Environmental Sciences Laboratory

Phytoremediation of the Nitrogen-Contaminated Subpile Soil at the Former Uranium Mill Tailings Site in Monument Valley, Arizona 2004 Status Report

December 2004

Prepared for U.S. Department of Energy Grand Junction, Colorado





Work Performed Under DOE Contract No. DE–AC01–02GJ79491 for the U.S. Department of Energy Approved for public release; distribution is unlimited.

DOE-LM/GJ768-2004 ESL-RPT-2004-07

Phytoremediation of the Nitrogen-Contaminated Subpile Soil at the Former Uranium Mill Tailings Site in Monument Valley, Arizona

2004 Status Report

December 2004

Prepared by

Environmental Research Laboratory University of Arizona Tucson, Arizona

and

Environmental Sciences Laboratory U.S. Department of Energy Grand Junction, Colorado

Contents

Execut	ive Summary	. 1
1.0	Introduction	. 1
2.0	Site Description	2
3.0	Irrigation Volumes and Soil Moisture	. 2
4.0	Total Annual Plant Growth, Survival and Nitrogen Uptake	. 5
5.0	Soil Nitrate and Ammonium Concentrations	7
6.0	Denitrification Studies	11
7.0	Quality Control and Assurance	13
8.0	Recommendations	13
9.0	References	13

Figures

Figure 1.	Soil Moisture Profiles Across All Zones and All Months at Depth for Years 2000
	Through 2004 at the Monument Valley Phytoremediation Plot
Figure 2.	Mean Annual Moisture Content Across all Zones, Months, and Depths at the
	Monument Valley Phytoremediation Plot. Error bars are the standard error of the mean.
	Dissimilar values indicate that the means are significant at $alpha = 0.05$
Figure 3.	Average Soil Moisture Profile Across all Four Zones at Depth for all Hydroprobe
	Measurements Obtained Throughout Years 2000, 2001, 2002 and 2004 at the
	Monument Valley Phytoremediation Plot. Error bars represent the standard error of the
	mean
Figure 4.	Correlation of Plant Volume and Dry Weight of Fourwing Saltbush at the Monument
	Valley Phytoremediation Plot Based on Data Compiled From Years 2000-2002 and
	2004
Figure 5.	Growth of Fourwing Saltbush Plants at the Monument Valley Phytoremediation Plot,
	2000 Through 2004 on the Basis of Dry Biomass Per Plant
Figure 6.	Growth of Fourwing Saltbush Plants at the Monument Valley Phytoremediation Plot,
	2000 to 2004, on the Basis of Plant Canopy Volume (top) and Plant Ground Cover
	(bottom). Error bars show standard errors of the mean7
Figure 7.	Mean Soil Nitrate and Ammonium Concentrations Across all Depths for Each Zone for
	Years 2000 through 2004 at the Monument Valley Phytoremediation Plot. Error bars
	represent standard error of the mean. (See Tables 1 and 2 for details)
Figure 8.	Mean Soil Nitrate and Ammonium Concentrations Profiles with Depth Across All
	Zones for Each Year at The Monument Valley Phytoremediation Plot. Error bars
	represent standard error of the mean. (See Tables 1 and 2 for details)10

Tables

Table 1. Comparison of Harvested Biomass Data From the Monument Valley Phytoremediation
Plot for Years 2000, 2001, and 2004. Standard error of the mean is reported in
parentheses, n.a. is not available
Table 2. Annual Mean Soil NO ₃ ⁻ N Concentrations from Four Zones at Six Depths for the
Monument Valley Phytoremediation Plot. Depths are in meters. Concentrations are expressed as $mg kg^{-1} NO_2$ N Values in parenthesis are the standard error of the
mean (SEM)
Table 3. Annual Mean Soil NH ₄ -N Concentrations from Four Zones at Six Depths for the
Monument Valley Phytoremediation Plot. Depths are in meters. Concentrations are
expressed as mg kg ⁻¹ NH ₄ -N. Values in parenthesis are the standard error of the mean
(SEM)
Table 4. Most Probable Number (MPN) of Denitrifiers and Denitrification Enzyme Activity
(DEA) Rates of N ₂ O Production at the Monument Valley Phytoremediation Plot. Data
are an average of samples collected at two different soil depths (0-1 m and 3-4 m). For
most cases $n = 8$ and the value in parentheses is the standard error of the
mean (SEM)
Table 5 Microcosm Rates of N_2O Production for the Monument Valley Phytoremediation Plot at
Field Moisture ($\mu g/kg/dav$) With and Without Acetylene ($n = 7$ or 8) Data are an
$\frac{1}{100}$ which and $\frac{1}{100}$ with and $\frac{1}{1000}$ with and $\frac{1}{10000}$ with and $\frac{1}{10000000000000000000000000000000000$
average of samples (50-100 g seven son) confected at two different son depuis (0-1 in $and 2.4 \text{ m}$). For most assage $n = 8$ and the value in parentheses is the SEM [12]
and 5-4 m). For most cases, $n = \delta$ and the value in parentineses is the SEW 12 Table (In give Sector Scill N O Elem (by (by (by) Magnetic parents of the Magnetic parent Valles)
Table 0. In-stitu Surface Soli N ₂ O Flux ($kg/na/yr$) Measurements at the Monument Valley
Phytoremediation Plot

Executive Summary

Results are presented from the past 5 years of characterizing and monitoring the feasibility of using phytoremediation as a remedy for nitrate and ammonium contamination in the subpile soil area at the Monument Valley, Arizona, Uranium Mill Tailings Radiation Control Act (UMTRCA) site. A 1.6 hecatre (ha) phytoremediation plot at the site was irrigated to an approximate depth of 0.24 meter (m) per year for the first 3 years. The plot was not irrigated in 2003 and only 0.1 m of water was applied in 2004. Plant canopy cover and volume doubled between 2002 to 2004, reaching 48% cover across all four irrigation zones and approaching 75% cover for zone 4 by September 2004. The decreased irrigation rate and an increased evapotranspiration (ET) rate, combined with the lack of irrigation during 2003, resulted in a drying of the soil profile. Nitrate removal via denitrification essentially ceased during the 2004 growing season, presumably because of lower soil moisture levels. However, denitrifying microbial activity was still higher in 2004 for soil samples taken from the plot than for samples taken from an adjacent non-irrigated site off the plot.

These data suggest that the rate of nitrate removal is highly dependent on soil moisture content, plant water use and the ammonia oxidation rate. Denitrification dominates under moist conditions, but activity decreases as the soil profile dries out. To maintain a high nitrate removal rate with a full plant cover, we recommend increasing the irrigation rate during the 2005 growing season.

1.0 Introduction

The purpose of this report is to document results thus far of an ongoing phytoremediation pilot study at the Monument Valley, Arizona, Uranium Mill Tailings Radiation Control Act (UMTRCA) site, and to recommend improvements for the coming year. The Monument Valley UMTRCA site is a former uranium ore processing operation located roughly 30 miles southeast of Mexican Hat, Utah, in Arizona. In 1994, the U.S. Department of Energy (DOE) completed a mandated remediation of radioactive materials, soil and tailings, from the site. However, the soil directly beneath the former tailings pile, the subpile soil, remained contaminated with significant levels of ammonium (NH₄) and nitrate (NO₃⁻). Both NO₃⁻ and NH₄ concentrations (ranging from $45-1,060 \text{ mg kg}^{-1}$ and $0-273 \text{ mg kg}^{-1}$, respectively) were thought to be contributing to contamination of an adjacent alluvial aquifer extending to the north.

A phytoremediation feasibility study has been underway at the site since 1998. It was envisioned that plantings of fourwing saltbush could utilize the NO_3^- and NH_4 in the subpile soil when irrigation water was provided to stimulate plant growth and microbial activity. The phytoremediation strategy for the subpile soil remediation plot is based on the concept of "deficit irrigation:" sufficient water is applied to produce good plant growth, but the amount supplied is less than the potential ET of the planting. Little or no water discharges past the rooting zone, thus cutting off and removing the source of the plume. This strategy works because the potential ET is very high in the Monument Valley desert, where plants are able to extract available water quickly yet persist under conditions of water deficit. The effectiveness of this strategy requires monthly monitoring of changes in the soil water profile, and annual measurements of soil nitrate and ammonia concentrations, and plant growth and nitrogen uptake.

2.0 Site Description

In 1999, a rectangular test plot (ca. 160 m by 100 m) was established consisting of a series of drip irrigation lines laid on the soil surface, and spaced 2 m apart with 2 m between emitters within the irrigation lines. The plot was divided into four irrigation zones, each containing approximately 970 plants, mainly *Atriplex canescens* (fourwing saltbush). The irrigation system was designed so that each plant received approximately 2 to4 L hr⁻¹ for 2 hr d⁻¹, 5 days per week during the growing season (April through September). Water was pumped from an uncontaminated well containing 380 mg l⁻¹ total dissolved solids (TDS) and monitored with a flow meter placed in the main line. The irrigation system was designed to apply approximately 720 liters (L) per plant, or 0.36 m yr⁻¹ over the plot each year, but flow meter readings indicated that the actual volume of water applied ranged from 0.3-0.1m. The subpile soil was not irrigated or monitored during the 2003 growing season. Irrigation and monitoring commenced again for the 2004 growing season.

From August 1999 to April 2000, a total of 20 thin-walled polyvinyl chloride (PVC) hydroprobe ports, one in the center of each irrigation zone, were installed to monitor the soil moisture profile. Each port extended from the ground surface to a depth of 4.6 m or to bedrock where the soil was shallow. Soil moisture measurements were obtained with a 503 DR Campbell Nuclear Neutron Hydroprobe (Campbell Pacific Nuclear International, Inc) at 0.5 or 1 foot intervals from each port. These data were used to infer if water was leaching below the root zone. From April 2000 to November 2004, soil moisture was measured monthly each year, except during 2003.

3.0 Irrigation Volumes and Soil Moisture

The total depth of water applied over the entire plot from April through September each year from 1999 to 2002 ranged from 0.2 to 0.3 m but decreased considerably during the 2004growing season to an average of only 0.1 m. Emitter flow meter records indicate that water application rates had decreased from between 2.5 to 4.6 L hr⁻¹ per plant in June 2000 to approximately 0.6 to 2.3 L hr⁻¹ per plant in June 2004. On a number of occasions during the 2004 growing season, the irrigation system or generator failed to operate properly, hence less water was applied to the plot.

When the hydroprobe ports were installed in September 1999, most of the soil profile was relatively dry, with moisture contents ranging from 0.02 to 0.10 g cm⁻³. However, probe measurements taken at one port at depths of 3 to 4 m indicated nearly saturated conditions (0.20 to 0.23 g cm⁻³) (Figure 1). From 2000 to 2002, moisture levels throughout the profiles were essentially static, even during months without irrigation. During the course of the study, mean annual moisture content values increased only slightly to approximately 0.11 g cm⁻³ (42% saturation; Figure 2), with the exception of the dry year 2004 when the moisture content dropped significantly (P < 0.05). In general, the plot was driest within the top 1 m of soil and wettest near the bottom of the profile (Figures 1 and 3). After 3 years of deficit irrigation, only a few ports were saturated at the 4-m depth and these were in the same vicinity as the initial bottom-saturated port upon commencement of the study. By 2004, the soil profile had dried considerably, especially within the first 3 m of the soil profile. The soil drying may be explained by (1) lack of irrigation during the growing season of the previous year, (2) the application of less water to the plot during this growing season, and (3) an increase in the ET rate because of increased plant growth and canopy cover over the past 2 years.



Figure 1. Soil Moisture Profiles Across All Zones and All Months at Depth for Years 2000 Through 2004 at the Monument Valley Phytoremediation Plot



Figure 2. Mean Annual Moisture Content Across all Zones, Months, and Depths at the Monument Valley Phytoremediation Plot. Error bars are the standard error of the mean. Dissimilar values indicate that the means are significant at alpha = 0.05.



Figure 3. Average Soil Moisture Profile Across all Four Zones at Depth for all Hydroprobe Measurements Obtained Throughout Years 2000, 2001, 2002 and 2004 at the Monument Valley Phytoremediation Plot. Error bars represent the standard error of the mean.

4.0 Total Annual Plant Growth, Survival and Nitrogen Uptake

In September for three consecutive years from 2000 to 2002 and again in 2004, plant growth was estimated by measuring survival, canopy volume, ground cover area, and productivity for the shrubs in the study. Overall survival rate was 94% in September 2004, a 1% decrease from 2002. This survival rate is partially attributed to new seedling emergence at the emitters where plants were missing during previous years. Although irrigation was much less (0.124 m total) for the last 2 years, plant survival was not significantly affected.

Plant height and width were measured for every other plant in 2000 (n = ca. 2000) and every 5th plant in 2001, 2002, and 2004 (n = ca. 800). Plant canopy volume was calculated from the mean length of two cross-sectional widths and the height of each plant by using the formula for a hemisphere ($2/3 \pi r^3$). Individual plant canopy was calculated based on the projected area of canopy on the ground using the area of a circle (πr^2); the average plant width used to estimate the radius (r). Aboveground biomass data were estimated by destructive harvesting of 20 *A. canescens* plants each year, from 2000 to 2002, and harvesting of 4 plants in 2004. The relationship between dry weight and canopy volume was determined for each year and was used to extrapolate biomass production for the entire plot. Plant dry weight was highly correlated to plant volume ($r^2 = 0.935$, Figure 4). The mean biomass for plants in 2004 was 1.96 kilogram (kg) per plant, more than double the mean biomass observed in 2002 (Figure 5). On the basis of the current number of live plants on the plot (3,710 plants), the aboveground biomass is approximately 7,272 kg.

Total plant N was measured in pulverized tissue samples on a CNS analyzer by the Water Quality Center Laboratory at the University of Arizona. The average N content of the 2000, 2001, and 2004 tissue was 1.66% N (Table 1). Approximately 121 kg N was present in the aboveground tissue; an estimate based on the average and current standing biomass on the plot. Although plant growth was initially slow, especially on a portion of the plot that exhibited a chemical stain (visible on aerial photographs), plant growth continues to increase throughout the plot (Figure 6). Apparently, the contamination in this area is confined to a shallow layer just beneath the soil surface, and plants began to grow rapidly once their roots penetrated through to deeper soil. At the end of the 2004-growing season, plant cover over the entire plot approximated 48%, and the standing crop biomass was estimated to be approximately 4,545 kg ha⁻¹. This vigorous and prolific plant growth facilitates a deficit water balance over the field, preventing further leaching of nitrate during irrigation. Presumably, the plants are effectively using all of the water applied to the field. The potential evapotranspiration (ET_o) for 48% plant cover is 0.720 m of water, whereas in 2004, one-tenth of that amount was applied (0.124 m, see Figure 3), which is much less than the plants can utilize.

Table 1. Comparison of Harvested Biomass Data From the Monument Valley Phytoremediation Plot for
Years 2000, 2001, and 2004. Standard error of the mean is reported in parentheses, n.a. is not available.

Year Parameter	2000 n=20	2001 n=18	2004 n=4
Total N	1.24 (0.104)	2.00 (0.173)	2.17 (0.22)
Nitrate	664.63 (0.155)	3,179.00 (505.82)	n.a.
Dry Weight	0.128 (0.065)	0.643 (0.115)	4.604 (1.344)



Figure 4. Correlation of Plant Volume and Dry Weight of Fourwing Saltbush at the Monument Valley Phytoremediation Plot Based on Data Compiled From Years 2000-2002 and 2004



Figure 5. Growth of Fourwing Saltbush Plants at the Monument Valley Phytoremediation Plot, 2000 Through 2004 on the Basis of Dry Biomass Per Plant





Figure 6. Growth of Fourwing Saltbush Plants at the Monument Valley Phytoremediation Plot, 2000 to 2004, on the Basis of Plant Canopy Volume (top) and Plant Ground Cover (bottom). Error bars show standard errors of the mean.

5.0 Soil Nitrate and Ammonium Concentrations

Subpile soils were sampled at multiple depths throughout the rooting zone at 20 locations each year for three consecutive years from September 2000 to September 2002, and again in September 2004. Soil NO_3^- concentrations decreased by 50% during the first 3 years (P < 0.001), from an average of 160 mg kg⁻¹ at the start of irrigation to just over 80 mg kg⁻¹ by the end of the third full growing season (Figure 7; Table 2). This trend ceased in 2004 with NO_3^- -N staying constant from 2002. There was a 25% reduction in soil NH₄ concentration from 2000 to 2004 but, because of spatial heterogeneity, the decrease was not significant at P<0.05.



Figure 7. Mean Soil Nitrate and Ammonium Concentrations Across all Depths for Each Zone for Years 2000 through 2004 at the Monument Valley Phytoremediation Plot. Error bars represent standard error of the mean. (See Tables 1 and 2 for details).

Zone/Depth (m)	2000	2001	2002	2004	Number of Samples
Zone 1					
0.3	71.8 (37.6)	100.8 (41.1)	22.6 (37.1)	25.9 (23.1)	5
0.9	90.6 (25.2)	31.2 (10.5)	7.0 (5.3)	42.9 (34.7)	5, 5, 5, 4
1.8	186.7 (73.9)	52.0 (21.1)	9.3 (6.1)	36.1 (28.8)	3, 3, 4, 4
2.7	218.0 (103.0)	77.5 (60.5)	10.9 (16.3)	27.8 (25.0)	2, 2, 2, 3
3.6	235.0	23.0	48.1		1
4.5	302.0	46.0	57.3		1
			Zone 2		
0.3	92.6 (35.5)	112.6 (58.2)	44.1 (47.3)	61.6 (26.5)	5
0.9	154.4 (42.0)	44.0 (13.2)	51.1 (73.5)	39.7 (20.2)	5
1.8	111.2 (33.4)	69.8(34.3)	35.1 (24.1)	43.1 (36.4)	5
2.7	67.8 (24.6)	113.3 (35.8)	34.7 (22.1)	38.4 (29.7)	4, 4, 5, 4
3.6	77.0 (31.9)	90.7 (73.3)	49.5 (39.8)	61.5 (52.5)	3, 3, 3, 2
4.5	113.0 (12.0)	133.5 (35.5)	74.8 (10.8)	59.1 (17.3)	2, 2, 2, 4
			Zone 3		
0.3	126.0 (34.3)	95.0(30.1)	73.4 (53.4)	92.9 (36.9)	5
0.9	276.5 (141.1)	116.0 (52.2)	82.6 (54.4)	60.9 (26.9)	5
1.8	213.2 (64.4)	146.2(58.2)	106.0 (41.2)	137.7 (72.3)	5
2.7	180.4 (65.3)	119.0 (53.6)	84.7 (62.1)	147.9 (75.5)	5
3.6	123.6 (35.7)	95.7 (27.9)	58.6 (48.7)	104.1 (39.6)	5, 4, 4, 5
4.5	170.3 (28.8)	164.7(108.8)	107.0 (76.3)	229.1 (111)	4, 3, 5, 4
			Zone 4		
0.3	62.0 (13.8)	131.8 (40.9)	145.4 (104.5)	81.5 (55.3)	5
0.9	217.8 (138.4)	181.8(81.6)	74.9 (75.9)	122.6 (73.1)	5
1.8	173.4 (70.9)	170.8 (43.8)	88.2 (87.2)	134.5 (47.4)	5
2.7	286.6 (85.4)	185.6(55.6)	87.3 (97.1)	128.9 (39.9)	5
3.6	227.0 (118.8)	166.8(27.5)	312.7 (432.2)	156.7 (37.4)	5
4.5	322.6 (106.1)	240.8 (20.3)	283.8 (229.8)	181.6 (71.0)	5, 4, 5, 5
Average	164	116	82	91	

Table 2. Annual Mean Soil NO₃⁻-N Concentrations from Four Zones at Six Depths for the Monument Valley Phytoremediation Plot. Depths are in meters. Concentrations are expressed as mg kg⁻¹ NO₃⁻⁻N. Values in parenthesis are the standard error of the mean (SEM).

The NO₃⁻ (p = 0.002) and NH₄ (P < 0.001) concentrations increased significantly with soil depth for all sampling periods (Figure 8; Tables 2 and 3). However, the interaction term (Depth × Year) was not significant (P > 0.05) for either contaminant (NO₃⁻ or NH₄), indicating that no net downward movement occurred during the study. Between 2000 and 2002, total losses in NO₃⁻-N over a 4.6 m depth interval were 1,360 kg ha⁻¹ yr ⁻¹ (equivalent to 0.031 mg-N kg⁻¹ day⁻¹ on a soil weight basis). This volume was much more than could be accounted for by plant uptake alone.



Figure 8. Mean Soil Nitrate and Ammonium Concentrations Profiles with Depth Across All Zones for Each Year at The Monument Valley Phytoremediation Plot. Error bars represent standard error of the mean. (See Tables 1 and 2 for details).

Table 3. Annual Mean Soil NH ₄ -N Concentrations from Four Zones at Six Depths for the Mor	nument
Valley Phytoremediation Plot. Depths are in meters. Concentrations are expressed as mg kg ⁻¹	NH_4 -N.
Values in parenthesis are the standard error of the mean (SEM).	

Zone/Depth (m)	2000	2001	2002	2004	Number of Samples		
	Zone 1						
0.3	2.5 (1.2)	66.8 (53.5)	1.9 (0.83)	1.52 (0.51)	5		
0.9	44.9 (45.5)	121.9 (62.1)	10.3 (11.1)	5.82 (3.98)	5, 5, 5, 4		
1.8	102.7 (93.2)	146.7 (109.1)	57.8 (77.8)	85.3 (65.0)	3, 3, 4, 4		
2.7	111.0 (104.0)	52.0 (47.5)	12.7 (24.6)	105 (67.6)	2, 2, 2, 3		
3.6	56.0	43.0	7.5		1		
4.5	140.0	113.0	77.5		1		
		Z	one 2				
0.3	8.2 (2.0)	55.3 (42.9)	12.3 (18.9)	1.37 (0.51)	5		
0.9	155.2 (73.3)	110.8 (53.2)	93.3 (76.0)	74.6 (57.6)	5		
1.8	329.6 (60.9)	200.2 (50.2)	196.1 (152.0)	191.5 (80.9)	5		
2.7	287.0 (50.7)	226.1 (50.2)	257.0 (144.2)	230 (89)	4, 4, 5, 4		
3.6	310.0 (60.4)	255.3 (22.6)	220.8 (141.2)	227.5 (62.5)	3, 3, 3, 2		
4.5	360.0 (145.0)	349.5 (90.5)	290.0 (420.0)	251.7 (11.81	2, 2, 2, 4		
		Z	one 3				
0.3	109.6 (87.0)	116.1 (91.6)	131.4 (158.9)	95.8 (60.4)	5		
0.9	183.2 (113.6)	257.7 (87.5)	270.8 (219.5)	186.0 (81.8)	5		
1.8	397.6 (70.1)	258.9 (72.9)	332.0 (136.1)	205.1 (84.8)	5		
2.7	340.4 (49.2)	360.3 (48.9)	400.0 (31.62)	286 (58.0)	5		
3.6	431.2 (69.2)	380.3 (45.2)	410.0 (389.1)	307 (40.9)	5, 4, 4, 5		
4.5	432.0 (105.0)	206.8 (84.2)	460.0 (159.4)	320 (113)	4, 3, 5, 4		
		Z	one 4				
0.3	4.8 (1.2)	19.2 (5.0)	2.4 (1.8)	81.9 (79.5)	5		
0.9	11.4 (9.6)	19.9 (10.1)	92.5 (1788)	35.2 (19.7)	5		
1.8	90.5 (54.3)	94.1 (79.9)	101.8 (199.1)	77.9 (74.3)	5		
2.7	316.8 (167.0)	114.4 (100.8)	181.3 (221.6)	168.6 (108)	5		
3.6	203.0 (118.8)	206.1 (103.6)	234.7 (278.4)	175.1 (103)	5		
4.5	159.4 (103.7)	230.0 (90.4)	290.1 (278.5)	145.3 (120)	5, 4, 5, 5		
Average	191	168	173	148			

6.0 Denitrification Studies

A series of experiments were conducted by the University of Arizona's Environmental Research Laboratory (ERL) to confirm that denitrification was the likely mechanism of nitrate loss in the irrigated field. ERL measured Denitrification Enzyme Activity (DEA) and enumerated the Most Probable Number (MPN) of denitrifiers in soil samples collected at two depths (0 to 1 m and 3 to 4 m) on and off the irrigated field (Table 4). Soils collected from the nitrate-contaminated and irrigated portion of the site showed significantly higher (p = 0.04) DEA activity in the presence of acetylene and absence of oxygen when supplemented with additional nitrate and glucose than contaminated soil sampled from an adjacent, non-irrigated area off the plot. Similarly, significantly higher (p = 0.04) denitrifier MPN counts were found for samples taken from the irrigated test plot than in samples from off the plot. Combined, these data support the hypothesis of high denitrifying activity on the irrigated plot. It is important to note that both measurements

utilize a medium that is selective for a particular denitrifier population and activity. Hence, this measurement may not be representative of the *in-situ* microbial population and activity. Instead, these measurements represent a potential for denitrification and an estimate of the number of microorganisms in the soil capable of reducing nitrate under the conditions imposed by the assay.

Table 4. Most Probable Number (MPN) of Denitrifiers and Denitrification Enzyme Activity (DEA) Rates of N_2O Production at the Monument Valley Phytoremediation Plot. Data are an average of samples collected at two different soil depths (0-1 m and 3-4 m). For most cases, n = 8 and the value in parentheses is the standard error of the mean (SEM).

Date	DEA (µg	ı/kg/day)	MPN/g dry soil	
Date	On Plot	Off Plot	On Plot	Off Plot
January 2004	51.1 (± 10.6)	7.30 (± 1.91)	-	-
June 2004	37.4 (±13.6)	7.88 (± 4.19)	4,060 (± 2190)	0
July 2004	-	-	639 (± 171)	78.1 (± 30.1)
August 2004	2.86 (± 2.93)	0.84 (± 1.71)	124 (± 46.5)	130 (± 53.5)
September-2004	12.8 (±10.0)	2.19 (± 0.39)	503 (± 233)	50.2 (± 20.4)

(-) indicates assays that were either not conducted or have yet to be analyzed.

ERL also measured N₂O evolution in unsupplemented soil microcosms (Table 5). Similar to the denitrification potential measurements above, significantly higher (P < 0.05) rates of N₂O production were found in samples from on the irrigated plot than off the plot, regardless of sample depth (0-1 or 3-4 m). Furthermore, when these microcosms were incubated under a 10% acetylene headspace, N₂O production was inhibited (p = 0.03) compared to the control (incubations without acetylene). These findings suggest that N₂O is the likely end product of denitrification in this system and that microorganisms other than heterotrophic denitrifiers are responsible for nitrate transformations in this soil. Lastly, ERL's *in-situ* measurements (Table 6), with soil covers constructed of PVC equipped with syringe sampling ports, were also higher on the irrigated plot than off the plot by a factor of 10 to 20 (p = 0.004). ERL attributes the higher rates of denitrification to higher (0.01%, p = 0.02) moisture content on the irrigated plot than off the plot study.

Table 5. Microcosm Rates of N₂O Production for the Monument Valley Phytoremediation Plot at Field Moisture (μ g/kg/day) With and Without Acetylene (n= 7 or 8). Data are an average of samples (50-100 g sieved soil) collected at two different soil depths (0-1 m and 3-4 m). For most cases, n = 8 and the value in parentheses is the SEM.

Date	On Plot	Off Plot	On + acetylene	Off + acetylene
January 2004 ^a	0.07 (±0.02)	0.04 (±0.02)	-	-
June 2004	5.13 (±1.66)	0.29 (±0.07)	0.20 (±0.02)	0.17 (±0.02)
July 2004	0.18 (±0.08)	-0.001 (±0.003)	0.08 (±0.04)	-0.01 (±0.01)
August 2004	1.15 (±0.50)	0.02 (±0.007)	0.05 (±0.02)	0.01 (±0.01)
September-2004	0.04 (±0.02)	-0.03 (±0.01)	0.01(±0.01)	0.15 (±0.14)

^aSoil microcosms were all adjusted to 10 % moisture for 01-04 sampling event.

(-) indicates assays that were either not conducted or have yet to be analyzed.

Table 6. In-situ Surface Soil N₂O Flux (kg/ha/yr) Measurements at the Monument Valley Phytoremediation Plot

Date	On Plot (n = 9)	Off Plot (n=9)
January 2004	-	-
June 2004	0.702 (±0.21)	0.242 (±0.081)
July 2004	1.44 (±0.451)	0.376 (±0.101)
August 2004	1.48 (±0.460)	0.134 (±0.095)
September-2004	1.95 (±0.54)	0.141 (±0.15)

7.0 Quality Control and Assurance

Samples were extracted (generally in 1:10 in 1 M KCl or AlSO₄) and analyzed by a number of different methods and laboratories. In 2000 and 2001, ERL and Laboratory Consultants Ltd. determined NO₃⁻ and NH₄ concentrations colorimetrically with Hach test kits and with an ion-specific electrode, respectively; results were within 10%. For soil samples collected in 2002 and 2004, ESL analyzed the samples by extracting with potassium chloride (KCl), then measuring NO₃⁻ and NH₄ by ion chromatography and spectrophotometrically, respectively. In 2002, archived subpile samples from 2000 and 2001 were re-evaluated to verify results obtained from the subcontracted laboratory (Laboratory Consultants Inc.). Nitrate concentrations were quantified for 11 randomly selected soil samples. Statistical (Paired t-test) analysis indicated no significant difference between the means reported by Laboratory Consultants Inc. and those reported by ERL for the 2000 samples. In 2004, archived samples were analyzed on a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL). Results were within 10% of values from colorimetric and ion electrode assays.

8.0 Recommendations

An increase in irrigation rates on the plot is recommended, perhaps twice the original application rate (approximately 0.7 m per growing season) in order to maintain high denitrification rates in the field. Direct measurement of downward water movement using vadose-zone flux meters (Gee et al. 2002) is recommended to confirm that discharge and leaching of nitrate from the subpile soil profile is not occurring.

9.0 References

Bonham, C.D., 1989. *Measurements for Terrestrial Vegetation*, John Wiley and Sons, New York.

DOE (U.S. Department of Energy), 2002. *Phytoremediation of Nitrogen Contamination in Subpile Soils and in the Alluvial Aquifer at the Monument Valley, Arizona, Uranium Mill Tailings Site*, GJO-2002-312-TAR, U.S. Department of Energy, Grand Junction, Colorado.

DOE (U.S. Department of Energy), 2004. *Monument Valley Ground Water Remediation: Pilot Study Work Plan*, Draft, U.S. Department of Energy, Office of Legacy Management, Grand Junction, Colorado.

Gardner, W.H., 1986. "Water Content," pp. 493-554, in A. Klute (ed.) *Methods of Soil Analysis*, Part 1, 2nd Edition, Agronomy Monograph 9, Soil Science Society of America, Madison, Wisconsin.

Gee G.W., A.L. Ward, T.G. Caldwell, and J.C. Ritter. 2002. "A vadose zone water fluxmeter with divergence control." *Water Resources Research* 38(8):35-41.

Gilbert, R.O., 1987. *Statistical Methods for Environmental Pollution Monitoring*, Van Nostrand Reinhold Company, New York.

Maynard, D.G., and Y.P. Kalra, 1993. *Chapter 4: Nitrate and exchangeable ammonium nitrogen*, Lewis Publishers, CCR Press, Inc., Boca Raton, Florida.

Page, A.L, R.H. Miller, and D.R. Keeney (eds.), 1982. *Methods of Soil Analysis, Part 2 Chemical and Microbiological Properties*, Second Edition, Section 33-3 Extraction of Exchangeable Ammonium, Nitrate, and Nitrite, American Society of Agronomy, Madison, Wisconsin.

STO 210. *Environmental Sciences Laboratory Procedures Manual*, S.M. Stoller Corporation, under contract to U.S. Department of Energy, Grand Junction, Colorado, continuously updated.

Acknowledgements

This report was prepared for the Environmental Sciences Laboratory by Fiona Jordan, Casey McKeon, and Ed Glenn of the University of Arizona's Environmental Research Laboratory.