DISTRIBUTION OF DENITRIFYING AND SULFATE REDUCING BACTERIA WITHIN A RIPARIAN ZONE ALONG A MOUNTAIN STREAM

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ABSTRACT

The presence and activity of denitrifying bacteria as well as bacteria capable of reducing sulfate in one upland and five riparian soils of a mountain watershed in Wyoming were studied. Bacteria were enumerated from soil samples collected during summer along transects placed perpendicular to stream flow. Samples were taken at three depths within each plant community. Subsamples were frozen and later utilized to determine denitrification potential.

Higher counts of total heterotrophic aerobic bacteria, sulfate-reducing bacteria, denitrifying bacteria and denitrification potential existed in the upper 5 to 15 cm of soil than at 30 cm. Soils located close to the streams edge tended to have more bacterial activity than those further from the stream indicating that these soils may be important areas for nitrate and sulfate reduction. Soil organic matter and water content decreased with depth in all plant communities, and those closer to the stream contained more organic matter and water than those further from the stream.

STATEMENT OF PURPOSE

The purpose of this study was to document presence or absence of nitrate and sulfate reducing bacteria within riparian zones located in a mountain area of Wyoming. If present, the study was designed to see if numbers of bacteria varied with distance from the water table. Transect data were taken from stream edge to upland areas knowing that the water table would be further from the soil surface toward upland habitats. Upland habitat would therefore be supported by less soil water than moist and wet vegetation types near streams and lakes. In addition, variation in bacteria numbers present were correlated to soil depth, soil ph, and organic matter. Soil temperature was equivalent to stream water temperature and was discarded as a test parameter. Furthermore, soil temperature probes were not adequate to provide accurate data. Potential activity of organisms capable of reducing nitrate to nitrogen gas was evaluated by measuring the anaerobic by product, nitrous oxide, under laboratory conditions.

OBJECTIVES

The objectives of this study were to:

1. Document the distribution of denitrifying and sulfate reducing bacteria in a mountain riparian zone.
2. Correlate numbers of organisms to stream flow, water table, soil moisture, and soil organic matter.

RELATED RESEARCH

Riparian ecosystems are ecologically important vegetative communities (Odum 1978). Compared to upland vegetation types riparian zones in the Rocky Mountain States are relatively limited and often receive extensive user pressure by livestock, wildlife, and man (Busby 1978, Jahn 1978, Johnson 1978). These users are often accused of causing excessive damage to associated aquatic habitat, vegetation, and stream channel stability, which often causes a reduction of soil moisture, plant production, and species composition (Settergren 1977, Platts 1978, Haugen and Duff 1982). The riparian zones' location between upland and aquatic ecosystems represents a transition zone and consequently may be of particular importance in understanding the contribution of nutrients to nearby waters from the uplands (Kirbby 1978).

Numerous studies using vegetation, channel morphology, and sediment deposition show changes occurring in riparian zones due to livestock grazing (Cooper 1976, Duff and Cooper 1978, Robinson 1982, and Platts et al. 1983). Measurements of other authors have used bacteria indicative of fecal pollution for assessing ungulate grazing contribution to stream pollution (Morrison and Fair 1966, Skinner et al. 1974b, Bunkhouse and Gifford 1976, Speck 1981, Jawson et al. 1982, Skinner et al. 1984a). Bacteria other than those used to determine fecal contamination in streams also may be useful to predict user impact on riparian zones. Data from Skinner et al. (1984b) suggested denitrifying bacteria may be associated with stream bottom and bank areas in a mountain drainage

basin. These bacteria varied significantly downstream between areas of depression storage. Bacteria capable of reducing sulfate were also present. It is possible these organisms may be entering stream flow from riparian zones through bank-stream interflow (Morrison and Fair 1966). The purpose of this study was to document: 1) denitrifying and sulfate-reducing bacterial numbers present in riparian plant communities, 2) differences in numbers of denitrifying and sulfate-reducing bacteria between different riparian and upland plant communities, 3) variation in numbers of these bacteria with soil depth, and 4) if denitrification potential varied between plant communities and soil depth.

Denitrifying bacteria, which are faculative anaerobes, and sulfate-reducing bacteria, which are strict anaerobes, are common in many soils and waters (Gamble et al. 1977, Knowles 1982, Pfennig and Widdel 1981, Postgate 1979, 1984). Bacterial denitrification has been positively correlated with soil moisture and organic matter (Wijler and Delwiche 1954, Cady and Bartholomew 1960, McGarity 1961, Mahendrappa and Smith 1967, Craswell and Martin 1974, Delwiche and Bryan 1976, Craswell 1978, Smith and Tiedje 1979a, 1979b, Patten et al. 1980, and Knowles 1981a, 1981b, 1982). Sulfate-reducing bacteria proliferate under anaerobic conditions in soils (ZoBell 1958, 1963, Peck 1962, Campbell and Postgate 1965, Bremner and Steele 1978, and Postgate 1959, 1979, 1984). Soil moisture is pertinent to maintaining riparian zones. Wet soils reduce soil oxygen content and should therefore provide a better environment for the bacterial reduction of nitrate and sulfate. The documentation of the presence, population dynamics, and potential for

denitrifying bacteria may provide an additional way to monitor user impacts of riparian ecosystems.

METHODS AND PROCEDURES

Study Site: A 30 x 100 m study site was located along Telephone Creek approximately 40 m above a stream flow gauging station approximately 3170 m above sea level.

Telephone Creek is one of three main drainages within the Nash Fork Hydrologic Observatory of Southeastern Wyoming. The watershed is continuously monitored by the Wyoming Water Research Center (WWRC). Vegetation and drainage basin has been described by Skinner et al. (1974a). The area is typical of many high mountain watersheds in the Rocky Mountains. Monthly air temperature, precipitation, and stream flow at the site for 1983 are presented in Table 1.

The selected study area was occupied by three plant communities designated wet meadow, moist meadow, and upland. The wet and moist meadows, were considered representative of riparian soil types. The upland had shallower soils containing less organic matter and were brown in color compared to the dark organic rich riparian soils. In the wet meadow, Carex (Carex sp.) and Equisetum (Equisetum sp.) were dominant, whereas grasses were dominant in the moist meadow. Three transects, approximately 30 m apart were established within the study area. Each transact started at the edge of the low stream bank (Platts et al. 1983) and continued, perpendicular to the stream, upland through each vegetation zone. Once established total length along a straight line was determined for each transect. Sampling occurred at the center of each vegetative zone.

Table 1. Mean daily precipitation, air temperature, and stream flow by month for Mill Pond gauging station (WWRC 1983).

Month	Precipitation cm	Air Temperature °C	Stream flow $L\ sec^{-1}$
Jan	.33	-12.66	22.36
Feb	.30	-12.33	9.91
Mar	.64	-9.72	15.00
Apr	.41	-7.94	9.91
May	.56	-0.17	87.73
June	.28	3.83	789.00
July	.23	8.77	581.00
Aug	.15	10.11	190.74
Sep	.33	4.39	68.49
Oct	.18	- 2.11	82.92
Nov	.25	- 7.33	87.73
Dec	.28	- 9.88	52.07

Sampling: Samples were collected on August 15, 16, and 17, and again on September 12, 13, and 14, 1983. Sampling depths from soil surface were 5, 15, and 30 cm. One transect (nine samples) was collected and processed on each sampling day. A soil core was removed and subsamples containing approximately 0.5 kg at appropriate depths were removed, placed in labeled "Ziploc Bags", iced, and transported to the laboratory for analysis. Subsamples (25 to 30 g, fresh weight) of each 0.5 kg sample were aseptically removed, weighed, and placed into sterile blenders containing 225 ml of chilled (6°C) sterilized distilled H_2O. Duplicate subsamples (10 to 20 g) of each 0.5 kg sample were then weighed and placed in soil drying cans for soil moisture determinations. Remaining soil from each 0.5 kg sample was frozen at -12°C for future analysis. The distilled water and soil was homogenized using a Waring commercial blender at low speed (18,500 rpm) for two minutes which yielded the highest bacterial counts in preliminary experiments. Serial 10 fold dilutions were made in 0.1% sterile peptone water (Straka and Stokes 1957). Processing was accomplished within 7 h of collection.

Microbiology: Heterotrophic aerobic bacterial counts were obtained on modified Henrici agar (Stark and McCoy 1938), via the procedure described by Skinner et al. (1974a). Denitrifying bacterial populations were estimated by the most probable number (MPN) method used by Skinner et al. (1974a) but were incubated for 14 days instead of 7. Nitrous oxide (N_2O) production was measured using the methods of Swank and Caskey (1982) using thawed soils which had been frozen for two weeks. Nitrous oxide was measured using a Hewlett Packard 5730 A gas chromatograph GC equipped with a ^{63}Ni electron capture detector. Separation of N_2O was achieved using a 3 mm x 1.8 m Porapak Q 80/100

mesh (Supelco Inc.) column at 50°C. Carrier gas was 5% methane in argon (Ar) flowing at 60 ml per min. Detector temperature was 300°C and the injector temperature was 250°C. Injections were made with Hamilton (Hamilton Co., Reno, Nev.) microliter gas-tight syringes. Two certified gas standards (Scott Specialty Gases, Troy, Mi.) containing 99.61 ppm N_2O in Ar and 1069 ppm N_2O and 1012 ppm acetylene (C_2H_2) in Ar were used to prepare standard curves. Peak heights were used in quantitating amounts of N_2O produced from samples. Multiple injections from each sample were made and average values obtained. The amount of N_2O in the slurry was calculated and added to headspace N_2O using the procedure described by Tiedje (1982).

Sulfate-reducing bacterial populations were enumerated using methods described by Mara and Williams (1970).

Soil Physical Parameters: Soil water content, reported on a wet weight basis, was determined gravimetrically at 105°C for 24 h. All values reported for N_2O production and microbial population estimates are on a per g dry soil basis. Estimates of organic matter were obtained via loss-on-ignition (Davies 1974).

Soil pH was determined using a Perkin Elmer glass electrode pH meter following McLean's (1982) method as modified using a slurry containing 10 g air-dried soil and 50 ml of 0.01 M calcium chloride (CaCl) and was standardized using soils of known pH.

Statistical Analysis: Two-way analysis of variance (ANOVA) was used to describe differences in plant communities, depths, and dates of sampling for organic matter, H_2O content, pH, denitrifying bacteria, sulfate-reducing bacteria, heterotrophic aerobic bactera, and micrograms of N_2O produced (Steel and Torrie 1960). One way ANOVA was used to

indicate differences in depth within each plant community for the above mentioned bacterial and soil parameters. Significant contrasts between means were separated using Duncan's New Multiple Range Test (Steel and Torrie 1960). All results were compared at a 95% probability level. Paired t tests were used for comparing fresh and frozen soil N_2O production. Regression analysis was performed to determine correlations between parameters.

RESULTS AND DISCUSSION

Overall, data indicates two general findings: 1) bacterial numbers, organic matter, and water content were higher in the upper 5 to 15 cm than at 30 cm; and 2) these parameters were higher in soils of the wet and moist meadows than in the upland soils (Table 2). No significant differences were noted between the August and September sampling dates for all parameters tested. Streamflow on August 15, 16, and 17 was 231.2, 212.8 and 182.8 L sec^{-1} respectively, while September 12, 13, and 14 showed 64.5, 64.0 and 63.7 L sec^{-1} (WWRC 1983). Less streamflow did not decrease soil moisture as expected. This is likely due to shallow riparian soils over a perennial watertable which is maintained near the soil surface season long. Increased precipitation in September may also have helped maintain soil moisture (Table 1).

Nitrous Oxide Production: Nitrous oxide production (Table 2) was significantly greater at 5 cm than at 15 or 30 cm. The moist meadow produced more N_2O than either the upland or wet meadow soils overall, this may be attributed to the rather large amount of N_2O produced at the 5 cm depth in the moist meadow (Table 3). Data in Table 2 was lumped by sampling date, vegetation community, and depth to determine what sampling scheme should be used to monitor riparian zones for bacteria capable of reducing nitrate and sulfate. Although this procedure is not statistically sound, number of samples for depth and plant communities increase. Trends shown in Table 3 follow those in Table 2.

Table 2. Microbiological data obtained per g dry soil and soil physical properties for depths and plant communities. Telephone Creek study site.

Site	ug N_2O Produced	Log Viable Cells			Soil Physical Properties		
		Denitifying Bacteria	Sulfate-Reducing Bacteria	Heterotrophic Aerobic	Organic Matter(%)	Water Content (% Wet)	pH
Depth							
5 cm	[1]1.88a	5.33a	4.59	7.07	19.96	43.82	4.99a
15 cm	0.88	4.96ab	4.76	6.65	14.57	37.13	5.05ab
30 cm	0.11	4.09b	3.03	6.77	7.36a	25.44a	5.28b
Plant Communities							
Wet	0.87	5.12ab	4.96	7.08	21.68a	52.68a	5.22a
Moist	1.69a	5.24a	3.82	6.75	15.04b	40.94b	5.40a
Upland	0.31b	3.98b	2.58	6.65	5.17c	12.76c	4.71b

[1]Values with same letters or no letters indicate no significant differences at $\alpha = 0.05$, $n = 18$.

Table 3. Microbiological data per g dry soil and physical properties for riparian plant communities by depths. Telephone Creek study site.

Parameters	Depths cm	Wet Meadow Aug n-3	Wet Meadow Sep n=3	Wet Meadow Mean n=6	Moist Meadow Aug n=3	Moist Meadow Sept n=3	Moist Meadow Mean n=6	Upland Aug n=3	Upland Sept n=3	Upland Mean n=6
ug N_2O Produced	5.0	2.11	[1]1.36	1.74a	3.62a	3.41	3.51a	0.62	0.13	0.38
	15.0	0.68	0.78ab	0.73ab	1.08	1.83	1.46ab	0.78	0.14	0.46
	30.0	0.13	0.16b	0.15b	0.13	0.09	0.11b	0.09	0.08	0.09
Log Denitrifying Bacteria	5.0	5.42	5.20	5.32	5.66a	5.60	5.63a	4.03	3.43	3.83
	15.0	4.67	5.46	5.22	4.37	5.21	4.97	4.22	3.93	4.10
	30.0	4.36	4.33	4.35	3.90	3.18	3.68	4.27	3.14	4.00
Log Sulfate-Reducing Bacteria	5.0	5.26	4.30	5.01	4.25	4.19	4.22a	2.78	2.39	2.63
	15.0	4.25	5.50	5.23	3.45	3.48	3.46	2.76	2.60	2.69
	30.0	3.17	3.54	3.40	2.53	2.86	2.73	2.38	2.35	2.37
Log Heterotrophic Aerobic Bacteria	5.0	7.20	6.99	7.11	7.09	7.17	7.13a	7.03a	6.83	6.94a
	15.0	6.77	7.00	6.90	6.32	6.41	6.36	6.62	6.33	6.50
	30.0	6.11	7.47	7.19	6.07	5.75	5.94	6.16	6.19	6.18
Estimate of Organic Matter %	5.0	26.23	26.70	26.46	28.83a	26.06a	24.95a	8.63a	8.30a	8.47a
	15.0	28.37	21.46	24.92	13.46b	15.70ab	14.58b	4.50ab	3.93	4.22
	30.0	12.56	14.77	13.66	4.90c	6.26b	5.58c	2.63b	3.70	2.85
Water Content % (wet weight)	5.0	58.60	60.70	59.65	58.43a	54.73	56.58a	19.60	10.87	15.23
	15.0	56.37	57.33	56.85	42.70b	42.93	42.32b	15.53	8.90	12.22
	30.0	39.77	43.33	41.66	23.87c	24.00a	23.93c	12.73	8.93	10.83
pH	5.0	5.33	5.03	5.18	5.33	5.06	5.20	4.66	4.53	4.60
	15.0	5.30	5.06	5.18	5.50	5.33	5.42	4.43	4.70	4.57
	30.0	5.37	5.20	5.28	5.66	5.50	5.58	4.90	5.03	4.97

[1]Values with same letters or no letters indicate no significant difference at $\alpha = 0.05$.

Table 4. Nitrous oxide production, heterotrophic aerobic bacteria and physical properties for fresh and frozen soil samples.

Soil	Repetition	Nitrous Oxide Production	Heterotrophic Aerobic Bacteria	Soil Physical Properties		
		ug N_2O (g dry $soil^{-1}$)	Viable Cells x 10^7 (g dry $soil^{-1}$)	Organic Matter %	Water Content (% wet)	pH
Fresh	1	2.1	1.1	18.0	47.4	5.95
	2	14.88	2.4	30.0	60.9	6.00
	3	2.35	1.3	18.8	47.7	6.00
Frozen	1	1.10	1.2	19.4	48.7	5.90
	2	3.06	5.9	31.0	56.1	5.95
	3	0.67	1.0	18.7	48.0	6.00

Nitrous oxide production was not significantly affected by freezing of soil samples(Appendix D), yet data presented in Table 4 indicated that more N_2O was produced in fresh soils than those frozen for 2 weeks. These data support that of others with regards to the effects of organic matter and water content on denitrification (McGarity and Myers 1968, Focht et al. 1979, Delwiche 1981, Payne 1981). As soil organic matter increases so does the soil's water holding capacity and in turn denitrification (Wijler and Delwiche 1954, Cady and Bartholomew 1960, Craswell 1978, Smith and Tiedje 1979a, 1979b, Payne 1981, Rolston et al. 1982).

Denitrifying Bacteria: Focht (1978) suggested no relationships exist between denitrifying bacterial populations in soils and N_2O production whereas this study suggests such relationships do exist (Table 5). Values obtained for denitrifying bacteria were higher in the upper 5 to 15 cm than at 30 cm and upland did not contain as many denitrifying organisms as the moist or wet meadow soils (Table 2). The values obtained in this study appear about two log lower per gram of soil than those reported for Domino silty clay loam and a Ramona sandy loam both from California (Focht and Joseph 1973), yet the same trend, decreasing numbers with depth, was observed. It is possible that Focht and Joseph (1973) were using agricultural soils that may have been amended with N fertilizer or utilizable organic matter, thus increasing the potential for denitrifying bacteria (Focht and Verstraete 1977, Tiedje et al. 1982).

Sulfate-Reducing Bacteria: Sulfate-reducing bacteria appeared to be more abundant in the upper 15 cm than at 30 cm (Table 2) for all plant communities (Table 3). The data also suggested that higher counts of sulfate-reducing bacteria were found in the wet and moist meadow

Table 5. Correlation regression matrix for all soil microbial parameters and physical properties, north side Telephone Creek.

	Denitifying Bacteria	Sulfate-Reducing Bacteria	Heterotrophic Aerobic Bacteria	Nitrous Oxide Production	Organic Matter	Water Content	pH
Denitrifying Bacteria	1.000						
Sulfate-Reducing Bacteria	.5572	1.000					
Heterotrophic Aerobic Bacteria	.3917	.2972	1.000				
Nitrous Oxide Production	.7203	.1579	.3303	1.000			
Organic Matter	.4140	.1450	.3355	.4792	1.000		
Water Content	.4814	.2879	.3789	.4498	.8996	1.000	
pH	.1116	.0308	.0968	.2213	.2016	.3306	1.000

[1]Numbers represent correlation coefficients for one variable compared to another. Values approaching 1.0 indicate high degree of correlation.

soils than in the upland soils (Table 2). These data were consistent with those of other researchers who noted that sulfate-reducing bacteria are abundant in bogs, swamps, muds, and poorly drained soils because they proliferate under anaerobic conditions utilizing sulfate ($SO_4^{=}$) as the terminal electron accepter. (ZoBell 1958, 1963, Postgate 1959, 1965, 1979, 1984, Peck 1962, Campbell and Postgate 1965, Bremner and Steele 1978).

Total Heterotrophic Aerobic Bacteria: Plate counts on Henrici agar found that more bacteria were present at 5 cm than at 15 or 30 cm for all plant communities, and that higher counts were attained for wet meadow soils than moist meadow or upland soils (Table 2).

The design of this study was constructed to establish three different transects through the riparian system to obtain a more statistically sound estimate by lumping the data of the bacterial numbers at a sampling time. It was initially assumed that there would be no differences between transects, but this was not the case (Table 6). Statistical analysis of these data revealed some significant differences in bacterial numbers and soil physical properties between transects; thus the sensitivity of this study to detect differences between depths in the soil and vegetative communities was greatly reduced. The importance of this finding is that many more transects, or more intensive sampling of a transect must be taken to adequately describe the distribution of bacteria and soil physical parameters within riparian zones.

The Telephone Creek sampling site was chosen to represent streamside riparian communities and was sampled extensively. In contrast, a less extensive sampling scheme was initiated directly across Telephone

Table 6. Microbiological data obtained per g dry soil and soil physical properties for each transect placed within the Telephone Creek study site.

Vegetative Community	Transect (Reps.)	N_2O Produced	Log Viable Cells			Soil Physical Properties		
		ug N_2O	Denitrifying Bacteria	Sulfate-Reducing Bacteria	Heterotrophic Aerobic Bacteria	Organic Matter(%)	Water Content (% wet)	pH
Wet Meadow	1	1.41	5.78a	5.42	7.48a	31.98	60.40	5.23
	2	0.85	4.58	3.19	6.31	8.73a	36.13a	5.32
	3	0.35	3.61	3.93	6.57	24.33	61.52	5.10
Moist Meadow	1	[1]1.15ab	4.96	4.14	6.81	15.46	44.53	5.56
	2	3.42a	5.48	3.59	6.97	19.17	42.88	5.65
	3	0.51b	5.13	3.38	5.96	10.48	35.41	4.98a
Upland	1	0.70	4.16	2.64	6.78	6.37	13.60	4.80
	2	0.11	4.12	2.24	6.65	4.98	11.53	5.13
	3	0.11	3.23	2.73	6.46	4.18	13.15	4.20a

[1]Values with same letters indicate no significant differences at $\alpha = 0.05$, $n = 6$.

Creek within a riparian zone developed over alluvial soils deposited in a sub-alpine pond. There were four distinct plant communities. The wettest, Carex bog, adjacent to the pond, was saturated, and appeared to be floating. Willow bog, Potentilla bog, and moist meadow communities upslope were present respectively from the Carex bog inland. Moist meadow represented the driest site and was considered the most advanced successional stage for pond filling. Data obtained (Table 7) represent only two samplings of this area. It is interesting to note that some of the findings discussed earlier are also evident here.

Nitrous oxide production from alluvial soils under plant communities within the filling sub-alpine pond appeared to decrease with depth, and was in general higher in those vegetative communities containing more organic matter and higher water contents (Table 7). Denitrifying bacteria and sulfate-reducing bacteria also appeared to decrease with depth over all, yet did not exert the apparent finding as N_2O production did with regards to plant community (Table 7). Nitrous oxide production was noted to be less in the potentilla bog than in the other plant types. Although not statistically significant it appears that the potentilla community contained more denitrifying bacteria (Table 7). The carex bog appeared to contain more heterotrophic aerobic bacteria, sulfate-reducing bacteria and N_2O production potential than the other communities, yet appeared to have less denitrifying bacteria (Table 7).

Table 7. Microbiological data per g dry soil and physical properties for riparian vegetative communities on pond filling site.

Parameter	Depths cm[1]	Carex Bog	Willow Bog	Potentilla	Moist Meadow	Mean[3]
ug N_2O Produced	5.0	10.76a[4]	2.98	3.13	1.68	4.64a
	15.0	2.21	2.04	0.45	1.96	1.67
	30.0	0.00	0.69	0.09	1.90	0.67
	Mean[2]	4.32a[5]	1.90ab	1.22b	1.85ab	
Log Denitrifying Bacteria	5.0	5.08	4.98	6.57	5.22	6.01
	15.0	4.51	5.59	5.45	5.34	5.36
	30.0	4.52	5.19	3.87	5.05	4.97
	Mean	4.79	5.33	6.14	5.22	
Log Sulfate-Reducing Bacteria	5.0	5.80	5.10a	4.86a	5.33	5.42
	15.0	5.74	4.61b	4.65ab	4.69	5.23
	30.0	4.89	3.37c	2.63b	4.48	4.44
	Mean	5.62a	4.75	4.59	4.99	
Log Heterotrophic Aerobic Bacteria Count	5.0	7.68	6.32	7.93	7.53	7.63
	15.0	8.51	6.44	6.83	7.51	7.96
	30.0	7.06	6.65	6.35	7.01	6.85
	Mean	8.10	6.49	7.50	7.41	
Estimate of Organic Matter %	5.0	79.20	45.40a	51.25a	19.80a	48.91a
	15.0	74.25	22.25ab	9.75	11.85	29.50
	30.0	69.40	6.70b	2.35	8.60	21.76
	Mean	74.25a	24.80b	21.12bc	13.42c	

Table 7. (continued)

Parameter	Depths cm[1]	Carex Bog	Willow Bog	Potentilla	Moist Meadow	Mean[3]
Water Content % (wet weight)	5.0	87.65	71.05	59.05	34.15	62.98a
	15.0	86.90	55.35	34.60	30.10	51.74b
	30.0	85.85	28.40	18.40	26.00	39.66c
	Mean	86.80a	51.60b	37.35c	30.08c	
pH	5.0	5.70	5.90a	5.30	5.30	5.55a
	15.0	5.55	6.25ab	5.40	5.30	5.63ab
	30.0	5.80	6.70b	5.60	5.35	5.86b
	Mean	5.68a	6.28b	5.43c	5.32d	

[1]Values for depths are averages of two samplings (September 8, and August 7, 1983).
[2]Mean values for vegetation communities are averages of six samplings (three depths each sampled twice).
[3]Mean values for depths are averages of eight samplings (four vegetative communities each sampled twice).
[4]Numbers with same letters or no letters indicate no significant differences between depths at α = 0.05, n = 2.
[5]Mean values with same letter or no letters indicate no significant differences between vegetative communities or depths at α = 0.05, n = 6.

SUMMARY AND CONCLUSIONS

The occurrence of denitrifying and sulfate-reducing bacteria within the riparian soils of a high mountain watershed was analyzed to determine whether their presence and distribution was related to plant community and soil depth. Denitrification potential, denitrifying bacteria, sulfate-reducing bacteria, and heterotrophic aerobic bacterial numbers were determined for three depths (5, 15, and 30 cm) in five riparian soils and one upland soil. The study was carried out within the Medicine Bow National Forest approximately 50 km west of Laramie, Albany County, Wyoming at an elevation of about 3170 m.

The findings of this study can be summarized as follows:

1) Nitrous oxide production was higher at 5 cm than at 15 or 30 cm for the soils of riparian habitat in a high mountain watershed.
2) Nitrous oxide production appeared greater in soils containing more organic matter, higher water contents, and located nearer to the stream.
3) Denitrifying, sulfate-reducing, and heterotrophic aerobic bacteria appeared more abundant at 5 to 15 than at 30 cm depths, and, in general, increased with proximity to stream side.
4) Organic matter content and water content increased with decreasing depth and proximity to the streams edge.

The greater apparent abundance and activity of microorganisms in close proximity to the stream's edge indicates that these soils could be of importance in protecting and regulating nutrient inputs to the stream from adjacent lands. The microbial aspects studied here if continuously monitored may provide researchers and land managers with information pertaining to activities such as grazing of livestock, wildlife and recreational use on adjacent uplands. Changes in soil moisture because of decrease in streamflow or increased user activity may well be detected by further studying these organisms and water relationships in riparian zones.

LITERATURE CITED

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APPENDIX A

FORMULAS APPLIED DURING ANALYSIS

FORMULAS APPLIED DURING ANALYSIS

1. Percent H_2O (wet weight)

   weight wet soil - weight oven dry soil (105°C 24 h) = weight dry soil

   weight dry soil - weight wet soil = weight water in soil

   weight water in soil - weight wet soil X 100 = % water (wet weight)

2. To attain organisms per g dry soil

   1) Wet weight soil X % water content (as a decimal) = weight water in soil

   2) Wet weight soil - weight water in soil = weight dry soil

   3) $\frac{\text{g dry soil}}{\text{g wet soil + 225 ml (in blender)}} = \frac{1}{X}$ (first dilution)

   4) First dilution (X) X value obtained (less one power of 10) = $\frac{\text{organisms}}{\text{g dry soil}}$

   example:

   Henrici counts = 137×10^5 and 139×10^5 average = $138 \times 10^5 = 1.38 \times 10^7$

   Soil used (wet weight) 31.8 g into 225 ml distilled H_2O (in blender)

   Soil water content (% wet) 64.9%

   31.8 X .649 = 20.64 g H_2O in soil

   31.8 g wet soil - 20.64 g H_2O = 11.16 g dry soil

$$\frac{11.16 \text{ g dry soil}}{31.8 \text{ g wet soil} + 225 \text{ ml}} = .04346 = \frac{1}{23.007} \text{ (first dilution)}$$

$23.007 \times 1.38 \times 10^6 = 3.17 \times 10^7$ organisms/g dry soil

3. Procedures for N_2O calculations

formula $M = CG (Vg + Vl \alpha)$

where: M = Total amt. N_2O in water and gaseous phases

Cg = [N_2O in gas phase] ppm or nl/cc

Vl = Volume liquid

Vg = Volume gas (headspace)

α = Bunsen absorption coefficient at 20°C $\alpha \simeq .588$ (Tiedje 1982)

example:

$M = 94$ nl/cc N_2O (ppm) (52 cc + 87 cc (.588))

$M = 94$ nl N_2O (103.156) = 9696.664 nl N_2O

9696.664 nl = 9.696 ul

9.696 ul N_2O - 26.3 g dry soil

.368 ul N_2O/g dry soil

given:

a. Volume liquid = 87 cc

b. Volume gas = 52 cc

c. 94 ml N_2O produced

d. Soil contained 47.4% H_2O

e. Used 50.0 g wet soil

f. Thus used 26.3 g dry soil

4. Procedure for conversion of ul N_2O into ug N_2O (Petrucci 1982)

use n = PV/RT where: n = number moles

P = Pressure (atmosphere)

V = Volume N_2O L

R = .0821 L atm $mole^{-1} {}^\circ K^{-1}$

T = Temp °K

example:

On November 4, 1983, the GC work was done for this example. The atmospheric pressure was 23 inches of HG = 584.2 mm Hg, and .368 ml N_2O was produced per g dry soil.

1 atm = 760 mm Hg.

$$\text{atm} = \frac{584.2 \text{ mm Hg}}{760 \text{ mm Hg}} = .7687 \text{ atm}$$

$$n = \frac{.769 \text{ atm } (.368 \times 10^{-6} \text{ L } N_2O)}{.0821 \text{ L atm mole}^{-1} \ (293^\circ K) \ {}^\circ K^{-1}}$$

$$n = \frac{.769 \text{ atm } (.368 \times 10^{-6} \text{ L } N_2O) \text{ mole } {}^\circ K}{.0821 \text{ L atm mole}^{-1} \ (293^\circ K)}$$

$$n = \frac{2.829 \times 10^{-7} \text{ mole } N_2O}{24.0553} = 1.176 \times 10^{-8} \text{ moles } N_2O$$

$$g\ N_2O = 1.176 \times 10^{-8} \text{ moles } N_2O \times \frac{44 \text{ g } N_2O}{\text{mole } N_2O}$$

$$= 5.17 \times 10^{-7} \text{ g } N_2O = .517 \text{ ug } N_2O \text{ per g dry soil}$$

where: N = 14.0 g/mole

O = 16.0 g/mole

N_2O = 44 g/mole

ug = 10^{-6} g

APPENDIX B

DENITRIFICATION AND THE MEASUREMENT OF N_2O PRODUCTION BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

DENITRIFICATION AND THE MEASUREMENT OF N_2O PRODUCTION BY ELECTRON CAPTURE GAS CHROMATOGRAPHY

Denitrifying bacteria are facultative aerobes (Payne, 1981, Delwiche 1981), and are common in fresh waters and soils (Atlas and Barthe 1981). Denitrification is the dissimilatory reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to the gaseous oxides nitric oxide (NO) and nitrous oxide (N_2O), with the final end product nitrogen gas (N_2) via the sequence:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

(Payne 1973, Delwiche 1981, Knowles 1982, Tiedje 1982). The chief end products of denitrification are N_2O and N_2 (Letey et al. 1980, Rolston et al. 1978, Terry and Tate 1980, Ryden and Lund 1980). Denitrification by the above pathway can be done biologically only by bacteria (Focht 1978, Tiedje 1982, Delwiche 1981). The denitrifying bacteria utilize NO_3^- or NO_2^- as a terminal electron acceptor when oxygen (O_2) is limiting (Delwiche and Bryan 1976).

The accurate measurement of denitrification using gas chromatography has been well established (Blackmer and Bremner 1977, Denmead et al. 1979, Ryden et al. 1979, Matthias et al. 1980, Ryden and Lund 1980, Hutchinson and Mosier 1981, Mosier et al. 1981, Jury et al. 1982, Lensi and Chalant 1982, Swank and Caskey 1982, Sadykov et al.

1983). The use of acetylene (C_2H_2) has been shown in inhibit nitrification, another source of atmospheric N_2O (Blackmer et al. 1980, Aulakeh et al. 1982), and does not provide a carbon source for denitrifying bacteria (Yeoman and Beauchamp 1982). Acetylene blocks the reduction of N_2O to N_2, thus the measurement of N_2O in the presence of C_2H_2 provides a direct measurement of denitrification (Ryden 1982).

Modified methods of Swank and Caskey (1982) were employed to measure N_2O production. Soil samples were frozen (-12°C) for 30 to 35 days prior to G.C. analysis. The frozen samples were thawed at 20°-22°C 12 h before being processed. Nitrous oxide production was measured in 10% C_2H_2 v/v inhibited slurries prepared by adding 50 ml of a solution containing .32 g potassium nitrate (KNO_3), 1.0 g glucose, and .25 g chloramphenicol (Sigma Chemical Co., St. Louis, MO.) per L H_2O to 50 g (wet weight) soil in 125 ml Erlenmyer flasks. Chloramphenicol was used to inhibit protein synthesis in procaryotic cells, thus N_2O production rates reflect the maximum activity of preexisting denitrifying enzymes present at the start of the experiment (Swank and Caskey 1982). The flasks were then stoppered. The rubber stoppers used were pierced with two sections of glass tubing (0.5 mm ID). At the top of one tube was a small section of rubber tubing extending approximately 2.5 cm above and below the glass tubing. To the top of the other glass tube was attached tubing from O_2 free Ar (Linde Specialty Gases). Argon is passed into the flask for 5 min. while swirling the slurry to remove as much O_2 as possible. The other tube is left open during this purging process

(Figure B1A). The exhaust tube was then clamped tight, developing positive pressure within the flask (Figure B1B). The tube to the Ar was then removed (equalizing pressure) and a serum stopper was quickly inserted into the exposed glass tube (Figure B1C). This process results in an air-tight anerobic environment within the flasks. Ten percent of the headspace was then removed using a gas tight syringe (Hamilton Co., Reno, NV) and an equal amount of C_2H_2 (Linde) injected back into the headspace using the same syringe. The flasks were then incubated for 2 h at 22°C ± 2°C in a PsycroTherm Controlled Environment Incubator Shaker (New Brunswick Scientific, Edison NJ) at 300 rpm. After incubation the flasks were transported to the USDA Honey Bee Pesticide Lab, about 5 minutes away, for G.C. analysis.

Nitrous oxide was measured using a Hewlett Packard 5730 A gas chromatograph equipped with a ^{63}Ni electron capture detector. Separation of N_2O was achieved using a 3 mm X 1.8 m Parapak Q 80/100 mesh (Supelco Inc.) column at 50°C. Carrier gas was 5% methane in Ar flowing at 60 ml per min. Detector temperature was 300°C and injector temperature was 250°C. Injections were made with Hamilton (Hamilton Co., Reno, NV) microliter gas-tight syringes. Two certified gas standards (Scott Specialty Gases, Troy, MI) containing 99.61 ppm N_2O in Ar and 1069 ppm N_2O and 1012 ppm C_2H_2 in Ar were used to prepare standards curves. Nitrous oxide retention time under the above conditions was about 1.77 min. Steam-sterilized soils produced no peaks at or around 1.77 min. indicating no N_2O production. Peak heights were

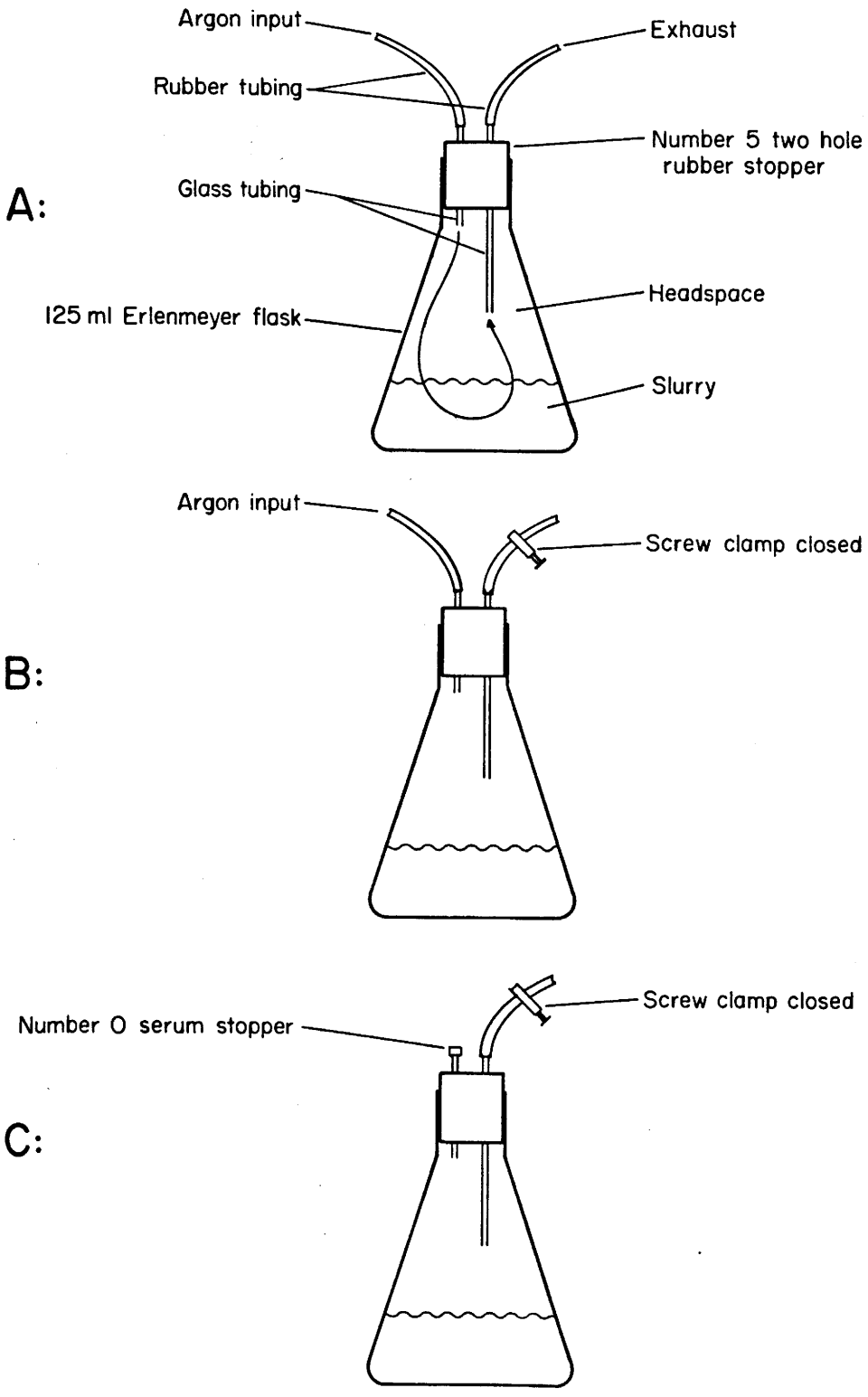

Figure B1. Flask used to measure denitrification potential. A: O_2 free Ar is used to purge flask, B: purging process complete, screw clamp closed, positive pressure, C: pressure released and anaerobic environment maintained with serum stopper.

used in quantitating amounts of N_2O produced. Multiple injections from each sample were made and average values obtained. It is recommended that at least 5 min. be allowed to pass between injections so that peaks from one injection do not show up on the next injection. Typical chromatograms resulting from this procedure are presented in Figures B2 and B3.

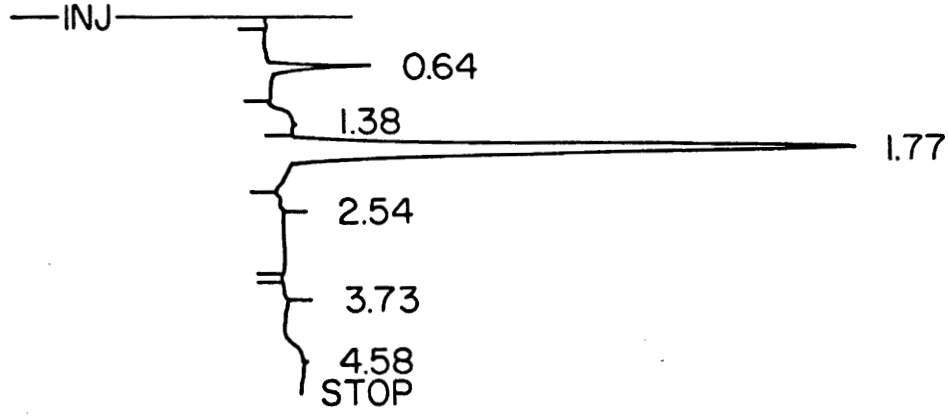

Figure B2. Chromatogram resulting from 5.0 μl injection of 1069 ppm N_2O in Ar, attenuation 64, 30 mv/min, N_2O retention time was 1.77 min.

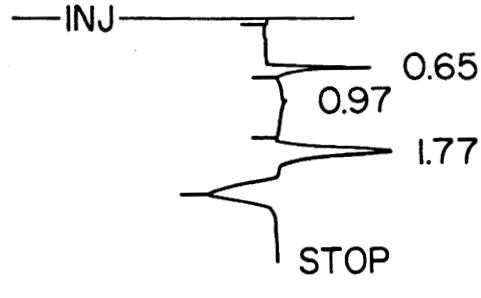

Figure B3. Chromatogram resulting from 5.0 μl injection of atmosphere from soil sample of wet meadow at a depth of 15 cm, attenuation 64, 30 mv/min, N_2O retention time was 1.77 min.

APPENDIX C

RAW DATA FOR SOIL MICROBIOLOGY OF THE NORTH AND SOUTH SIDE OF TELEPHONE CREEK, 1983

RAW DATA FOR SOIL MICROBIOLOGY NORTH AND SOUTH SIDE TELEPHONE CREEK 1983

(Values are g dry soil^{-1})

Date of Sampling	Atmospheric Pressure date of C.C. Work	Vegetative Community	Soil Depth cm	Log Viable Cells: MPN Denitrifying Bacteria	Log Viable Cells: Sulfate-Reducing Bacteria	Log Viable Cells: Heterotrophic Aerobic Bacteria	ug N_2O Produced	Soil Physical Properties: pH	Soil Physical Properties: Organic Matter %	Soil Physical Properties: Water Content (% wet)
8-15-83 (Middle transect North side)	.772 atm	Wet Meadow	5	5.89	5.72	7.51	3.97	5.1	23.9	64.9
			15	4.98	4.62	7.18	1.56	5.1	30.5	61.6
			30	4.48	3.36	6.26	0.38	5.5	23.1	52.2
		Moist Meadow	5	5.49	5.57	7.28	4.34	5.5	24.1	55.6
			15	3.41	3.86	6.39	0.82	5.7	15.7	46.0
			30	3.52	2.53	5.96	0.23	5.8	6.4	23.0
		Upland	5	4.38	2.86	7.20	1.58	4.7	13.1	23.2
			15	4.23	3.11	6.79	2.21	4.7	5.7	17.6
			30	4.08	1.97	6.00	0.20	5.0	2.5	9.7
8-16-83 (East transect North side)	.771 atm	Wet Meadow	5	4.15	3.59	6.80	2.02	5.4	13.4	44.7
			15	4.61	2.08	5.92	0.12	5.6	6.8	32.7
			30	4.59	3.04	6.15	0.00	5.6	3.2	30.0
		Moist Meadow	5	5.48	3.96	7.23	4.89	5.7	29.3	63.6
			15	4.67	2.95	6.49	2.25	5.8	14.7	40.7
			30	4.15	2.46	6.34	0.14	5.8	5.6	25.1
		Upland	5	3.54	2.85	6.87	0.22	5.0	8.1	21.7
			15	4.49	1.49	6.63	0.06	5.4	3.5	10.6
			30	4.63	0.00	6.38	0.00	5.6	2.8	12.1

Appendix C (continued)

Date of Sampling	Atmospheric Pressure date of G.C. Work	Vegetative Community	Soil Depth cm	Log Viable Cells: MPN Denitrifying Bacteria	Log Viable Cells: Sulfate-Reducing Bacteria	Log Viable Cells: Heterotrophic Aerobic Bacteria	ug N_2O Produced	Soil Physical Properties: pH	Soil Physical Properties: Organic Matter %	Soil Physical Properties: Water Content (% wet)
8-17-83 (West transect North side)	.772 atm	Wet Meadow	5	4.23	4.04	6.70	0.35	5.3	48.0	78.2
			15	3.54	4.08	2.30	0.36	5.2	47.8	74.8
			30	2.41	3.04	5.85	0.02	4.9	11.4	37.1
		Moist Meadow	5	5.88	3.88	6.00	1.54	4.8	18.1	56.1
			15	4.34	2.52	5.87	0.18	5.0	10.0	38.4
			30	3.84	2.59	5.59	0.03	5.4	2.7	23.5
		Upland	5	3.65	2.59	6.93	0.06	4.3	4.7	13.9
			15	3.20	2.62	6.30	0.06	4.1	4.3	18.4
			30	3.00	2.80	5.97	0.06	4.1	2.6	16.4
9-12-83 (Middle transect North side)	.772 atm	Wet Meadow	5	5.67	4.75	7.36	1.15	5.1	30.4	64.2
			15	5.87	5.98	7.38	1.08	5.2	18.4	62.0
			30	4.79	3.63	6.94	0.03	5.4	19.7	57.5
		Moist Meadow	5	5.36	4.54	7.15	1.26	5.2	18.5	60.2
			15	3.51	3.46	6.25	0.23	5.5	16.7	45.3
			30	2.23	2.91	5.98	0.05	5.7	11.4	37.1
		Upland	5	3.87	2.65	8.04	0.10	4.6	9.8	11.9
			15	4.43	1.76	7.04	0.08	4.8	3.9	8.8
			30	3.32	0.48	7.04	0.04	5.0	3.2	10.35

Appendix C (continued)

Date of Sampling	Atmospheric Pressure date of G.C. Work	Vegetative Community	Soil Depth cm	Log Viable Cells: MPN Denitrifying Bacteria	Log Viable Cells: Sulfate-Reducing Bacteria	Log Viable Cells: Heterotrophic Aerobic Bacteria	ug N_2O Produced	Soil Physical Properties: pH	Soil Physical Properties: Organic Matter %	Soil Physical Properties: Water Content (% wet)
9-13-83 (East transect North side)	.762 atm	Wet Meadow	5	2.89	3.41	6.38	2.34	4.9	19.1	52.9
			15	5.11	3.04	5.99	0.64	5.0	15.0	51.0
			30	3.30	2.62	5.50	0.00	5.2	1.5	17.5
		Moist Meadow	5	5.98	3.92	7.45	8.06	5.4	39.5	59.4
			15	5.69	3.53	6.72	5.00	5.6	20.4	47.2
			30	3.63	3.11	5.78	0.07	5.7	5.5	21.3
		Upland	5	2.83	1.75	6.82	0.11	4.9	9.1	10.6
			15	2.91	2.34	6.51	0.18	5.2	3.3	6.6
			30	1.51	1.46	6.41	0.08	5.6	3.1	7.6
9-14-83 (West transect North side)	.758 atm	Wet Meadow	5	3.34	3.25	6.60	0.60	5.1	30.6	65.0
			15	3.26	4.15	6.72	0.63	5.0	31.0	59.0
			30	2.15	3.76	6.08	0.19	5.0	23.1	55.0
		Moist Meadow	5	4.30	3.53	6.45	0.90	4.6	20.2	44.5
			15	2.94	3.43	5.66	0.26	4.9	10.0	36.3
			30	1.86	1.84	5.11	0.15	5.1	1.9	13.6
		Upland	5	1.54	2.36	6.41	0.19	4.1	6.0	10.1
			15	2.95	2.97	6.32	0.16	4.1	4.6	11.3
			30	3.32	2.81	6.00	0.14	4.5	2.9	8.8

Appendix C (continued)

Date of Sampling	Atmospheric Pressure date of G.C. Work	Vegetative Community	Soil Depth cm	Log Viable Cells: MPN Denitrifying Bacteria	Log Viable Cells: Sulfate-Reducing Bacteria	Log Viable Cells: Heterotrophic Aerobic Bacteria	ug N_2O Produced	Soil Physical Properties: pH	Soil Physical Properties: Organic Matter %	Soil Physical Properties: Water Content (% wet)
8-8-83 (South side)	.768 atm	Carex Bog	5	4.91	5.49	7.50	9.22	5.5	83.0	86.5
			15	4.72	5.97	7.41	4.41	5.4	74.9	87.6
			30	4.77	4.67	7.26	0.00	6.1	73.5	88.8
		Willow Bog	5	4.00	5.11	6.60	2.02	6.1	54.1	71.6
			15	5.78	4.73	6.38	1.74	6.2	28.0	69.7
			30	5.49	3.64	6.56	1.25	6.5	10.9	41.4
		Potentilla Bog	5	6.08	4.86	7.09	1.02	5.5	39.2	64.9
			15	5.48	4.43	6.85	0.22	5.4	10.5	38.5
			30	4.67	2.53	5.93	0.12	5.6	1.6	19.5
		Moist Meadow	5	5.34	5.59	7.23	1.56	5.2	19.6	38.5
			15	4.64	4.72	7.20	2.06	5.3	12.4	36.7
			30	5.18	4.68	7.17	2.17	5.4	10.3	31.0

Appendix C (continued)

Date of Sampling	Atmospheric Pressure date of G.C. Work	Vegetative Community	Soil Depth cm	Log Viable Cells: MPN Denitrifying Bacteria	Log Viable Cells: Sulfate-Reducing Bacteria	Log Viable Cells: Heterotrophic Aerobic Bacteria	ug N_2O Produced	Soil Physical Properties: pH	Soil Physical Properties: Organic Matter %	Soil Physical Properties: Water Content (% wet)
9-7-83 (South side)	.770 atm	Carex Bog	5	5.20	5.98	7.81	12.31	5.9	75.6	88.8
			15	4.11	5.15	8.74	3.02	5.7	73.4	86.2
			30	3.90	5.04	6.68	0.00	5.5	65.3	82.9
		Willow Bog	5	5.26	5.08	5.18	5.96	5.7	36.7	70.5
			15	5.25	4.45	6.49	2.35	6.3	16.5	41.0
			30	3.08	2.43	6.73	0.13	6.8	2.5	15.4
		Potentilla Bog	5	6.80	4.86	8.20	6.25	5.1	63.3	53.2
			15	5.43	4.80	6.81	0.69	5.4	9.0	30.7
			30	5.00	2.71	6.56	0.05	5.5	3.1	17.3
		Moist Meadow	5	5.04	4.62	7.72	1.79	5.4	20.0	29.8
			15	3.18	4.67	7.69	1.85	5.3	11.3	23.5
			30	4.87	4.08	6.76	1.63	5.3	6.9	21.0

APPENDIX D

T-TEST FOR COMPARING TWO SAMPLE MEANS WITH PAIRED OBSERVATIONS FOR STATISTICAL VERIFICATIONS FOR TABLE 4, COMPARISON OF FRESH AND FROZEN SOILS

T-TEST FOR COMPARING TWO SAMPLE MEANS WITH PAIRED OBSERVATIONS

STATISTICAL VERIFICATION FOR TABLE 4, COMPARING FRESH AND FROZEN SOILS

Parameter	Soil	Sum	Mean	Variance	St. Dev.	Total Sum of Squares	Corrected Sum of Squares	D.F.	T Value	95% Confidence Interval for the Mean Difference Limit 1	Limit 2
N_2O Production	Fresh	19.39	6.46	53.14	7.29						
	Frozen	4.83	1.61	1.62	1.27						
						143.65	72.99	2	1.39	19.86	-10.15
Heterotrophic Aerobic Bacteria	Fresh	4.80	1.60	0.49	0.70						
	Frozen	8.10	2.70	7.69	2.77						
						12.35	8.72	2	-0.91	4.09	- 6.29
Organic Matter	Fresh	66.80	22.27	45.01	6.71						
	Frozen	69.10	23.03	47.72	6.91						
						2.97	1.21	2	-1.71	1.16	- 2.69
Water Content	Fresh	156.00	52.00	59.43	7.71						
	Frozen	152.80	50.93	20.14	4.49						
						24.82	21.41	2	.564	9.19	- 7.06
pH	Fresh	17.95	5.98	.0008	.0025						
	Frozen	17.85	5.95	.028	.050						
						.005	.0016	2	2.00	.7717	- 0.7050

APPENDIX E

ANALYSIS OF VARIANCE TABLES FOR THE NORTH SIDE SOILS OF TELEPHONE CREEK

THREE FACTOR ANALYSIS OF VARIANCE FOR NITROUS OXIDE PRODUCTION BY VEGETATION COMMUNITY, DEPTH, AND DATE OF SAMPLING FOR SOILS (NORTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	45.813	5	9.163	4.665	.002*
VEGETATION COMMUNITIES	17.502	2	8.751	4.455	.019*
DEPTHS	28.051	2	14.025	7.141	.002*
DATE OF SAMPLING	.260	1	.260	.133	.718
2-WAY INTERACTIONS	16.885	8	2.111	1.075	.402
VEGCOM X DEPTH	15.391	4	3.848	1.959	.122
VEGCOM X DATE	.681	2	.340	.173	.842
DEPTH X DATE	.814	2	.407	.207	.814
3-WAY INTERACTIONS	.977	4	.244	.124	.973
VEGCOM X DEPTH X DATE	.977	4	.244	.124	.973
EXPLAINED	63.675	17	3.746	1.907	.051
RESIDUAL	70.706	36	1.964		
TOTAL	134.381	53	2.535		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.

THREE FACTOR ANALYSIS OF VARIANCE FOR MPN DENITRIFYING BACTERIA BY VEGETATION COMMUNITY, DEPTH, AND DATE OF SAMPLING FOR SOILS (NORTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	653756.895	5	130751.379	2.903	.027*
VEGETATION COMMUNITIES	269758.459	2	134879.229	2.995	.063
DEPTHS	378368.050	2	189184.025	4.200	.023*
DATES OF SAMPLING	5630.386	1	5630.386	.125	.726
2-WAY INTERACTIONS	429259.629	8	53657.454	1.191	.331
VEGCOM X DEPTH	342199.915	4	85549.979	1.899	.132
VEGCOM X DATE	7444.164	2	3722.082	.083	.921
DEPTH X DATE	79615.550	2	39807.775	.884	.422
3-WAY INTERACTIONS	48032.896	4	12008.224	.267	.897
VEGCOM X DEPTH X DATE	48032.896	4	12008.224	.267	.897
EXPLAINED	1131049.419	17	66532.319	1.477	.159
RESIDUAL	1621464.207	36	45040.672		
TOTAL	2752513.626	53	51934.219		

* SIGNIFICANTAT THE 0.95 PROBABILITY LEVEL.

THREE FACTOR ANALYSIS OF VARIANCE FOR SULFATE-REDUCING BACTERIA BY VEGETATION COMMUNITY, DEPTH, AND DATE OF SAMPLING FOR SOILS (NORTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	128139.142	5	25627.828	1.169	.343
VEGETATION COMMUNITIES	94055.677	2	47027.839	2.145	.132
DEPTHS	30651.869	2	15325.935	.699	.504
DATES OF SAMPLING	3431.595	1	3431.595	.157	.695
2-WAY INTERACTIONS	121512.902	8	15189.113	.693	.695
VEGCOM X DEPTH	56849.655	4	14212.414	.648	.632
VEGCOM X DATE	7203.832	2	3601.916	.164	.849
DEPTH X DATE	57459.415	2	28729.707	1.310	.282
3-WAY INTERACTIONS	113222.533	4	28305.633	1.291	.292
VEGCOM X DEPTH X DATE	113222.533	4	28305.633	1.291	.292
EXPLAINED	362874.577	17	21345.563	.974	.505
RESIDUAL	789266.904	36	21924.081		
TOTAL	1152141.481	53	21738.519		

THREE FACTOR ANALYSIS OF VARIANCE FOR TOTAL HETEROTROPHIC AEROBIC BACTERIA BY VEGETATION COMMUNITY, DEPTH, AND DATE OF SAMPLING FOR SOILS (NORTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	122367.111	5	24473.422	1.322	.277
VEGETATION COMMUNITIES	60832.448	2	30416.224	1.643	.207
DEPTHS	52893.448	2	26446.724	1.429	.253
DATES OF SAMPLING	8641.215	1	8641.215	.467	.499
2-WAY INTERACTIONS	100412.723	8	12551.590	.678	.707
VEGCOM X DEPTH	39808.601	4	9952.150	.538	.709
VEGCOM X DATE	27729.581	2	13864.791	.749	.480
DEPTH X DATE	32874.541	2	16437.271	.888	.420
3-WAY INTERACTIONS	62248.341	4	15562.085	.841	.508
VEGCOM X DEPTH X DATE	62248.341	4	15562.085	.841	.508
EXPLAINED	285028.175	17	16766.363	.906	.573
RESIDUAL	666292.460	36	18508.124		
TOTAL	951320.635	53	17949.446		

THREE FACTOR ANALYSIS OF VARIANCE FOR SOIL ORGANIC MATTER BY VEGETATION COMMUNITY, DEPTH, AND DATE OF SAMPLING (NORTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	4104.825	5	820.965	11.006	.001*
VEGETATION COMMUNITIES	2586.414	2	1293.207	17.337	.001*
DEPTHS	1518.221	2	759.111	10.177	.001*
DATES OF SAMPLING	.190	1	.190	.003	.960
2-WAY INTERACTIONS	424.540	8	53.067	.711	.680
VEGCOM X DEPTH	365.394	4	91.349	1.225	.318
VEGCOM X DATE	37.627	2	18.814	.252	.778
DEPTH X DATE	21.518	2	10.759	.144	.866
3-WAY INTERACTIONS	42.542	4	10.635	.143	.965
VEGCOM X DEPTH X DATE	42.542	4	10.635	.143	.965
EXPLAINED	4571.907	17	268.936	3.605	.001*
RESIDUAL	2685.287	36	74.591		
TOTAL	7257.193	53	136.928		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.

THREE FACTOR ANALYSIS OF VARIANCE FOR WATER CONTENT BY VEGETATION COMMUNITY, DEPTH, AND DATE OF SAMPLING FOR SOILS (NORTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	18955.573	5	3791.115	37.676	.001*
VEGETATION COMMUNITIES	15573.936	2	7786.968	77.386	.001*
DEPTHS	3322.311	2	1661.156	16.508	.001*
DATE OF SAMPLING	59.325	1	59.325	.590	.448
2-WAY INTERACTIONS	1459.505	8	182.438	1.813	.107
VEGCOM X DEPTH	1275.731	4	318.933	3.170	.025*
VEGCOM X DATE	130.545	2	65.272	.649	.529
DEPTH X DATE	53.229	2	26.615	.264	.769
3-WAY INTERACTIONS	7.711	4	1.928	.019	.999
VEGCOM X DEPTH X DATE	7.711	4	1.928	.019	.999
EXPLAINED	20422.788	17	1201.340	11.939	.001*
RESIDUAL	3622.480	36	100.624		
TOTAL	24045.268	53	453.684		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.

THREE FACTOR ANALYSIS OF VARIANCE FOR SOIL PH BY VEGETATION COMMUNITY, DEPTH, AND DATE OF SAPMLING (NORTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	5.545	5	1.109	7.504	.001*
VEGETATION COMMUNITIES	4.541	2	2.271	15.365	.001*
DEPTHS	.848	2	.424	2.868	.070
DATES OF SAMPLING	.156	1	.156	1.054	.311
2-WAY INTERACTIONS	.579	8	.072	.489	.856
VEGCOM X DEPTH	.244	4	.061	.414	.798
VEGCOM X DATE	.260	2	.130	.881	.423
DEPTH X DATE	.074	2	.037	.249	.781
3-WAY INTERACTIONS	.065	4	.016	.110	.978
VEGCOM X DEPTH X DATE	.065	4	.016	.110	.978
EXPLAINED	6.188	17	.364	2.463	.011*
RESIDUAL	5.320	36	.148		
TOTAL	11.508	53	.217		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.

APPENDIX F

ANALYSIS OF VARIANCE TABLES FOR THE SOUTH SIDE SOILS OF TELEPHONE CREEK

TWO FACTOR ANALYSIS OF VARIANCE FOR NITROUS OXIDE PRODUCTION BY VEGETATION COMMUNITY AND DEPTH FOR SOILS (SOUTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	121.717	5	24.343	10.323	.001*
VEGETATION COMMUNITIES	42.700	3	14.233	6.036	.010*
DEPTHS	79.016	2	39.508	16.753	.001*
2-WAY INTERACTIONS	66.905	6	11.151	4.728	.011*
VEGCOM X DEPTH	66.905	6	11.151	4.728	.011*
EXPLAINED	188.622	11	17.147	7.271	.001
RESIDUAL	28.299	12	2.358		
TOTAL	216.920	23	9.431		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.

TWO FACTOR ANALYSIS OF VARIANCE FOR DENITRIFYING BACTERIA BY VEGETATION COMMUNITIES AND DEPTHS FOR SOILS (SOUTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	10904817.231	5	.218E+07	1.973	.155
VEGETATION COMMUNITIES	6794044.048	3	.226E+07	2.048	.161
DEPTHS	4110773.182	2	.206E+07	1.859	.198
2-WAY INTERACTIONS	13053367.284	6	.218E+07	1.968	.150
VEGCOM X DEPTH	13053367.284	6	.218E+07	1.968	.150
EXPLAINED	23958184.515	11	.218E+07	1.970	.130
RESIDUAL	13267478.250	12	.111E+07		
TOTAL	37225662.765	23	.162E+07		

TWO FACTOR ANALYSIS OF VARIANCE FOR SULFATE-REDUCING BACTERIA BY VEGETATION COMMUNITY AND DEPTH FOR SOILS (SOUTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	793270.340	5	158654.068	3.189	.046*
VEGETATION COMMUNITIES	572639.157	3	190879.719	3.836	.039*
DEPTHS	220631.183	2	110315.591	2.217	.152
2-WAY INTERACTIONS	194694.215	6	32449.036	.652	.689
VEGCOM X DEPTH	194694.215	6	32449.036	.652	.689
EXPLAINED	987964.554	11	89814.959	1.805	.162
RESIDUAL	597097.043	12	49758.087		
TOTAL	1585061.597	23	68915.722		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.

TWO FACTOR ANALYSIS OF VARIANCE FOR TOTAL HETEROTROPHIC AEROBIC BACTERIA, BY VEGETATION COMMUNITY AND DEPTH FOR SOILS (SOUTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	8321986.437	5	.166E+07	1.056	.431
VEGETATION COMMUNITIES	5467923.250	3	.182E+07	1.156	.367
DEPTHS	2854063.187	2	.143E+07	.905	.430
2-WAY INTERACTIONS	9693955.812	6	.162E+07	1.025	.455
VEGCOM X DEPTH	9693955.812	6	.162E+07	1.025	.455
EXPLAINED	18015942.250	11	.164E+07	1.039	.471
RESIDUAL	18921524.250	12	.158E+07		
TOTAL	36937466.500	23	.161E+07		

TWO FACTOR ANALYSIS OF VARIANCE FOR SOIL ORGANIC MATTER BY VEGETATION COMMUNITIES AND DEPTHS (SOUTH SIDE TELEPHONE CREEK 1983)

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	16889.329	5	3377.866	65.853	.001*
VEGETATION COMMUNITIES	13759.098	3	4586.366	89.413	.001*
DEPTHS	3130.231	2	1565.115	30.513	.001*
2-WAY INTERACTIONS	1394.399	6	232.400	4.531	.013*
VEGCOM X DEPTH	1394.399	6	232.400	4.531	.013*
EXPLAINED	18283.728	11	1662.157	32.404	.001*
RESIDUAL	615.530	12	51.294		
TOTAL	18899.258	23	821.707		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.

TWO FACTOR ANALYSIS OF VARIANCE FOR SOIL WATER CONTENT BY VEGETATION COMMUNITIES AND DEPTHS (SOUTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	13604.761	5	2720.952	31.164	.001*
VEGETATION COMMUNITIES	11429.935	3	3809.978	43.637	.001*
DEPTHS	2174.826	2	1087.413	12.455	.001*
2-WAY INTERACTIONS	1431.188	6	238.531	2.732	.065
VEGCOM X DEPTH	1431.188	6	238.531	2.732	.065
EXPLAINED	15035.948	11	1366.904	15.656	.001
RESIDUAL	1047.730	12	87.311		
TOTAL	16083.678	23	699.290		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.

TWO FACTOR OF ANALYSIS OF VARIANCE FOR PH BY VEGETATION COMMUNITIES AND DEPTHS FOR SOILS (SOUTH SIDE TELEPHONE CREEK 1983).

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	3.767	5	.753	15.723	.001*
VEGETATION COMMUNITIES	3.341	3	1.114	23.243	.001*
DEPTHS	.426	2	.213	4.443	.036*
2-WAY INTERACTIONS	.378	6	.063	1.313	.323
VEGCOM DEPTH	.378	6	.063	1.313	.323
EXPLAINED	4.145	11	.377	7.863	.001*
RESIDUAL	.575	12	.048		
TOTAL	4.720	23	.205		

* SIGNIFICANT AT THE 0.95 PROBABILITY LEVEL.