Denitrification Rates and Associated Soil Characteristics of Wetlands Created on Oxidized and Reduced Mine Spoil in East Texas[1].

by

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Abstract Recovery of wetland function is the primary goal of wetland creation and restoration. Denitrification is a wetland function and part of the nitrogen (N) biogeochemical cycle in which nitrate (NO_3^-) and nitrite (NO_2^-) forms of N are converted to gaseous forms and lost to the atmosphere. Measurement of denitrification rate may therefore be an important tool for evaluating wetland function. This study examines denitrification rates and associated soil variables on wetlands created on lignite mine spoil in East Texas. Wetlands created on oxidized and reduced mine spoil were selected as study sites. Soil cores were removed from recently-created (age 4-8 years), older-created (age 10 years) and reference (natural) wetlands. Denitrification was quantified using an acetylene inhibition/gas chromatography method. Soil texture, pH, total N, and organic matter content were also measured. Soil pH range from 4.5 to 7.8 and varied by age and spoil type ($\alpha \leq 0.05$). Total N ranged from 342 to 1564 mg kg^{-1} and varied only by spoil type. Organic matter content varied by spoil type with values ranging from 1.2 % to 3.0 %. Denitrification rate did not differ among wetlands and ranged from 0.2 kg N ha^{-1} yr^{-1} to 105 kg N ha^{-1} yr^{-1}. Older-created and recently-created wetlands on both oxidized and reduced mine spoil had denitrification rates similar to natural wetlands. Denitrification appears to function as well in wetlands created on mine spoil as in natural wetlands.

Introduction

Wetland creation is an important component of reclamation, both ecologically and legally. Wetland creation implies a restoration of wetland functions. Sediment retention, wildlife habitat, flood attenuation, aquifer recharge, and biogeochemical cycling are commonly recognized as wetland functions (Mitsch and Gosselink 1993). Denitrification is also an important wetland function that improves water quality (Brinson 1993). Denitrification is the primary process leading to environmentally benign removal of nitrogen (N) from wetlands.

When N enters a wetland, it is utilized by a variety of microorganisms and plants. In denitrification, bacteria (*Pseudomonas, Bacillus, Micrococcus, Achromobacter,* and other genera) reduce nitrate (NO_3^-) to dinitrogen (N_2) and other N gases which are lost to the atmosphere (Tiedje 1982, Groffman et al. 1996, Murray et al. 1995). These facultative anaerobes switch from using oxygen (O_2) during aerobic respiration, to the use of NO_3^- for respiration when soils become anaerobic. Enzymes catalyze this reduction of NO_3^- to nitrite (NO_2^-) then to nitrous oxide (N_2O) and finally N_2. Acetylene (C_2H_2) stops this enzymatic pathway after N_2O has been produced. Since these microbes are ubiquitous, natural and created wetlands can function in a similar manner, although wetlands designed and constructed for water treatment tend to receive higher levels of NO_3^- and achieve higher rates of denitrification (Gale et al. 1993, Duncan and Groffman 1994, Alberston and Coughenour 1995). Denitrification is influenced by soil abiotic and biotic factors such as temperature, pH, texture, redox potential, the nature and amount of organic matter, soluble N, dissolved oxygen, microorganism populations, and plant cover (Bodelier et al. 1996, Eriksson and Weisner 1997, Groffman and Hanson 1997). Denitrification slows or ceases at very low temperatures (Christensen and Tiedje 1990).

Methods

Created wetlands were selected from TXU Mining (formerly Texas Utilities Mining Company) lignite surface mine reclamation sites at Beckville, Tatum, and Oak Hill, in East Texas. All wetlands had been delineated for Section 404,

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Clean Water Act requirements. Wetland creation at Beckville and Tatum mines occurs on spoils derived from mixing overburden with topsoil and produces a mine spoil which is generally reduced in nature. Wetlands at Oak Hill originate on four foot haul-back, the top four feet of pre-mine soil, and is oxidized in nature. These mitigation wetlands are created in depressional areas, typically contiguous with ephemeral streams, and are commonly associated with aquatic features (ponds). Microtopographical differences are constructed by machine, and appropriate species of bottomland hardwood seedlings are planted in and around wetland areas. Three undisturbed natural "reference" wetlands were selected on the Tatum site. Six recently-created and six older-created wetlands were selected, with three of each age class on "reduced" (Beckville and Tatum) and "oxidized" (Oak Hill) mine spoil. Older-created wetlands were established in 1989 (age 10 years), while recently-created wetlands were established between 1991 and 1995. Mature bald cypress (*Taxodium distichum*), planer tree (*Planera aquatica*), overcup oak (*Quercus lyrata*), button bush (*Cephalanthus occidentalis*) and green ash (*Fraxinus pennsylvanica*) dominated the reference wetlands. Grasses (*Panicum sp, Paspalum sp*), sedges (*Cyperus sp*), rushes (*Juncus sp*), cattail (*Typha sp*), black willow (*Salix nigra*), and eastern Baccharis (*Baccharis halimifolia*) dominated the created wetlands.

Wetlands were sampled during April, July, and October 1999 (periods one, two and three). Three soil samples and three intact cores were taken from randomly selected areas in each wetland during each sampling period. During periods one and two, samples were removed from flooded areas. During period three, soil samples were taken from relatively dry areas in many wetlands due to drought conditions in East Texas. Soil cores were taken using a wetland soil sampler which produced intact 10 cm diameter by 15 cm long cores. A straight bladed spade was used to retrieve sample cores when heavy clay textures or dry conditions made the wetland soil core sampler ineffective. Soil cores were placed in PVC sleeves, capped and stored at 4 C°. Soil texture, pH, organic matter, total N, NO_3^-, and ammonium (NH_4^+) were measured by the Stephen F. Austin State University Soils Testing Laboratory using air dried samples. Organic matter was determined using a loss on ignition technique (Ben-Dor and Banin 1989, Nelson and Sommers 1996) and pH was measured in a 2:1 water soil slurry using a pH meter. Total-N was measured using the Kjeldahl method (Bremner 1996) while NH_4^+ was determined using a colorimetric technique (Baethgen and Alley 1989). NO_3^- was measured using a salicylic acid method (Cataldo et al. 1975, Vendrell and Zupancic 1990).

To determine the denitrification rate, soil cores were maintained at field moisture status and warmed to seasonal temperatures (25, 30, and 15 C°, measured *in situ*). Cores were amended with three milligrams NO_3^- - N in solution prior to incubation and made air-tight using silicone caulk. A volume of 125 ml acetylene (C_2H_2) generated from calcium carbide (CaC_2)was injected into each core through a rubber septum-stopper using a 25 ml gas tight syringe and eight inch needle. Five 25 ml injections were made at several levels within a core to ensure the diffusion of C_2H_2 throughout the core. After injection of C_2H_2 cores were allowed to equilibrate for two hours, then initial headspace gas samples (5 μl) were drawn to determine N_2O concentration. Prior to sample removal, cores were vigorously shaken to ensure equilibrium between soil and headspace gases (Aulakh and Doran 1991). The gas samples were analyzed for N_2O concentration using an SRI 8610C gas chromatograph (SRI Instruments Torrance, CA) equipped with an ^{63}Ni electron capture detector operating at 343 C° and a GS-Q 30m capillary column (J&W Scientific Folsom, CA) cooled in an ice bath. Eight to twelve hours after initial sampling, additional headspace samples were withdrawn and analyzed for N_2O. Water and gas volume were determined in each core, and used with appropriate Bunsen coefficients to determine the amount of N_2O produced (Tiedje 1982). The rate of N_2O production was determined by dividing the amount of N_2O produced by the length of time between injections. The rate of N_2O production is assumed to equal the rate of denitrification.

Statistical Analysis

Each sample period was analyzed independently using Statistical Analysis Software (SAS) procedures (SAS Institute 1989). Analysis of variance was conducted for a completely randomized design with three replications. Each wetland was considered a plot. Wetland age and initial substrate state (oxidized mine spoil or reduced mine spoil) were independent variables. Denitrification rate, soil texture, pH, total N, and organic matter were dependent variables. Created wetlands were compared to natural (reference) wetlands using Student's t test.

Results and Discussion

More differences were detected by spoil type than by age (Table 1). Little variation by age class was apparent within created wetlands; pH differed by age during period one and denitrification rate differed by age by age during period 2 (Table 2). Created wetlands were most different in period three. Wetlands on reduced mine spoil had higher pH, total N and organic matter than wetlands on oxidized mine spoil during sampling period three (Table 3). Recently-created wetlands on reduced mine spoil also had significantly higher pH than reference wetlands for all sampling periods (Table 4). Reference wetlands contained significantly more organic matter than wetlands recently-created on oxidized mine spoil during periods one and two and older-created wetlands on oxidized mine spoil during periods two and three (Table 5). Reference wetlands and wetlands on reduced mine spoil had similar levels of organic matter (Table 5), while wetlands on oxidized mine spoil had significantly less organic matter than wetlands on reduced mine spoil during sample period two and three (Table 3). Reference wetlands had higher total N levels than created wetlands during sample periods one and two (Table 6). On average, reference wetlands had higher N levels than all other wetlands (Table 7). Wetlands on oxidized mine spoil have lower total N levels than wetlands on reduced mine spoil during period three. Reference wetlands had higher total N than wetlands on oxidized mine spoil during periods one and two. Recently-created wetlands on reduced mine spoil wetlands in period one and older-created wetlands on reduced mine spoil wetlands in period two had significantly less total N than reference wetlands. Total N was significantly different between spoil types during period three, but not during periods one and two. Denitrification rate was not significantly different ($\alpha \leq 0.05$) between reference and created wetlands (Table 8). Among created wetlands denitrification rate was not significantly different (Table 1). Lower denitrification rates during period three are probably related to lack of soil moisture. Due to the short incubation length, the denitrification rates are most properly viewed as potential rates. The actual yearly denitrification rates are probably lower in these wetlands.

pH variations within created wetlands are most likely due to differences in spoil material. Oxidized mine spoil originates from local surface soils which are highly weathered, and acidic in nature. Denitrification is most efficient near neutral pH, and N_2O becomes the predominant denitrification product at lower pH levels (Thomsen et al. 1994). In the wetlands on oxidized mine spoil, at the pH levels recorded (below pH 6), the reduction of N_2O is likely inhibited (Parkin et al. 1985, Gale et al. 1993, Thomsen et al. 1994). A few "hot spots", including a sub-sample with pH 2.6, occurred in oxidized mine spoil; pH at this level may completely inhibit denitrification (Parkin et al. 1985). No inhibition of the reduction of N_2O to N_2 should occur in wetlands created on reduced mine spoil since pH is near neutral. Unless NO_3^- is in excess, N_2 should be the predominate denitrification end product in wetlands created on reduced mine spoil (Gale et al. 1993, Thomsen et al. 1994). Additional study may be warranted to determine if N_2O is the predominant denitrification product in reference wetlands and wetlands on oxidized spoil as a result of low pH. Since denitrification rates were similar over a range of pH values, including the low pH values of the natural "reference" wetlands, pH is most likely not a barrier to the establishment of the denitrification function within these wetlands.

Lower organic matter levels in wetlands created on oxidized mine spoil in comparison to wetlands on reduced mine spoil was unexpected. If productivity and decomposition rates are similar within created wetlands, organic matter levels should be similar. Higher levels of organic matter may originate from lignite coal and other carbon rich materials incorporated in mixed overburden (reduced mine spoil) (Waggoner 1993, Stewart 1996). Since the oxidized mine spoil represents only the top four feet of soil material, incorporation of organic matter from lignite is unlikely in the oxidized mine spoil. Studies on mixed overburden have encountered higher levels of organic matter due to lignite in spoil material (Waggoner 1993, Stewart 1996) Additionally, lignite is very resistant to decay, relative to other forms of organic carbon, and can be expected to persist for long periods. Future studies should examine soluble carbon (C) or microbial biomass C as this may be a better indicator of carbon availability for denitrification (McCarty and Bremner 1993, Duncan and Groffman 1994). Lack of variation by wetland age class (within created wetlands) is also an interesting problem. This lack of variation may indicate a lack of distinction in the arbitrary age classes used. Another possibility is that organic matter amounts rapidly achieve a basic level, then only climb slowly. Organic matter levels appear sufficient to support denitrification, and are near expected values for riparian wetlands with bottomland forests (Mitsch and Gosselink 1993).

Table 1. Probability of a greater F values for main effects of age and spoil type for selected dependent variables.

		Period 1	Period 2	Period 3
	age	0.381	0.045*	0.496
denitrification rate	spoil type	0.142	0.240	0.988
	age X spoil	0.297	0.130	0.372
	age	0.041*	0.088	0.169
PH	spoil type	0.001*	0.058	0.023*
	age X spoil	0.365	0.359	0.822
	age	0.675	0.349	0.615
total N	spoil type	0.062	0.063	0.028*
	age X spoil	0.367	0.367	0.487
	age	0.623	0.101	0.467
organic matter	spoil type	0.797	0.005*	0.018*
	age X spoil	0.324	0.101	0.954

* means are significantly different @ $\alpha \leq 0.05$ level for a variable within a sample period.

Table 2. Mean denitrification rate, pH, total N, and organic matter within created wetlands by age class for each sampling period.

		Period 1	Period 2	Period 3
denitrification rate	recently-created	83	60*	20
(kg N ha^{-1} yr^{-1})	older-created	68	43	9
PH	recently-created	6.8 *	6.8	6.7
	older-created	6.0	5.9	5.8
total N	recently-created	437	514	782
(mg N kg^{-1})	older-created	466	446	695
organic matter	recently-created	2.0	2.1	2.4
(percent)	older-created	2.8	1.6	1.9

* means are significantly different @ $\alpha \leq 0.05$ level for a variable within a sample period.

Table 3. Mean denitrification rate, pH, total N, and organic matter within created wetlands by spoil type for each sampling period.

		Period 1	Period 2	Period 3
denitrification rate	oxidized	62	47	14
(kg N ha^{-1} yr^{-1})	reduced	89	56	15
PH	oxidized	5.5 *	5.8	5.5 *
	reduced	7.2	6.9	7.0
total N	oxidized	371	394	516 *
(mg N kg^{-1})	reduced	607	566	960
organic matter	oxidized	2.2	1.2 *	1.3 *
(percent)	reduced	2.6	2.4	3.0

* means are significantly different @ $\alpha \leq 0.05$ level for a variable within a sample period.

Table 4. Soil pH by wetland age class and spoil type and p value for wetland class vs. reference by period.

		Period 1		Period 2		Period 3	
		pH	p > |t|	pH	p > |t|	pH	p > |t|
Natural		5.1		4.9		4.5	
Oxidized							
	recently-created	5.7	0.068	6.5	0.068	5.9	0.125
	older-created	5.3	0.773	5.1	0.659	5.0	0.054
Reduced							
	recently-created	7.8	0.003	7.1	0.005	7.4	0.0002
	older-created	6.6	0.353	6.6	0.030	6.6	0.060

Table 5. Soil organic matter (percent) by wetland age class and spoil type and p value for wetland class vs. reference by period.

		Period 1		Period 2		Period 3	
		om	p > |t|	om	p > |t|	om	p > |t|
Natural		3.7		3.0		4.0	
Oxidized							
	recently-created	1.1	0.014	1.2	0.019	1.5	0.053
	older-created	3.3	0.943	1.2	0.027	1.0	0.042
Reduced							
	recently-created	2.9	0.419	2.9	0.772	3.2	0.549
	older-created	2.2	0.550	1.9	0.113	2.8	0.355

Table 6. Total N (mg N kg^{-1}) by wetland age class and spoil type and p value for wetland class vs. reference by period.

		Period 1		Period 2		Period 3	
		total N	p > |t|	total N	p > |t|	total N	p > |t|
Natural		1447		1343		1564	
Oxidized							
	recently-created	342	0.001	395	0.017	499	0.062
	older-created	400	0.017	392	0.019	533	0.103
Reduced							
	recently-created	683	0.044	633	0.053	1064	0.311
	older-created	531	0.136	499	0.026	856	0.185

Table 7. Average total-N, Ammonium-N, and Nitrate-N (mg N kg^{-1}) by spoil type and wetland age.

		Ammonium-N	Nitrate-N	total-N
Natural		7.3	4.0	1451
Oxidized				
	recently-created	3.1	2.1	412
	older-created	3.6	1.2	441
Reduced				
	recently-created	7.2	2.1	793
	older-created	6.5	1.4	628

Table 8. Denitrification rate (kg N $ha^{-1} yr^{-1}$) by wetland age class and spoil type and p value for wetland class vs. reference by period.

	Period 1		Period 2		Period 3	
	rate	p > |t|	rate	p > |t|	rate	p > |t|
Natural	90		59		0.15	
Oxidized						
recently-created	61	0.307	49	0.591	13	0.374
older-created	64	0.659	45	0.369	16	0.086
Reduced						
recently-created	105	0.248	70	0.384	28	0.379
older-created	72	0.608	42	0.387	1.9	0.401

Levels of total N were higher in wetlands on reduced mine spoil than on oxidized mine spoil. A significant portion of the N in reduced wetlands may arise from geologic N integrated into spoil material, especially lignite coal (Li and Daniels 1994, Hons and Hossner 1980). Little N incorporated from lignite is plant available, and the nitrification potentials of mined soils was extremely low (Hons and Hossner 1980). Higher levels of total N in reference wetlands is likely due to long term accumulation.

Denitrification rates were comparable with results from Groffman and Hanson (1997), but were much lower than rates reported by Gale et al. (1993). Gale et al. examined wetlands used for waste water treatment, so it is not unexpected that wetlands in this study had lower rates of denitrification. With the amount of variation is other characteristics, it is surprising denitrification did not vary significantly. Based on these observations, denitrification function can become established in a wide range of spoil conditions. Denitrification appears to function as well in these created wetlands as in natural reference wetlands.

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