

# Measuring Denitrification Enzyme Activity in Soils under Pastures Using Membrane Inlet Mass Spectrometer



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## Introduction

Denitrification is mainly the biological process of transforming  $\text{NO}_3^-$  to end products  $\text{N}_2\text{O}$  and  $\text{N}_2$ . Size and potential activity of the denitrifying organisms in association with environmental factors regulate its rates. Denitrification enzyme activity (DEA) is measured with non-limiting substrates and under anaerobic conditions for a few hours to several days to attain denitrification potential at the time of soil/water sampling. Due to high background of atmospheric  $\text{N}_2$ , denitrification measurement is generally performed using acetylene block or stable isotope techniques. Membrane Inlet Mass Spectrometer (MIMS), measuring  $\text{N}_2$ ,  $\text{Ar}$ ,  $\text{O}_2$  and other noble gases by auto-degassing water samples, was investigated as a potential alternative method to assay DEA in soil slurry/sediments.

## Materials and Methods

Experiments were conducted with soils of A, B and C horizons and groundwaters collected from pastures (grass and clover-grass) to obtain optimal  $\text{NO}_3^-$  and available carbon (C) rates to initiate DEA. For this, 30 g soil followed by deionized water (*He* purged) was taken in 160ml glass bottle and sealed with a rubber stopper without any air entrapments. N as potassium nitrate (0 to 120  $\text{mg kg}^{-1}$  soil) and C as glucose (0 to 240  $\text{mg C}$ ) plus 30  $\text{mg NO}_3^- \text{N, kg}^{-1}$  soil each were added. Incubation was carried out under water in dark conditions at 21°C for six hours. Water for each treatment was sampled in exetainers and kept it under water in a cold room at 4°C before analysis using MIMS. A second experiment was carried out to see the denitrification potential of subsoil (C horizon) with simulated unsaturated and saturated conditions plus groundwater at 12°C and those were treated with 30  $\text{mg NO}_3^- \text{N}$ , and 60  $\text{mg glucose-C, kg}^{-1}$  dry soil).

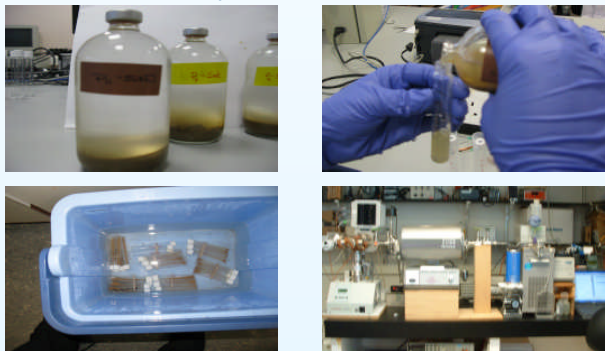


Fig. 1 showing (L to R) incubation of sediment-groundwater, collection, storage, and analysis using MIMS.

## Results and Discussion

Peaks for  $\text{N}_2/\text{Ar}$  ratio, representing denitrification, varied between soils under two land uses and also between soil depths within the land use, being higher in surface over subsurface soils (Fig. 2). Soils under grass-clover required generally a small amount of  $\text{NO}_3^- \text{N}$  (15  $\text{mg kg}^{-1}$  soil) and glucose-C (60  $\text{mg kg}^{-1}$  soil) for the short-term DEA assay. By contrast, the amount of N and C varied largely within horizons under ryegrass, ranging from 30 to 120  $\text{mg NO}_3^- \text{N}$ , and 60 to 120  $\text{mg glucose-C, kg}^{-1}$  soil.

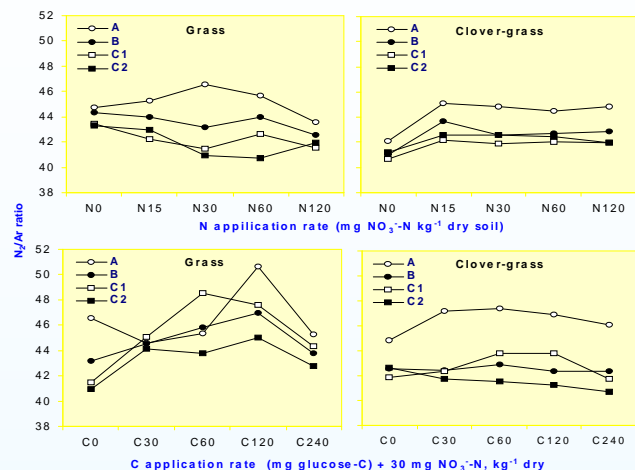


Fig. 2. Short-term DEA at variable  $\text{NO}_3^-$  and C rates, receiving  $\text{NO}_3^-$  at 30  $\text{mg N, kg}^{-1}$  dry soil.

During the 14 days incubation, presumably higher pre-oxidative state of unsaturated subsoil showed a lower denitrification activity initially compared to the saturated one (Fig. 3). The maximum  $\text{N}_2/\text{Ar}$  was generally lower with  $\text{NO}_3^- \text{N}$ , relating again to higher oxidative state and the possible C limitation, than the control. The application of N and C together had a higher denitrification potential (DP) over time than the other two treatments, achieving peak fluxes within a short period.

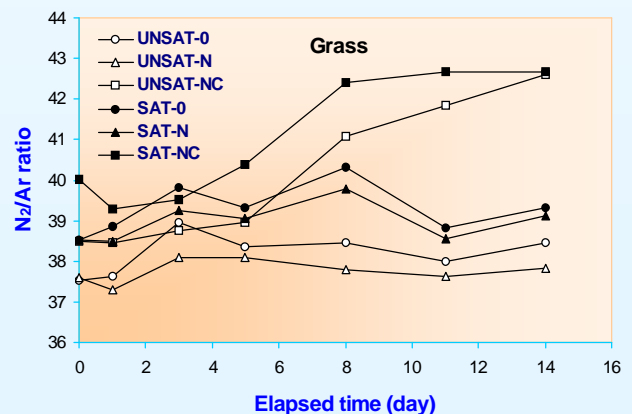


Fig. 3. Denitrification potentials in unsaturated (UNSAT) and saturated (SAT) subsoils + groundwater

## Conclusions

The DEA was mainly limited by substrates and that the availability of C as an energy source could override the temporary oxidative state occurring due to  $\text{NO}_3^-$  addition. The soil having high mineralization potential might require less substrate to activate denitrifier activity, with lower being in the subsoils. Saturated sediments had larger DP than unsaturated conditions. Results imply that MIMS can measure DP in soils/sediments at coupled 30  $\text{mg NO}_3^- \text{N}$  and 60  $\text{mg available C, kg}^{-1}$  soil.

