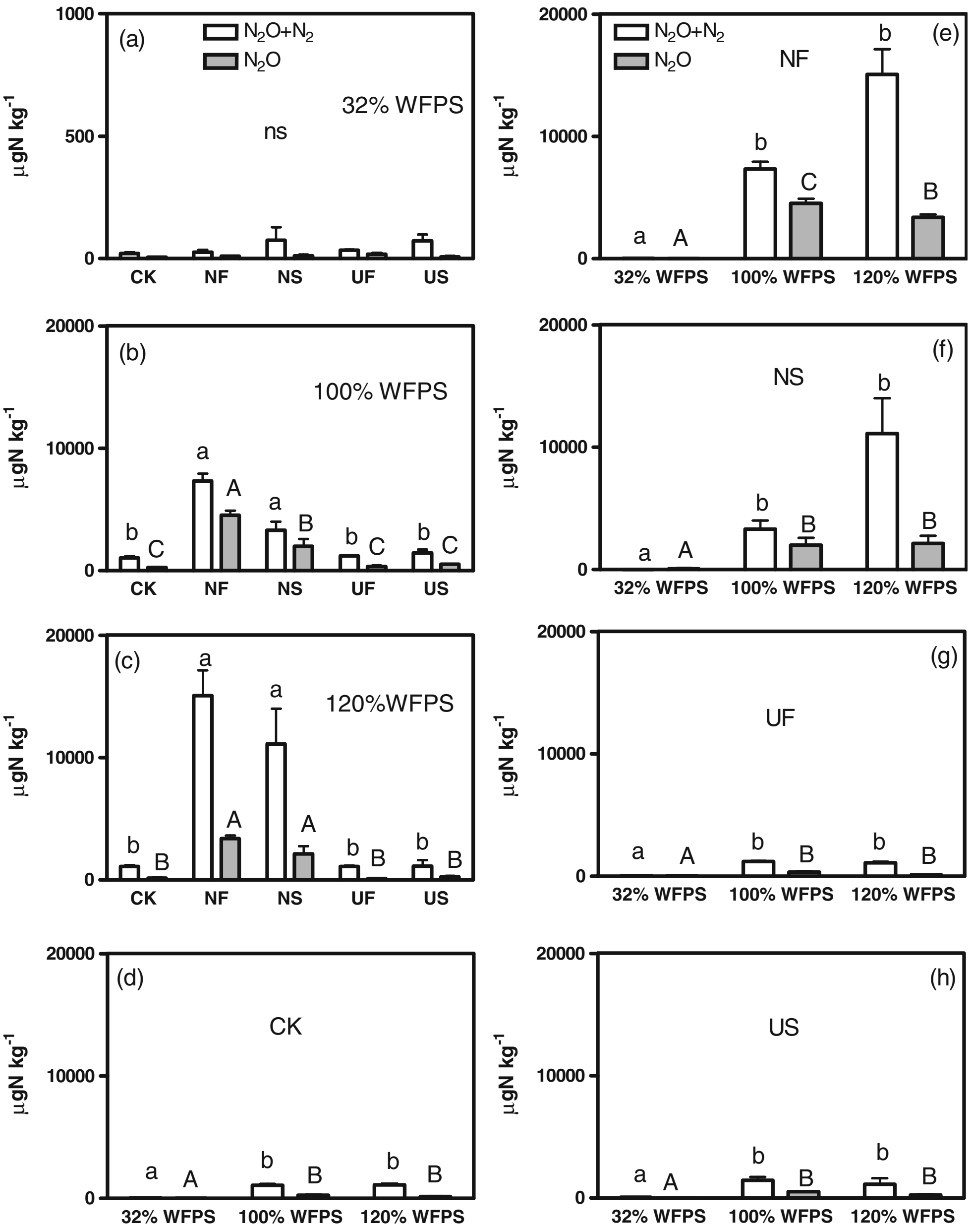

Fig. 1 Cumulative emissions of N_2O+N_2 and N_2O in soil at 32% WFPS (**a**), 100% WFPS (**b**), or 120% WFPS (**c**). Unfertilized soil CK (**d**), soil fertilized with a single full application of KNO_3 NF (**e**), soil fertilized with a split application of KNO_3 NS (**f**), soil fertilized with a single full application of urea UF (**g**), and soil fertilized with a split application of urea US (**h**). Values are the mean of three replicates, whereas *bars* represent the mean standard error. *Lower letters* indicate significant differences in cumulative N_2O+N_2 emissions ($p<0.05$), whereas *capital letters* indicate significant differences in cumulative N_2O emissions ($p<0.05$). Scale of **a** is different from scales of the other plots so as to improve data presentation

fertilization treatments at 32% WFPS (p=0.49; Fig. 1a). At 100% WFPS, cumulative N_2O emissions with the full application of KNO_3 were significantly higher than with the other fertilization treatments (p<0.0001; Fig. 1b). At 120% WFPS, cumulative N_2O emissions were the highest from soils fertilized with KNO_3 (p<0.0001; Fig. 1c).

$N_2O/(N_2O+N_2)$ ratio of daily emissions presented a high variability between treatments and along time, with extreme values of 0.00 and 3.27 (Table 1). The effect of different moisture on this ratio changed with time, but significant differences only appeared on the first day; the highest ratio was observed at 100% WFPS (p=0.003; Table 1). Average $N_2O/(N_2O+N_2)$ ratio values were significantly affected by moisture levels (p=0.015) and were lowest under 120% WFPS. The fertilization treatments had no significant effects on daily (p=0.38; Table 1) or average (p=0.47) $N_2O/(N_2O+N_2)$ ratio values (Table 1).

Soil pH was significantly related to cumulative N_2O+N_2 (p=0.02, r=0.33) and N_2O emissions (p=0.03, r=0.32) and to the $N_2O/(N_2O+N_2)$ ratio (p=0.04, r=−0.29).

Discussion

Emissions of N_2O were very low after urea application, similar to those of the unfertilized soil (Fig. 1g and h). Stehfest and Bouwman (2006) reported significantly higher N_2O emissions from soils fertilized with a NO_3^--based fertilizer (CAN) than from soils treated with other fertilizers. These low emissions with urea applications in our work are not probably related to the nitrification process, as similar NO_3^- amounts were observed in the various treatments during incubation (data not shown); probably, they depend on the low pH values after urea application, which can negatively affect denitrification rates.

The split application of N fertilizers decreased N_2O emissions with respect to a single initial application only when KNO_3 was applied to a saturated soil, at 100% WFPS (Fig. 1b). Although split applications of KNO_3 reduced mean levels of N–NO_3^- at all moisture contents (data not shown), it is probable that the aeration conditions of the soil at 32% WFPS or at 120% WFPS have limited the effect of fertilizer application frequency on soil N_2O emissions. The low N_2O emissions after N-urea application did not allow detecting differences between the application frequencies. Our results contradict the report by Weier (1999) who observed that cumulative N_2O emissions in urea-fertilized soils were higher with split applications than with a single dose application. Probably, by increasing the frequency of gas samplings and/or with longer incubation periods than those of our study, it might be possible to detect differences in the cumulative N_2O emissions between the two modalities of applying the fertilizer, as the persistence of NO_3^- in soils may favor its reduction to N_2O when conditions are favorable.

The $N_2O/(N_2O+N_2)$ ratio values were affected by the moisture level, being generally the highest under 100% WFPS. It is difficult to explain the lower ratio at 32% WFPS, as even trace amounts of O_2 can inhibit nitrous oxide reductase activity (Zumft 1997). Under oversaturating conditions, the presence of a superficial water layer limited

Table 1 The $N_2O/(N_2O+N_2)$ ratio values as affected by the different moisture and fertilization treatments during the experiment and weighed averaged by the number of days elapsed between measurements

WFPS	Fertilization	Day 1	Day 6	Day 9	Day 14	Day 21	Weighed average
32	UF	0.73B (0.46)	2.75 (2.14)	0.50 (0.27)	0.54 (0.27)	0.67 (0.67)	1.02A (0.10)
	US	0.33B (0.33)	0.23 (0.15)	0.62 (0.59)	0.31 (0.20)	0.00 (0.00)	0.30A (0.00)
	NF	0.45B (0.02)	0.83 (0.17)	0.63 (0.59)	0.38 (0.31)	0.23 (0.15)	0.50A (0.11)
	NS	0.59B (0.02)	0.67 (0.67)	0.27 (0.16)	0.48 (0.42)	0.33 (0.33)	0.46A (0.11)
	CK	0.00B (0.00)	0.33 (0.33)	0.32 (0.12)	0.33 (0.18)	0.11 (0.11)	0.25A (0.12)
100	UF	3.27A (2.79)	0.43 (0.18)	0.21 (0.08)	0.07 (0.04)	0.12 (0.07)	0.58A (0.13)
	US	1.35A (0.55)	1.12 (0.97)	0.16 (0.63)	1.48 (1.25)	0.89 (0.56)	1.12A (0.06)
	NF	0.82A (0.62)	0.55 (0.22)	0.73 (0.30)	0.42 (0.07)	0.31 (0.08)	0.68A (0.28)
	NS	0.93A (0.20)	1.08 (0.51)	0.65 (0.34)	1.02 (0.34)	0.83 (0.52)	0.82A (0.04)
	CK	0.99A (0.72)	0.17 (0.15)	0.15 (0.14)	0.08 (0.07)	0.06 (0.05)	0.21A (0.07)
120	UF	0.10B (0.04)	0.15 (0.04)	0.06 (0.01)	0.03 (0.01)	0.05 (0.03)	0.07B (0.03)
	US	0.16B (0.05)	0.16 (0.06)	0.19 (0.10)	0.17 (0.09)	0.06 (0.02)	0.15B (0.07)
	NF	0.31B (0.04)	0.31 (0.08)	0.30 (0.05)	0.19 (0.06)	0.12 (0.07)	0.24B (0.12)
	NS	0.29B (0.09)	0.28 (0.12)	0.18 (0.07)	0.31 (0.18)	0.26 (0.22)	0.28B (0.00)
	CK	0.14B (0.03)	0.18 (0.09)	0.14 (0.05)	0.09 (0.01)	0.07 (0.02)	0.12B (0.03)

Capital letters indicate significant differences (p<0.05) in log-transformed $N_2O/(N_2O+N_2)$ ratio values between moisture treatments; no significant effects (p>0.05) of fertilization treatments or of the interaction effect moisture × fertilization were detected. Values in parentheses show the mean standard error (n=3) oxygen diffusion to soil, thus promoting the consumption of secondary electron acceptors as N_2O (Ciarlo et al. 2007).

The $N_2O/(N_2O+N_2)$ ratio averaged 0.2 in unfertilized soil and 0.5 in fertilized soil (Table 1), and these values were not statistically significant probably because of their high variability. Dittert et al. (2005) and Hong et al. (2002) found significant increases in the $N_2O/(N_2O+N_2)$ ratio after the application of N fertilizers. Nitrogen fertilization decreased soil pH values, which were inversely correlated with the $N_2O/(N_2O+N_2)$ ratio. Thus, it may be possible that the not significant increase in the $N_2O/(N_2O+N_2)$ ratio due to N fertilization was caused by the inhibition of nitrous oxide reductase activity due to the increased soil acidity (Blackmer and Bremner 1978). Indeed, we observed that N_2O emissions were low in soil fertilized with urea at high moisture conditions, and this fertilizer caused the strongest reduction in soil pH.

Conclusions

Gaseous N emissions were enhanced by the application of KNO_3 at high moisture levels. Split applications of KNO_3 decreased N_2O emissions compared to a single initial application of the same fertilizer only when soil was saturated but not waterlogged. Conversely, N_2O emissions were unaffected by the application frequency when urea was applied.

The $N_2O/(N_2O+N_2)$ ratio was significantly affected by moisture levels, being highest under 100% WFPS. Fertilization treatments did not significantly affect $N_2O/(N_2O+N_2)$ ratio at any moisture content, thus confirming the second hypothesis.

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