

CHANGES IN DENITRIFICATION RATE AND N₂O/N₂ RATIO WITH VARYING SOIL MOISTURE CONDITIONS IN NEW ZEALAND PASTURE SOILS

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Abstract

Denitrification is the primary process of N₂O production in temperate grassland soils and accounts for 60% of the total N₂O emissions globally. There are various soil and environmental factors that regulate denitrification and affect denitrification rate (DR) and N₂O/ N₂ ratio. Among these, soil moisture is the most important. Generally, DR increases and N₂O/N₂ ratio decreases with increasing soil water content. However, the effect of changing soil moisture on DR and N₂O/N₂ ratio may vary with the type of soil, its nutrient status and the management practices followed on the farm. The interrelationships among the various factors affecting DR are not very well quantified. Therefore, the current study was planned to investigate the effect of soil moisture on DR and N₂O/N₂ ratio in five different New Zealand pasture soils with varying physical and chemical characteristics such as soil texture, total porosity, pH, NO₃⁻ and NH₄⁺ content, total nitrogen (TN), total carbon (TC), microbial biomass carbon (MBC) and denitrification enzyme activity (DEA). The experiment involved incubation of surface (0-10cm) and subsurface (10-20cm) soil samples at field capacity (FC) and complete saturation at constant temperature (25°C). DR and N₂O/N₂ ratio were estimated using an acetylene inhibition (AI) technique.

Denitrification rates were higher in soils incubated at saturation than in soils incubated at FC. Similarly, the N₂O/N₂ ratio decreased in soils when incubated at saturation as compared to FC. The extent of these increases in DR and decreases in N₂O/N₂ ratio with increasing moisture content varied among the soils due to differences in NO₃⁻ and NH₄⁺ content, MBC and DEA of the soils.

Introduction

Denitrification is an anaerobic microbial process which converts N oxides to inert N₂ and in the process emits NO and N₂O as gaseous byproducts (Firestone 1982; Tiedje 1988). Grassland systems are one of the principal sources of N₂O emission and denitrification is one of the major processes contributing to N₂O production from grassland soils, both globally and in temperate grasslands (Mosier *et al.* 1998; Kroeze *et al.* 1999; Saggarr *et al.* 2009). Studies have been conducted firstly to measure N loss from agricultural soils, including grazed pastures and secondly to assess the effect of soil and environmental factors on denitrification rate (DR) (Focht 1974; Linn and Doran 1984; Myrold and Tiedje 1985; Colbourn 1993; de Klein and van Logtestijn 1994a, b; Luo *et al.* 1994a, b, 1998, Zaman *et al.* 2008). These studies suggest DR is affected by soil texture, pH, soil moisture or oxygen content, temperature and availability of C and N. Interactions of these variables in soil affect the rate of denitrification and the relative production of N₂O and N₂. Generally, the DR is highest

when soil is warm and wet, and NO_3^- and C are available in sufficient amounts (Luo *et al.* 2000). Denitrification rates have been found to be highest in spring, summer and autumn in northern temperate agricultural soils (Ryden 1983; Parsons *et al.* 1991; de Klein and van Logtestijn 1996; Schnabel and Stout 1994) and in winter in New Zealand pasture soils (Ruz-Jerez *et al.* 1994).

Soil water content is key for controlling DR in pasture soils (de Klein and van Logtestijn 1994a; Jarvis *et al.* 1991; Myrold 1988), as it alters the aeration status and nutrient availability. The DR increases and $\text{N}_2\text{O}/\text{N}_2$ ratio decreases with increasing soil water content (Colbourn and Dowdell 1984; Vinther 1984). The synthesis of the enzymes involved in denitrification is affected by the O_2 availability and the concentration of NO_3^- and NO_2^- and this in turn affects the DR and the relative proportion of $\text{N}_2\text{O}/\text{N}_2$ produced during the process (Conrad 1996; McKenney *et al.* 2001). It has been reported that with increasing soil moisture, the concentration of nitrous oxide reductase increases in soil. This speeds up the reduction of N_2O and hence a greater amount of N_2 is produced, which not only increases the overall rate of denitrification but also decreases the $\text{N}_2\text{O}/\text{N}_2$ ratio (Davidson 1991; Miller *et al.* 2008). Work done by Pilot and Patrick (1972) and Klemetsson *et al.* (1991) suggested DR increases sharply when soil moisture exceeds a critical level. This critical soil water content was termed the threshold water content. DR seems to be unrelated to soil moisture below the threshold water content. However, the soil water threshold value varies according to the soil type. de Klein and van Logtestijn (1996) suggested the critical soil moisture level for denitrification in most soils is field capacity (FC). When soil moisture exceeds FC, the DR increases. Drury *et al.* (2003) reported that the rate of denitrification increases with increasing WFPS from 60% and reaches its maximum value with WFPS at 90%.

The DR in New Zealand grazed pasture soils is highly variable both spatially and temporally. It has been reported to vary from 0.10 to 42 $\mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil d}^{-1}$ (Luo *et al.* 1998, 2000) and from 3.4 to 19.3 $\text{kg N ha}^{-1} \text{ yr}^{-1}$ (Ruz-Jerez *et al.* 1994) and this places limitations on the estimation of DR. The factors controlling denitrification, their interactions and variations are still not well understood in New Zealand pasture soils. The current experiment was therefore, planned to study the effect of changing soil moisture on DR and $\text{N}_2\text{O}/\text{N}_2$ ratio in New Zealand pasture soils. It was anticipated that the experiment would enable us to develop a deeper understanding of the denitrification process by assessing the relative production of N_2O and N_2 with increasing soil moisture and relating it to soil characteristics. This understanding would then aid in developing techniques to reduce N_2O emitted through denitrification by converting it to N_2 .

Methodology

Collection of soil samples: For the current study, 5 New Zealand dairy pasture soils varying in soil physical and chemical characteristics and DEA were selected (Table 1). Soil cores (2.5 cm diameter and 10 cm long) were collected from two soil depths (0-10 and 10-20 cm) from six random locations on each farm between August and December 2010. Soil cores were taken to the laboratory, sieved through a 2mm sieve and stored in plastic bags at 4°C until used for analyses.

Determination of DEA: Samples (10g) of field moist, sieved (2mm) soil were placed in 125ml conical flasks and 20ml of deionised water and 8ml of nutrient solution was added to make a slurry. The nutrient solution was prepared by dissolving 0.45g KNO_3 , 0.77g D-glucose and 2mg chloramphenicol in 1L of deionised water. This provided 22 $\mu\text{g NO}_3^- \text{ g}^{-1}$ soil and 250 $\mu\text{g C g}^{-1}$ soil. The flasks were sealed air tight using suba seals. Air was scrubbed

from the flasks by flushing with N₂ gas to create anaerobic conditions. Ten percent of the headspace volume of the flask was replaced by purified C₂H₂, and the syringe was flushed 2-3 times to ensure proper mixing of C₂H₂ into the soil sample.

Gas samples for time 0 were taken immediately and transferred to 11ml evacuated vials. Then all the flasks were placed on a shaker set at 125 rpm and incubated at 25°C in a constant temperature room. Thereafter, gas samples were taken after 1hr, 2hr, 4hr, and 6hr of incubation. A 5ml gas sample was collected from each flask each time and the same amount of N₂ gas was replaced into the flasks. The collected samples were diluted to 25ml by adding 20ml of N₂ gas and then were transferred to 11ml evacuated vials. Gas samples were analysed by gas chromatography (GC) for N₂O production in each flask. A Shimadzu GC 17 A (Japan) equipped with back flush system was used. This had a ⁶³Ni- electron capture detector operating at column, injector and detector temperatures of 55, 75 and 330°C respectively.

Calculations of DEA: The measured volumetric N₂O concentrations were used to calculate the amount of N₂O contained in the water and gas phases as follows:

$$\text{DEA } (\mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil hr}^{-1}) = \text{N}_2\text{O production rate (N}_2\text{O-N at } T_{n+1} - \text{N}_2\text{O-N at } T_n) \\ (\mu\text{L N}_2\text{O-N kg}^{-1} \text{ soil hr}^{-1}) \times \text{density of N}_2\text{O (1.8264 } \mu\text{g } \mu\text{L}^{-1} \text{ at normal temperature} \\ (25^\circ\text{C) and pressure (1 atm))}$$

$$\text{N}_2\text{O production rate } (\mu\text{L-N}_2\text{O-N kg}^{-1} \text{ soil hr}^{-1}) = (\text{analysed N}_2\text{O concentration}) \times (V_g + \\ V_l \alpha) \times \text{Dilution factor} / (\text{weight of soil (kg)} \times \text{incubation time (hr)})$$

where: Analysed N₂O concentration = N₂O concentration (μL L⁻¹) of the diluted gas in the storage vials; V_g = volume (L) of gas phase in the incubation flasks; V_l = volume (L) of liquid phase (soil + water) in the incubation flasks; α, the Bunsen absorption coefficient = 0.544; Weight of soil = 10g (0.01kg); Dilution factor = (5ml sample + 20 ml N₂ gas) / (5ml sample) = 25/5 = 5; T_n and T_{n+1} are two incubation times

Incubation of soils at field capacity (FC) and saturation: Six replicate samples (50 g) of soil at FC and at saturation from the 0-10 and 10-20 cm soil depths were transferred into plastic containers having 1 mm pores for exchange of gases.

The soil water contents were maintained by spraying deionized water at 2-hour intervals during the day. On the following day each plastic container was transferred into a 1L Agee jar, and sealed tightly. In half of the jars 10% of the headspace volume was filled with purified C₂H₂ (to stop the conversion of N₂O to N₂). Incubations were carried out for 24 hours at 25°C. The amount of water needed to wet the soils to FC was determined using the suction plate method (Loveday 1974). Soil samples were saturated with water on the suction plate and the plate was fitted to a bubble tower with a suction pump set at a pressure of 0.1 bars. The assembly was kept undisturbed for 24 hours to pump out the excess water absorbed by soils. After 24 hours gravimetric soil water content was determined by drying the wet soil at 105°C for 24 hours. This soil water content corresponds to the amount of water in the soils at FC.

Table 1: Characteristics of soils collected for the experiment (n=6±SEM)

Soil Name	Texture Class	Geographic Location	pH	TN (mg kg ⁻¹ soil)	TC (mg kg ⁻¹ soil)	MBC (g kg ⁻¹ soil)	NH ₄ ⁺ (mg kg ⁻¹ soil)	NO ₃ ⁻ (mg kg ⁻¹ soil)	Total Porosity (%)	DEA (µg N O-N kg ⁻¹ soil ⁻¹ hr ⁻¹)	
										0-10 cm	10-20 cm
Paparua	Silt Loam (ZL)	43°38'15.97"S, 172°28'13.81"E	6.0 ± 0.06	3.6 ± 0.08	40.0 ± 0.58	0.59 ± 0.06	3.5 ± 0.4	13.4 ± 3.1	63 ± 0.8	2035 ± 64	150 ± 6
Manawatu	Fine Sandy Loam (FSL)	40°22'58.26"S, 175°32'21.65"E	5.9 ± 0.05	5.1 ± 0.26	51 ± 1.97	0.97 ± 0.07	3.0 ± 0.3	47.3 ± 3.1	70 ± 0.9	941 ± 89	39 ± 10
Mayfield	Silt Loam (ZL)	43°38'30.12"S, 171°43'47.28"E	4.8 ± 0.08	4.4 ± 0.17	44 ± 1.78	0.44 ± 0.08	103.9 ± 4.5	117.6 ± 9.6	65 ± 0.5	727 ± 60	20 ± 2
Lismore	Stony Silt Loam (STZL)	43°53'17.44"S, 171°38'28.43"E	5.7 ± 0.03	3.8 ± 0.14	38 ± 0.78	0.66 ± 0.05	4.42 ± 0.3	61.5 ± 8.8	72 ± 0.7	352 ± 36	13 ± 3
Otorohonga	Silt Loam (ZL)	38°11'19.70"S, 175°12'35.67"E	5.6 ± 0.02	8.4 ± 0.14	83 ± 1.10	0.46 ± 0.05	4.09 ± 0.4	6.0 ± 0.7	83 ± 0.8	70 ± 32	14 ± 3

Gas Sampling and analysis: A 10-ml gas sample was collected from each jar after 0, 3, 6, 9, 12, 24 and 48 hours of incubation and an equal amount of air was replaced to maintain the same gas pressure inside the jars. The collected gas samples were diluted to 25 ml by adding 15ml of N₂ gas and transferred to evacuated glass vials. Gas samples were analysed by gas chromatography (GC) for N₂O production in each flask as described previously.

Calculations of Denitrification Rate: The measured volumetric N₂O concentrations from the C₂H₂ treatment were calculated from the amount of N₂O contained in the collected gas samples as follows:

$$\text{N}_2\text{O production rate } (\mu\text{L N}_2\text{O-N kg}^{-1}\text{ soil hr}^{-1}) = (\text{Analysed N}_2\text{O concentration}) \times \text{Dilution factor} / (\text{weight of soil (kg)} \times \text{incubation time (hr)})$$

$$\text{DR } (\mu\text{g N}_2\text{O-N kg}^{-1}\text{ soil hr}^{-1}) = \text{N}_2\text{O production rate (N}_2\text{O-N at T}_{n+1} - \text{N}_2\text{O-N at T}_n) (\mu\text{L N}_2\text{O-N kg}^{-1}\text{ soil hr}^{-1}) \times \text{density of N}_2\text{O (1.8264 } \mu\text{g } \mu\text{L}^{-1} \text{ at normal temperature (25}^\circ\text{C) and pressure (1 atm))}$$

where: Analysed N₂O concentration = N₂O concentration (μL L⁻¹) of the diluted gas in the storage vials; Dilution factor = (10ml sample+ 15 ml N₂ gas)/ (10ml sample) = 25/10 = 2.5; T_n and T_{n+1} are two incubation times

Estimation of amount of N₂ produced = Amount of N₂O from acetylene treated jars - Amount of N₂O from jars with no acetylene.

Estimation of N₂O/N₂ ratio = Amount of N₂O produced in the jars/ Amount of N₂ calculated in the above equation

Soil Characteristics: The collected soil samples were analysed for mineral N (NO₃⁻ and NH₄⁺), total N (TN), total carbon (TC), pH, Olsen P, microbial biomass carbon (MBC), total porosity and water filled pore space (WFPS). Soil NO₃⁻-N and NH₄⁺-N were determined by 1 hr soil extraction with 2 M KCl solution at soil extract ratio of 1:5, and subsequent analysis of the filtrate colorimetrically using an automatic analyzer method (Downes 1978). TN and TC were determined by combustion using a LECO CNS-1000 (Bremner 1996; Nelson and Sommers 1996). Soil pH was measured in a 1:2.5 (w/w) soil to water mixture using a PHM 83 Autocal pH meter after incubating the mixture overnight. Olsen P was determined in 0.5M NaHCO₃ soil extracts (Olsen *et al.* 1954), by the phosphomolybdate method of (Murphy and Riley 1962) using a Spectrophotometer PU 8625 UV/VIS at 712nm. MBC was determined using the chloroform fumigation-extraction technique described by Vance *et al.* (1987). The amount of C in the extracts was determined by the potassium dichromate oxidation method of Jenkinson and Powlson (1976) in which an aliquot of soil extract was added to a mixture of sulphuric acid and orthophosphoric acid and boiled under refluxing condition for 30 min. The excess of dichromate was titrated with ferrous ammonium sulfate. The MBC contents in the extracts were calculated by subtracting the amounts of C in the unfumigated samples from the amounts of C in the fumigated samples.

Total porosity of soil samples was calculated from the equation:

$$TP = 1 - \frac{P_B}{P_p}$$

where: TP is the total porosity, P_B is the soil bulk density (g cm^{-3}), and P_p the soil particle density (2.65 g cm^{-3})

The WFPS of soil as a percentage was calculated from equation:

$$\text{WFPS} = \left(\frac{\theta_m \cdot P_B}{\rho_{\text{H}_2\text{O}} \cdot TP} \right) \times 100$$

where: θ_m is gravimetric water content (g/g), P_B is the soil bulk density (g cm^{-3}), $\rho_{\text{H}_2\text{O}}$ is density of water (g cm^{-3}) and TP is the total porosity.

Statistical Analysis: The data on the effect of soil water content on DR and $\text{N}_2\text{O}/\text{N}_2$ ratio was analysed using Minitab 16 in one way and two-way ANOVA. Multiple comparisons among the means were made using the Tukey's Studentized Range Test at 5% significance level. The relationship among DR, N_2 emissions and soil characteristics were determined using correlation and multiple regression analysis.

Results and Discussion

The DR was calculated from the total amount of N_2O produced in the C_2H_2 treated jars. The DR at FC in surface soils (Figure 1) ranged from 4 to $33 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ with the maximum DR in the Manawatu FSL. The DR at FC in the Manawatu SL is significantly different than in the other four soils (Table 3). In sub-surface soils the DR at FC ranged from 4 to $8 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ with no discernible differences among the soils. The DR at saturation ranged from 19 to $50 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ in surface soils with the maximum DRs in the Manawatu FSL and Paparua ZL soils. The DRs in these two soils were significantly different than in the other three soils (Tables 2 and 3). The DR ranged from 4 to $16 \mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ in sub-surface soils.

In all the soils DR at saturation was significantly higher than DR at FC (results of statistical analysis not shown). Also the DR, both at saturation and FC, was significantly higher in surface soils (0-10cm) than in sub-surface soils (10-20cm) (Tables 2 and 3). A correlation analysis was performed to identify the relationship between soil chemical parameters with DR at FC and saturation. The results of the correlation analysis (Table 4) suggested DR at FC was significantly correlated to soil depth, NO_3^- content, DEA and MBC. DR at saturation was significantly and strongly correlated to soil depth, moderately to NO_3^- content, DEA, pH, MBC, TN and TC. To relate DR to soil chemical factors, multiple linear regression analysis was performed using the best sub-set model. The results of the regression analysis (Table 5) suggest 45% of the variation in DR at FC was explained by soil depth, NO_3^- content, NH_4^+ content and DEA. Similarly, 85% of the variation in DR at saturation is explained by soil depth, MBC, TC, TN, pH, DEA and NO_3^- content with NO_3^- content, soil depth, DEA and pH having significant effects on DR at saturation.

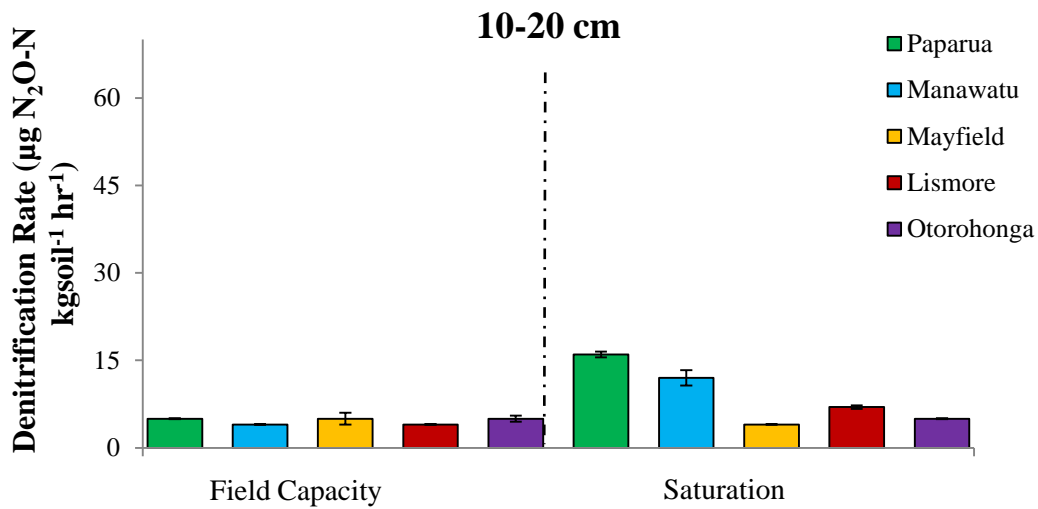
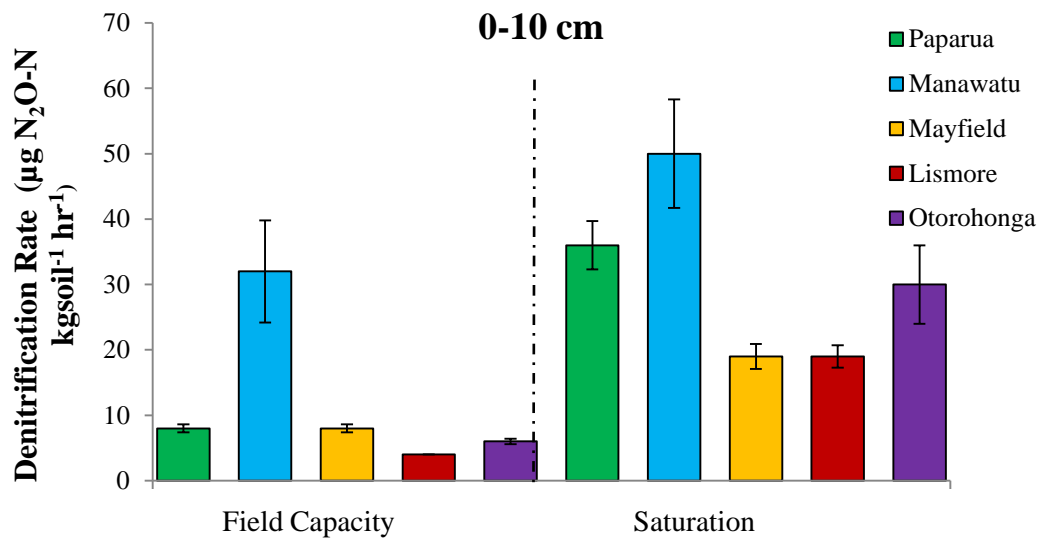


Figure 1: Denitrification rate (N₂O + N₂) at FC and saturation (error bars denote SEM)

Table 2: Two way ANOVA of the DR in different soil types and different soil depths.

	Source	DF	p
DR FC	Soil Type	4	0.0001
	Soil Depth	1	0.0001
	Interaction	4	0.0001
DR Saturation	Soil Type	4	0.0001
	Soil Depth	1	0.0001
	Interaction	4	0.0001

Table: 3 One way ANOVA (Tukey's studentized test) of the DR in 5 different soils. (DR values with the same letter are not significantly different ($P < 0.05$))

Soil name	DR (FC) 0-10	DR (FC) 10-20	DR (Sat) 0-10	DR (Sat) 10-20	N ₂ (FC) 0-10	N ₂ (Sat) 0-10
Paparua	b	a	a	a	c	a
Manawatu	a	a	a	a	a	ab
Mayfield	b	a	b	ab	b	c
Lismore	b	a	b	ab	b	cd
Otorohonga	b	a	b	ab	c	cd

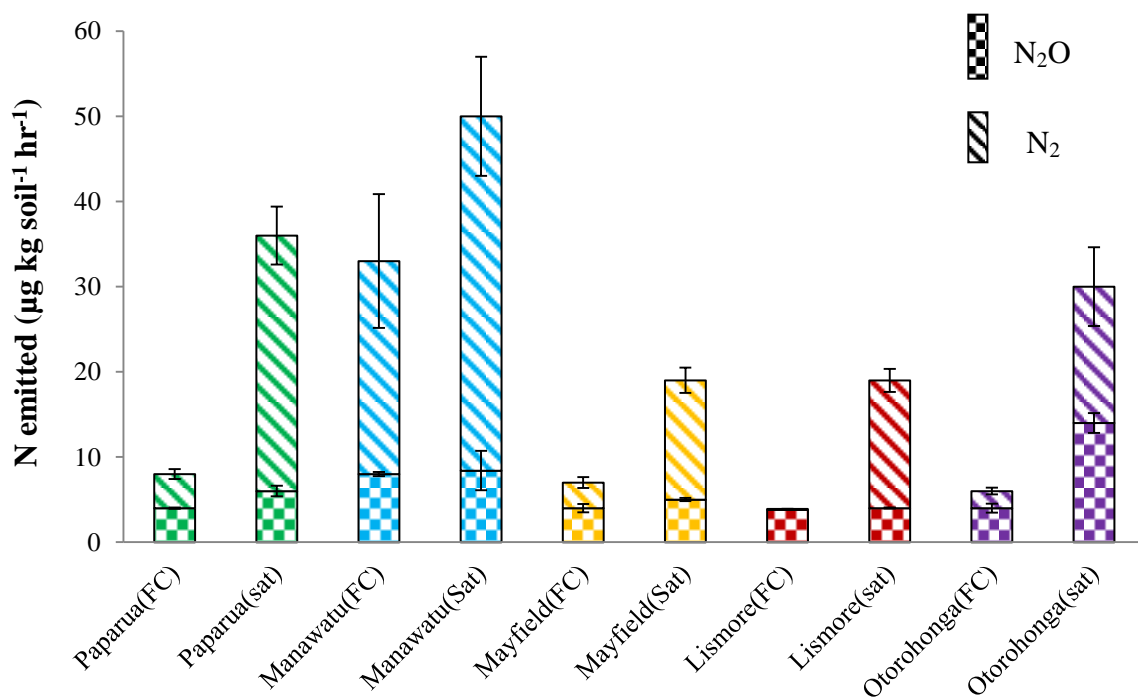


Figure 2: Amounts of N₂O and N₂ emitted from soils at FC and saturation (0-10cm) (error bars denote SEM)

Although the DR at saturation was significantly greater than the DR at FC in all the soils, the magnitude of this difference in DR varied between the soils. The increase in DR with increasing soil moisture was consistent with the work done by Bremner and Shaw (1958), Grundmann and Rolaton (1987), Myrold (1988) and Ruz-Jerez *et al.* (1994) who have suggested that soil water is the major factor influencing the rate of denitrification. de Klein and van Logtestijn (1996) measured DR in laboratory and field incubations using three contrasting soils (sand, loam and peat) and found increases in DR in all three soils with increasing soil water filled pore space (WFPS). With increasing soil moisture content the number of anaerobic microsites increases (Smith and Arah 1990) and the solubility of available substrates like NO₃⁻ and C also increases (de Klein and van Logtestijn 1996). These changes in turn affect the denitrifier enzyme activity and thereby increase DR in soils incubated at higher moisture contents (Miller *et al.* 2008).

In a laboratory incubation study of New Zealand pasture soils, Luo *et al.* (1999) observed higher DRs in soils incubated at saturation than in field moist soils incubated as controls. The increase in DR was greater in saturated soils amended with NO_3^- and C than in the saturated soils alone. Similarly, Luo *et al.* (2000) observed an increase in DR in soils with irrigation but it has been reported that this increase in DR can vary with soil texture or soil type (Groffman and Tiedje 1989, 1991). Nommik and Larsson (1989) found no positive relationship between DR and irrigation or soil water in sandy soils, which have fewer anaerobic zones than finer textured soils. Similarly, Sexstone *et al.* (1985) found a stronger positive relationship between soil water and DR in clay loam soils as compared to sandy loams. Finer texture soils have comparatively smaller pores than coarse texture soils resulting in higher water retention and higher prospects of anaerobic sites for denitrification. The soils used in the current experiment did not vary greatly in soil texture, with only one sandy loam soil and the other four with silt loam texture. Therefore no significant relationship was observed between the soil texture and DR at FC and saturation in the current study. At higher moisture contents the activity of nitrous oxide reductase enzyme increases in soils Miller *et al.* (2008) and therefore, soils with inherently higher DEA (Manawatu FSL and Paparua ZL) have higher DR at saturation than other soils.

At FC the rate of N_2O emission from five soils ranged from 4 to 8 $\mu\text{g N}_2\text{O-N kg}^{-1} \text{ soil h}^{-1}$ (Figure 2). In all the soils, these N_2O emission rates increased at saturation but these increases were small in most of the soils and it was only in the Otorohonga soil that the difference in N_2O emission rates between FC and saturation was statistically significant. The large increase in N_2O emission rate in the Otorohonga soil may be because this soil has the highest porosity. With higher porosity there may have been more aerobic sites, resulting in higher N_2O emission in this soil as compared to others. The amounts of N_2 emitted from surface soils at FC ranged from 0.1 to 25 $\mu\text{g N}_2\text{-N kg soil}^{-1} \text{ h}^{-1}$ with virtually no measureable N_2 produced in the Lismore soil at FC, and ranged from 14 to 42 $\mu\text{g N}_2\text{-N kg}^{-1} \text{ soil h}^{-1}$ at saturation. N_2 production increased in each soil with increasing soil moisture content, but the extent of the increase varied among the soils. In the regression analysis (Table 5), 71% of the variation in N_2 production at FC was explained by NO_3^- content, Olsen P, MBC and DEA with significant effects of Olsen P and MBC on N_2 production at FC. Similarly, 96% of the variation in N_2 production at saturation could be explained by NO_3^- content soil pH, TN and DEA with significant effects of NO_3^- content, pH and DEA. The DEA of the soils was strongly related ($p=0.0001$) to the N_2 production at saturation. The Manawatu FSL and Paparua ZL soils produced more N_2 at saturation and these soils also had higher DEA and MBC than other soils. The variation in inherent microbial enzyme activity in these soils appeared to result in variable N_2 emissions at the same moisture level.

The $\text{N}_2\text{O}/\text{N}_2$ product ratio of denitrification (Table 6) for soils incubated at FC ranged from 0.3 to 38.0 in surface soils with the lowest product ratio in the Manawatu FSL and the highest in the Lismore STZL. The extremely high $\text{N}_2\text{O}/\text{N}_2$ ratio in Lismore STZL results from the near zero N_2 emission from this soil at FC. The variation in $\text{N}_2\text{O}/\text{N}_2$ ratio in the other soils was much smaller (0.3 – 2.9). The product ratio of denitrification at saturation ranged from 0.2 to 0.9 in surface soils with the lowest product ratios for Manawatu FSL and Paparua ZL. At saturation, the $\text{N}_2\text{O}/\text{N}_2$ ratio was significantly correlated with DEA and MBC (Table 4) and 82% of the variation in $\text{N}_2\text{O}/\text{N}_2$ ratio at FC was explained by MBC, pH, NO_3^- , NH_4^+ content and DEA with significant effects of MBC, pH and NH_4^+ content (Table 5). Similarly, 95% of the variation in $\text{N}_2\text{O}/\text{N}_2$ ratio at saturation was explained by NO_3^- content, NH_4^+ content, OP, MBC and DEA, with significant effects of DEA and NO_3^- content. As there was

little change in N_2O production in these soils as moisture content changed from FC to saturation, the changes in N_2O/N_2 ratio were driven by changes in N_2 production with soil moisture. The variation in product ratio for these soils at the same moisture level is due to the differences in their NO_3^- content, NH_4^+ content, Olsen P, MBC nutrient status, DEA and WFPS. The Manawatu FSL had the lowest variation in N_2O/N_2 ratio from FC to saturation and this could be due to annual effluent irrigation on the farm from where the soil was collected. This practice may have affected the soil factors affecting DEA and MBC in this soil leading to a lower N_2O/N_2 ratio. In contrast, Lismore STZL soil with highest variation in N_2O/N_2 ratio from FC to saturation had the lowest DEA and MBC of all the soils. The allophanic Otorohonga ZL soil had the highest porosity among all the soils and had the highest N_2O/N_2 ratio at saturation.

In a laboratory incubation study using a N^{15} labeling technique, Clough *et al.* (2004) reported an increase in both N_2O and N_2 fluxes from soils incubated at saturation as compared to FC. The product ratio of N_2O/N_2 (recalculated from the flux ratio) was higher at FC and decreased with time under saturated conditions. The higher N_2O/N_2 ratio of soils at FC than saturation could be due to more aerobic conditions at FC which may have prevented N_2O from being reduced further to N_2 (Firestone *et al.* 1979). In a seasonal field trial in a permanent pasture Rudaz *et al.* (1999) found increases in both N_2O and N_2 fluxes and decrease in N_2O/N_2 ratio with increasing soil moisture from 60% WFPS and above (recalculated from their results) which was in accordance with the results found by others (Drury *et al.* 1992; Skiba *et al.* 1993; Bandibas *et al.* 1994; Maag and Vinther 1996). These are in agreement with the results of the current experiment. They also suggest emission of N_2 is favored as compared to N_2O with increasing soil moisture and thus soils show decreases in N_2O/N_2 ratio with increases in soil moisture.

The textures of soils used in current experiment only range from fine sandy loam (Manawatu) to silt loam (remaining soils), and therefore do not allow any insights into the effect of soil texture on the relationships between soil moisture and DR, N_2 production or N_2O/N_2 ratio. Other studies (de Klein and van Logtestijn 1996; Groffman and Tiedje 1989, 1991) have observed significant effects of soil texture change on the relationship between DR and soil moisture. However, the soils used in current study do have a range of other chemical characteristics and DEA. The statistical analysis revealed the relationships between soil depth, total porosity, pH, NO_3^- , NH_4^+ content, Olsen P, MBC and DEA on DR, N_2 emissions and N_2O/N_2 ratio at high soil moisture contents.

Denitrification being a biochemical process is driven mostly by the soil factors affecting microbial activity in soil. Therefore, a possible reason for the differences in DR and N_2O/N_2 ratio among these soils at same moisture is due to the differences in the microbial activity. The Manawatu FSL and Papanua ZL with higher DEA and MBC have higher DR and produce more N_2 at saturation than the rest of the soils.

Table 4: Pearson's correlation coefficients between DR and N₂ emission rate and soil factors

Variable		r	p
DR(FC)	DEA	0.535	0.0001
	MBC	0.452	0.0001
	Soil depth	-0.356	0.0050
	NO ₃ ⁻	0.310	0.0160
DR(Sat)	Soil Depth	-0.719	0.0001
	DEA	0.672	0.0001
	MBC	0.637	0.0001
	TC	0.385	0.0020
	TN	0.332	0.0090
	pH	0.429	0.0180
	NO ₃ ⁻	0.420	0.0210
N ₂ O/N ₂ (FC)	MBC	0.519	0.0030
	NO ₃ ⁻	0.489	0.0060
N ₂ O/N ₂ (Sat)	DEA	0.595	0.0010
	MBC	0.365	0.0380

Table 5: Multiple linear regression analysis using best sub-set model of DR and N₂ emission rates against soil factors at FC and saturation.

Variables	Parameters	Equation (Model)	R ² adj
DR (FC)	NO ₃ ⁻ (p=0.002), DEA (p=0.098), NH ₄ ⁺ (p=0.441), soil depth (p=0.455)	DR (FC) = 0.077 Soil Depth + 0.157 NO ₃ ⁻ - 0.0644 NH ₄ ⁺ + 0.00208 DEA	0.45
DR (Sat)	Soil depth (p=0.012), DEA (p=0.030), pH (p=0.040), NO ₃ ⁻ (p=0.044), MBC (p=0.076), TN (p=0.235), TC (p=0.387)	DR(Sat) = - 1.37 Soil Depth + 14.3 MBC - 2.87 TC + 33.2 TN + 5.33 pH + 0.00582 DEA - 0.0250 NO ₃ ⁻	0.85
N ₂ (FC) (0-10cm)	MBC (p=0.010), OP (p=0.016), DEA (p=0.175), NO ₃ ⁻ (p=0.688)	N ₂ (FC) = - 1.25 NO ₃ ⁻ + 1.31 OP + 9.88 MBC - 2.52 DEA	0.71
N ₂ (sat) (0-10cm)	NO ₃ ⁻ (p=0.000), pH(p=0.000), DEA (p=0.017), TN (p=0.483)	N ₂ (Sat)= - 0.0135 NO ₃ ⁻ - 0.0516 pH - 0.0142 TN + 0.0462 DEA	0.96
N ₂ O/N ₂ (FC) (0-10cm)	MBC (p=0.020), pH (0.0001), NH ₄ ⁺ (p=0.005), NO ₃ ⁻ (p=0.002), DEA (0.668)	N ₂ O/N ₂ (FC) = - 4.54 NH ₄ ⁺ + 1.23 NO ₃ ⁻ + 0.079 DEA + 0.967 MBC - 0.578 pH	0.82
N ₂ O/N ₂ (Sat) (0-10cm)	DEA (p= 0.0001), NO ₃ ⁻ (p=0.013), NH ₄ ⁺ (p=0.144), OP (p=0.422), MBC (p=0.930)	0.511 DEA + 0.0573 NO ₃ ⁻ - 1.72 NH ₄ ⁺ - 0.0470 OP + 0.031 MBC	0.95

Table 6: N₂O/N₂ ratios of soils with changing soil moisture condition

	At FC	At saturation
Surface (0-10 cm) soil	N₂O/N₂	N₂O/N₂
Paparua	1.5 ± 0.41	0.2 ± 0.04
Manawatu	0.3 ± 0.04	0.2 ± 0.02
Mayfield	2.3 ± 0.77	0.3 ± 0.04
Lismore	38.0 ± 4.42	0.3 ± 0.02
Otorohonga	2.9 ± 0.47	0.9 ± 0.19

Conclusions

The results of the current experiment confirm earlier findings that soils have higher DRs and lower N₂O/N₂ ratios at saturation than at FC. The effect of changing the soil moisture was more pronounced in surface soils (0-10 cm) as compared to subsoils (10-20 cm). The effect of soil moisture on DR, N₂ emissions and N₂O/N₂ depends directly and indirectly on the physical, chemical and biological characteristics of soils. The positive interaction of soil NO₃⁻, NH₄⁺, pH, Olsen P, and MBC at higher moisture content affects the microbial enzyme activity and thus controls the DR and N₂O/N₂ in soils. Soils with varying inherent physical, chemical and microbial status will show variation in DR and N₂O/N₂ at the same moisture level. It is recommended that further experiments be conducted to study the factors affecting total denitrification and N₂O/N₂ ratio in DEA rich soil such as Manawatu FSL. Also the effect of soil texture on DR could be studied by designing experiments using soils with a wider range of texture classes than in the current study.

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