ASSESSMENT OF DENITRIFICATION POTENTIALS OF GRAZED PASTURE SOILS

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In pasture systems, grazed by dairy and beef cattle, large fluxes of both urine N and C occur in the soil associated with urine patches. Various field and lysimeter studies have shown that urine patches enriched in N, whilst covering only 30% of the grazing area per annum are estimated to create 50% of the pasture growth, 50% of the N leached and 68 % of the N lost to the atmosphere by volatilisation (55%) and denitrification (13%). Leaching losses of NO_3-N to water and incomplete denitrification releasing N_2O-N to atmosphere impact adversely on the environment. Complete denitrification under soil conditions where micro-organisms can create low redox potential has the potential to reduce both nitrate leaching to waterways and greenhouse gas emissions.

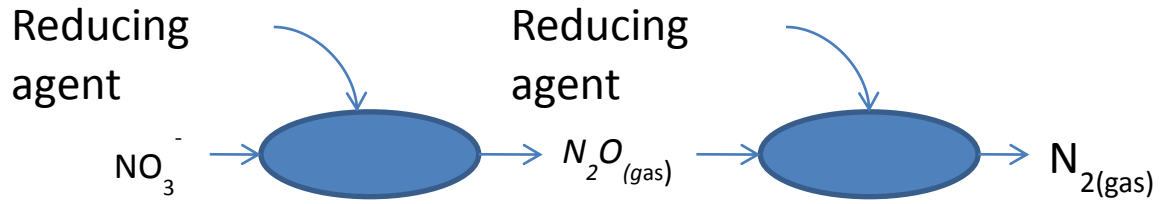
Our knowledge of the spatial distribution of highly denitrifying conditions in soils is limited, and would be improved by extensive field surveys. One limiting factor constraining the extent of field surveys is the lack of a rapid technique to assess denitrification activity and potential for a soil horizon.

This paper summarizes field work undertaken to evaluate whether soil Vis-NIR spectroscopy can be used to predict both denitrification activity and potential activity.

Introduction

The return of urine to grazed dairy pasture is an important part of the nutrient cycle and pasture production system, increasing pasture production by concentrating nutrients in urine patch's stimulating plant growth. However, the excess of organic nitrogen in the form of urea deposited back to pasture poses a risk to the environment due to leaching of nitrate and gaseous emissions of ammonia and nitrous oxide.

Denitrification of nitrate in soils to di-nitrogen gas (N_2), while producing nitrous oxide (N_2O) as an intermediate, has the potential in the subsoil below the root zone to significantly reduce nitrate leaching loses to ground and surface water.



The extent of denitrification potential in New Zealand pastoral soil and vadose zones requires investigation and rapid techniques to allow the spatial and temporal measurements. Direct Vis-NIR spectroscopy on soil cores is one method which may allow detailed mapping of the de-nitrification potential using on-site calibrations of activity and dissolved organic carbon distributions.

This paper discusses the development of a rapid, time effective denitrification enzyme activity assay (DEA) using a vacuum bag technique and Vis-NIR analysis to asses DEA spatial variability and effect on denitrification under field conditions.

The new methods were field tested on two Massey university farms with contrasting soils, in conjunction with current projects. The Tuapaka hill country sheep and beef farm provided a high organic matter soil (Ramiha-Brown soil intergrade) associated with a catchment run off, green house gas and pugging trial. The Moginie sheep unit provided a low organic matter pallic soil(Tokomaru silt loam) with undisturbed and recently cultivated soil. The Moginie sheep unit site combined with a long term carbon sink biochar trial, measuring soil carbon changes over time. These site measurement of DEA using the bag incubation and Vis-NIR in combination with the associated trial data allowed the determination of spatial variability in DEA and correlation factors attributing to DEA such as; dissolved organic carbon (DOC), mineral –N and soil moisture.

Methodology

Denitrification Enzyme Activity (DEA)

The capacity of a soil to reduce nitrate to nitrous oxide and then di-nitrogen has been assessed using an enzyme kinetic approach where the maximum rate (V_{max}) for nitrate reduction is measured during a soil incubation with excess nitrate and reducing carbon (glucose) in a anoxic inert atmosphere of N_2 containing acetylene to prevent the full reduction of nitrate to di-nitrogen. With the reduction of N_2O blocked by the acetylene, the rate of de-nitrification during the incubation was measured by the rate of N_2O production.

In the traditional method a soil sample of 5 to 10 grams is placed in a 125 ml erlenmeyer flask with 25 ml of nutrient solution containing glucose, nitrate and biostat (chloramphenicol, CAP) (Smith and Tiedje 1979). The flask is then plugged with a septum and purged with three volumes of N_2 gas using a fine needle by syringe. Following the purging of the flask 10% acetylene is added to inhibit the reduction of N_2O to N_2 and placed on a shaker. The headspace gas is then sampled periodically to determine the production of N_2O and thus the denitrification rate.

The fixed volume and rigidity of the flask makes it difficult to fully purge the incubation with N_2 and remove the oxygen (O_2) which may delay denitrification. In addition it has been observed during some incubation's that anaerobic fermentation may occur increasing the internal pressure in the flask, which adds to the errors in measurement as the internal gas volume at atmospheric pressure is assumed in the calculations. The purging of the air from the incubation flask is also time consuming and not able to fully remove the initial air.

To overcome these limitations a vacuum bag incubation technique has been developed, allowing the soil sample to be rapidly degassed under low vacuum (-60kPa) and replaced with an inert gas environment. The vacuum bag also allows lager soil samples to be incubated lowering the DEA detection levels, which is important with low activity subsoils.

Vacuum bag DEA incubation

In this method the weighed fresh soil (5 to 20g) is placed in a low gas permeable vacuum pouch (100x 285mm Cas-Pak EVA Vacuum Pouch) fitted with a rubber grommet and three way stopcock. The top of the bag is heat sealed and the bag evacuated through the stopcock followed by the addition of acetylene (25 ml), N_2 gas (120 ml) and 50 ml of nutrient solution containing, nitrate, labile carbon (DOC or glucose) and biostat (chloramphenicol, CAP). The initial gas sample is then drawn through the stopcock (25ml) and compressed into a 12 ml vac-vial for analysis of N_2O via GC with ion capture detector. The bags were then incubated on a horizontal shaker at 25^{o}C with further gas samples taken at 3hr intervals.

Initial method development found that the change in soil to solution ratio between 1:10 soil to solution and 1:2.5 (Table 1) had no effect on observed DEA. However the orientation of the incubation bag on the horizontal shaker and level of CAP addition produced significant effects on observed DEA.

Table 1. Effect of soil : solution ratio on DEA incubations using vacuum bag technique with 50ml volume of solution of 300 mg C (as glucose): 200 mg NO_3-N: 4mg CAP per litre. Following incubation for 3 hours at 25^{o}C, shaken (125 rpm) in the vertical position.

Soil: Solution	DEA ($\mu g\ N_2O\text{-}N\ kg^{-1}\ soil\ hr^{-1}$)	Mean	SD
1:10	1673		
1:10	1145	1409	373
1:5	1422		
1:5	1690	1556	188
1:2.5	1471		
1:2.5	1746	1608	194

The shaking of the incubation bag in the horizontal position (lying flat) significantly reduced observed DEA activity compared to bags in the vertical position (standing), while DEA tended to reduce with CAP additions greater than 10 mg/l (Figure 2).

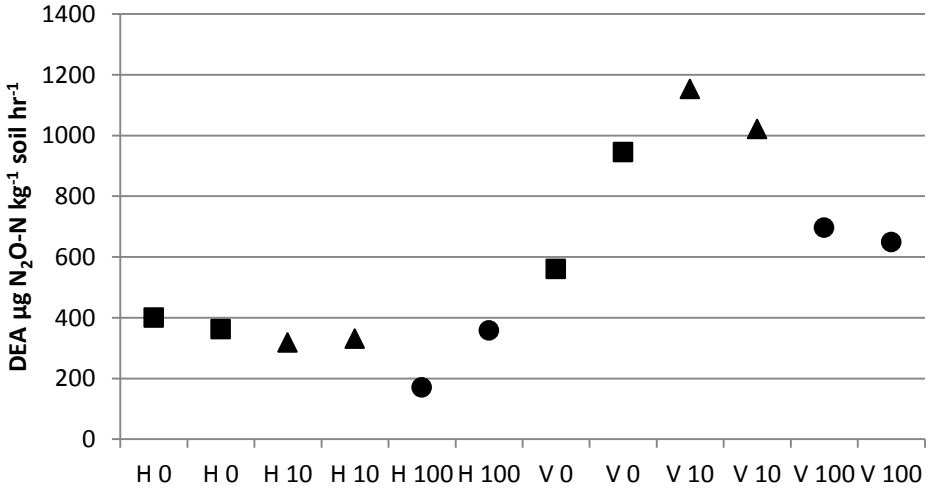


Figure 2 Denitrification enzyme activity assayed using the bag technique as a function of shaking orientation, horizontal-H (flat) and vertical-V (standing) and chloramphenicol concentration of 0, 10 and 100mg/l.,for Tokomaru silt loam 0-5 cm (10g) incubated in 50ml of 300 mg C (as glucose) and 200mg NO_3-N per litre for 3 hours at 25 ^{o}C.

The bag method has introduced a confounding factor which is not found in the rigid glass flask method as the orientation and mixing intensity appear to have a major effect on the DEA results, with the well mixed horizontal orientation resulting in low DEA levels compared to the vertical poorly mixed orientation. This requires more in-depth investigation regarding the usefulness of DEA incubation in determining soil denitrification potential whether carried out in a flask or bag.

Application of in the bag DEA analysis and Vis-NIR spectral analysis for assessment of de-nitrification rate and N_2O emissions from field sites

The spatial distribution of DEA can potentially be measured using Vis-NIR spectral analysis of soil cores with site and time specific DEA calibration, using incubations of a small subset on the total scanned cores. This calibration was carried out for two sites on Massey university farms, Tuapaka (Ramiha-Brown soil intergrade) hill country beef and sheep farm and Moginie (Tokomaru silt loam - Pallic soil) sheep farm.

The Tuapaka site measurements were conducted alongside surface run-off and pugging trials with applied urine in quadrates and gas chambers to monitor N_2O emissions. The trial isolated areas with three degrees of treading damage of nil, light and heavy to which urine was applied with non-urine controls. 43mm diameter cores to a depth of 600mm were taken from the area in the quadrates outside the gas chambers on day 1 and 14 following urine application and transported to the lab in 50mm PVC tubes. The cores were then scanned using an ASD FieldSpec3Vis-NIR spectroradiometer at 1cm increments along the outer surface. As the initial scanning point had to avoid the top edge of the core, due to light scattering effects, the first scan point started 2-5mm from the upper edge of the core. This point was then referred to as depth zero. The unscanned 2-5 mm on the surface were analysed separately from the 0- 5cm depth as not to contaminate the calibration of the Vis-NIR with un-scanned material. The remaining cores was sectioned into 5-10,10-20,20-30,30-40cm depths, dried and weighed for bulk density analysis and divided into vertical sections for DEA, water content and mineral N extraction in 2M KCl. The DEA samples were immediately taken, sealed, evacuated and refrigerated prior to incubation on the following day when 84 DEA's were performed in one day by with a team of four people to reduce delays in gas samples between analysis.

Results

Vis-NIR reflectance estimation of DEA

The analysis of the Vis-NIR spectral data and DEA showed a good correlation between the predicted DEA and measured values (Figure 3), making Vis-NIR spectral reflectance a useful method for the estimation of soil DEA.

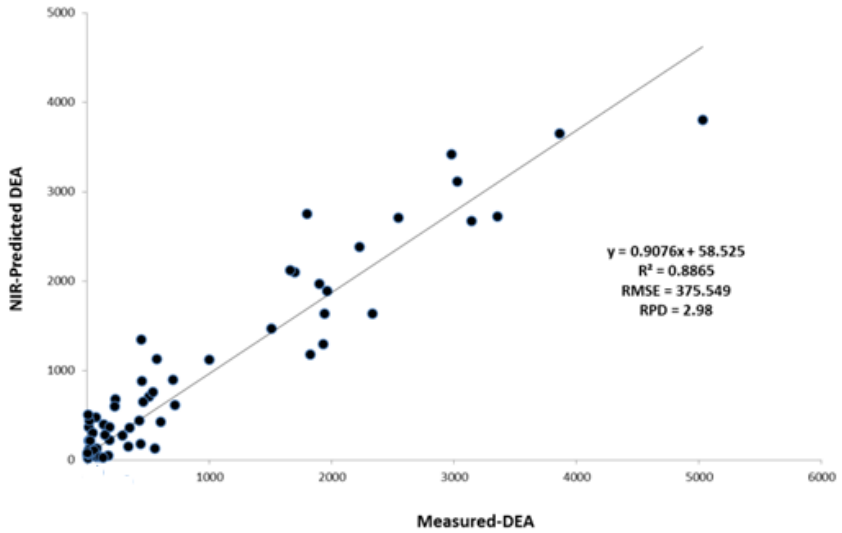


Figure 3 Comparison of measured DEA and predicted DEA from NIR spectral analysis for the Tuapaka site.

Spatial variability of DEA

The spatial variability of DEA with depth and between sites (Figure 4) as measured using the bag incubation technique, showed the highest DEA activity at the soil surface with a rapid decrease with depth, in all soils. This appears to be related to soil carbon and biological activity following similar trends between sites and with soil depth. On the Moginie site (Tokomaru silt loam) the renewal of pasture with its associated cultivation to 20 cm produced a redistribution of DEA to depth with the inversion and mixing of the upper soil profile.

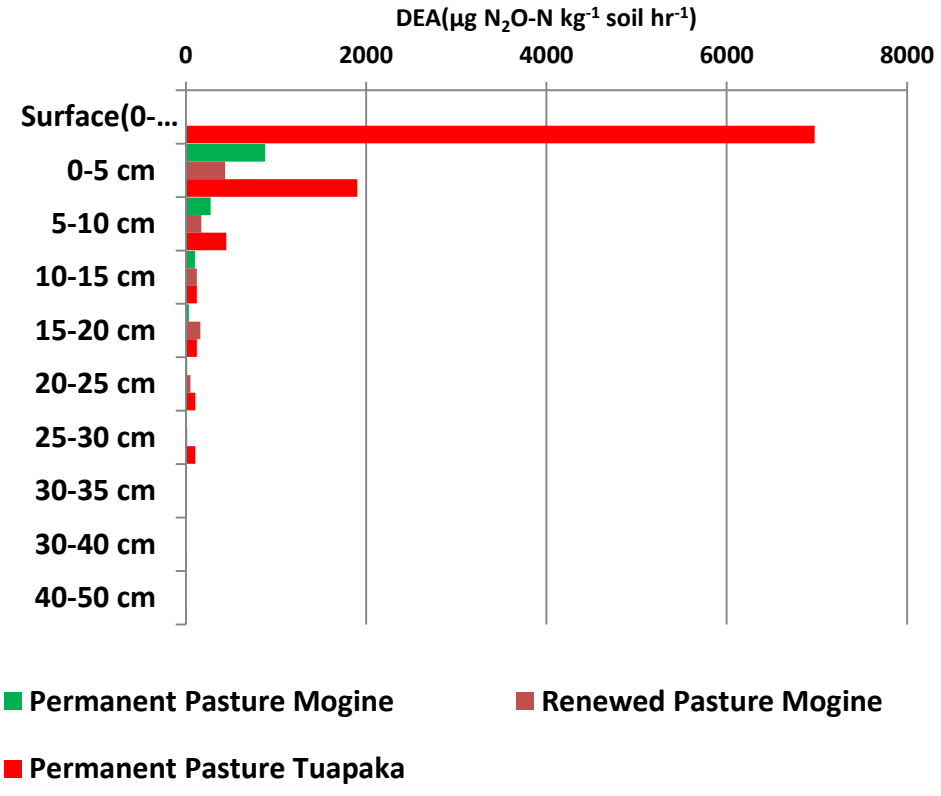


Figure 4. Distribution of DEA in soil profiles from Moginie and Tuapaka sites

Relationship between DEA soil properties and observed field N_2O

N_2O fluxes from the Tuapaka site were measured on day 1 and day 14 following urine application. Heavy rainfall occurred during these two measurments, resulting in a change in oxidation state throughout the soil profile, observed as a transformation of the red iron oxide coatings on soil particles in the soil profile to a reduced grey coloured state (i.e. anoxic conditions). The change was also associated with an increase in N_2O emissions from the urine affected areas and a drop in DEA.

The increase in N_2O emissions did not correlate with the DEA measurements at the surface or 0-5cm depth (Figure 5), but showed a good correlation with mineral N, DOC (as indicated by UV absorption of 2M KCl extracts at 210nm) and specific water content (Figure 6).

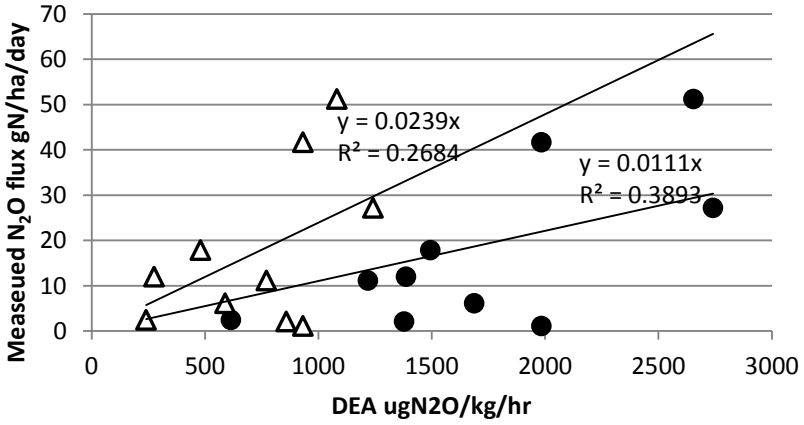


Figure 5. Correlation between measured DEA at the surface(●) and 0-5cm (Δ) soil depth to N_2O flux for the Tuapaka Site, 14 days following urine application.

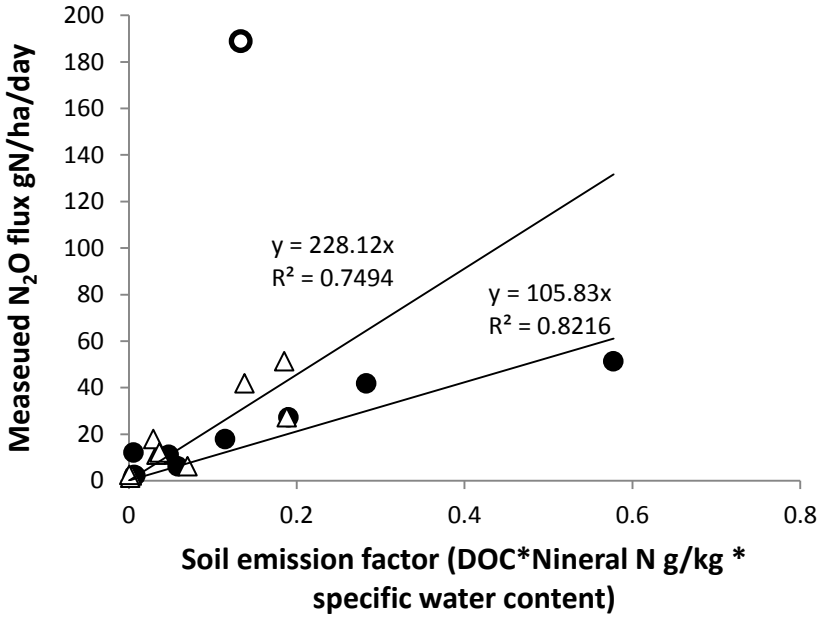


Figure 6. Correlation between combined soil emission factors (dissolved organic matter, mineral N and specific water content) at the surface(●) and 0-5cm (Δ) soil depth to N_2O flux for the Tuapaka Site, 14 days following urine application.

These results show that N_2O emissions are driven by surface substrate concentration for both nitrification and subsequent denitrification.

Conclusion

The measurement of DEA in the field using both in the bag incubation or Vis-NIR reflectance proved to be useful tools in assessment of DEA spatial distribution allowing rapid assessment of soil denitrification potential.

Analysis of the spatial distribution of DEA with depth at two sites has shown that DEA decreases rapidly down the soil profile associated with decreasing soil C content.

In terms of surface emissions of N_2O, measured DEA gave a poor correlation with the measured N_2O fluxs as denitrification appeared to be more limited by dissolved organic carbon and mineral-N, with specific water content as an indicator of anoxic conditions.

Further work is required to assess those factors contributing to denitrification, particularly in regards to subsurface denitrification which can potentially minimize the risk of nitrate leaching to both surface and ground waters.

Reference

Smith, M. S. and J. M. Tiedje (1979). Phases of denitrification following oxygen depletion in soil. Soil Biology & Biochemistry **11**(3): 261-267.