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Denitrification and Bacterial Numbers in Riparian Soils of a Wyoming Mountain Watershed

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Abstract

The presence and activity of denitrifying bacteria as well as bacteria capable of reducing sulfate in 1 upland and 5 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 3 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 stream's 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.

Riparian ecosystems are ecologically important vegetative communities (Odum 1978). Compared to upland vegetation types, riparian zones in the Rocky Mountain States are relatively limited in area 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 (Kirby 1978).

Numerous studies using vegetation, channel morphology, and sediment deposition show changes occurring in riparian zones due to livestock grazing (Duff and Cooper 1978, and Platts et al. 1983). Other authors have used bacteria indicative of fecal pollution for assessing ungulate grazing contribution to stream pollution (Morrison and Fair 1966, Jawson et al. 1982, Skinner et al. 1984a). Bacteria other than those used to determine fecal contamination in streams 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 were the only population out of several monitored to show significant response downstream even with settling of organisms because 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 facultative anaerobes, and sulfate-reducing bacteria, which are strict anaerobes, are common in many soils and waters (Gamble et al. 1977, Knowles 1982, Postgate 1984). Bacterial denitrification has been positively correlated with soil moisture and organic matter (Smith and Tiedje 1979a, 1979b; Patten et al. 1980; and Knowles 1982). Sulfate-reducing bacteria proliferate under anaerobic conditions in soils (Peck 1982, Bremner and Steele 1978, and Postgate 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. This work was carried out to determine if the presence, population dynamics, and potential for denitrifying bacteria could, with future research, provide an additional way to monitor user impacts of riparian ecosystems. User impacts that may decrease soil moisture could change the soil/oxygen balance in riparian zones and reduce bacteria numbers or anaerobic related activity. A reduction could occur prior to changes in vegetation, water quality, or wildlife diversity observed through current monitoring methodology. Consequently, change in these bacteria or biological activity may provide early warning to successional change in riparian eocsystems.

Materials and Methods

Study Site

A 30 × 100 m study site was located along Telephone Creek approximately 40 m above a stream flow gauging station approximately 3,170 m above sea level.

Telephone Creek is 1 of 3 main drainages within the Nashfork Hydrologic Observatory of Southeastern Wyoming. The watershed has been continuously monitored by the Wyoming Water Research Center (WWRC) since the mid 1960's. Vegetation and drainage basin have been described by Skinner et al. (1974). The area is typical of many high mountain watersheds in the Rocky Mountains. Monthly air temperature, precipitation, and stream

Table 1. 1983 mean monthly precipitation air temperature and stream flow 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
Jun	.28	3.83	789.00
Jul	.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

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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: Denitrifying Bacteria	Log Viable Cells: Sulfate-Reducing Bacteria	Log Viable Cells: Heterotrophic Aerobic Bacteria	Soil Physical Properties: Organic Matter(%)	Soil Physical Properties: Water Content (% Wet)	Soil Physical Properties: pH
Depth							
5 cm	*1.88 a	5.33 a	4.59	7.07	19.96	43.82	4.99 a
15 cm	0.88	4.96 ab	4.76	6.65	14.57	37.13	5.50 ab
30 cm	0.11	4.09	3.03	6.77	7.36 a	25.44 a	5.28 b
Plant Communities							
Wet	0.87 ab	5.12 ab	4.96	7.08	21.68 a	52.68 a	5.22 a
Moist	1.69 a	5.24 a	3.82	6.75	15.04 b	40.94 b	5.40 a
Upland	0.31 b	3.98 b	2.58	6.65	5.17 c	12.76 c	4.71 b

*Values with same letters or no letters within columns, depths, and plant communities indicate no significant differences at $\alpha = 0.05$, n = 18.

flow at the site for 1983 are presented in Table 1.

The selected study area was occupied by 3 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. The riparian soils have been described as cryaqualls and histosols. The upland soils were described as being primarily Endlich. In the wet meadow, Carex (*Carex* sp.) and Equisetum (*Equisetum* sp.) were dominant; grasses and rushes (*Juncus* sp.) were dominant in the moist meadow; grasses, forbs, and subalpine forest were characteristic of the uplands. Three transects, approximately 30 m apart and 30 m long were established within the study area. Each transect started at the edge of the low stream bank (Platts et al. 1983) and continued, perpendicular to the stream, upland through each vegetation zone, and total length along a straight line was determined. Sampling occurred at the center of each vegetative zone.

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 (9 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.

In the laboratory, 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 were homogenized using a Waring commercial blender at low speed (18,000 rpm) for 2 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. (1974). Denitrifying bacterial populations were estimated by the most probable number (MPN) method used by Skinner et al. (1974) but were incubated for 14 days instead of 7. Nitrous oxide (N_2O) production was measured following methods of Swank and Caskey (1982) using thawed soils which had been frozen for 2 weeks. Nitrous oxide was measured using a Hewlett Packard 5730 A GC equipped with a ^{63}Ni electron capture detector. Separation of N_2O was achieved using a 3mm × 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 Speciality Gases, Troy, Mich.) containing 99.61 ppm N_2O in Ar and 1,069 ppm N_2O and 1,012 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 bacteria, 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 indicate 2 general findings: (1) bacterial numbers, organic matter, and water content were higher in the upper 5 to 15 cm than at 30 cm; (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. Stream flow 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 stream flow did not decrease soil mois-

ture as expected. This is likely due to shallow riparian soils over a perennial watertable 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 when data were combined from all plant communities. 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).

Nitrous oxide production was not significantly affected by freezing of soil samples for 2 weeks. These data support that of others with regard 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 (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 does (Table 4). 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 g of soil than those reported for Domino silty clay loam and a Ramona sand 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 indicate that higher counts of sulfate-reducing bacteria were found in the wet and moist meadow 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. (Peck 1962, Bremner and Steele 1978 Postgate, 1984).

Total Heterotrophic Aerobic Bacteria

Plate counts on Henrici agar showed 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).

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

		Wet Meadow			Moist Meadow			Upland		
Parameters	Depths cm	Aug. n=3	Sept. n=3	Mean n=6	Aug. n=3	Sept. n=3	Mean n=6	Aug. n=3	Sept. n=3	Mean n=6
ug N_2O Produced	5.0	2.11	*1.36 a	1.74 a	3.62 a	3.41	3.51 a	0.62	0.13	0.38
	15.0	0.68	0.78 ab	0.73	1.08	1.83	1.46 ab	0.78	0.14	0.46
	30.0	0.13	0.16 b	0.15 b	0.13	0.09	0.11 b	0.09	0.08	0.09
Log Denitrifying Bacteria	5.0	5.42	5.20	5.32	5.66 a	5.60	5.63 a	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	4.22 a	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	7.13 a	7.03	6.83	6.94 a
	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	28.83 a	26.06 a	24.95 a	8.63 a	8.30 a	8.47 a
	15.0	28.37	21.46	24.92	13.46 b	15.70 ab	14.58 b	4.50 ab	3.93	4.22
	30.0	12.56	14.77	13.66	4.90 c	6.26 b	5.58 c	2.63 b	3.70	2.85
Water Content % (wet weight)	5.0	58.60	60.70	59.65	58.43 a	54.73	56.58 a	19.60	10.87	15.23
	15.0	56.37	57.33	56.85	42.70 b	42.93	42.32 b	15.53	8.90	12.22
	30.0	39.77	43.33	41.66	23.87 c	24.00 a	23.93 c	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

*Values with same letters or no letters indicate no significant difference between depths at $\alpha = 0.05$.

Table 4. Correlation regression matrix for all soil microbial parameters and physical properties, north side Telephone Creek.

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

*Numbers represent correlation coefficients for one variable compared to another.

The design of this study was constructed to establish 3 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 assumed that there would be no differences between transects; this was not the case (Table 5). Statistical analysis of these data found that there were 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 bacteria and other 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 Creek within a riparian zone developed over alluvium soils deposited in a subalpine pond. There were 4 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 6) represent only 2 samplings of this area. It is interesting to note that some of the findings discussed earlier are also evident here.

Table 5. 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	5.78 a	5.42	7.48 a	31.98	60.40	5.23
	2	0.85	4.58	3.19	6.31	8.73 a	36.13 a	5.32
	3	0.35	3.61	3.93	6.57	24.33	61.52	5.10
Moist Meadow	1	*1.15 ab	4.96	4.14	6.81	15.46	44.53	5.56
	2	3.42 a	5.48	3.59	6.97	19.17	42.88	5.65
	3	0.51 b	5.13	3.38	5.96	10.48	35.41	4.98 a
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	4.20

*Values with same letter or no letters indicate no significant differences between transects at $\alpha = 0.05$, n = 6.

Table 6. Microbiological data per g dry soil and soil physical properties for riparian vegetative communities on pond filling site.

Parameter	Depths cm	Carex Bog	Willow Bog	Potentilla	Moist Meadow	Mean^{ooo}
ug N_2O Produced	5.0	10.76 a***	2.98	3.13	1.68	4.64 a
	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**	4.32 a****	1.90 ab	1.22 b	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	5.10 a	4.86 a	5.33	5.42
	15.0	5.74	4.61 b	4.65 ab	4.69	5.23
	30.0	4.89	3.37 c	2.63 b	4.48	4.44
	Mean	5.62 a	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	45.40 a	51.25 a	19.80 a	48.91 a
	15.0	74.25	22.25 ab	9.75	11.85	29.50
	30.0	69.40	6.70 b	2.35	8.60	21.76
	Mean	74.25 a	24.80 b	21.12 bc	13.42 c	
Water Content % (wet weight)	5.0	87.65	71.05	59.05	34.15	62.98 a
	15.0	86.90	55.35	34.60	30.10	51.74 b
	30.0	85.85	28.40	18.40	26.00	39.66 c
	Mean	86.80 a	51.60 b	37.35 c	30.08 c	
pH	5.0	5.70	5.90 a	5.30	5.30	5.55 a
	15.0	5.55	6.25 ab	5.40	5.30	5.63 ab
	30.0	5.80	6.70	5.60	5.35	5.86 b
	Mean	5.68 a	6.28 b	5.43 c	5.32 d	

*Values for depths are averages of two samplings (September 8 and August 7, 1983).
**Mean values for vegetative communities are averages of six samplings (four vegetative communities each sampled twice).
^{ooo}Mean values for depths are average of eight samplings (four vegetative communities each sampled twice).
***Numbers with same letters or no letters indicate no significant differences between depths at $\alpha = 0.05$, n = 2.
****Mean values with same letter or no letters indicate no significant differences between vegetative communities or depths at $\alpha = 0.05$, n = 6.

On the filling pond N_2O production appeared to decrease with depth, and was in general higher in those vegetative communities containing more organic matter and higher water contents (Table 6). 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 6). 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 6). 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 6).

Summary and Conclusions

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 riparian soils than in upland soils.

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 stream's edge.

The presence, as well as higher number, of faculative and anaerobic bacteria in riparian soils studied here indicate the organisms could be of importance in protecting and regulating nutrient inputs to streams 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 stream flow or increased user activity may well be detected by further studying these organisms and water relationships in riparian zones.

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