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ORIGINAL PAPER

The effect of moisture on nitrous oxide emissions from soil and the $N_2O/(N_2O+N_2)$ ratio under laboratory conditions

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

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Abstract Nitrous oxide (N_2O) contributes to greenhouse effect; however, little information on the consequences of different moisture levels on $N_2O/(N_2O+N_2)$ ratio is available. The aim of this work was to analyze the influence of different soil moisture values and thus of redox conditions on absolute and relative emissions of N_2O and N_2 at intact soil cores from a Vertic Argiudoll. For this reason, the effect of water-filled porosity space (WFPS) values of soil cores of 40, 80,100, and 120% (the last one with a 2-cm surface water layer) was investigated. The greatest N_2O emission occurred at 80% WFPS treatment where conditions were not reductive enough to allow the complete reduction to N_2. The $N_2O/(N_2O+N_2)$ ratio was lowest (0–0.051) under 120% WFPS and increased with decreasing soil moisture content. $N_2O/(N_2O+N_2)$ ratio values significantly correlated with soil Eh; redox conditions seemed to control the proportion of N gases emitted as N_2O. N_2O emissions did not correlate satisfactorily with $N_2O/(N_2O+N_2)$ ratio values, whereas they were significantly explained by the amount of total N_2O+N_2 emissions.

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

N. Bartoloni
Cátedra de Métodos Cuantitativos Aplicados, FAUBA,
Capital Federal, Argentina

G. Rubio
Cátedra de Fertilidad y Uso de Fertilizantes, FAUBA,
Capital Federal, Argentina

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Introduction

Dinitrogen (N_2) gas is the end product of denitrification, and nitrous oxide (N_2O) is the by-product with important harmful environmental consequences because of its contribution to greenhouse effect. In addition, N_2O is the main source of stratospheric nitric oxide, which damages the ozone layer (Crutzen 1979). N_2O is also produced by nitrification, but N_2O emissions are considered to be more driven by reduction than by oxidation processes in soil (Bergsma et al. 2002).

Tiedje (1988) suggested that in aerobic systems, oxygen availability is the main limiting factor of denitrification, whereas in anaerobic systems, NO_3^- availability may be limiting. Both soil redox status and oxygen diffusion are affected by soil moisture, but there are contradictory results in the literature. Dobbie and Smith (2003) found the greatest N_2O fluxes from pasture soils at water-filled porosity space (WFPS) values higher than 60% when NO_3^- concentration was nonlimiting. However, Bøckman and Olfs (1998) proposed that N_2O formation is greater when soil WFPS ranges between 40 and 80%. Marinho et al. (2004) found that the maximum N_2O fluxes occurred several days after strong rain events, with significant correlation coefficients (r=0.84–0.94) between N_2O emissions and precipitations during 1 week before measurements. Data of rice-based agricultural systems showed that N_2O emissions were greatest at not continuously flooded fields (Xing and Zhu 1997). McSwiney et al. (2002) concluded that N_2O production is greatest under conditions that are suboptimal for nitrifiers and denitrifiers.

Few information exist about the consequences of different moisture levels on $N_2O/(N_2O+N_2)$ ratio. Granli and Bøckman (1994) suggested that the N_2O mole fraction produced by denitrification could increase by aeration. Entic soils from Canada displayed greater $N_2O/(N_2O+N_2)$ ratio values with WFPS lower than 30% with respect to soils at WFPS higher than 50% (Elmi et al. 2003); at the latter WFPS values, probably a greater reduction of N_2O to N_2 occurred. Weier et al. (1993) found smaller $N_2O/(N_2O+N_2)$ ratio values with the greatest moisture levels, although water saturation was not tested. Bandibas et al. (1994) found that N_2O emissions were greater in saturated than in flooded soils, but they did not measure N_2 emissions. Hofstra and Bouwman (2005) have suggested that wetland rice systems are more prone to denitrification than those in upland and grassland systems. It is important to underline that in Argentina, rice cultivation is mainly carried out in soils with Vertic properties. Despite the extensive research, the effect of either soil moisture or a superficial flooding water layer on both N_2O and N_2 emissions is not clear.

We have hypothesized that the presence of a superficial water layer would increase the reduction of N_2O to N_2, and therefore, the $N_2O/(N_2O+N_2)$ ratio under flooding conditions would be lower than nonflooding. The aim of this work was to analyze the influence of different moisture and thus of redox conditions on absolute and relative emissions of N_2O and N_2 with intact soil cores from a Vertic Argiudoll.

Materials and methods

Soil sampling and handling

Two hundred and four undisturbed soil cores were sampled within PVC cylinders of 10 cm length and 5 cm of internal diameter from the surface horizon of a Vertic Argiudoll, Martín Fierro series, located at the INTA Castelar experimental station (S 34°36′20″, W 58°40′20″). The study area was covered by a native grassland. The cylinders were randomly extracted from a 3×3-m area, sealed on the base, conducted to the laboratory, and put into plastic jars. The fresh soil had the following properties: clay 247 g kg^{-1}, silt 587 g kg^{-1}, sand 166 g kg^{-1}, $N–NO_3^-$ 30.7 mg kg^{-1}, $N–NH_4^+$ 6.1 mg kg^{-1}, total organic C 25.9 g kg^{-1}, total N (Nt) 2.4 g kg^{-1}, pH H_2O 6.07, and WFPS at field capacity 42.71%.

The soil of cylinders was air-dried for 20 days when the soil moisture was close to 24% WFPS in all cylinders. The behavior of soil moisture can be particularly important because denitrifying enzymes are differentially induced by wetting, and this can affect denitrification products (Bergsma et al. 2002).

Soil treatments and incubation

The experiment was conducted under laboratory conditions, with temperature ranging between 18 and 22°C. Treatments were randomly assigned to the soil cores in a completely randomized design, and each treatment was replicated three times. The following different soil moisture contents were reached by adding distilled water to soil cores: 40, 80, 100, and 120% WFPS, with the last treatment involving a 2-cm surface water layer. Water was added so as to avoid soil alteration and gas trampling. Cylinders containing soils at 120% WFPS treatment had in their superior part an open PVC cylinder (5 cm high and with an internal diameter a little bigger than the soil cylinder) so as to maintain a superficial water layer of 2 cm during the experiment. Soil moisture was maintained constant by adding water lost by evaporation.

Analyses

Denitrification losses (N_2O+N_2) were measured by the acetylene blockage technique (Yoshinari et al. 1977). In this study, we consider N_2O emission the N_2O which had left the soil–floodwater system, whereas the water-dissolved N_2O was not taken into consideration. Gas emission was measured from the same soil cores throughout. Each replication included a pair of intact soil cores, one incubated with acetylene and the other one without acetylene. N_2O emission was the value determined without acetylene, whereas the N_2O emitted with acetylene represented N_2O+N_2 emission. Cylinders were incubated within plastic jars with hermetic covers with a rubber septum for the injection of acetylene and the collection of gaseous samples. 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 were accumulated within the jars for 24 h before they were determined; jars were left open between measurements. Triplicate gas samples (2 ml) were taken from the headspace of the jars by using disposable syringes, and they were immediately analyzed by the Gaseous Chromatograph Agilent 6890 (Palo Alto, CA, USA) with ECD detector and capillary column Carboplot and using helium (He) gas as carrier; the oven, injector, and detector temperatures were 100, 100 and 250°C, respectively. Nitrogen emissions were measured at 0, 2, 7, 14, and 21 days. Cumulative emissions were calculated by averaging daily rates between two consecutive measurements and multiplying the average emission rate by the number of days elapsed between measurements.

In addition to the two soil cores for gas determinations, each replicate at each determination time involved three

additional soil cores that were incubated so as to carry out chemical analysis. Before chemical analysis, the samples were air-dried, ground, and sieved (2-mm mesh size). The pH was measured in soil–water relationship 1:2.5 (Thomas 1996).

Soluble organic C (WSOC) was extracted by shaking soil suspensions (10 g in 20 ml 0.5 M K_2SO_4) for 30 min in horizontal shaker; then, the supernatant was vacuum-filtered through Whatman No. 42 filter previously rinsed with distilled water. Soluble organic C of the filtered solution was analyzed by the modified dichromate oxidation method (Nelson and Sommers 1982), which involved the oxidation of 3 ml of the filtered extract with 1.5 ml 0.06 N potassium dichromate and 3 ml of concentrated H_2SO_4. The residual dichromate was titrated with 0.03 N Mohr's salt.

Redox potential (Eh) was measured with specific platinum combination electrode (Digital Ionalyzer/501, Orion Research, Boston, MA, USA) at a constant depth of 3 cm (Patrick et al. 1996).

Nitrate–N was determined by extracting a 20-g sample from each soil core with 100 ml 0.25% $CuSO_4$ + 0.01 M BO_3H_3 solution; the soil solution was filtered, and the N–NO_3^- content was determined colorimetrically by the hydrazine-reduction method (Carole and Scarigelli 1971) without drying or sieving.

Statistical analysis and calculations

N_2 emissions were obtained by subtracting N_2O emission without acetylene to N_2O emission with acetylene (Ryden et al. 1979), and then the $N_2O/(N_2O+N_2)$ ratio was then calculated.

Data were analyzed by the Statistical Analysis System (SAS) package (SAS Institute Inc. 1985). Absolute and relative gas emissions and daily and cumulative emissions were log-transformed to obtain their normality due to the asymmetry. Simple and multiple regression analyses between the total emissions (N_2O+N_2), N_2O emissions, and $N_2O/(N_2O+N_2)$ ratio and the different soil chemical measured variables were performed with PROC REG procedure of SAS. Variance analysis with PROC MIXED procedure of SAS package assessed differences in daily N_2O+N_2 and N_2O emissions and $N_2O/(N_2O+N_2)$ ratio between different treatments. A repeated-measures model was chosen because the measurements were made in the same soil cores throughout the incubation, and *sphericity* and *compound symmetry* conditions were met. In this way, incubation day factor was straightforwardly analyzed. Differences in cumulative and daily N_2O+N_2 and N_2O emissions between treatments were evaluated through conventional variance analysis with PROC GLM procedure of SAS package. Afterwards, a multiple comparison test (least significant difference) was made to see which treatments really differed.

Results

N_2 emissions accounted in average for 78% of the total emissions and were significantly correlated with total (N_2O+N_2) emissions ($p<0.0001$, $R^2=0.86$). Indeed, total N emissions followed a similar pattern to N_2 emissions (Fig. 1a,b). Total emissions were high at the beginning of the incubation and decreased during the incubation (Fig. 1a), with the exception of the 40% WFPS treatment that displayed low and relatively constant values throughout the experiment. Total losses significantly increased ($p<0.05$) by increasing WFPS values (Fig. 1a, Table 1), reaching the higher values under 120% WFPS. Interaction between moisture levels and incubation time was not detected. Average daily fluxes during the studied period were of 2.26, 12.42, 17.31, and 28.47 μg N kg^{-1} of soil for 40, 80, 100, and 120% WFPS treatments, respectively. Cumulative total N emissions at 40% WFPS were significantly lower ($p=0.02$) than those at 80, 100, and 120% WFPS, and the latter values were statistically similar.

N_2O emissions on the first day of incubation were statistically different from the rest of the incubation days ($p<0.005$), with a high initial outburst in the N_2O emission which differed depending on the moisture level (Fig. 1c). This effect was more relevant at the flooded situation (120% WFPS), with a marked reduction during incubation. After 10 days of incubation, N_2O increased both under 80 and 100% WFPS (Fig. 1c). Under prevailing aerobic conditions (40% WFPS), N-N_2O emissions were low and practically constant after the small initial outburst. N_2O emissions presented daily averages of 0.53, 5.84, 2.58, and 1.18 μg N kg^{-1} of soil for the 40, 80, 100, and 120% WFPS treatments, respectively. Daily N_2O emissions were significantly different (Table 1) between different moisture levels, with no existing interaction between this moisture levels and incubation time ($p=0.52$). Overall daily emissions of this gas were statistically highest at 80% WFPS ($p<0.05$). N_2O emissions under 100% WFPS treatment were significantly greater than those under 40% WFPS ($p=0.06$) and than those under 120% WFPS, although this last difference was statistically significant only after 14 days ($p=0.07$). Cumulative N_2O emissions along the incubation were statistically similar between treatments ($p=0.31$, Table 1).

In spite of a highly variable pattern, 40% WFPS treatment generally presented the greatest $N_2O/(N_2O+N_2)$ ratio values ($p=0.0008$). The $N_2O/(N_2O+N_2)$ ratio values increased with time, after a small initial decrease, under 80 and 100% WFPS (Fig. 1d). The ratio was lowest (0–0.051) under 120% WFPS. Average $N_2O/(N_2O+N_2)$ ratio values

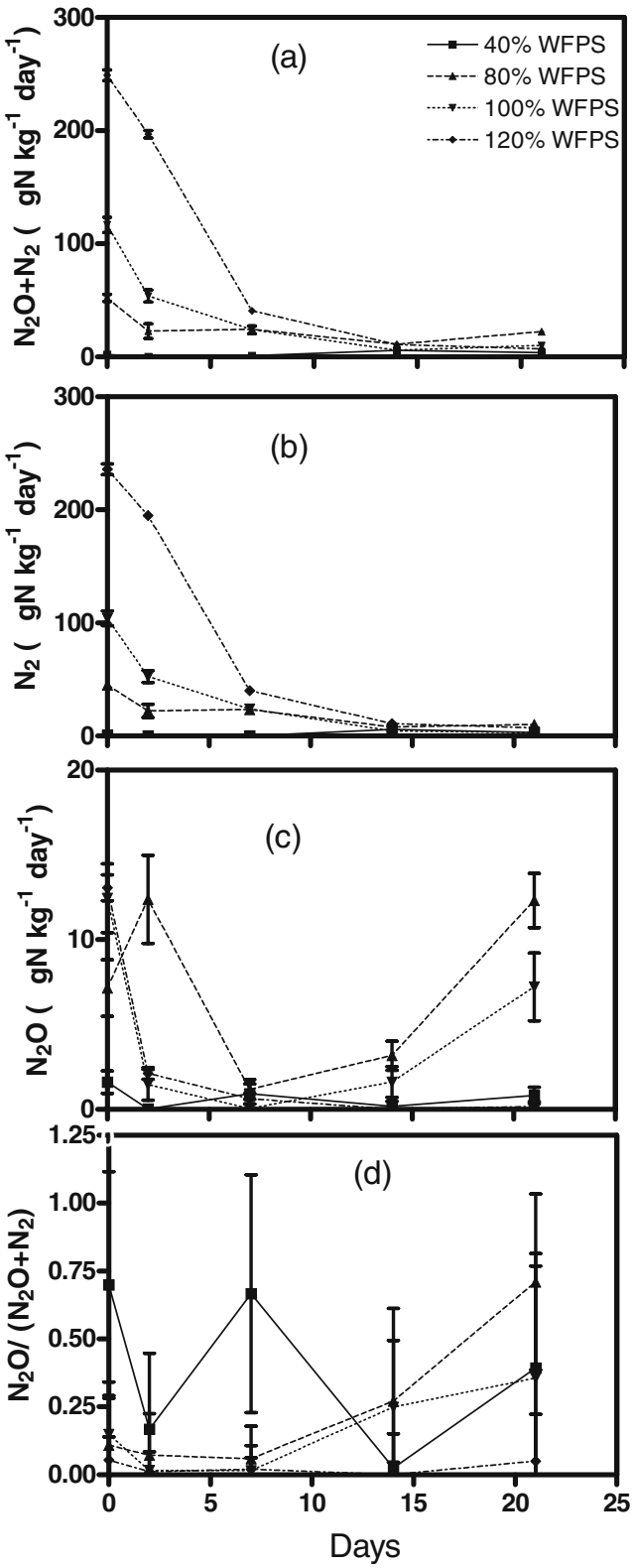

Fig. 1 N_2O+N_2 emissions (**a**), N_2 emissions (**b**), N_2O emissions (**c**), and $N_2O/(N_2O+N_2)$ ratio (**d**) during the 21-day incubation period. *Points* plotted are the means of three values, with *vertical bars* showing the standard errors

Table 1 Variance analysis of the moisture level and day of incubation upon daily and cumulative N_2O+N_2 and N_2O emissions and $N_2O/(N_2O+N_2)$ ratio

Variable effect	*F* value	*p* value
N_2O+N_2		
Moisture level	10.94	0.003
Incubation day	1.37	0.26
Moisture *X* day	0.75	0.68
N_2O		
Moisture level	7.94	0.008
Incubation day	6.06	0.0009
Moisture *X* day	0.91	0.52
$N_2O/(N_2O+N_2)$		
Moisture level	16.85	0.008
Incubation day	3.87	0.42
Moisture *X* day	2.91	0.65
Cumulative N_2O+N_2		
Moisture level	8.08	0.02
Cumulative N_2O		
Moisture level	1.44	0.31

for the entire incubation were 0.32, 0.27, 019, and 0.13, for 40, 80, 100, and 120% WFPS, respectively, being statistically different only under 40 and 120% WFPS ($p=0.04$).

N_2O emissions showed a linear and positive relationship with total denitrification emissions ($p=0.04$, $R^2=0.481$), (Fig. 2a), whereas the relationship between N_2O emissions and $N_2O/(N_2O+N_2)$ ratio values was not consistent ($p=0.96$). The $N_2O/(N_2O+N_2)$ ratio displayed a negative relationship with total N emissions ($p=0.09$, $R^2=0.487$) (Fig. 2b).

N_2O emissions were not significantly related to any of the measured soil variables (Table 2). The averages of daily emissions for each moisture level showed a quadratic relationship with WFPS (Fig. 3). The $N_2O/(N_2O+N_2)$ ratio values were significantly and positively correlated with soil potential redox levels ($p=0.02$, $R^2=0.4$) (Table 2).

Multiple explanatory models of total emissions by denitrification and of $N_2O/(N_2O+N_2)$ ratio including variables such as WFPS, NO_3^-, WSOC, pH, and redox potential were significant ($p<0.05$), but with low explaining power ($R^2=0.41$ and 0.45, respectively), and for this reason, they are not presented in this work. Multiple regression model trying to explain N_2O emissions variability, including all the above-mentioned soil variables, was not significant ($p=0.4$). Moreover, no model of multiple linear regression satisfactorily explained the changes in the N_2O emissions.

Discussion

Total N_2O+N_2 emissions presented in this work (2.26–28.47 μg N kg^{-1} of soil day^{-1}) are in the same range of those reported by Sainz Rozas et al. (2001), who found

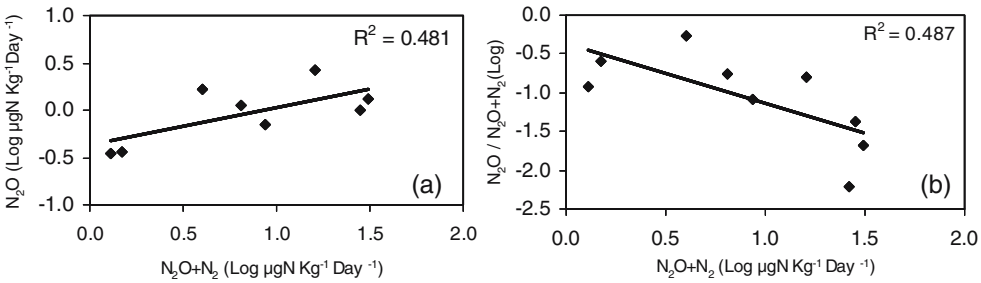

Fig. 2 Relationships between **a** N_2O emissions and denitrification losses and **b** between $N_2O/(N_2O+N_2)$ ratio and denitrification losses

denitrification losses of 14.64 µg N kg^{-1} day^{-1} at intact cores taken from a Typic Argiudoll from Argentina. Weier et al. (1993) found denitrification values as low as 2.9 µg N kg^{-1} day^{-1} at repacked soil cores taken from a Typic Argiudoll soil at 75% WFPS, but these emissions reached a value of 251 µg N kg^{-1} day^{-1} at 90% WFPS. Olde Venterink et al. (2002) have found that denitrification rates increased markedly when WFPS exceeded 60–80% and were the highest on the first day. We have found that the highest denitrification losses occurred at the highest moisture contents, thus confirming the close relationship between these emissions and redox conditions. However, the first day of the experiment presented both relatively high redox potential values (132–265 mV, data not shown), indicative of aerobic conditions, and the highest denitrification losses; this apparent contradiction may depend on the fact that NO_3^- is reduced to NO_2^- under Eh values as high as 300 mV (Rowell 1981); in addition, Eh values gave an average value of the bulk soil, and the presence of high potential redox values does not exclude the presence of hot spots where denitrification losses can occur (Parkin 1987) because these active microsites can have a reduction potential greater than the rest of the soil matrix.

N_2O emissions presented daily averages of 0.53, 5.84, 2.58, and 1.18 µg N kg^{-1} of soil for the 40, 80, 100, and 120% WFPS treatments, respectively. Carran et al. (1995) found N_2O emission values ranging from 6 to 9.05 µg N kg^{-1} day^{-1} at intact cores from silty loam soils with 70% WFPS, with soils having carbon and nitrogen contents at least twofold higher than those of the present work.

N_2O emissions displayed a high initial peak after the addition of water probably because the transition between aerobic and anaerobic conditions increased the formation of N_2O (Kester et al. 1997) due to differences in the synthesis and regulation of the enzymes involved in the denitrifying sequence (Zumft 1997). An increase in the N_2O production after the moistening of a dry soil has also been reported by Hao et al. (1988).

N_2O emissions were influenced by moisture treatments, and the greatest emissions occurred at 80 and 100% WFPS, coincident with other authors who showed that the greatest N_2O emission potential occurs at WFPS values close to 80% (Inubushi et al. 1996; Dobbie and Smith 2001, 2003; Khalil and Baggs 2005). The general relationship found between N_2O emissions and WFPS with a quadratic function shape (Fig. 3) enhances this concept, which is in agreement with the data presented by Dalal et al. (2003). However, other authors found a linear (Dobbie and Smith 2001) or exponential relationship (Dobbie and Smith 2003), where the maximum WFPS values were 90 and 100%, respectively.

Although denitrification is the main process generating N_2O, nitrification can also produce low amounts of this gas (Ritchie and Nicholas 1972). Acetylene inhibits at low concentrations the first step of autotrophic nitrification (Klemedtsson et al. 1988); thus, N_2O+N_2 emissions may be underestimated under well-aerated situations, as the 40% WFPS treatment in this report. However, the observed low N_2O emissions at 40% WFPS support the hypothesis that denitrification is more important than nitrification in producing N_2O.

Bandibas et al. (1994) proposed that the N_2O emission is affected by the relative emission $N_2O/(N_2O+N_2)$ ratio

Table 2 Determination coefficient R^2 between log-transformed N_2O, N_2O+N_2, $N_2O/(N_2O+N_2)$ ratio, and measured soil properties

Variable	WSOC	Eh	$N–NO_3^-$	pH
N_2O	0.008	0.06	0.19	0.3
N_2O+N_2	0.22	0.61*	0.73*	0.3
$N_2O/(N_2O+N_2)$	0.1	0.4*	0.25	0.01

*$P<0.05$

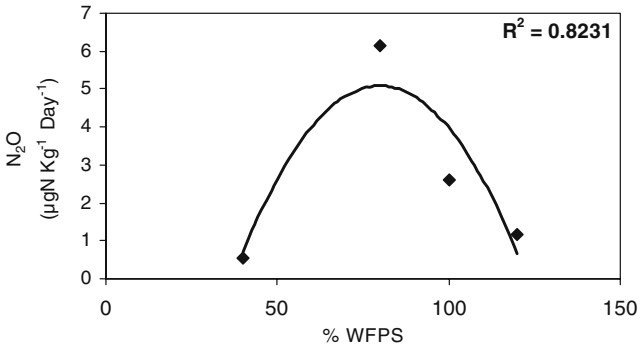

Fig. 3 Effect of WFPS values on average daily N_2O emissions

rather than by the denitrification activity. However, in our work, N_2O emissions were not significantly correlated with the $N_2O/(N_2O+N_2)$ ratio values, but they were significantly correlated ($p=0.04$) with the total N emissions.

N_2O emissions were poorly related to the analyzed soil chemical properties probably because they depend on the complex combination of temperature, soil structure, NO_3^- concentration, aeration, and moisture content, and each factor can affect denitrification and vary in space and time (Liang and Mackenzie 1997).

The low $N_2O/(N_2O+N_2)$ ratio values under 120% WFPS should probably be due to strong anaerobic conditions created by the presence of a superficial water layer, which promoted N_2O reduction to N_2, as suggested by Xu et al. (2004). Weier et al. (1993) also found lower $N_2O/(N_2O+N_2)$ ratio values under increasing moisture levels, although the greatest moisture contents analyzed did not reach soil saturation (90% WFPS). The important effect of reductive conditions were supported by the significant and positive relationship between $N_2O/(N_2O+N_2)$ ratio values and soil Eh values. In addition, the surface water layer probably limited N_2O upward diffusion, as suggested by Yan et al. (2000), and this probably stimulated N_2O reduction to N_2. Another possible explanation would be the greater sensitivity of the N_2O reductase than the other denitrifying enzymes to oxygen (Knowles 1982).

It is known that high NO_3^- concentrations inhibit N_2O reduction to N_2 (Gaskell et al. 1981; Bandibas et al. 1994). Schlegel (1992) explained this phenomenon by stating that NO_3^- is preferred as an electron acceptor with respect to N_2O. However, neither N_2O nor the $N_2O/(N_2O+N_2)$ ratio was related to soil NO_3^- levels probably because of the strong control of moisture exerted on these variables.

The $N_2O/(N_2O+N_2)$ ratio, as N_2O or total N emissions data, was not satisfactorily fitted by any of the used multiple regression model. Using multiple linear regression, Clayton et al. (1997) could only explain 28% of N_2O emissions. Probably, any variable controlling the N_2O emission can be a rate-limiting one at different moments depending on the particular conditions (Dobbie and Smith 2003).

In conclusion, maximum N_2O emissions were found when 80 to 100% of pores were occupied by water, where conditions were not reductive enough to carry out completely the denitrifying sequence. This led to higher $N_2O/(N_2O+N_2)$ ratio values under these situations than at 120% WFPS, confirming the proposed hypothesis. Soil Eh values significantly correlated with $N_2O/(N_2O+N_2)$ ratio, suggesting that this soil parameter regulates the proportion of N gases emitted as N_2O. N_2O emissions did not correlate satisfactorily with $N_2O/(N_2O+N_2)$ ratio values, whereas these emissions were significantly explained by total N emissions.

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