PATTERNS OF DENITRIFICATION IN RECLAIMED MINESPOILS IN THE ARID SOUTHWEST[1]

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Abstract.--Irrigation and occasional heavy rains or snowmelt may lead to anaerobic conditions in replaced and revegetated soils or spoils providing opportunities for nitrogen loss through denitrification. Using the acetylene block technique, samples of spoil and replaced topsoil from a surface coal mine in northwestern New Mexico were assayed for denitrification potential by enriching the sample with nitrate nitrogen and glucose. Generally, potential denitrification rates in revegetated topsoil areas were found to be low even under an anaerobic atmosphere and moist soil conditions. However, when "whole core" samples were assayed, relatively high rates of denitrification were observed in revegetated spoils that contained high concentrations of nitrate from mineralization of fossil nitrogen in the spoil material. Apparently, denitrification is highest where levels of nitrate are high and the spoil material is less permeable.

INTRODUCTION

In the practice of revegetation and reclamation of mined lands, the loss of available nitrogen from soil materials used for revegetation is counterproductive. Nitrogen can be lost from mine soil materials by erosion, ammonia volatilization, leaching, and denitrification. It seems unlikely that important quantities of nitrogen would be lost from mineland soil materials through denitrification in arid and semiarid areas of the western U.S. The availability of organic substrate, high concentrations of nitrate and high soil moisture conditions that form ideal conditions for denitrification occur infrequently in the west (Focht and Verstraete 1977, Westerman and Tucker 1978). However, where nitrate is available and organic carbon has accumulated in the rhizosphere or from litter, denitrification may be stimulated (Smith and Tiedje 1979). The possible effect of N_2O, a product of denitrification, on the vertical distribution of ozone in the atmosphere has focused considerable research attention on denitrification and significant advancement has been made in understanding the biochemistry and ecology of this process (Delwiche 1981, Payne 1981). However, too little information is available on the role of denitrification in the mine land revegetation process.

The objective of the work reported here was to evaluate the denitrification potential in spoils and revegetated soil materials at the San Juan Coal Mine near Farmington, New Mexico, both to determine the possibility for loss of nitrogen through this route and as an indicator of general microbial activity in the soil.

MATERIALS AND METHODS

Samples

The semiarid climate, soils, and vegetation of the San Juan coal mine area have been described previously (Fresquez and Lindeman 1983).

Samples of soil material from 2 to 3 year old spoil piles and a 3 to 4 year old topsoil stockpile were collected to a depth of 15 cm with a garden spade. An undisturbed (unmined) area and areas revegetated in 1974 through 1982 were also sampled. Areas revegetated in 1974 through 1976 were not covered with topsoil material (table 1). Samples from the undisturbed area and revegetated areas were taken to a depth of 30.5 cm with a 7.6 cm ID hand driven soil coring tube (Giddings Machine, Fort Collins, CO). Ten core samples were taken at arbitrary distances along transects through each revegetation area. Soil cores were

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Table 1.--Extractable mineral nitrogen and surface texture of soil and reclaimed spoil at the San Juan coal mine.

Site	NH_4-H (µg/g)	NO_3-N (µg/g)	Surface Texture
Undisturbed soil	[1]0.6 ± 0.2	1.3 ± 0.6	Loamy sand
Stored topsoil	1.2 ± 0.2	20.4 ± 10.1	Sandy loam
Spoil	8.7 ± 1.7	14.2 ± 4.9	Clay
Revegetated spoils:			
[2]1974	1.6 ± 1.2	50.7 ± 27.9	Clay
1975	11.3 ± 13.6	65.7 ± 25.0	Sandy loam
1976	10.4 ± 11.0	45.1 ± 16.3	Clay loam
Revegetated soils:			
1978	0.9 ± 0.2	2.9 ± 2.3	Sandy loam
1979	0.8 ± 0.2	3.3 ± 2.6	Sandy loam
1980	0.7 ± 0.2	2.6 ± 1.0	Sandy loam
1981	0.7 ± 0.2	1.4 ± 0.4	Sandy loam
1982	1.8 ± 1.6	24.9 ± 7.2	Sandy clay loam

[1] ± 90 percent confidence interval.
[2] Year reclaimed.

divided into 0 to 15 cm and 15 to 30 cm sections. All samples were placed in clean plastic bags and returned to the laboratory in insulated chests. In the laboratory, samples were sieved to pass a 2 mm screen and stored at 4°C.

Soil cores for whole core denitrification experiments were taken with 8.5 cm ID steel tubing to a depth of 30.5 cm. Three cores were collected from the undisturbed area and each of the 1976, 1978, and 1982 revegetation areas. These cores were also transported to the laboratory in insulated chests and stored at 4°C until the denitrification experiments were performed.

Soil Analyses

Subsamples were extracted for mineral nitrogen analyses using 1:4 (weight:volume) slurries of soil in 1N KCl. Slurries were extracted on an orbital shaker at 150 rpm for 30 min, solids were removed by centrifugation at 600 x g for 10 min, and NO_3-N and NH_4-N were determined in the supernatant.

Ammonium nitrogen was determined using the indophenol method (Strickland and Parsons 1968), and nitrate was determined using the automated cadmium reduction procedure (APHA 1980). Denitrification potential was determined using the acetylene inhibition technique (Yoshinari et al. 1977). Ten grams of soil were weighed into a 70 ml serum bottle and saturated by adding 4.5 ml of a solution containing 1 percent (w/v) glucose and 2 g KNO_3 per liter. The serum bottle atmosphere was replaced with N_2 by flushing for 5 min. Six milliliters of the serum bottle head space gas was then withdrawn using a hypodermic syringe and 6 ml of acetylene were added, giving an acetylene content of about 10 percent. The sample was then incubated for approximately 24 hours. Actual incubation time for each sample was recorded. After incubation, the bottle head space was analyzed for N_2O using a Hewlett Packard Model 5750 gas chromatograph with high temperature electron capture detection (Rasmussen et al. 1976) and He as the carrier gas. The GC column, 2 m x 2.16 mm ID stainless steel packed with Porapak Q (Waters Associates, Milford, Mass.), was operated at 50°C. The gas chromatograph was programmed to recondition the column at 150°C for 5 min after each injection. Without reconditioning, unidentified, slow eluting compounds interfered with subsequent analyses. Carrier flow rate was 30 ml/min. The electron capture detector was operated at 300°C. Atmospheric levels of N_2O (≈ 0.5 ppm by volume) could be detected using this system. Calibration was done using a commercial standard (Scientific Gas Products, Denver, CO) containing 100 ppm N_2O in N_2.

Whole core denitrification was determined by using the acetylene inhibition technique as well. A schematic diagram of the soil core apparatus is shown in figure 1. The steel tube was closed on the bottom with a plastic cap and made gas tight with silicone grease. The top was closed with a rubber stopper. Rubber septa were placed in all of the locations shown in figure 1. Commercial grade acetylene was scrubbed through concentrated sulfuric acid to remove acetone, and through a $Ca(OH)_2$ suspension to remove acid fumes and then injected into the soil to produce a concentration of 15 percent in the soil atmosphere. Pore space was assumed to be 50 percent of the soil volume. Water was added to the surface and injected through each of the septa below the soil surface to saturate the soil. Air (61 ml/min) and acetylene (11 ml/min) were pumped through hypodermic needles into the headspace of the column and vented through a hypodermic needle through the septum as shown. This maintained an air atmosphere containing approximately 15 percent acetylene that was replaced approximately every 9 hours. Samples of the headspace gas were taken by inserting a hypodermic syringe needle through the vent septum, removing the vent needle, drawing 3.5 ml of gas, removing the hypodermic syringe needle, and replacing the vent needle. The soil columns were operated for up to 52 hours at room temperature (21 ± 3°C) in a laboratory hood.

Initial nitrifying potential of the undisturbed and revegetated soil materials was assessed using measurements of the ammonium oxidation rate. Substrate concentrations were kept low to avoid toxic effects and the analytical time frame was kept short enough to exclude the proliferation of the bacteria primarily responsible

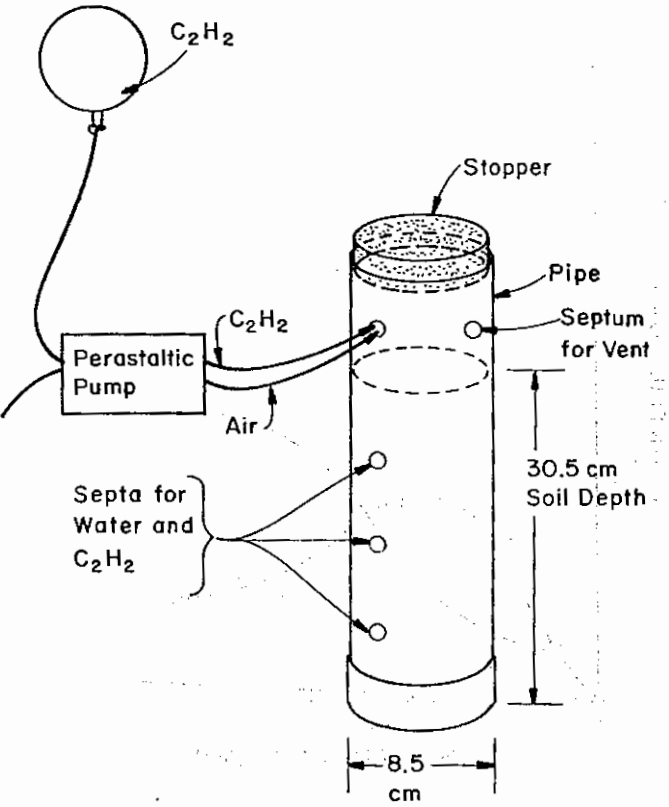

Figure 1.--Soil core denitrification apparatus.

Table 2.--Potential denitrification in undisturbed soil, stored topsoil, spoil, revegetated spoil, and revegetated soil at the San Juan coal mine.

Site	Average Denitrification Rate (ng N_2O-N/g·d) 0-15 cm depth	15-30 cm depth
Undisturbed soil	[1]11.4 ± 10.0	< 0.42
Stored topsoil	28.7 ± 13.8	-
Spoil	10.7 ± 10.4	-
Revegetated spoils:		
[2]1974	23.2 ± 12.0	3.8 ± 4.4
1975	10.0 ± 8.4	4.1 ± 3.7
1976	30.1 ± 9.1	< 0.34
Revegetated soils:		
1978	10.3 ± 2.5	6.0 ± 3.9
1979	23.5 ± 8.1	27.1 ± 25.7
1980	29.8 ± 8.4	9.8 ± 4.0
1981	26.0 ± 12.7	18.3 ± 15.8
1982	24.8 ± 8.7	17.5 ± 6.6

[1] ± 90 percent confidence interval.
[2] Year reclaimed.

for this activity. In this way, a measure for the preexisting enzymatic activity of these organisms was obtained (Belser and Mays 1980, E. L. Schmidt, Personal Communication, 1980).

To measure initial potential NH_4^+ oxidation activity, 12 g of soil were weighed into a 250 ml Erlenmeyer flask. Fifty milliliters of a 0.5 mM ammonium phosphate-buffer solution (167 mg K_2HPO_4/l, 3 mg KH_2PO_4/l, 6.6 mg $(NH_4)_2SO_4$/l, pH 8) was added. To each flask, 0.5 ml of 1 M $NaClO_3$ was added to block NO_2^- oxidation. The flasks were capped with aluminum foil and placed on an orbital shaker at 200 rpm and 21 ± 3°C. Over the next 24 to 30 hours, at 8-14 h intervals, three 5-7 ml samples of the soil slurry were poured from each flask into a test tube; $Ca(OH)_2$ (0.1 g) was added to coagulate suspended clay, and the samples were centrifuged at 600 x g for 10 min. A 2 ml portion of the supernatant was diluted to 50 ml and analyzed for NO_2^--N. A least-squares linear regression line was calculated to fit the nitrite concentration of the slurry over time. The slope of the regression line represented the rate of NO_2^--N production from ammonium ion.

RESULTS AND DISCUSSION

Potential Denitrification

Results of potential denitrification measurements are shown in table 2. Average potential denitrification rates were similar in the 0 to 15 cm depth of all of the soils tested. Apparently, denitrifying bacteria with similar activity were present in the surface of all of the soils. Results between individual samples within each site were, in general, highly variable with coefficients of variation exceeding 100 percent in some cases.

Average potential denitrification rates were less uniform among the sites in the 15 to 30 cm depth. Rates were below detection in the undisturbed soil and 1976 revegetation areas while the 1979 revegetation area rate at this depth was not significantly different from that of the surface soil.

Even under these apparently idealized conditions, denitrification rates were low. Under field conditions where levels of nitrate and organic substrate are lower, the rate would be expected to be much lower.

Whole Core Denitrification

The results of whole core denitrification experiments are illustrated in figures 2 through 4. Highest rates of denitrification were observed in cores from the 1976 revegetation area soil material (fig. 2). One core denitrified at rates in excess of 40 ng/cm^2/h after 36 h incubation. Two of the 1976 cores exhibited lag times of approximately 12 h before appreciable rates of denitrification developed and did not reach the maximum rate until 36 h. The third core, however, exhibited a maximum rate in approximately 10 h. The maximum in the third core was followed by declining denitrification rates over the remainder

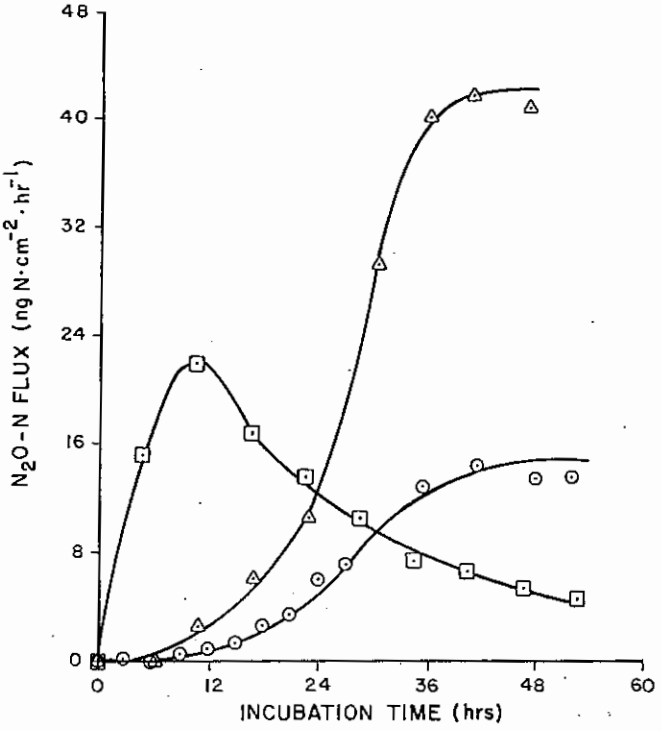

Figure 2. Denitrification kinetics in three whole core samples of minespoil revegetated in 1976.

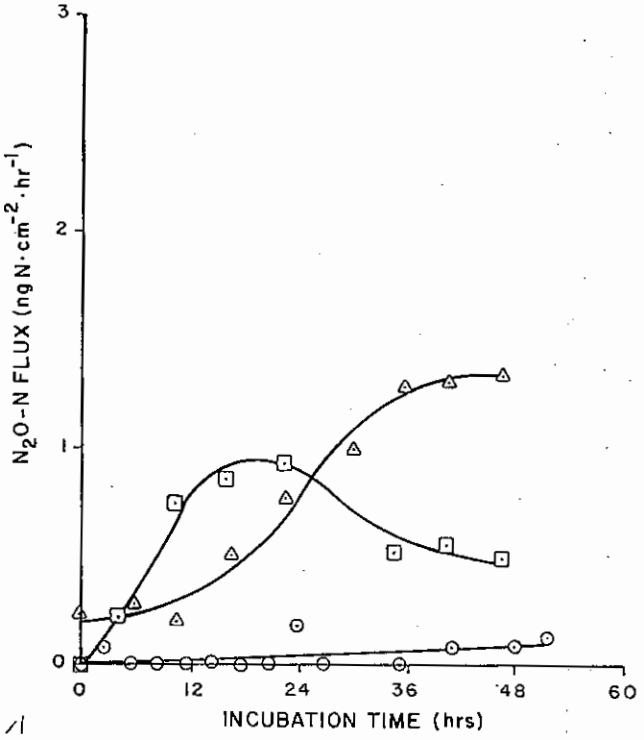

Figure 4. Denitrification kinetics in three whole core samples of undisturbed soil.

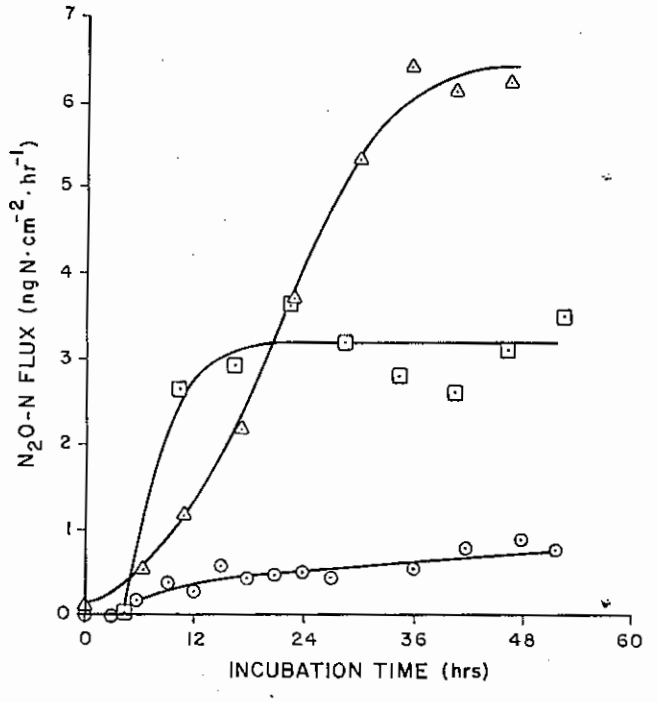

Figure 3.--Denitrification kinetics in three whole core samples of soil over minespoil which was revegetated in 1978.

of the experiment. This implies that this core had an existing denitrifying population which became active quickly and probably exhausted the available energy yielding substrate. Substrate may have been made available when the soil material was moistened, and without available substrate, the denitrification rate declined. It is anticipated that a similar declining rate would have been observed in other cores if the experiments had been continued.

Maximum denitrification rates were considerably lower in the replaced topsoil of the 1978 revegetation area (fig. 3) than in the 1976 soil (fig. 2). The kinetics in these cores were also dissimilar and emphasize the magnitude of the variability of soil microbial processes in revegetation areas.

Denitrification rates were essentially nil in all of the cores from the 1982 revegetation area (results not shown).

Cores from the undisturbed soil also showed variable kinetics, and maximum rates of denitrification were low (fig. 4). This loamy sand soil probably remains well aerated even when wet and contains low amounts of nitrate (table 1) and, therefore, does not support high denitrification activity.

In contrast, the fine textured, relatively high nitrate soil material of the 1976 revegetation area has occasionally been anaerobic and supports considerably higher denitrification

activity. The initial nitrification (ammonium oxidation) rate was significantly higher ($P \leq 0.05$) in the 1976 soil material than in any of the other sites tested (fig. 5), indicating that nitrogen processing in this site probably proceeds at a relatively high rate when environmental conditions are appropriate. Total soil nitrogen at this site is also relatively high, and mineral nitrogen pools are probably maintained through mineralization of fossil nitrogen in the reclaimed spoil material (Reeder and Berg 1977).

The lack of measurable denitrification in cores of 1982 revegetated soil material was surprising considering the relatively high nitrate nitrogen concentrations in this material (table 1) and its demonstrated potential for denitrification (table 2). This result suggests that denitrification is limited by substrate availability in the 1982 site. Appreciable amounts of labile organic matter may not have accumulated in this relatively recently revegetated area.

The apparent low rates of denitrification from all the sites tested indicate that denitrification is not a major pathway for loss of soil nitrogen from San Juan Coal Mine soil materials. Climatic conditions severely limit the amount of time when the soil environment is conducive to denitrification even at the most active sites.

SUMMARY AND CONCLUSIONS

Potential denitrification rates were low and similar in surficial spoil material, stockpiled topsoil, revegetated spoil, and revegetated replaced topsoil regardless of age. Soil material 15 to 30 cm below the surface tended to be lower in potential denitrification activity than surface materials. Variability in potential denitrification activity was very high within sampling sites.

Whole core denitrification kinetics and maximum rates were also extremely variable both between and within sites. Highest rates of denitrification were observed in a fine textured, revegetated mine spoil that contained relatively high amounts of nitrate and total nitrogen, and that had relatively high initial nitrification potential. Whole core denitrification rates were essentially nil in the most recently revegetated area studied, and were probably limited by the amount of available organic substrate. Sandy loam soils, including an undisturbed soil, with lower concentrations of nitrate had considerably lower denitrification rates than the revegetated spoil.

Generally, nitrogen losses from denitrification at the San Juan mine appear to be limited both by microbial activity and the semiarid environment.

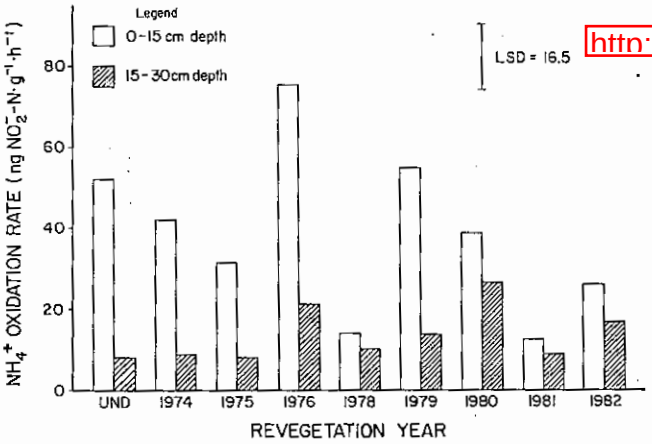

Figure 5. Initial $NH4^+$ oxidation rates in revegetated mine soils and undisturbed soil.

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