Characterization of Oxidation-Reduction Processes in Constructed Wetlands for Swine Wastewater Treatment

A. A. Szögi, P. G. Hunt, E. J. Sadler, D. E. Evans

Abstract. *Constructed wetlands designed and properly operated for treatment of swine wastewater may enhance oxidation-reduction processes and nutrient treatment performance. The objective of this investigation was to characterize soil wetland processes related to nitrogen (N) treatment (nitrification-denitrification) and phosphorus (P) removal using soil oxidation-reduction potential (ORP) data. We evaluated three surface-flow wetland systems constructed for treatment of swine wastewater in Duplin Co., North Carolina, in 1992. Each system consisted of two 3.6- × 33.5-m cells connected in series. The three systems were planted to bulrushes, cattails, and agronomic crops (soybean in saturated soil culture and flooded rice), respectively. Soil aerobic/anaerobic conditions were determined by monitoring soil ORP at 18 sites using platinum (Pt) electrodes. Three monitoring sites were established in each wetland cell. Each site consisted of five Pt electrodes at three soil depths (0.02, 0.05, and 0.10 m) and a reference electrode. A data logger was used for hourly acquisition of soil ORP and temperature records. Hourly ORP data were averaged on a 24-h basis and corrected to standard hydrogen electrode readings (Eh). Frequency analysis of daily soil Eh showed that bulrush and soybean cells were moderately reduced (+100 < Eh < +300 mV) and anaerobic (Eh < +300 mV) about 70% of the time. However, cattail and rice cells were anaerobic 100% of the time and had reduced (-100 < Eh < +100 mV) to highly reduced (Eh <-100 mV) soil conditions. These results indicate that different wetland plant species promote distinct anaerobic and reducing soil conditions. Outflow concentration of ammonia-N (NH_3-N) and soluble P increased with increasing ORP values for bulrush and soybean-rice wetland cells due to lower temperatures during fall and winter, but not for cattails. Denitrification enzyme activities and ORP indicated that soils in bulrush wetlands promoted better conditions for nitrification-denitrification than cattails or rice soils. However, equivalent NH_3-N removal rates (4.8-5.6 kg ha^{-1} d^{-1}) for cattails and bulrush suggested that treatment occurred mostly in the water column for cattails rather than the wetland soil. Prevalent anaerobic soil conditions and soluble P outflow concentrations determined rather poor P retention capacity for all three wetlands.*

Keywords. *Nitrification, Denitrification, Nitrogen, Phosphorus, Redox potential, Soil reduction, Wetlands.*

Under flooding conditions, soils rapidly become anaerobic and oxygen is depleted. Flooding of soil has a marked effect on oxygen content and behavior of nutrients in natural and constructed wetlands (Hammer, 1989; Mistch and Gosselink, 1993; Kadlec and Knight, 1996). Oxygen content measurement is needed for assessment of anaerobic conditions and associated nutrient treatment processes. However, the rapid decrease of oxygen in flooded soil precludes direct measurement of this element under field conditions. Instead, *in situ* measurement of oxidation-reduction potential (ORP) using platinum electrodes is used as an indirect field method to characterize oxygen status and intensity of oxidation-reduction processes in flooded soils (Bohn, 1971; Faulkner et al., 1989).

Oxidation-reduction processes are characterized by the loss (oxidation) or gain (reduction) of electrons. When present, oxygen acts as the preferred electron acceptor for aerobic microbial respiration. Once oxygen is consumed, the alternative electron acceptor for anaerobic microbial respiration is nitrate followed in sequence by manganese oxide, iron oxide, sulfate, and finally carbon dioxide (Patrick et al., 1985). For instance, a soil at pH 7 is considered anaerobic and moderately reduced when nitrate reduction occurs at an ORP < +300 mV (Reddy and Patrick, 1984; Kralova et al., 1992). The biological reduction of nitrate, denitrification, is an important water purification process that transforms nitrate into nitrogen gas. This process is highly active in constructed wetlands for swine wastewater treatment (Hunt et al., 2003). Oxidation-reduction conditions are also important for the capacity of constructed wetlands to retain inorganic phosphorus. While phosphate is not directly involved in oxidation-reduction reactions in flooded soils, phosphate solubility is related to the oxidative status of iron. Thus, ORP < +120 mV may cause the reduction of ferric-iron oxides into soluble ferrous-iron and the release of associated inorganic phosphorus (Gambrell and Patrick, 1978; Faulkner and Richardson, 1989).

Constructed wetlands have been successfully used around the world for municipal wastewater treatment applications for more than two decades (Gersberg et al., 1986; Kadlec and

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The authors are **Ariel A. Szögi,** Soil Scientist, **Patrick G. Hunt, ASAE Member,** Soil Scientist, **Edward J. Sadler, ASAE Member,** Soil Scientist, and **Dean E. Evans,** Agricultural Engineer, USDA-ARS Coastal Plains Soil Water, and Plant Research Center, Florence, South Carolina. **Corresponding author:** Ariel A. Szögi, USDA-ARS, 2611 W. Lucas St., Florence, SC 29501-1242; phone: 843-669-5203; fax: 843-669-6970; e-mail: szogi@florence.ars.usda.gov.

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Knight, 1996). They have been more recently investigated for their use in animal wastewater treatment (Cronk, 1996; Knight et al., 2000; Hunt and Poach, 2001). Their primary role is to remove nitrogen via nitrification-denitrification processes before land application, thereby reducing the area of cropland required for wastewater application and N assimilation (Hunt et al., 2002). Management factors affecting water treatment processes and soil anaerobic status, such as pollutant strength, flooding time and depth, temperature, plant cover and composition, and oxidation-reduction conditions, are less documented for constructed wetlands that treat livestock wastewaters (Hunt and Poach, 2001).

The wetlands discussed in this article were constructed in 1992 to treat swine lagoon wastewater as part of a Water Quality Demonstration Project (WQDP) in Duplin County, North Carolina (Stone et al., 2002). Previous studies in these wetlands have shown relationships between nutrient removal performances and biological treatment processes (Szögi et al., 1994, 2000; Hunt et al., 2002, 2003; Poach et al., 2002). Although ORP results were reported in those studies, they were not discussed in detail. More detailed analyses and reporting of ORP measurements would allow better characterization and understanding of the physico-chemical and biological interdependence of nutrient treatment processes. The objectives of this study were to (1) examine intensity of soil oxidation-reduction and frequency of anaerobic conditions using *in situ* ORP data, (2) determine seasonal differences in ORP and anaerobic conditions, and (3) characterize N and P treatment processes in relation to ORP in constructed wetlands that treated swine lagoon wastewater.

Materials and Methods

Site Description

Three pilot surface-flow wetland systems were constructed for treatment of swine lagoon wastewater in Duplin Co., North Carolina, in 1992. The Natural Resource Conservation Service (USDA-NRCS) designed these systems to treat wastewater supplied by an adjacent anaerobic lagoon (USDA, 1991). The constructed wetlands consisted of six 3.6- × 33.5-m wetland cells arranged in three parallel sets of two end-to-end connected cells (fig. 1). The lengthwise slope of the wetland cells was 0.2%. The texture of the soil substrate was sandy loam (86% sand, 10% silt, and 4% clay). Each set of two cells was connected so that the first cell received the inflow of wastewater and the second cell collected the outflow from the first cell. The effluent from this second cell was accumulated in a tank and pumped back to the lagoon (fig. 1). Details of wetland construction were previously reported (Szögi et al., 2000; Hunt et al., 2002; Stone et al., 2002). Operation and treatment performance for the length of this study (July 1993 – June 1994) is presented in table 1.

Four cells were manually planted to native wetland vegetation (May 1992) with nursery-grown vegetative propagules. One set of two connected cells was planted to a plant community containing bulrushes [*Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R. Keller], *Scirpus cyperinus* (L.) Kunth, and [*Schoenoplectus tabernaemontani* (K.C. Gmel.) Palla] and rush (*Juncus effusus* L.); hereafter referred to as bulrush wetlands (cells 1 and 2). A second set of two cells was planted to a plant community containing two

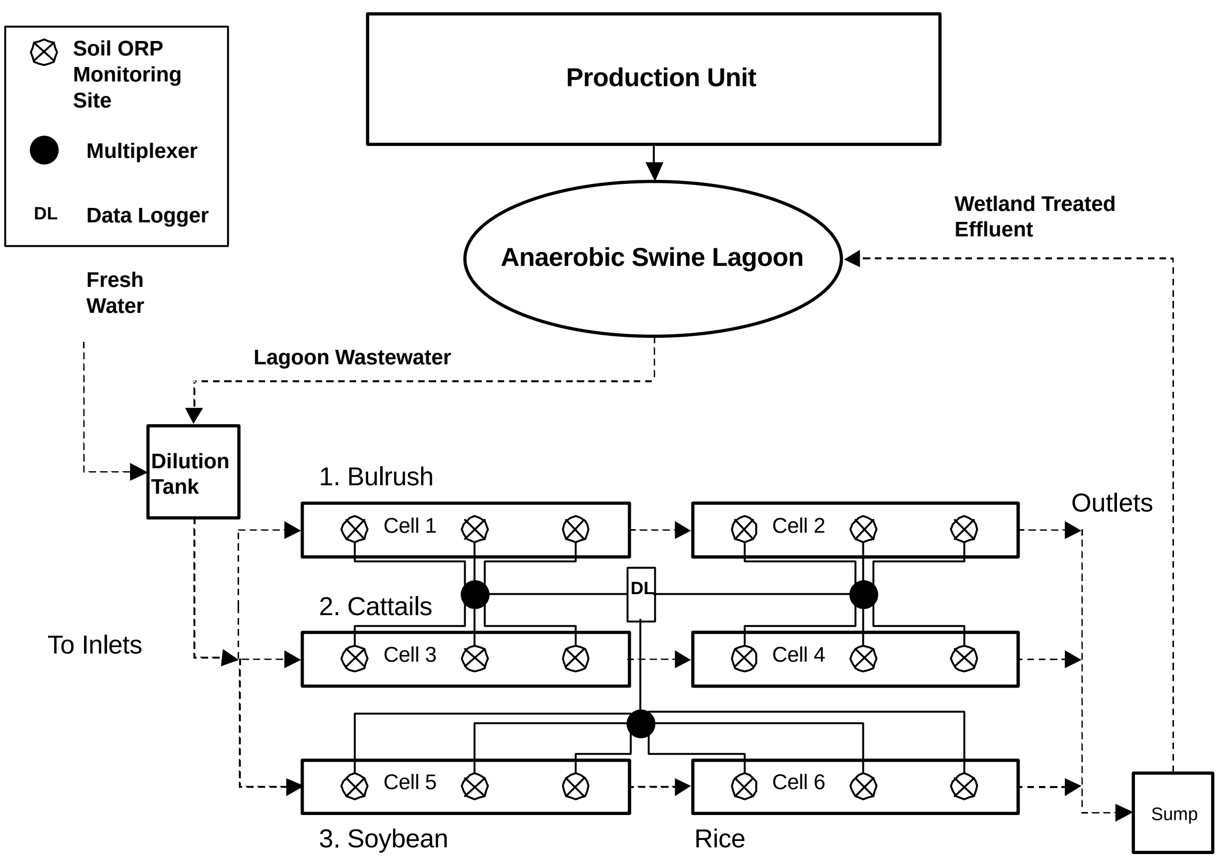

Figure 1. Conceptual schematic (not to scale) of the constructed wetland study showing the soil oxidation-reduction potential (ORP) monitoring system.

Table 1. Operation parameters and treatment performance for three constructed wetland systems in Duplin Co., North Carolina (1993-1994).

Wetland System	Hydraulic Load (m^3 d^{-1})	Mean Residence Time (d)	Maximum Water Depth (m)	Nitrogen Loading Rates[a] (kg ha^{-1} d^{-1})	Nitrogen Removal Rates (kg ha^{-1} d^{-1})
Bulrush	2.6	12.5	0.10	4.8 – 5.6[b]	4.8 – 5.5
Cattails	3.4	12.5	0.10	5.6 – 6.4	4.9 – 5.6
Soybean-Rice	2.2	6	0.15[c]	2.0 – 4.8	1.9

[a] Nitrogen loading rates based on ammonia-N.
[b] First and second value indicates mean loading and removal rates for year 1993 and 1994, respectively.
[c] Water depth in the rice cell only.

species of cattails (*Typha angustifolia* L. and *Typha latifolia* L.) and bur-reed (*Sparganium americanum* Nutt.); hereafter referred to as cattail wetlands (cells 3 and 4). A third set of two cells was planted to the agronomic crops soybean (*Glycine max* Merr.) in saturated soil culture and rice (*Oryza sativa* L.); hereafter referred to as soybean–rice wetlands (cells 5 and 6).

Flooding Regime

Both bulrush and cattail wetlands were flooded with diluted swine lagoon wastewater and kept under shallow water depth conditions (< 0.10 m) the same number of days (table 1). The soybean-rice wetland system experienced a different flooding regime. In cell 5, saturated-soil-culture soybean was grown on raised beds that were surrounded by 0.15-m deep furrows where water level was maintained about 0.05 m below the bed surface. In cell 6, which was graded to a 0.2% slope and planted to flooded rice, water level was maintained between 0.10- and 0.15-m depth (Szögi et al., 2000). Wastewater was applied regularly July-October 1993 and applied intermittently January-April 1994. Wastewater was not applied October-December 1993 and May-June 1994. However, the wetlands remained waterlogged. While water levels were shallow in the three wetlands, soil and water temperatures closely resembled air temperature (Hunt et al., 2002). For the period of this study, air temperatures had a mean monthly range of 4.5°C to 26.7°C and a median of 17.2°C.

ORP Monitoring System

Soil ORP was measured *in situ* at 18 sites in the constructed wetlands (fig. 1). Three monitoring sites were maintained in each wetland cell. Each site consisted of five platinum (Pt) electrodes and one reference electrode-salt bridge assembly. Duplicate Pt electrodes were inserted in the soil at a depth of 0.02 and 0.05 m. A fifth single Pt electrode was inserted at a depth of 0.10 m (fig. 2). The reference electrode-salt bridge was inserted in the soil to a depth of about 0.15 m. The leads of the electrodes (Pt and reference) were connected to 32-channel multiplexers (one per wetland system) using AMP® pin and socket connectors (Newark Electronics, Chicago, Ill.). Each multiplexer (AM 32 model, Campbell Scientific, Logan, Utah) handled the voltage signals from 30 Pt electrodes. The ORP was recorded using a CR7X Campbell Scientific Datalogger (Logan, Utah) powered by solar cell and a 12-V battery. The data logger also collected weather data (temperature, solar radiation, rainfall, relative humidity, and wind speed).

Electrode Construction

The ORP monitoring system used mercury junction Pt electrodes constructed according to Faulkner et al. (1989). The Pt tips of the electrodes were constructed using 1.2-cm length segments of 20-gauge (0.81-mm dia.) Pt wire. The body of the electrodes consisted of soda lime glass tubing (o.d. = 0.4 cm) cut to 15-cm length. Reference electrodes were single junction Ag/AgCl electrodes with epoxy body and filled with a gel electrolyte (Jenco Instruments, San Diego, Calif.). Each reference electrode was placed in a salt bridge column that contained a KCl-agar gel mixture. The salt bridge was used to avoid direct contact of the reference electrode with soil particles, which could plug the electrode tip, and to obtain rapid and accurate ORP readings (Veneman and Pickering, 1983). The body of the salt bridge column consisted of 1.91-cm-diameter PVC pipe cut into 50-cm length pieces. The salt bridge was connected to the reference electrode as shown in figure 2.

Electrode Testing

Prior to testing new or used Pt electrodes, Pt tips were cleaned using an electrode polish cleaner (Corning Cat. 477256, Corning Inc., Corning, N.Y.). The polish residue was rinsed off the Pt tip with distilled water. The Pt electrodes were tested three times: after construction, when connected to the data logger before field installation, and after being removed from the field. Electrodes were tested using quinhydrone pH-buffered solutions. These testing solutions were prepared by dissolving approximately 0.1-g quinhydrone in 50 mL of pH 4- and pH 7-buffer solutions, respectively (Jones, 1966). A calomel reference electrode was immersed in the solution together with the Pt electrode to be tested, then connected to a voltmeter. The measured ORP was compared to the standard potential of buffered quinhydrone solution at a given temperature and pH (Jones, 1966). If a potential difference greater than ±10 mV existed, the Pt electrode was rejected. The field electrodes were connected to the salt bridge tested with respect to a reference glass calomel electrode (Orion Model 7102SC, Beverly, Mass.) in a saturated potassium chloride solution. All Ag/AgCl reference electrodes tested had potential measurements with respect to the calomel electrode of 45±1 mV.

ORP Data Analysis and Interpretation

Voltage readings were logged every 5 min and averaged on an hourly basis. The hourly records were downloaded using a laptop computer. After downloading, hourly ORP records were averaged on a 24-h basis and transformed to standard hydrogen electrode (SHE) readings (Eh) using the following equation:

$$Eh = Ef + 200 \text{ mV} \tag{1}$$

where Eh is the corrected value of the redox potentials in mV with respect to SHE, and Ef is the voltage reading taken in the field in mV with respect to the Ag/AgCl reference.

The Ag/AgCl reference electrode had a difference of +200 mV with respect to the SHE. Though the SHE correction factor is temperature dependent, corrections for field temperatures were not made as the error involved from this source is relatively small compared to other errors in the measuring system (Patrick et al., 1996). Usually, it is necessary to make a second correction to convert Eh values

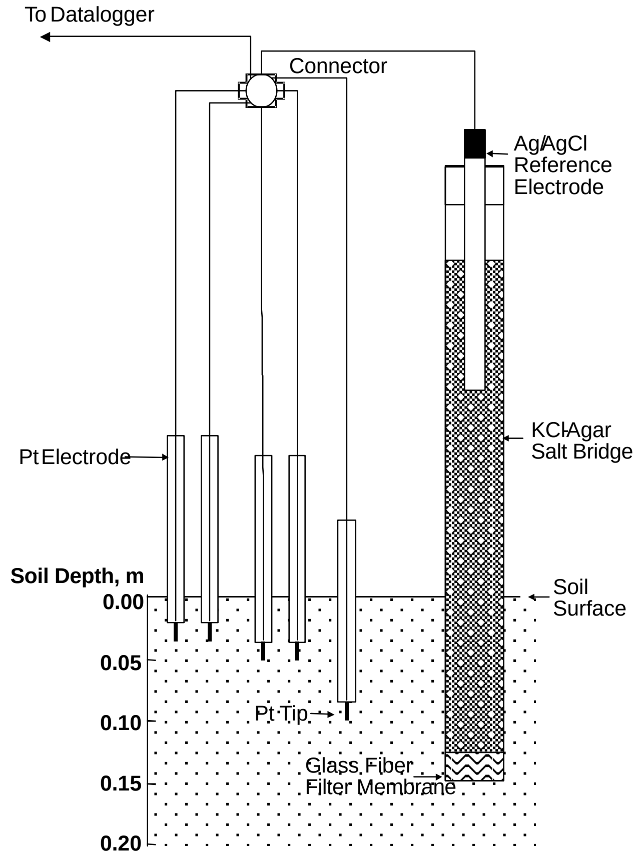

Figure 2. Schematic of one of the soil monitoring sites instrumented with platinum electrodes.

to standard pH 7 (-59 mV per unit of pH change) to interpret ORP data (Gambrell and Patrick, 1978). However, the Eh values were not corrected to standard pH 7 because soil pH measurements under flooded conditions of our study were close to neutrality, within a range of 6.43 to 7.25 units. Soil ORP values, at pH 7, found in the literature were used to characterize the soil in four classes: oxidized (> +300 mV), moderately reduced (+100 to +300 mV), reduced (-100 to +100 mV), or highly reduced (< -100 mV) (table 2).

Database management and statistical analyses including descriptive statistics, regression, and analysis of variance were performed with version 8.0 of SAS (SAS, 1999).

Water and Soil Analysis

Details of monitoring water quality and flow measurements have been previously reported (Szögi et al., 2000; Hunt et al., 2002). Wastewater analyses were performed according to Methods for Chemical Analysis of Water and Wastewater (APHA, 1992). The orthophosphate (PO_4-P) fraction that represents soluble phosphorus (SP) was determined by the automated ascorbic acid method (Standard Method 4500-P F) after filtration through a 0.45-μm membrane filter (Gelman type Supor-450, Pall Corp., Ann Arbor, Mich.). The same filtrate was used to measure ammonia-N (NH_3-N) by the automated phenate method (Standard Method 4500-NH_3 G). NH_3-N and PO_4-P constituted > 95% of both total N and total P concentration in the inflow and outflow of the three wetland systems.

Denitrification enzyme activity (DEA) was determined in field-moist soil samples collected at 2.5-cm depth from four

Table 2. Measured oxidation-reduction potentials (Eh) required to reduce oxidized forms of various alternative electron acceptors in waterlogged soil (adapted from Patrick et al., 1996).

Oxidized Form	Eh Range[a] (mV)	Reduced Form	Oxidation Status	Aeration Status
Oxygen (O_2)	> + 300	Water – H_2O	Oxidized	Aerobic
Nitrate (NO_3^-)	+100 to +300	Nitrogen (N_2)	Moderately reduced	Anaerobic
Manganic-Manganese (Mn^{4+})	+100 to +300	Manganous-Manganese (Mn^{2+})	Moderately reduced	Anaerobic
Ferric-Iron (Fe^{3+})	+100 to -100	Ferrous-iron (Fe^{2+})	Reduced	Anaerobic
Sulfate (SO_4^{2-})	< -100	Sulfide (S^{2-})	Highly reduced	Anaerobic
Carbon dioxide (CO_2)	< -100	Methane (CH_4)	Highly reduced	Anaerobic

[a] Eh measured at pH 7.

locations in each cell following procedures reported by Hunt et al. (2003). Each sample location received one of the following treatments: (A) non-amended control, (B) nitrate, (C) glucose, and (D) nitrate and glucose. The DEA was determined by the acetylene inhibition method to block the conversion of nitrous oxide to dinitrogen gas (Tiedje, 1994). Nitrous oxide was measured by gas chromatography (Hunt et al., 2003).

Three soil samples (0 to 20 cm depth) were obtained from each wetland cell in July 1993 and April 1994. Soil samples were transported on ice to the laboratory. Soil pH was measured in wet samples (1:1 soil to water mixture) using a combination electrode. Soil samples were air dried and ground to pass a 2-mm sieve. Extractable P and reducible iron and manganese associated to poorly crystallized oxides were extracted by mechanically shaking soil samples with an acidic ammonium oxalate buffer solution (1:20 w/v ratio) for 120 min (Houba et al., 1986). Iron and manganese concentrations were determined in filtered soil extracts by flame absorption spectrophotometry (AAS) using a Perkin-Elmer AAnalyst 300 analyzer (Perkin-Elmer, Norwalk, Conn.). Aliquots of the soil extract were digested with concentrated sulfuric acid in a digestion block to determine extractable soil P. Extractable P concentrations were determined by the ascorbic method adapted to digested extracts (Technicon Instruments Corp., 1977).

Results and Discussion

Intensity and Frequency of Anaerobic Soil Conditions

ORP measurements usually experience high variability among replicate readings because flooded soils seldom reach oxidation-reduction equilibrium due to the continual addition of electron donors such as oxidizable organic compounds (Bohn, 1971). For this reason, field ORP measurements are at best semi-quantitative according to Patrick et al. (1996). Despite this limitation, ORP is the best single indicator of the degree of anaerobiosis in flooded soil conditions (Gambrell and Patrick, 1978; Patrick et al., 1985; Faulkner and Richardson, 1989). Therefore, it was expected that our Eh readings show high coefficients of variation (CV > 60%) and wide ranges at three soil depths (0.02, 0.05, and 0.10 m) (table 3). Bulrush wetlands (cells 1 and 2) and

Table 3. Mean, median, coefficient of variation, and minimum and maximum values of daily oxidation-reduction potentials (Eh) at three soil depths for bulrush, cattails, and soybean-rice wetland systems.

Wetlands	Cell No.	Depth (m)	Mean[a] Eh (mV)	Median Eh (mV)	CV%[b] Eh (mV)	Min Eh (mV)	Max. Eh (mV)
Bulrush	1	0.02	142	261	152	-287	399
	1	0.05	189	306	105	-235	414
	1	0.10	115	198	157	-254	302
Bulrush	2	0.02	206	197	97	-261	474
	2	0.05	185	190	96	-280	419
	2	0.10	110	177	129	-266	356
Cattails	3	0.02	-157	-194	-60	-272	140
	3	0.05	-102	-128	-105	-272	201
	3	0.10	-95	-92	-87	-266	233
Cattails	4	0.02	-32	-38	-409	-275	552
	4	0.05	-28	-26	-431	-213	521
	4	0.10	-125	-145	-86	-217	443
Soybean	5	0.02	197	227	115	-258	503
	5	0.05	126	165	190	-270	526
	5	0.10	135	174	188	-262	580
Rice	6	0.02	-85	-68	-112	-271	131
	6	0.05	-90	-95	-96	-289	120
	6	0.10	-95	-75	-92	-284	117

[a] n = 315 days.
[b] CV% = Coefficient of variation expressed in percentage.

soybean (cell 5), with median Eh values between +165 and +306 mV, were less anaerobic and less reduced than cattails wetlands (cells 3 and 4) and rice (cell 6), which had negative median Eh values between -194 and -26 mV. A first conclusion from these results is that an increase of replicate readings to reduce high variability of ORP measurements may be necessary for proper assessment of anaerobic conditions. However, the large ORP variations found in this study can be explained by the soil spatial variability and response of ORP to flooding. Even within short distances, large variability in field measurements can be expected because of intrinsic soil heterogeneity. According to Patrick et al. (1996), the tips of the platinum electrodes are so small that the microzone around the electrode affects ORP readings. If the tip of the electrode is in contact with a plant root, an old root channel or an aerobic microsite, readings may not be representative of the bulk soil. With respect to ORP variability due to response to flooding, Patrick and Wyatt (1964) clearly showed that soil ORP changed from +600 to near -300 mV at the onset of flooding in response to the rapid use of O_2 and NO_3^- by microbes when there was enough readily decomposable organic matter (low molecular weight C compounds). Further depletion of readily decomposable organic matter or drop of flood water level due to drainage resulted in less soil reduction. We observed similar results as those of Patrick and Wyatt (1964) at the onset of flooding when ORP reached values near -200 mV in all cells during summer of 1993 (fig. 3). Plenty of organic matter supplied by plant biomass during summer sustained reducing soil conditions (Eh < 100 mV) in all three wetland systems. Later, during fall, winter, and early spring, less reduction occurred in bulrush (Eh > 100 mV) and soybean-rice (Eh > 50 mV) due to both depletion of readily decomposable organic matter and slower biological activity (fig. 3). Although lower temperatures slowed microbial activity,

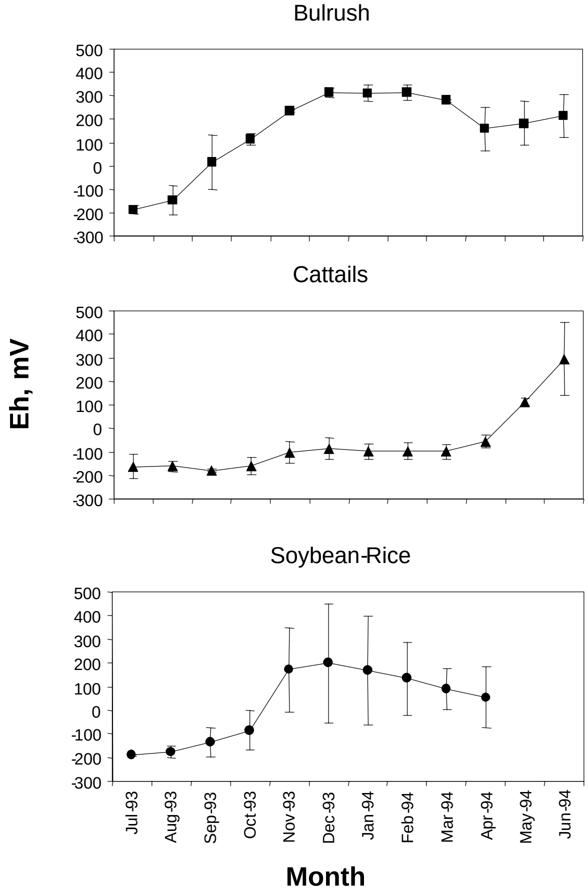

Figure 3. Mean monthly oxidation-reduction potential (Eh) for three wetland treatment systems. Error bars indicate ±1 standard error of the mean.

cattails remained reduced below 0 mV during fall and winter probably because their detritus had larger contents of readily decomposed organic matter than bulrush or rice. Cell 4 with cattails experienced the largest coefficients of variation (> -400%) at 0.02- and 0.05-m depth (table 3). This large variation was due to drop of water level that promoted moderated reducing soil conditions (+100 < Eh < +300 mV) during May and June 1994 (fig. 3).

Cumulative frequency distributions were used to discern how often constructed wetland soils were anaerobic. These distributions indicated that soils in cattail cells were anaerobic and reduced (Eh < +300 mV) almost 100% of the time and at any soil depth (table 4). On the other hand, bulrush cells experienced oxidative conditions within the upper soil layers (< 0.05 m). Frequencies of anaerobic and reduced conditions for bulrush were 61% to 73% at 0.02-m depth, 36% to 61% at 0.05-m depth, and 100% anaerobic at 0.10-m depth. There is no logical explanation to why bulrush cell 1 had a frequency of 36% for anaerobic conditions except for the possibility that most of the six electrodes installed at 0.05-m depth were giving non-representative readings due to microsite effects. Although both bulrush and cattail wetlands had almost the same flooding regime, cattail soil was more frequently anaerobic (100%) than bulrush soil according to table 4. For the soybean and rice cells, frequency analysis indicated large differences of ORP and anaerobic conditions between soybean and flooded rice soils. The least reduced was the soybean soil (cell 5) because it was saturated but not

Table 4. Frequency of anaerobic soil conditions (Eh < +300 mV[a]) by cell and soil depth in three constructed wetland systems (Duplin Co., N.C.).

		Depth (m)		
		0.02	0.05	0.10
Wetlands	Cell No.	Frequency (%)[b]	Frequency (%)[b]	Frequency (%)[b]
Bulrush	1	73	36	100
	2	61	61	100
Cattails	3	100	100	100
	4	100	100	100
Soybean	5	62	72	68
Rice	6	100	100	100

[a] Oxidation-reduction potential at which the soil is considered anaerobic.
[b] Frequency is the cumulative frequency of mean daily Eh values < +300 mV, n = 315 days.

flooded. Soybean cell anaerobic condition frequencies were between 62% and 72% for the 0.02-, 0.05-, and 0.10-m depths (table 4). In contrast, the rice cell was 100% anaerobic at any soil depth. Differences in soil anaerobic frequencies may be due to differences in the capacity of wetland plant species to transport O_2 through their leaves and stems to roots (Armstrong, 1964; Gersberg et al., 1986; Good and Patrick, 1987). Other researchers think that oxygen transfer to wetlands is controlled by atmospheric diffusion and that little oxygen is released from plant roots to the anaerobic surroundings (Brix, 1993; Wu et al., 2001). However, Reddy et al. (1990) found that higher Eh and lower frequency of anaerobic conditions is consistent with higher O_2 transport capacity of bulrush stems and roots relative to cattails. Our results are consistent with the hypothesis that different wetland plant species have distinct anaerobic and reducing soil conditions.

Seasonal Effects on ORP

Seasonal changes and management factors, such as temperature, solar radiation, duration of flooding, and water depth, have been shown to have a direct effect on soil ORP and greatly affect the treatment performance of constructed wetlands (Gumbricht, 1993; Hunt et al., 2002, 2003). However, changes in water quality after wetland treatment can be particularly difficult to interpret only in terms of ORP data because this parameter was correlated with temperature and depth of flooding.

Temperature effect on ORP was different for each wetland system. Monthly mean soil Eh values were very low (-250 to +100 mV) during the warm period (July-September 1993) for the three systems (figs. 3 and 4). From November 1993 to March 1994, Eh values were much less reduced in bulrush and soybean-rice cells (+100 to +250 mV). Instead, cattail cells remained reduced (Eh< +100 mV) from July 1993 to March 1994 and became moderately reduced (+100 < Eh < +300 mV) when water depth was lowered to 0.05-m depth in spring (April-June 1994) (fig. 4). The dependency of Eh on daily temperatures for bulrush, cattails, and soybean-rice wetlands was determined by regression analysis. Linear regression of Eh versus temperature was statistically significant for bulrush (Eh = 388.5 – 15.4 × Temp, $r^2 = 0.59$, n = 315, $p < 0.0001$) and soybean-rice (Eh = 214.5 - 12.3 ×

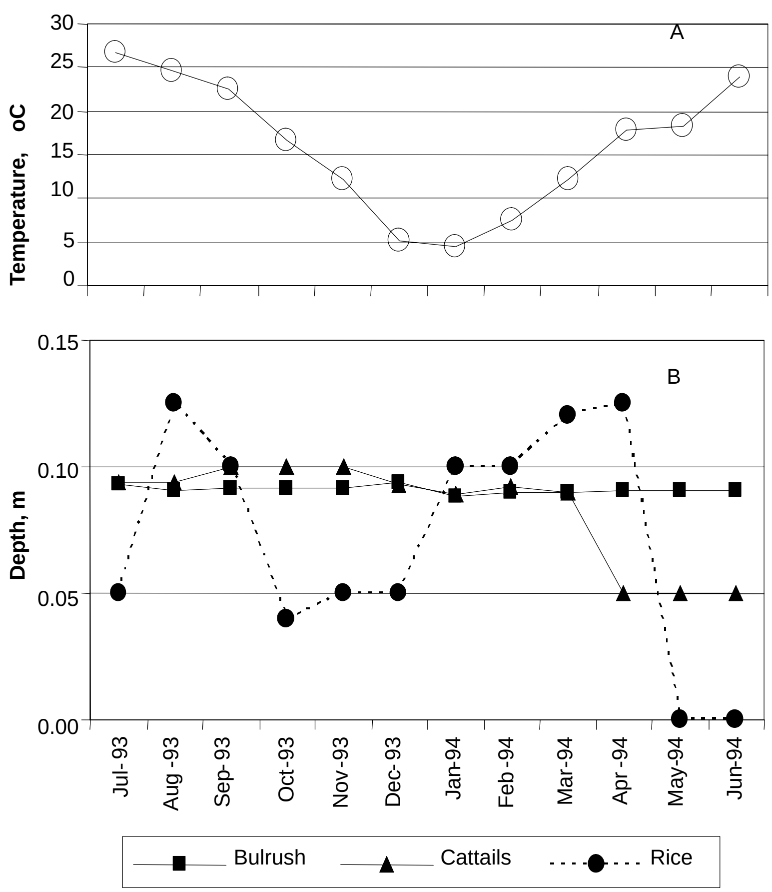

Figure 4. Seasonal changes in air temperature (A) and water depth (B).

Temp, $r^2 = 0.48$, n = 315, p < 0.0001), but not significant for cattails (Eh = -84.2 – 0.37 × Temp, $r^2 = 0.001$, n = 315, p = 0.57). With the exception of cattails, these regression equations clearly indicate that Eh decreases with increasing temperature under flooded soil conditions because warmer temperatures speed up the rates of biological processes.

Bulrush wetlands had very small fluctuations in flooding depth during the study (fig. 4). In cattails, significant changes in water depth occurred in spring (April 1994) when flooding depth decreased due to repair maintenance of the system. However, large fluctuations in the rice cell were due to water level management (fig. 4). Initially, water levels were kept below 0.05 m during rice seedling growth (July 1993). Water level was increased during the growth season (> 0.10 m) and dropped (< 0.05 m) by early October 1993 to harvest the rice. After harvest, the rice cell was flooded again with shallow water to promote a second short vegetative growth cycle before winter, after which the water depth was increased to > 0.10 m (January-May 1994). In May 1994, the cell was drained to prepare the soil for the next rice crop. These large water level fluctuations determined that this system received much lower NH_3-N loads (2.0 to 4.8 kg ha^{-1} d^{-1}) than bulrush and cattails (4.8 to 6.4 kg ha^{-1} d^{-1}) (table 1). Linear correlation of Eh versus flooding depth (D) was weak for bulrush with $r^2 = 0.20$ (Eh = 6287 – 67376 × D, n = 24, p < 0.05) and soybean-rice with $r^2 = 0.23$ (Eh = 97.8 – 1864 × D, n =18, p < 0.05). However, correlation between Eh and flooding depth was moderate for cattails with $r^2 = 0.57$ (Eh = 390 – 5473 × D, n = 24, p < 0.0001). These regressions confirm a moderate effect of water depth on ORP measurements, particularly in the cattails wetland.

Table 5 shows summary means and standard deviations for inflow and outflow nutrient concentrations for the three wetland systems. Effect of ORP on wetland treatment processes was assessed by linear regression using monthly mean outflow nutrient concentration and monthly mean Eh as dependent and independent variables, respectively. Regression results for NH_3-N and SP outflow concentrations as dependent variables versus Eh are presented in figures 5 and 6, respectively. Correlation of NH_3-N versus Eh was moderate in bulrush ($r^2 = 0.64$ in cell 1 and $r^2 = 0.22$ in cell 2), soybean ($r^2 = 0.40$), and rice ($r^2 = 0.53$). Except for cell 2, their respective regression lines had statistically significant regression coefficients (p < 0.05). However, a linear relationship between outflow NH_3-N concentration and Eh was very weak for cell 3 ($r^2 = 0.13$) and non existent for cell 4 ($r^2 = 0.004$) in cattail wetlands (fig. 5). These results indicate that NH_3-N treatment was affected by soil ORP in bulrush and soybean-rice wetlands but not in cattails wetlands. The lack of correlation between outflow NH_3-N concentration and ORP in cattail wetlands was due to the little variation on ORP values in the first nine months of the study (fig. 3).

Table 5. Concentration of ammonia-N (NH_3-N) and soluble phosphorus (SP) in inflow and outflow to bulrush, cattail and soybean-rice wetlands (July 1993 to June 1994).

		Outflow		
	Inflow ($mg\ L^{-1}$)	Bulrush ($mg\ L^{-1}$)	Cattails ($mg\ L^{-1}$)	Soybean-Rice ($mg\ L^{-1}$)
NH_3-N	34.4 ± 4.1[a]	2.0 ± 0.9	4.0 ± 1.3	0.6 ± 0.2
SP	7.8 ± 1.1	1.6 ± 0.5	2.6 ± 0.9	0.4 ± 0.2

[a] Mean and standard error of the mean, n = 12.

Correlation of SP versus Eh was moderate for bulrush in cell 1 ($r^2 = 0.62$) and cell 2 ($r^2 = 0.63$) but no correlation was found for cattails in cell 3 ($r^2 = 0.0022$) and cell 4 ($r^2 = 0.0016$) as shown in figure 6. Soluble P had a moderate correlation with Eh in the soybean cell ($r^2 = 0.36$) but it was very weak for rice ($r^2 = 0.12$). Regression models for both NH_3-N and SP predict that higher Eh values will result in higher outflow NH_3-N or SP concentrations. This result should be expected for both bulrush and soybean-rice wetland systems because Eh values increased with decreasing temperatures during fall and winter as we previously discussed.

Oxidation-Reduction Processes

Major Oxidation-Reduction Processes

Daily Eh data was summarized in four frequency classes as defined in table 2. These ORP classes relate to the activity of bacteria that derive energy for biosynthesis from oxidation of organic carbon compounds supplied by wastewater, dead plant tissues, and root exudates (Patrick et al., 1985). During their metabolism, bacteria obtain energy by oxidizing organic and inorganic compounds through several intermediate sequential steps. Measured ORP values in table 2 that characterize the gain (reduction) of electrons are described by selected generic oxidation-reduction half-reactions presented in table 6 (James and Bartlett, 2000). As long as oxygen is present, other elements are not used as electron acceptors in microbial respiration because their chemical reduction yields less energy than aerobic respiration. Eventually, flooded soils become anaerobic due to a lack of sufficient oxygen in the soil to serve as the sole electron acceptor for microbial respiration.

Although Eh classes described in table 2 help to assess oxidation-reduction intensity and the associated oxidation-reduction reactions, this assessment is semi-quantitative because one oxidized form is not completely reduced before the reduction of the next begins. Therefore, some overlapping between oxidation-reduction reactions may occur beyond the boundary class. Nevertheless, selected Eh classes reflect the experimental evidence that oxygen and NO_3^- are depleted before Fe^{3+} is reduced, and that SO_4^{2-} and CO_2 are not reduced in the presence of oxygen or NO_3^-(Patrick et al., 1985, 1996). In addition, when Eh is measured in function of depth, these Eh classes may help to characterize vertical ORP distribution in soils. Under flooded conditions, two different soil layers (a distinctly oxidized layer usually underlain by an anaerobic reduced layer) have been characterized (Patrick and Delaune, 1972). These conditions result in unique large ORP gradients in which aerobic or oxidized conditions occur in two distinctive zones: soil-floodwater interface and root zone (Reddy et al., 2000).

The exercise of dividing daily Eh values into four frequency classes (oxidized, moderately reduced, reduced, and highly reduced) by soil depth confirmed differences on ORP conditions among the three wetland systems already discussed in previous sections (fig. 7). Bulrush cells had most Eh observations concentrated in the oxidized (> +300 mV) and moderately reduced (+100 to +300 mV) classes at surface and subsurface soil layers (0.02- and 0.05-m depth). The deeper layer (0.10 m) had no observations in the aerobic class (> +300 mV). These results indicate O_2, NO_3, Mn, and Fe reduction were predominant processes in the surface and subsurface soil layers of the bulrush wetland. Similar

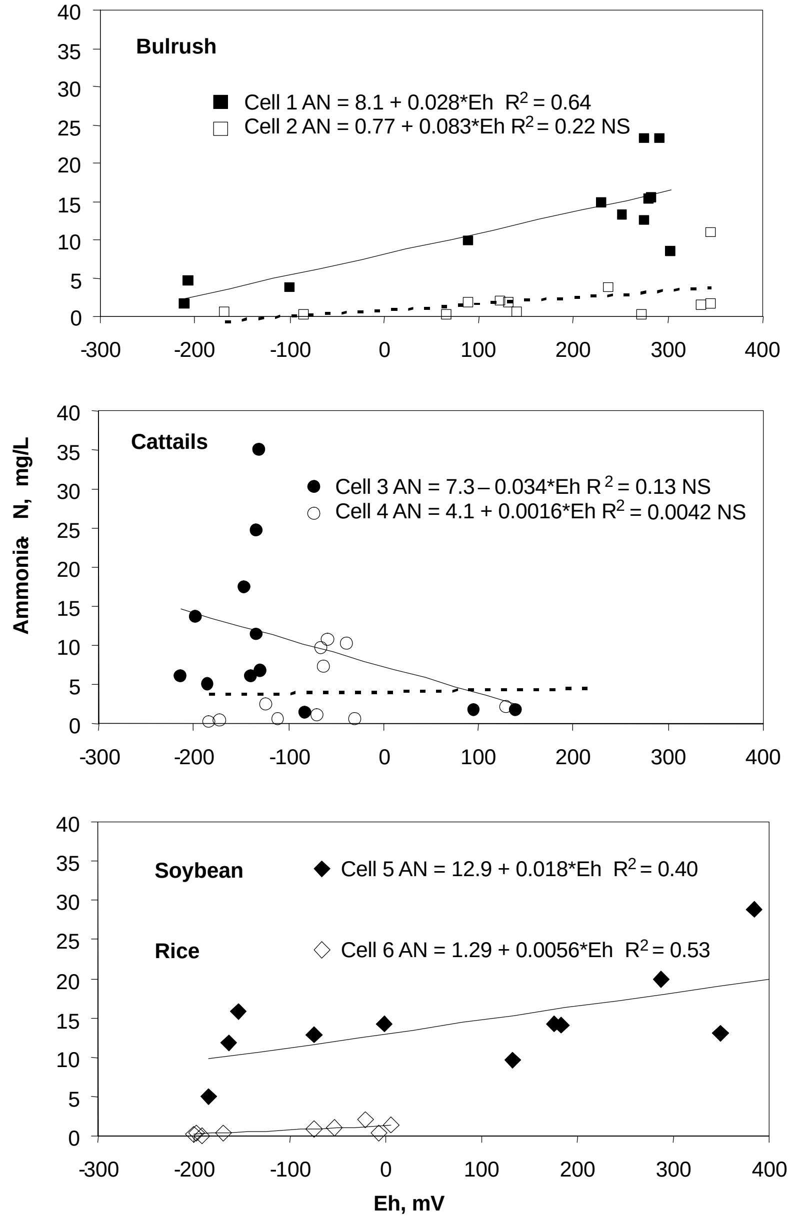

Figure 5. Relationship of ammonia-N outflow concentration vs. soil oxidation-reduction potential for bulrush, cattails, and soybean rice wetland systems. NS indicates correlation is non significant ($p < 0.05$).

reduction processes were prevalent in the soybean cell, ORP oxidation (> +300 mV) reached 0.10-m depth and observations were almost evenly distributed among all four classes at all three soil depths. Cattails and rice cells (3, 4, and 6) had Eh values mostly within the reduced (-100 to +100 mV) and highly reduced classes (< -100 mV). This highlighted prevalent reduced soil conditions, which caused Fe^{3+} and SO_4^{2-} reduction in cattails and rice cells. According to Patrick et al. (1985), SO_4^{2-} and CO_2 are reduced to sulfide and methane, respectively, only under strictly anaerobic conditions with Eh below -100 mV. Hydrogen sulfide was probably produced but not at the rate that it would be toxic to wetland plants as neither plant health decline nor characteristic odor in soil samples were observed. Methane formation, however, was not considered a major process in our wetland study. Whiting and Chanton (1993) estimated that roughly 3% of the net biomass production in wetlands escapes to the atmosphere as CH_4. Very low daily Eh values (< -200 mV) are required for the onset of CO_2 reduction to CH_4 (Patrick, 1981). Eh values < -200 mV represented a very small percentage (<5%) of all observations in any of the wetland systems of our study. The relationship between oxidation-reduction and nutrient removal processes is discussed in more detail in the subsequent nutrient treatment processes section.

Characterization of Nutrient Treatment Processes

Constructed wetlands have been shown to effectively remove NH_3-N from anaerobic lagoon-treated swine wastewater through biological nitrification-denitrification processes (Hunt et al., 2002, 2003). In flooded soils, both nitrification and denitrification can proceed simultaneously, and NH_3-N levels are greatly influenced by the presence of a thin, surface-oxidized aerobic and anaerobic soil layers (Reddy and Patrick, 1984). The fate of NH_3-N entering

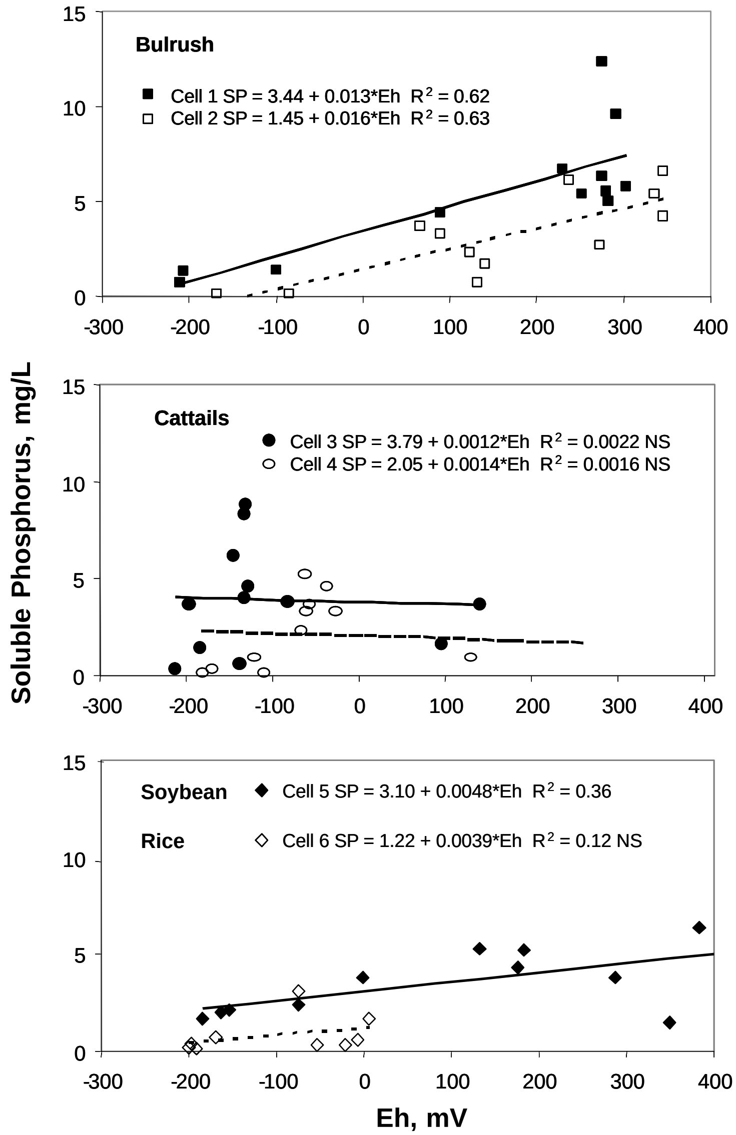

Figure 6. Relationship of soluble phosphorus outflow concentration vs. soil oxidation-reduction potential for bulrush, cattails, and soybean rice wetland systems. NS indicates correlation is non significant ($p < 0.05$).

Table 6. Selected oxidation-reduction half reactions and associated processes.

Half Reactions	Process
a(oxidants) + $be^- + cH^+ =$ d(Reductants) + eH_2O	Generalized oxidation-reduction reaction
$O_2 + 4e^- + 4H^+ = 2H_20$	Oxygen reduction – aerobic respiration
$NO_3^- + 5e^- + 6H^+ = ½N_2 + 3H_2O$	Nitrate reduction – denitrification
$MnO_2 + 2e^- + 4H^+ = Mn^{2+} + H_2O$	Manganese oxide reduction
$FeOOH + e^- + 3H^+ = Fe^{2+} + 2H_2O$	Iron oxide reduction
$SO_4^{2-} + 8e^- + 10H^+ = H_2S + 4H_2O$	Sulfate reduction – hydrogen sulfide emission
$CO_2 + 8e^- + 8H^+ = CH_4 + 2H_2O$	Carbon dioxide reduction – methanogenesis

wetlands has been assessed by examining if trace concentrations of NO_3-N appear in the effluent (Gersberg et al., 1986). However, when sufficient dissolved carbon is present, constructed wetlands are very efficient at promoting denitrification and NO_3-N is not found in the outflow (Reed, 1993). Therefore, denitrification enzyme activities (DEA) were used to determine potential denitrification. Results of the DEA assays presented in figure 8 indicate that nitrate was the most limiting factor because the DEA values from glucose treatment did not differ from the control treatment, and the nitrate plus glucose treatment did not differ from the nitrate amendment. Differences in denitrification rates were statistically significant ($P < 0.05$) between wetland systems when soil was amended with nitrate. Bulrush had the largest denitrification rate (479 ng N $g^{-1}h^{-1}$) with respect to cattails (138 ng N g^{-1} h^{-1}) and soybean-rice (290 ng N $g^{-1}h^{-1}$). Our ORP data shown in fig. 4 indicate that soils in bulrush wetlands and soybean cell sustained oxidized (Eh > +300 mV) and moderately reduced (+300 < Eh < +100 mV) conditions at 0.02-m depth, which are characteristic of

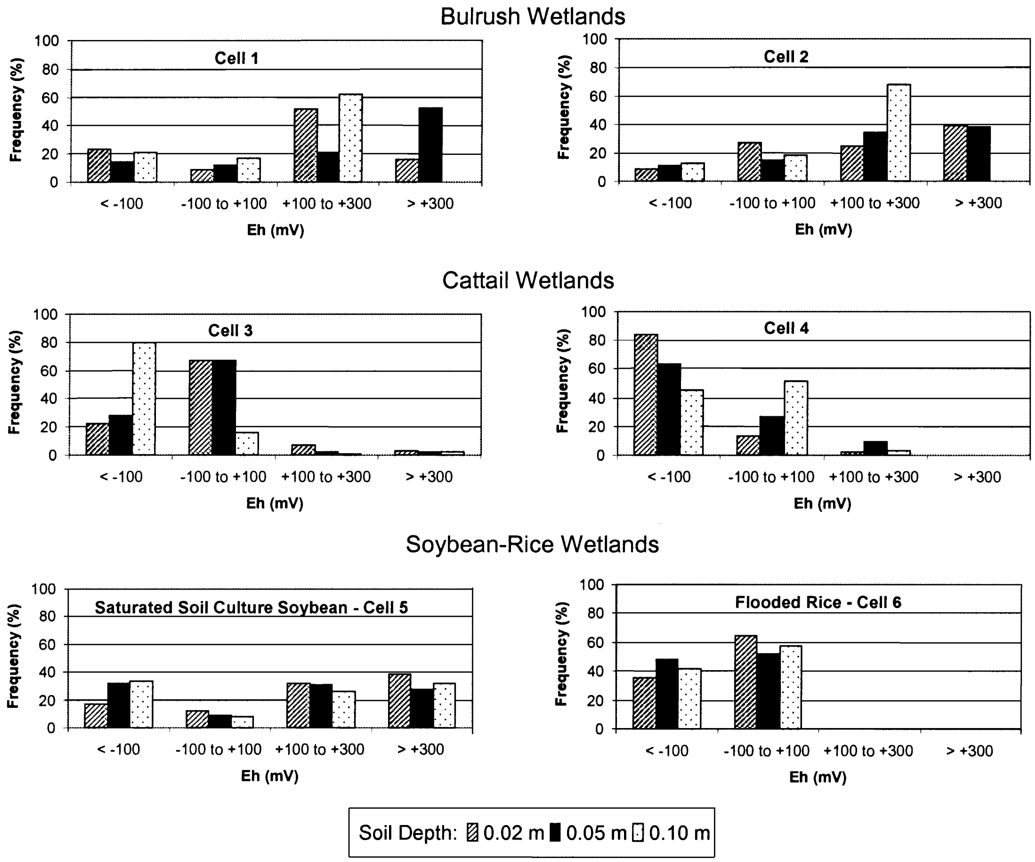

Figure 7. Frequency of oxidation-reduction potential (Eh) measured per wetland cell and soil depth. The frequencies were classified in four classes: oxidized (> +300 mV), moderately reduced (+100 to +300 mV), reduced (-100 to +100 mV), or highly reduced (< -100 mV).

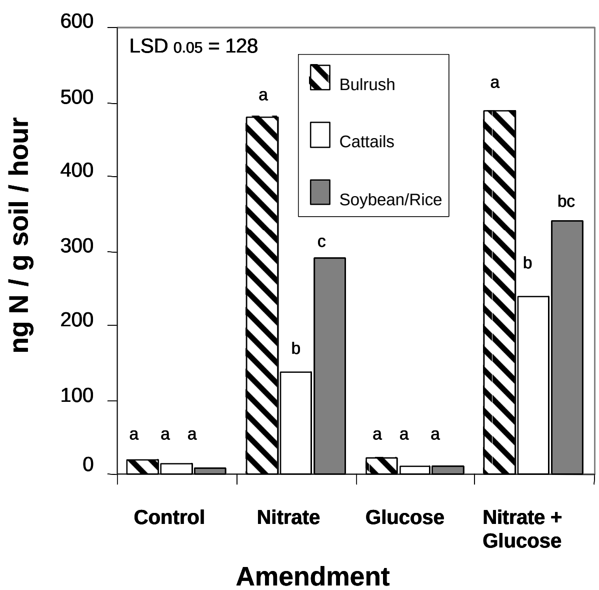

Figure 8. Mean denitrification potential as influenced by soil amendment for three constructed wetland systems. Means for each wetland system followed by the same letter are not significantly different ($p < 0.05$) within the same amendment.

nitrification-denitrification conditions in wetland soils (Reddy and Patrick, 1984; Kralova et al., 1992, Reddy et al., 2000). On the other hand, persistent reduced ($-100 < Eh < +100$ mV) and highly reduced conditions ($Eh < -100$ mV) in the cattails and the rice soil at 0.02-m depth reflect both intense anaerobic conditions and limited nitrification. From this information, we concluded that flooded soils planted to bulrush could contribute to better conditions for nitrification-denitrification processes than soils planted to cattails or flooded rice. However, NH_3-N removal rates (table 1) are almost the same for bulrush (4.8 to 5.5 kg ha^{-1} d^{-1}) and cattails (4.9 to 5.6 kg ha^{-1} d^{-1}). In cattails, DEA assay of flocs (particles formed in the water column by aggregation of smaller sediment particles) samples with nitrate amendment treatment indicated denitrification rates 10-times higher than those of nitrate amended wetland soil samples (T.A. Matheny, unpublished data, 2003. Florence, S.C.: USDA-ARS). This DEA result is an indication that nitrification-denitrification processes in cattails and rice cells probably took place in the surface water and the majority of the treatment occurred in the water column rather than in the wetland soil.

Removal of inorganic P from the water column depends on sorption capacity, pH, and ORP conditions of the flooded soil (Patrick et al., 1985). In flooded soils at pH < 7, P is retained in phosphate form by ferric-iron oxides, but oxidation-reduction conditions affect their retention capacity. Therefore, P retention potential may be predicted in wetland soils by determining reducible iron, and manganese oxides and associated extractable P from acid oxalate extractions (Syers et al., 1973; Richardson, 1985; Shahandeh et al., 2003). Oxidation-reduction conditions are very important because wetland soils with Eh < +120 mV may cause reduction of Fe^{3+} and Mn^{4+} oxides into soluble $Fe^{2+}$and Mn^{2+}, and subsequently releasing iron and manganese-associated P (Gambrell and Patrick, 1978; Sahandeh et al., 2003).

Table 7. Soil pH, reducible iron and manganese, and extractable phosphorus content, and outflow soluble phosphorus concentration in bulrush, cattails, and soybean-rice wetland systems.

Wetland System	pH	Reducible Fe ($mg\ kg^{-1}$)	Reducible Mn ($mg\ kg^{-1}$)	Extractable P ($mg\ kg^{-1}$)	Outflow SP ($mg\ L^{-1}$)
Bulrush	7.05±0.03[a]	683 ± 32	3.6 ± 0.4	104 ± 9	1.6 ± 0.5
Cattails	6.76±0.10	728 ± 39	4.4 ± 0.3	66 ± 3	2.6 ± 0.9
Soybean - Rice	7.06±0.13	561 ± 48	6.1 ± 1.0	92 ± 12	0.4 ± 0.2

[a] Mean and standard error of the mean, n = 12.

Results presented in table 7 show that reducible iron was about 100- to 200-fold (561 to 683 mg kg^{-1}) higher than reducible manganese concentrations (3.6 to 6.1 mg kg^{-1}) and it undoubtedly controlled the potential release of P from soil into the water column. Although cattail wetlands had the largest reducible Fe content (728 mg kg^{-1}), they had the lowest extractable P content (66 mg kg^{-1}) and the highest mean SP outflow concentration (2.6 mg L^{-1}) of all three wetland systems. The bulrush soil had lower reducible iron content (683 mg kg^{-1}), higher extractable P (104 mg kg^{-1}) and lower SP outflow concentration (1.6 mg L^{-1}) than cattails. According to t-tests for differences between two means, differences between cattails and bulrush were significant for extractable P but not for extractable Fe ($p < 0.05$, n = 12). In soybean-rice wetlands, reducible Fe (561 mg kg^{-1}), and extractable P (95 mg kg^{-1}) contents were not significantly different from those of cattails or bulrush ($p < 0.05$, n = 12), but this system had the lowest SP outflow (0.4 mg L^{-1}). Reducible Fe and extractable P data support the hypothesis that cattails with ORP median Eh values < +100 promoted the dissolution of Fe oxides and consequently released extractable P to the water column. Although oxidizing conditions (Eh > +300) favorable for P retention by Fe-oxides occurred in bulrush cell 1 and the soybean cell, prevalent anaerobic conditions in both bulrush cell 2 and the rice cell (Eh < +100 mV) determined rather poor P retention capacity for both bulrush and soybean-rice wetland systems.

CONCLUSIONS

- Median and mean Eh values at three soil depths showed that bulrush wetlands and soybean cell were less anaerobic and reduced than cattail wetlands and rice cell. Cumulative frequency distributions showed that cattails and rice cells were anaerobic (Eh < +300 mV) 100% of the time at three soil depths (0.02, 0.05, and 0.10 m). Bulrush cells had lower frequencies (36% to 73%) of anaerobic soil conditions within the topsoil to 0.05-m soil depth, but 100% anaerobic at 0.10-m depth. The least anaerobic was the soybean cell (62% to 72%) at any soil depth because its soil was continuously saturated but not flooded. These results indicate that different wetland plant species promote distinct anaerobic and reducing soil conditions.
- Water depth and seasonal temperature changes had an effect on intensity of anaerobic conditions, which affected outflow nutrient concentration in the bulrush and soybean-rice wetlands. Instead, Eh values in cattail wetlands were affected only by water depth ($r^2 = 0.57$). Eh values increased with lower temperatures for the bulrush ($r^2 = 0.59$) and soybean-rice ($r^2 = 0.48$) wetlands, but not for cattails ($r^2 = 0.001$). This determined that the outflow concentrations of NH_3-N and SP for bulrush and soybean-rice wetland cells increased with increasing ORP values due to lower temperatures during fall and winter. In cattails, the poor relationship between temperature, ORP and NH_3-N outflow concentrations suggest that the majority of the wastewater treatment occurred in the water column rather than the wetland soil because NH_3-N removal rates were almost the same for cattails and bulrush.
- Persistent reduced and highly reduced soil conditions (Eh < +100) in cattail and rice cells determined limited denitrification due to restricted nitrification in the flooded soil according to DEA assays. However, soils in the bulrush had significant higher denitrification potential than cattail and rice because they were moderately reduced (+100 < Eh < +300 mV) and less intensely anaerobic than soils in cattail and rice cells. Therefore, flooded soils planted to bulrush could promote better conditions for nitrification-denitrification processes than soils planted to cattails or flooded rice. Outflow SP concentrations and prevalent anaerobic soil conditions indicated that reduction of ferric-iron oxides and release of associated inorganic P from soil solid-phase into the water column occurred in the three wetland systems. These results confirmed rather poor P retention capacity of all three constructed wetlands.

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