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Denitrification in a Soil under Wheat Crop in the Humid Pampas of Argentina

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Abstract

The need to accurately estimate gaseous nitrogen losses from soils is required to have a better understanding of the processes involved as well as soil and environmental conditions, and management practices contributing to these emissions. The objective was to quantify the denitrification rate using undisturbed cores with acetylene, as related to nitrogen (N) fertilization rate in a spring wheat crop (*Triticum aestivum L.*) under conventional tillage. Soil denitrification losses remained low throughout most of the growing season, when water-filled pore space (WFPS) was below 60%, ranging from 0.79 to 447.3 g N_2O-N $ha^{-1}\cdot day^{-1}$ in the fertilized plot and was less than 47.3 g N_2O-N $ha^{-1}\cdot day^{-1}$ in the control. Denitrification rates were the highest when N fertilizer was applied after frequent and intensive rain. A good correlation was found between the logarithm of the daily denitrification rate and WFPS (r = 0.67, n = 90); however the NO_3-N concentration was not a good indicator (r = 0.21, n = 90). Cumulative N_2O-N losses by denitrification averaged 3.5 and 0.9 kg N_2O-N ha^{-1} in the fertilized and unfertilized treatment, respectively, during a period of 4 months this difference was not significant. Most N_2O-N losses occurred early in the spring; therefore sampling schedules need to focus on this period.

Keywords

Nitrogen, Urea-N, Losses, Water Content, Soluble Organic Carbon

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1. Introduction

The intensive use of natural resources by man, especially non-renewable natural resources has led to a gradual degradation of environmental quality, compromising the sustainability of ecosystems. One consequence of this intensification is global warming, caused by increasing concentration of atmospheric greenhouse gases such as nitrous oxide (N_2O). Comparatively, N_2O has a global warming potential 298 times higher than CO_2 over a 100-year time horizon [1]. This gas accounts for about 8% of the global annual emissions of anthropogenic greenhouse gases, and its concentration has increased considerably over the past few decades and continues to increase at an annual rate of 0.25% [1].

Nitrous oxide is produced in soils by microbial transformations during nitrification and denitrification processes [2]. Nitrification is the dominant process contributing to N_2O emissions at water-filled pore space (WFPS) between 35% and 60% [3] while denitrification increases with increasing WFPS above 60% [4]. Therefore, denitrification is considered as the predominant pathway responsible for N_2O production, particularly under humid climates, and in very wet and waterlogged soils [5] [6]. Soil water content is commonly identified as the most important regulator of soil denitrification [7], but this process is also controlled by nitrate (NO_3-N) concentration, available carbon (C) and other soil properties such as temperature and pH [8]. Due to the complex interactions among these factors, large temporal and spatial variations of N_2O emissions are usually observed in cropland soils.

The soil and crop management practices can also regulate the N_2O emissions through its effect on the mentioned soil properties. One agricultural management practice is the application of nitrogen (N) fertilizers that provides substrate for denitrification. Several studies have shown that the N_2O emissions from agricultural soils increase with N application [9]-[12]. Worldwide, IPCC [1] has estimated that the emission factor is 1% of applied N. Zhang *et al*. [13] reported N losses by denitrification that increase with the fertilization rate, ranging from 0.28% to 0.49% of the applied N fertilizer in a winter wheat crop. In contrast, a study that evaluated the effects of water table management on denitrification during the corn growing season, showed no consistent differences between 120 and 200 kg N ha^{-1} rates [14]. The synchrony between the supply and demand of N is an important factor in determining the availability of soil N and release of N_2O from agricultural soils. Fertilizer N application prior to crop planting results in increased soil N with no N uptake by plant and greater potential of N_2O emissions. However, if the N fertilizer is used efficiently by the crop, for example by adjusting N applications to crop needs, less N_2O should be generated and released to the atmosphere. In fact, in study conducted on maize crop under no-tillage, denitrification losses were greater when urea was applied at planting than those when fertilizer was applied at six leaf stage (V6) [10].

The Pampas region is the main producer of maize, wheat, sunflower and soybeans in Argentina and is one of the most important agricultural areas in the world [15], with climatic and soil conditions suitable for grain crop production. This region includes several sub-regions, one of which is the southeastern area of Buenos Aires province, with a cropped area of 0.7 million ha and a wheat production of 2.5 million Mg [16]. Field experiments conducted in that area have shown that the wheat crop yield has increased an average rate of 38 $kg \cdot ha^{-1} \cdot yr^{-1}$, as a result of increments in applied N; however the N recovery efficiency in grain plant was low, ranging on average from 32% to 41% [17], depending on the timing of N fertilizer application and weather conditions. Then, it is probably that some mechanism of N loss is occurring in the soil and may help to explain the low N use efficiency by plants. In fact, the southeast wheat belt is characterized by a high probability of rainfall during the fallow and the early stages of the wheat crop periods, along with low evaporative demand [18]. This situation combined with availability of NO_3-N from mineralization of organic N during the fallow period or from applied N fertilizer at sowing, likely results in favorable conditions for denitrification and associated N_2O emissions.

In Argentina, the total greenhouse gas emissions were estimated at 238702.9 Gg of CO_2 equivalents in 2000. Of this amount, N_2O emissions from agricultural activities accounted for approximately 43% [19]. The estimation of Argentina's greenhouse gas emissions is based on the IPCC methodology, which includes gas emission factors based on international gas emission studies. Research on N_2O emissions in Argentina is crucial in order to set up an appropriate national inventory of greenhouse gases and to calculate the emission factors based on specific experimental measurements that could then be included in the IPCC methodology [20]. Therefore, more field investigation on N_2O fluxes, about regulating factors and processes that contribute to N emissions, is needed to assess their contribution to the N cycling in order to adopt management practices leading to reduced emissions from agricultural soils.

Therefore, the aims of this study were: 1) to quantify the denitrification rate related to N fertilization rate and 2) to identify the soil factors, such as water content and NO_3-N concentration that control this process, in a wheat crop under conventional tillage.

2. Methods and Materials

2.1. Site Description

The denitrification measurements and the field experiment were conducted at the Balcarce-Experimental Station of the National Institute of Agricultural Technology (INTA), located in the southeastern area of Buenos Aires province, Argentina (37˚45'S lat. 58˚18'W long.; 130 m above sea level). The soil is a complex of a fine, mixed, thermic Typic Argiudoll and a fine, illitic, thermic Petrocalcic Paleudoll (USDA Soil Clasification). It has a loam texture, with an organic matter content of 58.4 g·kg^{-1}, 23.7 cmolc·kg^{-1} of cation-exchange capacity and a pH of 5.8 (ratio soil:water, 1:2.5) in the top soil (0 - 20 cm depth).

The region has a humid-subhumid mesothermal climate with maximum and minimum monthly mean temperatures of 27.4˚C and 3.1˚C in January and July, respectively. The 40-yr average annual precipitation is 922.4 mm, 45% of which occurs during the growing season of wheat. Air temperature as well as precipitation during the experiment is shown in **Figure 1**.

2.2. Experimental Design

The overall field experiment was designed to compare the effects of two tillage systems: no tillage (NT) and conventional tillage (CT), each with two rates of N fertilization: 0 and 120 kg N ha^{-1}. The experimental design was a split-plot arrangement set as a randomized complete block with three replications. Tillage systems were applied to the main plots while the two fertility treatments were applied to the subplots. However, measurements of denitrification fluxes were only made on the CT treatment. The CT consisted of disking to mix crop residues into the soil, one moldboard plowing to the depth of 20 cm followed by one to three disking to the depth of 8 to 10 cm, before wheat planting date. Plots were seeded with wheat (*Triticum aestivum L.*) in the second week of July and it was harvested early in December.

Nitrogen fertilizer was applied as urea at a rate of 120 kg N ha^{-1} and it was broadcasted on the soil surface, three days before wheat planting. At sowing, the experiments were fertilized with P as triple superphosphate at a rate of 31 kg P ha^{-1}.

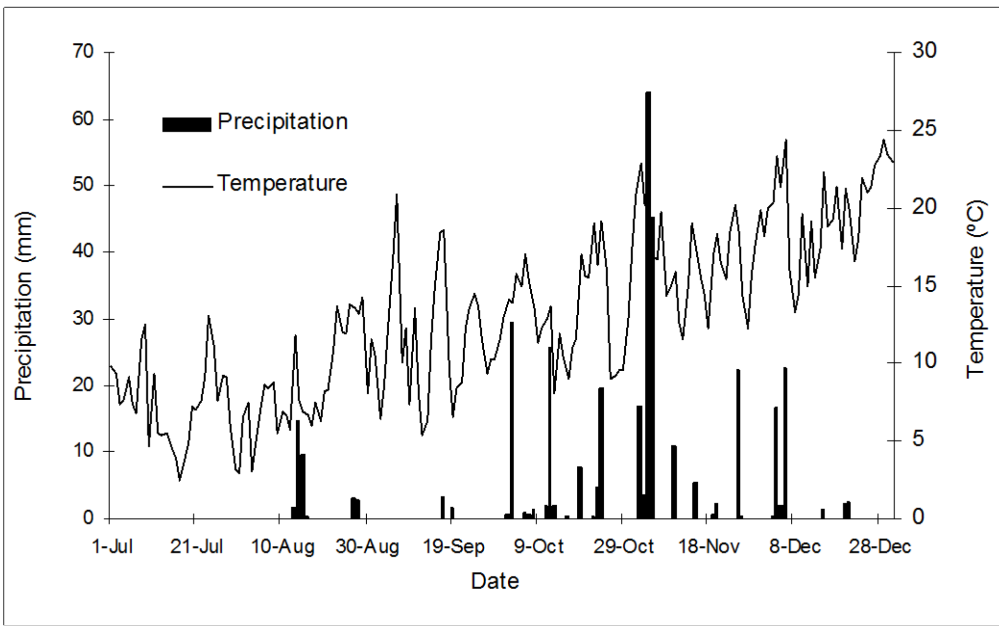

Figure 1. Rainfall and daily mean air temperatures during the growing season of spring wheat (July-December).

2.3. Denitrification Measurements

Denitrification rate measurements were made approximately weekly, during the wheat growing season, from early August (16 August) to mid-December (15 December) by the acetylene inhibition method [21]. Eight intact soil cores (4.2 cm in diameter by 15 cm long) were randomly taken from between rows in each plot using polyvinyl chloride cylinders (PVC) of 20-cm length. The cylinders were immediately brought to the lab, and both ends were capped with rubber stoppers, the upper stopper has a rubber septum for gas sampling. Approximately 10% of the headspace volume in the cylinder was removed using syringe and then an equivalent volume of acetylene (generated from calcium carbide and distilled water) was injected to the headspace. The cores were then incubated for 24 hours, outside in the shade. Gas samples were removed from each cylinder after 0 and 24 hours of incubation and subsequently stored in evacuated vials. The N_2O concentration in a 1 mL gas sample was determined using a 5890 series-II Hewlett Packard (Palo Alto, CA) gas chromatograph equipped with a Porapak Q column at 35˚C and a ^{63}Ni-electron capture detector (ECD). The injector was set at 50˚C and the ECD at 300˚C, and the carrier gas was N_2 flowing at a rate of 15 $mL \cdot min^{-1}$.

Daily denitrification rates were calculated as the change in N_2O concentration over the time using linear regression analysis. Denitrification rates were corrected for the N_2O dissolved in the liquid phase using the Bunsen absorption coefficient [22]. Cumulative N_2O losses over 4 months were calculated by interpolating linearly the daily denitrification rates between consecutives sampling dates and integrating the area.

2.4. Soil Measurements

At each date of denitrification rate measurement, the surface soil (0 - 15 cm depth) was sampled by collecting soil cores beside each cylinder for determination of NO_3-N and gravimetric water content. Soil NO_3-N was extracted by shaking 20 g of field moist soil with 80 mL of 0.5 M K_2SO_4 solution (extractant:soil ratio of 4:1, w:w) for 1 hour at 200 rpm, and then filtering through filter paper (Whatman N˚42). Filtrates were kept frozen until analyzed by steam distillation [23]. Gravimetric soil moisture was determined after oven drying of fresh subsamples at 105˚C for 24 hours.

Soil samples (0 - 15 cm depth) for water-extractable organic C (WEOC) were taken monthly during the wheat growing season. The method of WEOC determination was adapted from Mebius [24]. Briefly, 10 g of fresh soil sample was shaken with 20 mL of distilled water for 30 min (140 rpm) and centrifuged at 19,500 g for 5 min. The supernatant was filtered through a 0.22 um membrane filter (Millipore Corp.) and analyzed for total organic C by dichromate oxidation in the presence of H_2SO_4, involving boiling and refluxing conditions during 30 min at 150˚C. The excess of dichromate was titrated with ferrous ammonium sulfate.

Soil bulk density was determined by the cylinder method [25]. Undisturbed soil cores (5 cm in diameter by 5 cm long) were taken from 0 to 15 cm depth. On average, bulk density was 1.20 $Mg \cdot m^{-3}$ in the CT plots.

Gravimetric water content was converted to WFPS using the equation:

$$\text{WFPS} = (\theta * \delta) / f$$

where: θ = gravimetric water content; δ = soil bulk density; f = total soil porosity. Soil porosity was calculated from the average bulk density, assuming a particle density of 2.65 $Mg \cdot m^{-3}$.

2.5. Statistical Analysis

The denitrification rate values were checked for normality using the Shapiro-Wilk test [26], and they were $\log_{10}$ transformed prior to statistical analysis due to the skewed distribution and unequal variance. The effect of fertilization rate on daily denitrification fluxes, WFPS, and NO_3-N and WEOC contents was evaluated for each sampling date using the General Linear Model (GLM) procedure of SAS statistical program [27]. The PROC CORR function of SAS was used to determine the Pearson correlation coefficients between denitrification rates and NO_3-N content using the individual data of each sampling date. A probability level of $p = 0.05$ was used to indicate significant differences.

3. Results and Discussion

3.1. Air Temperature and Precipitation

Over the 4-month evaluation period, the mean daily air temperature was 14.0˚C ± 5.0˚C, and ranged from 3˚C (3

August) to 24.4˚C (6 and 29 December). The coldest month was August with air temperatures below 10˚C, and the warmest months were November and December with average temperatures equal or above 16.8˚C (**Figure 1**). During the evaluation period, 38 days received precipitation, approximately 28% of the total period. Precipitations were more frequent and intensive in November and December, and less frequent in September. The lowest total precipitation occurred in September with only 4.9 mm of rainfall while the highest total precipitation was in October and November with 94.1 and 171.5 mm, respectively (**Figure 1**).

3.2. Soil Mineral Nitrogen, Water-Filled Pore Space and Water Extractable Organic Carbon

There was a significant effect of the fertilized treatment on NO_3-N concentration in the 0 to 15 cm depth. Application of N fertilizer resulted in higher NO_3-N concentrations ($p < 0.05$) in August, September, October and 17 November compared with the unfertilized treatment. Nitrate concentration was more than twice in the fertilized soil in comparison with the control soil. However, on 3, 8 and 22 November and during December, there were no differences ($p > 0.05$) in NO_3-N level between both treatments (**Figure 2**).

The maximum value of soil NO_3-N concentration of the control was 22.10 mg N kg^{-1} at the beginning of the growing season, and gradually decreased to low values in December, below 5.88 mg N kg^{-1} (**Figure 2**). Previous study conducted in these soils also showed relatively low inorganic N contents early in the growing season of wheat related to the fertilized treatment [28]. This initial concentration could be attributed to mineralization and subsequent nitrification of organic N from previous crop residues. For this site, the mineralized organic N estimated during a wheat season under CT was 65 kg N ha^{-1} [29].

The highest content of NO_3-N (82.22 mg N kg^{-1}) was found shortly after 1 month fertilizer application (**Figure 2**) and represents 108 kg N ha^{-1} (subtracted the control), about 90% of the applied N. This result indicates that urea hydrolysis and NH_4 oxidation was almost completed during this time. Ferrari [30] reported that soils from the study area exhibit high urease activity, 43.8 mg N $kg^{-1}\cdot h^{-1}$, and thus high potential for urea hydrolysis. On the other hand, Navarro *et al.* [31] measuring nitrification rates under laboratory conditions, found that there was no delay in the nitrification process even with the highest concentration of ammonium, suggesting a high density of nitrifying microorganisms. This peak in NO_3-N content was followed by a sharp decrease, probably caused by a combination of immobilization, plant uptake and gas emission by denitrification/ nitrification. A second fall in inorganic N in mid-October coincided with the early stem elongation to anthesis wheat stages, the period during which the crop can accumulate up to 75% of total N in above-ground biomass at maturity [32] [33]. At wheat physiological maturity, the soil N content reached values similar to those measured in the control.

The WFPS was not affected ($p > 0.05$) by N application but fluctuated with time. It decreased from August to September, increased rapidly in response to rainfall events in October and November, and again decreased thereafter. The WFPS peaked on 16 August, 13 October and 8 November, reaching values higher than 50% (**Figure 3**). These values of water content correspond to aeration conditions that encourage N_2O production by both

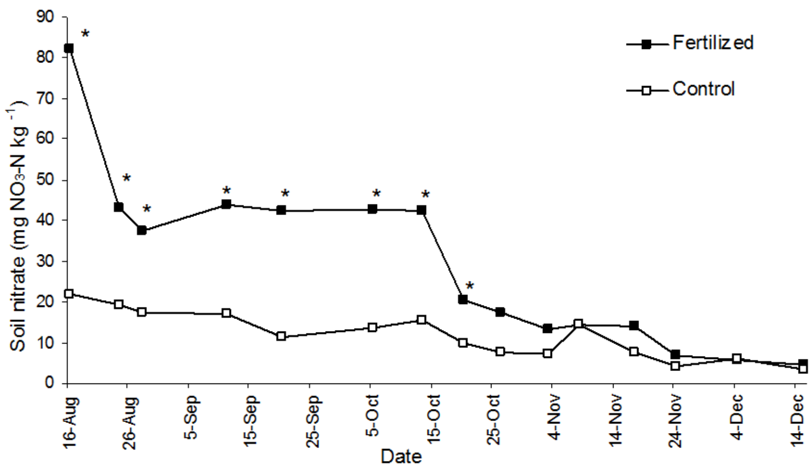

Figure 2. Soil nitrate (NO_3-N) content in the 0 to 15 cm depth for the control (without N) and fertilized treatments during the growing season of spring wheat. Asterisks indicate significant differences between both treatments ($p < 0.05$).

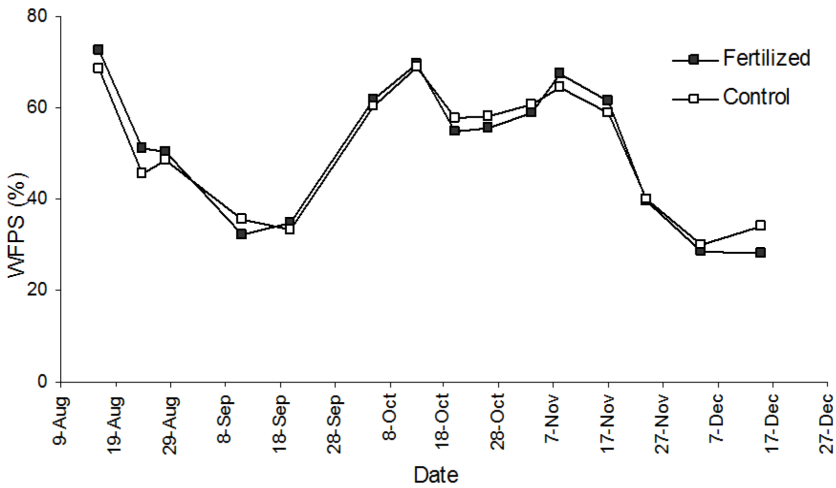

Figure 3. Distribution of soil water-filled pore space (WFPS) in the 0 to 15 cm depth for the control and fertilized treatments throughout the wheat growing season.

nitrification and denitrification [34]. The highest value of WFPS, 70%, occurred immediately after several consecutive days of rain, corresponding to gravimetric water content of 0.36 $g \cdot g^{-1}$ which is higher than the water content at field capacity. Towards the end of the wheat growing season, the WFPS was about 28%, close to the water content at permanent wilting point.

The mean concentration of soil WEOC was higher in the unfertilized treatment than in the fertilized treatment (**Table 1**), but the differences were only significant ($p < 0.05$) in November. Trends toward lower WEOC in the fertilized treatment than in the unfertilized treatment suggest that WEOC in fertilized soils might have been metabolized at a greater rate. The WEOC concentrations remained relatively unchanged and low during the plant growth, ranging between 12.52 and 32.13 mg C kg^{-1} (**Table 1**). Similar results were found by Elmi *et al.* [14] who reported that WEOC concentration was relatively uniform, ranging from 10 to 30 $mg \cdot kg^{-1}$ (15 cm depth) in a corn field. The WEOC is a dynamic pool C, controlled by several physical and biological mechanisms. Sorption and desorption are two key processes for WEOC stabilization and production in soils, soluble root exudates and microbial processes can also contribute with dissolved C, and plants and microorganisms can consume WEOC [35]. Then, the variety of factors determining the concentration of WEOC indicates that is difficult to explain its variation over the time in the soil.

3.3. Daily Denitrification Rates

Denitrification rates varied greatly throughout the wheat growing season, being more variable in the fertilized treatment (**Figure 4**). Daily rates of denitrification ranged from 0.776 to 472.83 ng N_2O-N $cm^{-2} \cdot day^{-1}$ in the unfertilized treatment and from 0.789 to 4472.00 ng N_2O-N $cm^{-2} \cdot day^{-1}$ in the fertilized treatment. Most of the time, denitrification rates were relatively low but on occasions increased to high values as 4472.00 ng N $cm^{-2} \cdot day^{-1}$ on 13 October. This irregular pattern of denitrification rates has been shown by other experiments [36] [37] and was often the result of complex interactions among weather conditions, soil properties and management practices, each having specific effects on denitrification. In fact, the coefficients of variation (CV) of daily measurements ranged from 9 to 157% indicating a high spatial and temporal variability. Several studies showed a large variability of denitrification rates with coefficients of variation ranging from 70% to 379% when they were measured using intact cores and under field conditions [38]-[40].

Throughout August the fertilized treatment emitted more N_2O-N than the unfertilized treatment but the daily denitrification rate was only significantly different on 24 August ($p < 0.05$). During this month, denitrification rate was relatively low (<500 ng N_2O-N $cm^{-2} \cdot day^{-1}$) even though the NO_3-N content was high, particularly in the fertilized treatment, and the WFPS ranged from 50% to 70%. This means that other factors such as temperature and/or availability of C, independent to inorganic N and water content, controlled N_2O-N losses by denitrification. Previous studies indicate that C availability is the most important limiting factor controlling denitrification, even in soils with low NO_3-N contents [41] [42]. According to Burton and Beauchamp [43], soluble organic

Table 1. Water-Extractable Organic Carbon (WEOC) concentration in soil from the control and fertilized treatments in the wheat growing season.

Date	WEOC (mg C kg^{-1})	
	Control	Fertilized
August	23.80 a	22.45 a
September	32.13 a	21.73 a
October	13.20 a	12.52 a
November	17.89 a	14.13 b
December	22.20 a	20.25 a

Different letters within each row indicate significant differences ($p < 0.05$) between treatments.

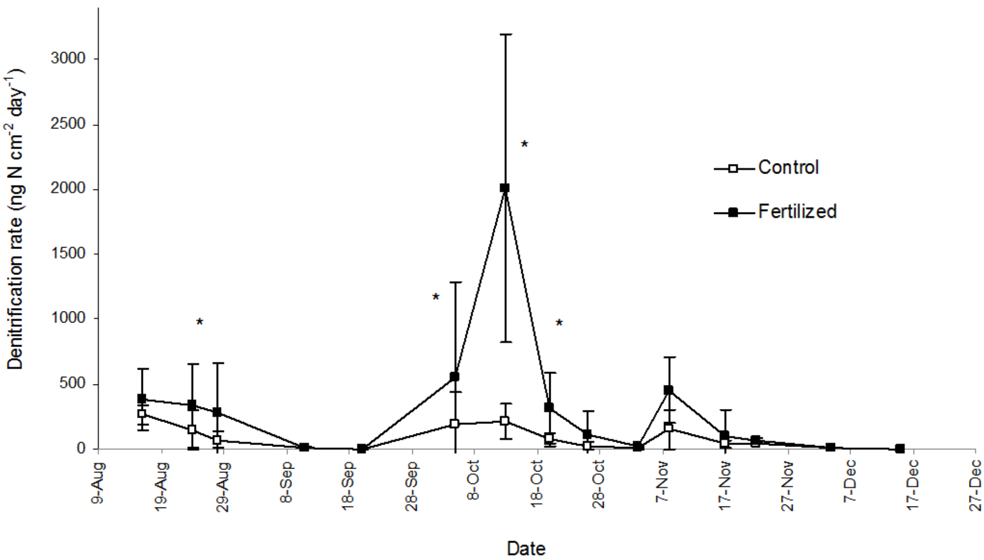

Figure 4. Daily denitrification rates (ng N_2O-N $cm^{-2}\cdot day^{-1}$) measured during the growth period of spring wheat. Vertical bars represent standard errors. Asterisks indicate significant differences between fertilized and nonfertilized treatments ($p < 0.05$).

C contents greater than 60 - 80 mg C kg^{-1} are required for denitrification. Since the WEOC levels measured during the growing season were less than 32.13 mg C kg^{-1}, it is possible that C was limiting this process during the crop growth. However, because the WEOC was measured each month and no more frequently, its concentration may not reflect accurately the effect on denitrification rate.

After this initial period, the daily denitrification rate decreased and was very low (<20 ng N_2O-N $cm^{-2}\cdot day^{-1}$) during September. Soil water content was low, approaching 33% WFPS during the dry phase from 28 August through 20 September, when the total rainfall was 9.6 mm. However, a second peak of N_2O occurred on 13 October following an increase in WFPS. The highest peak was recorded in the fertilized soil, after several rainfall events reflected in a WFPS of 70%, and coinciding with an average NO_3-N content of 25.35 mg N kg^{-1}. The denitrification rate of the fertilized soil was more than nine times the rate of the control soil, suggesting that N_2O-N production was driven mainly by NO_3-N availability since all the other measured soil properties (available C and water content) were similar in both treatments. Previous studies have shown that NO_3-N would not be the limiting factor unless all other parameters were optimized [44]. Then, NO_3-N could be limiting when concentrations are lower than 5 - 10 mg N kg^{-1} in a clay loam soil [45], 20 mg N kg^{-1} under grassland soil [46] or 40 mg N kg^{-1} in maize under no-tillage [10]. A small N_2O-N peak was observed on 8 November in the fertilized treatment, but the emissions were not statistically different ($p > 0.05$) between both treatments. Towards the end of the wheat growing season, WFPS and NO_3-N concentration dropped to about 30% and 6 mg N kg^{-1}; respectively, while the corresponding denitrification rate decreased to less than 12.7 ng N_2O-N $cm^{-2}\cdot day^{-1}$. This result was probably consequence to depletion of soil NO_3-N pool by plant uptake in combination with low water content leading to cessation of denitrification.

The active denitrification during spring appears to have been associated mainly with high soil moisture contents. It has been recognized that O_2 concentrations can affect both synthesis and activity of denitrifying enzymes [47]. Denitrification increases with increases in WFPS and the maximum emission of N_2O-N by denitrification occurs at WFPS values >60% [34] [48]. A high correlation between the logarithm of daily denitrification rate and WFPS ($r = 0.67$, $n = 90$) was found, although nearly all WFPS values are below 60% during the measurement period. On the other hand, although NO_3-N concentration has been recognized to play a significant role in the regulation of denitrification process [49], it was not a good indicator of the logarithm of the denitrification rate because the relationship between both variables was weak ($r = 0.21$, $n = 90$). Denitrification is maybe more influenced by the diffusion of the NO_3-N to the active denitrification sites even at a high NO_3-N concentration [45] than by the concentration of available NO_3-N.

3.4. Cumulative N_2O Losses

Cumulative losses of N_2O-N for the 4-month period were lower for the unfertilized treatment compared with the fertilized treatment, but this difference was not statistically significant ($p > 0.05$). The high variability in N_2O-N rates probably overrides the statistical significances of the means. During the wheat season, the fertilized soil had a cumulative N_2O-N loss of 3.5 kg N_2O-N ha^{-1} whereas it was 0.9 kg N_2O-N ha^{-1} in the unfertlized soil (**Figure 5**). The results agree with those of Skiba *et al.* [11], and Granli and Bockman [50] who found higher total N_2O losses from the fertilized treatment compared with the control. The emissions of N_2O-N by denitrification expressed as a percentage of applied N fertilizer were low, 2%, after subtraction of N_2O-N emissions attributable to the control. For the non-fertilized plots, total N_2O-N losses were also low but this confirms that there are emissions even without N fertilization due to the potential for N mineralization of this soil.

Most cumulative N_2O losses in the wheat crop, about 82%, occurred early in the spring probably because of high precipitation, NO_3-N availability and higher air temperature. This indicates the importance of this period for the evaluation of total N_2O-N losses from wheat crop, in temperate climate zones.

3.5. Denitrification and Its Relationship to Other Loss Processes

In this study, denitrification measurements were performed on a site where other aspects of the N cycle were measured. A previous study reported that ammonia volatilization losses from this soil were low, on average 1.69 kg N ha^{-1}, which represented 1.5% of the N fertilizer when 120 kg N ha^{-1} of urea was applied at sowing of wheat

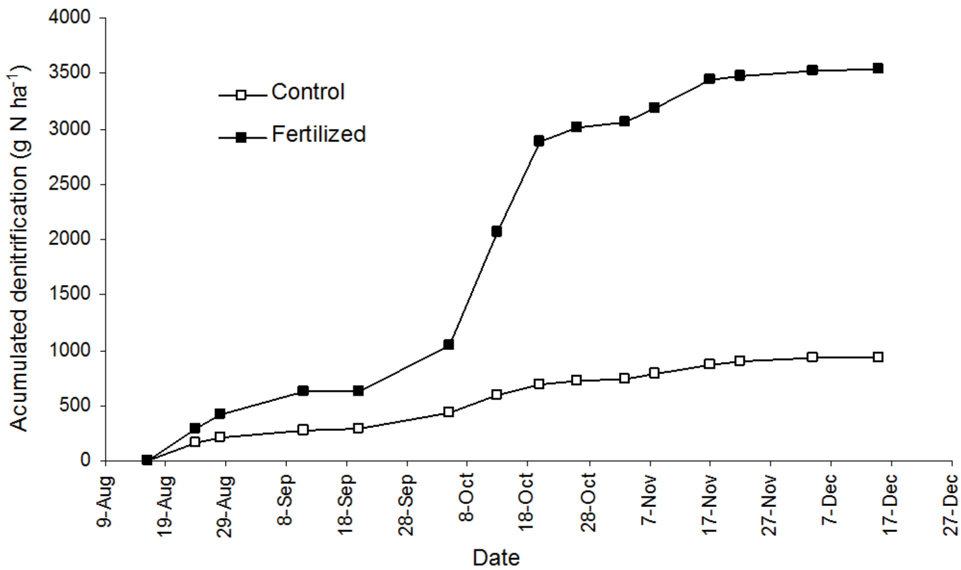

Figure 5. Cumulative N_2O emissions (g N_2O-N ha^{-1}) by denitrification throughout the growing season of wheat (August-mid December) from the control and fertilized treatments.

crop [28]. Using Ceres-Wheat model, it was predicted that NO_3-N losses by leaching ranged from 12 to 62 kg N ha^{-1} for rates of 0 to 175 kg N ha^{-1}; respectively, while denitrification losses fluctuated between 1.2 and 3.9 kg·N·ha^{-1} depending on the N rate at sowing of wheat [17]. According to this information, the denitrification process was expected to be a minor pathway of N loss. In fact, the results of this research support this conclusion since N_2O-N rates measured by acetylene were comparable to those predicted by the model, 0.9 kg N ha^{-1} and 3.5 kg N ha^{-1} in the control and fertilized plot; respectively. However, the lack of measurements during the first days immediately after N fertilizer application may have resulted in an underestimation of the cumulative N_2O-N loss as emissions could have occurred during that period. In addition, weather conditions during the study were drier (350 mm) than the median over the 1971-2003 period (489 mm). This means that a median year could lead to higher N_2O emissions than those measured in this study due to a higher rainfall.

4. Conclusions

The peaks of denitrification rate in the wheat crop were observed when N fertilizer was applied and rain was more frequent and intensive. A high correlation was found between daily denitrification rate and WFPS (r = 0.67, n = 90), however, the NO_3-N soil concentration was not a good indicator of denitrification rate (r = 0.21, n = 90). The accumulative N_2O loss under spring wheat was not significantly different between the fertilized and control treatments. This is due to the high measured variability of denitrification rates. Denitrification process does not appear to be a major pathway for loss of N from this crop.

Denitrification rates were small considering that they represent 2% of the N added to the soil. Most cumulative N_2O-N losses occurred early in the spring (82%). Therefore sampling schedules need to focus during this period for wheat crop, in temperate climate regions.

References

[1] IPCC (2006) Guidelines for National Greenhouse Gas Inventories Third Authors/Experts Meeting: Industrial Processes and Product Use, Washington D.C., 27-29 July 2004.

[2] Firestone, M.K. and Davidson, E.A. (1989) Microbiological Basis of NO and N_2O Production and Consumption in Soils. In: Andreae, M.O. and Schimel, D.S., Eds., *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere*, John Willey and Sons, New York, 7-21.

[3] Bateman, E.J. and Baggs, E.M. (2005) Contributions of Nitrification and Denitrification to N_2O Emissions from Soils at Different Water-Filled Pore Space. *Biology and Fertility of Soils*, **41**, 379-388. http://dx.doi.org/10.1007/s00374-005-0858-3

[4] Drury, C.F., Zhang, T.Q. and Kay, B.D. (2003) The Non-Limiting and Least Limiting Water Ranges for Soil Nitrogen Mineralization. *Soil Science Society of American Journal*, **67**, 1388-1404. http://dx.doi.org/10.2136/sssaj2003.1388

[5] Parton, W.J., Mosier, A.R. and Schimel, D.S. (1988) Rates and Pathways of Nitrous Oxide Production in a Shortgrass Steppe. *Biogeochemistry*, **6**, 45-48. http://dx.doi.org/10.1007/BF00002932

[6] Skiba, U., Smith, K.A. and Fowler, D. (1993) Nitrification and Denitrification as Sources of Nitric Oxide and Nitrous Oxide in a Sandy Loam Soil. *Soil Biology and Biochemistry*, **25**, 1527-1536. http://dx.doi.org/10.1016/0038-0717(93)90007-X

[7] Coyne, M.S. (2008) Biological Denitrification. In: Schepers, J.S. and Raun, W.R., Eds., *Nitrogen in Agricultural Systems*, American Society of Agronomy, Crop Science Society of America. Soil Science Society of America, Madison, 202-254. http://dx.doi.org/10.2134/agronmonogr49.c7

[8] Firestone, M.K. (1982) Biological Denitrification. In: Stevenson, F.J., Ed., Nitrogen Agricultural Soils. Agron. Monogr. 22. American Society Agronomy, Crop Science Society of America and Soil Science Society of American, Madison, 289-326.

[9] Grant, R.F., Pattey, E., Goddard, T.W., Kryzanowski, L.M. and Puurveen, H. (2006) Modeling the Effects of Fertilizer Application Rate on Nitrous Oxide Emissions. *Soil Science Society of American Journal*, **70**, 235-248. http://dx.doi.org/10.2136/sssaj2005.0104

[10] Sainz Rozas, H., Echeverría, H.E. and Picone, L.I. (2001) Denitrification in Maize No-Tillage: Effect of Nitrogen Rate and Application Time. *Soil Science Society of American Journal*, **65**, 1314-1323. http://dx.doi.org/10.2136/sssaj2001.6541314x

[11] Skiba, U., Fowler, D. and Smith, K. (1994) Emissions of NO and N_2O from Soils. *Environmental Monitoring and Assessment*, **31**, 153-158. http://dx.doi.org/10.1007/BF00547191

[12] Stehfest, E. and Bouwman, L. (2006) N_2O and NO Emission from Agricultural Fields and Soils under Natural Vegeta-

tion: Summarizing Available Measurement Data and Modeling of Global Annual Emissions. *Nutrient Cycling in Agroecosystems*, **74**, 207-228. http://dx.doi.org/10.1007/s10705-006-9000-7

[13] Zhang, Y.M., Chen, D.L., Zhang, J.B., Edis, R., Hu, C.S. and Zhu, A.N. (2004) Ammonia Volatilization and Denitrification Losses from an Irrigated Maize-Wheat Rotation Field in the North China Plain. *Pedosphere*, **14**, 533-540.

[14] Elmi, A.A., Astatkie, T., Madramootoo, C., Gordon, R. and Burton, D. (2005) Assessment of Denitrification Gaseous End Products in the Soil Profile under Two Water Table Management Practices Using Repeated Measures Analysis. *Journal of Environmental Quality*, **34**, 446-454. http://dx.doi.org/10.2134/jeq2005.0446

[15] Satorre, E.H. and Slafer, G.A. (1999) Wheat Production Systems of the Pampas. In: *Wheat*: *Ecology and Physiology of Yield Determination*, Food Products Press, New York, 333-348.

[16] Reussi Calvo, N., Sainz Rozas, H., Echeverría, H. and Berardo, A. (2013) Contribution of Anaerobically Incubated Nitrogen to the Diagnosis of Nitrogen Status in Spring Wheat. *Agronomy Journal*, **105**, 321-328. http://dx.doi.org/10.2134/agronj2012.0287

[17] Barbieri, P.A., Rozas, H.S. and Echeverría, H.E. (2008) Time of Nitrogen Application Affects Nitrogen Use Efficiency of Wheat in the Humid Pampas of Argentina. *Canadian Journal of Plant Science*, **88**, 849-857. http://dx.doi.org/10.4141/CJPS07026

[18] Calviño, P.A. and Sadras, V.O. (2002) On-Farm Assessment of Constraints to Wheat Yield in the South-Eastern Pampas. *Field Crops Research*, **74**, 1-11. http://dx.doi.org/10.1016/S0378-4290(01)00193-9

[19] Gobierno Argentino (2007) Segunda Comunicación Nacional del Gobierno Argentino a la Convención Marco de las Naciones Unidas sobre Cambio Climático. Proyecto BIRF No. TFO51287. http://www.fundacionbariloche.org.ar

[20] Taboada, M.A. and Cosentino, V.R.N. (2012) Emisión de óxido nitroso (N_20) desde suelos agrícolas. *XIX Congreso Latino Americano de la ciencia del suelo*, *XXIII Congreso argentino de la Ciencia del suelo*, 16-20 abril 2012, Resumen en actas.

[21] Yoshinari, T., Hynes, R. and Knowles, R. (1977) Acetylene Inhibition of Nitrous Oxide Reduction and Measurement of Denitrification and Nitrogen Fixation in Soil. *Soil Biology and Biochemistry*, **9**, 177-183. http://dx.doi.org/10.1016/0038-0717(77)90072-4

[22] Tiedje, J.M. (1982) Denitrification. In: Page, A.L., Ed., *Methods of Soil Analysis*: *Part* 2. *Microbiological and Biochemical Properties*, Agron. Monogr. 9, American Society of Agronomy and Soil Science Society American, Madison, 1011-1026.

[23] Keeney, D.R. and Nelson, D.W. (1982) Nitrogen Inorganic Forms. In: Page, A.L., Ed., *Methods of Soil Analysis Part* 2 *Chemical and Microbiological Properties*, American Science of Agronomy and Soil Science Society of America, Madison, 643-693.

[24] Mebius, L.J. (1960) A Rapid Method for the Determination of Organic Carbon in Soil. *Analytica Chimica Acta*, **22**, 120-124. http://dx.doi.org/10.1016/S0003-2670(00)88254-9

[25] Blake, G.R. and Hartge, K.H. (1986) Bulk Density. In: Klute, A., Ed., *Methods of Soil Analysis*, *Part* 1, 2nd Edition, Agron. Monogr. 9, American Society of Agronomy and Soil Science Society of America, Madison, 363-375.

[26] Shapiro, S.S. and Wilk, M.B. (1965) An Analysis of Variance Test for Normality (Complete Samples). *Biometrika*, **52**, 591-611. http://dx.doi.org/10.1093/biomet/52.3-4.591

[27] SAS Institute Inc. (1988) User's Guide: Statistics. Version 6.03 ed. SAS/STAT. SAS Inst., Cary.

[28] Videla, C.C., Ferrari, J.L., Echeverria, H.E. and Travasso, M.I. (1996) Transformaciones del nitrógeno en el cultivo de trigo. *Ciencia del suelo*, **14**, 1-6.

[29] García, F. and Fabrizzi, K. (2001) Dinámica del nitrógeno en ecosistemas agrícolas: Efecto de la siembra directa. Siembra directa en el Cono Sur. Programa cooperativo para el desarrollo tecnológico agroalimentario y agroindustrial del Cono Sur. Instituto Interamericano de Cooperación para la Agricultura, Procisur, Montevideo, 299-323.

[30] Ferrari, M.H. (1995) Hidrólisis de la urea en suelos de la region pampeana. Tesis Ing. Agr. Facultad de Ciencias Agrarias, UNMdP, Balcarce.

[31] Navarro, C.A., Echeverría, H.E., Gonzalez, N.S. and Iglesias, M.A. (1980) Cinética de las reacciones de amonificación y nitrificación en algunos suelos de Argentina. *IX Reunión Argentina de la Ciencia del Suelo*, 15 al 20 septiembre 1980, 431-437.

[32] Abbate, P.E. and Andrade, F.H. (2006) Los nutrientes del suelo y la determinación del rendimiento de los cultivos de grano. In: Echeverría, H.E. and García, F.O., Eds., *Fertilidad de suelos y fertilización de cultivos*, Editorial INTA, 43-65.

[33] Baethgen, W.E. and Alley, M.M. (1989) Optimizing Soil and Fertilizer Nitrogen Use for Intensively Managed Winter Wheat. I. Crop Nitrogen Uptake. *Agronomy Journal*, **81**, 116-120.

[34] Linn, D.M. and Doran, J.W. (1984) Effect of Water Filled Pore Space on Carbon Dioxide and Nitrous Oxide Produc-

tion in Tilled and Non-Tilled Soils. *Soil Science Society of America Journal*, **48**, 1267-1272. http://dx.doi.org/10.2136/sssaj1984.03615995004800060013x

[35] Neff, J.C. and Asner, G.P. (2001) Dissolved Organic Carbon in Terrestrial Ecosystems: Synthesis and a Model. *Ecosystems*, **4**, 29-48. http://dx.doi.org/10.1007/s100210000058

[36] Hénault, C., Grossel, A., Mary, B., Roussel, M. and Léonard, J. (2012) Nitrous Oxide Emission by Agricultural Soils: A Review of Spatial and Temporal Variability for Mitigation. *Pedosphere*, **22**, 426-433. http://dx.doi.org/10.1016/S1002-0160(12)60029-0

[37] Mathieu, O., Lévêque, J., Hénault, C., Milloux, M.J., Bizouard, F. and Andreux, F. (2006) Emissions and Spatial Variability of N_2O, N_2 and Nitrous Oxide Mole Fraction at the Field Scale, Revealed with ^{15}N Isotopic Techniques. *Soil Biology and Biochemistry*, **38**, 941-951. http://dx.doi.org/10.1016/j.soilbio.2005.08.010

[38] Folorunso, O.A. and Rolston, D.E. (1984) Spatial Variability of Field Measured Denitrification Gas Fluxes. *Soil Science Society of America Journal*, **48**, 1214-1219. http://dx.doi.org/10.2136/sssaj1984.03615995004800060002x

[39] Parkin, T.B., Kaspar, H.F., Sextone, A.J. and Tiedje, J.M. (1984) A Gas-Flow Soil Method to Measure Field Denitrification Rates. *Soil Biology and Biochemistry*, **16**, 323-330. http://dx.doi.org/10.1016/0038-0717(84)90026-9

[40] Parsons, L., Scott Smith, M. and Murray, R.E. (1991) Soil Denitrification Dynamics: Spatial and Temporal Variations of Enzyme Activity, Populations and Nitrogen Gas Loss. *Soil Science Society of America Journal*, **55**, 90-95. http://dx.doi.org/10.2136/sssaj1991.03615995005500010016x

[41] Drury, C.F., McKenney, D.J. and Findlay, W.I. (1991) Relationships between Denitrification, Microbial Biomass and Indigenous Soil Properties. *Soil Biology and Biochemistry*, **23**, 751-755. http://dx.doi.org/10.1016/0038-0717(91)90145-A

[42] Weier, K.L., Doran, J.W., Power, J.F. and Walters, D.T. (1993) Denitrification and the Dinitrogen/Nitrous Oxide Ratio as Affected by Soil Water, Available Carbon and Nitrate. *Soil Science Society of America Journal*, **57**, 66-72. http://dx.doi.org/10.2136/sssaj1993.03615995005700010013x

[43] Burton, D.L. and Beauchamp, E.G. (1985) Denitrification Rate Relationship with Soil Parameters in the Field. *Communications in Soil Science and Plant Analysis*, **16**, 539-549. http://dx.doi.org/10.1080/00103628509367626

[44] Vinther, F.P. (1984) Total Denitrification and the Ratio between N_2O and N_2 during the Growth of Spring Barley. *Plant and Soil*, **76**, 227-232. http://dx.doi.org/10.1007/BF02205582

[45] Ryden, J.C. (1983) Denitrification Loss from a Grassland Soil in the Field Receiving Different Rates of Nitrogen as Ammonium Nitrate. *Journal of Soil Science*, **34**, 355-365. http://dx.doi.org/10.1111/j.1365-2389.1983.tb01041.x

[46] Myrold, D.D. and Tiedje, J.M. (1985) Establishment of Denitrification Capacity in Soil: Effects of Carbon, Nitrate and Moisture. *Soil Biology and Biochemistry*, **17**, 819-822. http://dx.doi.org/10.1016/0038-0717(85)90140-3

[47] Dendooven, L. and Anderson, J.M. (1994) Dynamics of Reduction Enzymes Involved in the Denitrification Process in Pasture Soil. *Soil Biology and Biochemistry*, **26**, 1501-1506. http://dx.doi.org/10.1016/0038-0717(94)90091-4

[48] Davidson, E.A. (1991) Fluxes of Nitrous Oxide and Nitric Oxide from Terrestrial Ecosystems. In: Rogers, J.E. and Whitman, W.B., Eds., *Microbial Production and Consumption of Greenhouse Gases*: *Methane*, *Nitrogen Oxide*, *and Halomethanes*, ASM Press, Washington DC, 219-235.

[49] Tiedje, J.M. (1988) Ecology of Denitrification and Dissimilatory Nitrate Reduction to Ammonium. In: Zehnder, A.J.B., Ed., *Biology of Anaerobic Microorganisms*, John Wiley and Sons, New York, 179-244.

[50] Granli, T. and Bøckman, O.C. (1994) Nitrous Oxide from Agriculture. *Norwegian Journal of Agricultural Sciences*, **12**, 1-128.