Nutrient assimilation and root porosity responses of aquatic macrophytes to application of dairy effluents using constructed wetlands
by Bryce Reed Romig

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Rehabilitation
Montana State University

Abstract:
This study was developed to determine the effect of two liquid dairy waste loading concentrations on wastewater nutrient removal by four north-temperate wetland species, Nebraska sedge (Carex nebraskensis Dewey), beaked sedge (Carex rostrata Stokes), broad-leaved cattail (Typha latifolia L.), and panicled bullrush (Scirpus microcarpus Presl); and, to evaluate the role of root aerenchyma in wastewater treatment. Microcosms were planted with these four species and were treated with two concentrations (30% and 70%) of liquid dairy waste. Unplanted microcosm units receiving only H2O served as controls. Plants were grown for one month prior to applications of wastewater. . Soil waters were sampled from ceramic cups embedded in the soil medium at three soil depths (15, 30, 45 cm) prior to each application of effluent. Water was analyzed for pH, total Kjeldahl-nitrogen (TKN), nitrate nitrogen (N03-N) , and chemical oxygen demand (COD). Plants were harvested and measured for biomass production, root porosity, and total nitrogen at the end of the treatment cycle. Results showed differences ($p < 0.05$) in plant root porosity that corresponded to different levels of effluent concentration. No correlation was observed between percent root porosity in the wetland species and the level of contaminant removed. No differences were observed in soil pore water TKN at the 30 and 45 cm depths; however, significant differences were observed at the 15 cm depth. Soil surface TKN also was significant with respect to effluent concentration and plant treatment. Microcosms receiving the low concentration (70% dilution) showed stabilization of N level over the two month period while units receiving the high concentration (30% dilution) showed increased levels of nitrogen (N) . The results of this study suggested that volume of root porosity was not a determinant factor in plant efficiency of waste modification. The effects of plant uptake on nutrient removal from applied wastes also were inconclusive. However, wastewater modification occurred chiefly in the upper 15 cm of soil and was affected by plant rooting depth and effluent concentration. These results suggest wetlands are capable of removing N and organic constituents from livestock wastes provided threshold loading rates are not exceeded.

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Land Rehabilitation

MONTANA STATE UNIVERSITY
Bozeman, Montana

June 1993

APPROVAL

of a thesis submitted by

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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VITA

Bryce Reed Romig was born in Albuquerque, New Mexico, the second son of Donald A. Romig and Edith L. Powers on the 20th day of August, 1964. Raised in Santa Fe, New Mexico, Bryce graduated from Santa Fe High School in 1982 to enter the University of Idaho in Moscow, Idaho the fall of the same year. He graduated *cum laude* in May of 1986 with a Bachelors degree in Forest Resources Science from the College of Forestry Wildlife and Range. Post-education work in mine reclamation activities in south-central Idaho and management of a native seed outlet for the plants of the southwestern United States culminated in the pursuit of a Master's Degree in Land Rehabilitation from Montana State University.

ACKNOWLEDGEMENTS

I acknowledge the financial assistance of the Montana Water Resource Center in conjunction with a grant from the United States Geological Survey. Many thanks to Dr. Robyn Tierney, Animal and Range Science Department, Montana State University for encouragement and assistance throughout my graduate activities. Thank you to the other members of my graduate committee Dr. Frank Munshower, Dennis Neuman, and Dr. Jon Wraith.

To Edith Powers, Donald Romig, Ann Girand, Ken, Doug, Kate and extended family, thank you for undying support and inspiration. I also wish to extend a personal note of thanks to Peggy Wright, whose lessons will remain the strongest.

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ABSTRACT

This study was developed to determine the effect of two liquid dairy waste loading concentrations on wastewater nutrient removal by four north-temperate wetland species, Nebraska sedge (*Carex nebraskensis* Dewey), beaked sedge (*Carex rostrata* Stokes), broad-leaved cattail (*Typha latifolia* L.), and panicled bullrush (*Scirpus microcarpus* Presl); and, to evaluate the role of root aerenchyma in wastewater treatment. Microcosms were planted with these four species and were treated with two concentrations (30% and 70%) of liquid dairy waste. Unplanted microcosm units receiving only H_2O served as controls. Plants were grown for one month prior to applications of wastewater. Soil waters were sampled from ceramic cups embedded in the soil medium at three soil depths (15, 30, 45 cm) prior to each application of effluent. Water was analyzed for pH, total Kjeldahl-nitrogen (TKN), nitrate nitrogen (NO_3^--N), and chemical oxygen demand (COD). Plants were harvested and measured for biomass production, root porosity, and total nitrogen at the end of the treatment cycle. Results showed differences ($p \leq 0.05$) in plant root porosity that corresponded to different levels of effluent concentration. No correlation was observed between percent root porosity in the wetland species and the level of contaminant removed. No differences were observed in soil pore water TKN at the 30 and 45 cm depths; however, significant differences were observed at the 15 cm depth. Soil surface TKN also was significant with respect to effluent concentration and plant treatment. Microcosms receiving the low concentration (70% dilution) showed stabilization of N level over the two month period while units receiving the high concentration (30% dilution) showed increased levels of nitrogen (N). The results of this study suggested that volume of root porosity was not a determinant factor in plant efficiency of waste modification. The effects of plant uptake on nutrient removal from applied wastes also were inconclusive. However, wastewater modification occurred chiefly in the upper 15 cm of soil and was affected by plant rooting depth and effluent concentration. These results suggest wetlands are capable of removing N and organic constituents from livestock wastes provided threshold loading rates are not exceeded.

OBJECTIVES

This study was developed to investigate liquid wastes generated by dairy cattle and the treatment of these wastes using constructed wetland treatment systems. First, the removal of contaminants from simulated constructed wetland systems planted with four wetland plant species was studied following application of two concentrations of liquid dairy effluents. Second, plant rooting depth and root aerenchyma volume were studied to evaluate the role of rhizosphere oxygenation on transformation of N species and removal of chemical oxygen demand (COD) under saturated soil conditions.

The objectives of this research were:

1) to evaluate the effect of two feedlot effluent loading concentrations on nutrient uptake by four temperate wetland species;

2) to determine the role of root aerenchyma in the removal of pollutants from dairy runoff.

Two hypotheses based on these objectives were developed.

1) Removal of water borne pollutants by wetland systems increases with the size and volume of cortical air tissue in the roots of wetland plants.

2) The level of pollutant removal by an individual wetland plant increases with increased rhizosphere oxygenation.

REVIEW OF LITERATURE

Livestock Wastes Impacts on Health and Environment

Agricultural developments include areas where livestock are concentrated for feeding and transport. Wastes generated at these facilities are often in vulnerable hydrologic settings, and may impair water quality. Wastes include feces, urine, unused food products, bedding material, nitrogen (N), phosphorous (P), trace metals from mineral supplements, trace amounts of antibiotics, detergents, and high levels of organic matter (Viraraghavan and Kikkeri 1990). The chemical and physical properties in the solid and liquid fraction of livestock wastes are summarized by the USDA (1978). These properties vary with type of animal and nutritional management (Kirchmann and Witter 1992).

The effects of livestock waste on water quality are well documented. Ammonia (NH_3), a primary N species in dairy effluents, is toxic to freshwater aquatic life and fish (EPA 1976). High levels of organic matter and biochemical oxygen demand (BOD) also are associated with fish kills. Organic loading and algal growth contribute to O_2 depletion (Richardson and Davis 1987). Nutrient loading further damages aquatic habitat through eutrophication. The BOD of livestock wastes is several times that of human sewage (Long and Painter 1991). Pathogens in livestock waste can contaminate estuarine habitats and render shellfish and fish unfit for consumption (National Research Council 1972).

Nitrate contamination of groundwater is a potential health threat in many industrial and agricultural areas (Keeney 1982, Adriano et al. 1971). Additionally, there is significant movement of NO_3^- through soil profiles and into groundwater under feedlots, corrals, and heavily fertilized fields (Adriano et al. 1971). Manure disposal on fallow croplands also poses a serious hazard to groundwater pollution.

Several sources (Barnes and Bliss 1983, National Research Council 1972, Wright and Davison 1964) cite the health risks of NO_3^- including methanoglobinemia and formation of carcinogenic nitrosamines. Methanoglobinemia results from the reduction of NO_3^- to NO_2^-. Nitrites oxidize hemoglobin to methemoglobin, a compound that does not carry oxygen (O_2). Mammalian infants are particularly susceptible because of low body weight and the presence of nitrate-reducing bacteria in the upper gastrointestinal tract. More complete explanations of NO_3^- toxicity are offered by Wright and Davison (1964). The EPA standard for NO_3^- in drinking water is 10 mg l^{-1} (EPA 1976).

Current Management of Livestock Wastes

Manures are beneficial to nutrient cycling in the plant root zone. One method commonly used to dispose of livestock wastes is application of manures onto cropland. Unfortunately, this approach is not a long-term solution to waste disposal.

Land applications of manure increased soil surface organic matter (Mathers and Stewart 1974, Kuo 1981, Chang et al. 1991), subsurface electrical conductivity and sodium (Kuo 1981, Chang et al. 1991), increased subsurface concentrations of NH_4^+ (Kuo 1981), and decreased soil pH (Chang et al. 1991). Concentrated manures can exceed the ability of the plant-soil system to cycle nutrients. Thus, selection of appropriate soil types for disposal is critical for land-based disposal of livestock wastes (Kuo 1981).

Chang et al. (1991) and Mathers and Stewart (1974) found evidence of leaching of soluble salts and NO_3^- beyond most plant's rooting depths. They concluded that, in some agricultural soils, potential pollution exists from directly applied manure. Repeated annual applications of manures to soil causes a buildup of salts sufficient to lower soil productivity (Wallingford et al. 1975). Mathers and Stewart (1974) also cautioned against manure applications supplying excess N (salts). Clearly, land disposal of manures as a long-term solution to livestock waste management is not advisable under most soil conditions. Given the problems associated with concentrated livestock production, livestock wastes must be handled in such a way as to protect surface and groundwater quality.

The Wetland Alternative

The use of wetland and vegetated terrestrial systems to treat wastewater has been the subject of numerous publications and recent research (Reed et al. 1988). Young et al. (1980) used vegetated buffer strips for reduction of coliform organisms from feedlot runoff. Wetlands also function as filters, anaerobic and aerobic bioreactors, and mediums for exchange and transformation of wastewater constituents. Wetlands have key ecological features which have led many in the past ten years to investigate their potential in providing advanced treatment or polishing of secondary wastewater. Reed and Bastian (1979) indicated that impacts caused by organic loading are relatively small in wetlands receiving secondary wastewater. However, wetlands may be used to improve water quality with respect to N, suspended solids, BOD, P (Kadlec 1987a, Hurry and Bellinger 1990), coliform bacteria (Bavor et al. 1987), metals, and some organic pollutants (Stowell et al. 1981). Wetland plant species concentrate and in some cases assimilate heavy metals and radioactive materials (Wolverton 1987a).

Regulatory considerations under EPA for treatment of wastewater using wetland systems were presented in Davis and Montgomery (1987). Cost comparisons of constructed wetland systems to contemporary wastewater facilities have been made by Crites and Mingee (1987), Duffer (1982), Wolverton (1987b), and Richardson and Davis (1987). In general, wetland treatment systems are cheaper to build and maintain than other types of sewage treatment systems (Cooper et al. 1989). Lower nutrient concentrations than those normally attained in conventional advanced waste treatment processes can be achieved using constructed wetlands (Swindell and Jackson 1990). In referring to wetland research as inflow-outflow "black box" studies, Reddy and DeBusk (1987) observed that the significance of vegetated wetlands in contaminant removal varied with treatment system design, which in turn depended on the desired contaminant removal goals. Further, the variability in N removal rates as reported by different researchers arises from differences in design criteria, substrate of the aquatic system (Weber and Tchobanoglous 1986) and other factors that may effect the growth of microorganisms (Reddy et al. 1989).

Aquatic Plants in Wastewater Treatment

The role of aquatic plants in wastewater treatment systems has been discussed by many researchers. Ideally, wetland plants should have rapid growth rates, high nutrient contents, and high standing crop (Reddy and DeBusk 1987). DeBusk and Ryther (1987) suggested that large biomass production, efficient assimilation of carbon, rapid growth rate in dense stands, and ease of vegetative propagation are also important growth characteristics. However, Reddy and DeBusk (1987) argued that nutrient removal via plant uptake is governed more by nutrient concentration in the plant tissues than by plant productivity. Nutrient assimilative capacity of emergent macrophytes also is affected by wastewater composition, loading rate, water depth, sediment characteristics, O_2 transfer to root zone, bio- and physio-chemistry at the root-water-sediment interface, plant density, plant cultural practices, and climate (Reddy and DeBusk 1987). Aquatic plant roots provide a medium for microbial growth, filtration of solids, translocation of O_2, and improvement of soil permeability (Wood 1990). Bacterial activity is further enhanced by O_2 release from plant roots (Armstrong and Armstrong 1988).

Boyd and Hess (1970) and Wolverton (1987a) suggested that wetland plants may be harvested as forage. Wood (1990) stated that management of wetland wastewater treatment systems may include selective harvest to control plant community diversity and dominance. Nitrogen removal may be facilitated by harvest of plant biomass or manipulation of hydraulic retention (Whitehead et al. 1987). Gersberg et al. (1986) and Wolverton (1982) also noted that artificial wetland systems may be managed to include biomass production.

The Wetland System as a Wastewater Treatment Reactor

A review of biogeochemical processes in wetland ecosystems with an overview of littoral O_2 and inorganic carbon assimilation is given by Carpenter and Lodge (1986).

Bacterial metabolism and physical sedimentation improve water quality in wetlands and function much like conventional activated sludge and trickling filters. Microbially mediated chemical transformations largely dictate the success of constructed wetland systems (Gersberg et al. 1986).

Wetland and aquatic plants are not active in chemical transformations, but provide a physical structure to the aquatic environment that enhance root-microbial symbiosis (Wolverton 1987b) and wastewater treatment capability (Tchobanoglous 1987). Aquatic plants supply O_2 to nitrifying microorganisms in microzones adjacent to roots and thus play an important role in N removal (Brix 1987). These rhizosphere interactions are perhaps best described by Reddy et al. (1989): "Ammonium (NH_4^+) in the anaerobic zone of the sediment diffuses either into the root zone or the overlying water column as a result of concentration gradients. Some of the NH_4^+ is taken up directly by aquatic macrophytes and part is oxidized to NO_3^-. The NO_3^- formed is either taken up by the plant or diffused into adjacent anaerobic zones where it is denitrified."

Research has identified additional considerations in the design of wetland systems for water quality improvement. The roles of evaporation (Wood 1990), substrate effects on retention time (Wood 1990, Brix 1987, McIntyre and Rhia 1991), and methods of effluent application (Wood 1990, Rogers et al. 1990) also have been studied. Boyd (1969) found evidence that aquatic macrophyte based treatment systems primarily designed as living substrate for microbial activity were effective in reducing suspended solids and BOD. Soils deep in the profile are thought to have little influence on N removal as one study (Wittgren and Sunblad 1990) found 85% loss of N in the surface 20 cm of soil. Kadlec (1987a) stressed the importance of algas and retention time for particle settling. Gersberg et al. (1986) found that treatment of N in trench type systems by bacterial metabolism requires an additional carbon source. System washout occurs when loading rate exceeds the assimilative capacity of the nitrifying-denitrifying bacteria in the system (Prakasam and Loehr 1972, Barnes and Bliss 1983).

Differences in pollutant removal efficiency caused by temperature variation are reviewed by Viraraghavan and Kikkeri (1990). Brix (1989) stated that plant cover, heat from microbial activity and influent wastewater temperature may help prevent the freezing of systems in the winter. Wood (1990), however, showed the temperature variations in wastewater during winter have little effect on effluent quality. An increase in effluent retention time must accompany a decrease in temperature to obtain results that are similar to those observed at higher temperatures (Tchobanoglous 1987).

Wetland Soils and Soil Redox Status

Knowledge of the soil oxidation-reduction condition and the measurement of redox potential (E_h) are valuable tools for interpreting soil nutrient fluxes and changes in nutrient availability. Ponnamperuma (1984) found that the oxidation-reduction potential of interstitial waters in flooded soils and sediments may explain changes in concentration of ecologically important ions (plant nutrients). Armstrong et al. (1990) and Bouldin et al. (1974) related oxidation status of soils to predicting the presence of N species and noted that a mosaic of oxidized and unoxidized zones may be present in wetland soils. Anaerobic conditions occur when the transport processes for O_2 fail to keep pace with soil biological and chemical processes (Veen 1988).

Variations in redox electrode readings may result because of electrode construction, placement of electrodes, and microsite differences (Cogger et al. 1992). While theoretical justifications of the measurements are made by several authors (Ives and Janz 1961, Bohn 1971), there are no widely accepted methods of measuring and interpreting field redox potentials (Cogger et al. 1992). Several researchers discuss the use of platinum microelectrodes for *in-situ* redox measurements (Mueller et al. 1985, Faulkner et al. 1989). Redox measurements are more reliably interpreted in evaluating hydric soils (Cogger et al. 1992). Thus, E_h frequently is used as an index of reducing or oxidizing condition in wetlands and wetland sediments.

This measure has been particularly useful, albeit controversial, in understanding the role of plants in modification of sediment chemistry. Vegetation shifts between vascular and non-vascular plants leads to corresponding shifts in sediment redox (Jaynes and Carpenter 1986). Haraguchi (1991) presented an argument against the role of plants in influencing sediment redox, and related oxidation-reduction potentials to properties of the substrate. Carpenter et al. (1983) related E_h to root zone oxygenation and corresponding decrease in soil water COD. Armstrong et al. (1990) demonstrated that *Phragmites* can raise soil redox considerably during a single growing season. Armstrong (1964) suggested redox condition may play a role in distribution of plant species. While Wium-Anderson and Anderson (1972) found no seasonal variation in redox potential, Cogger et al. (1992) found field redox potentials varied by season and with flooding and draining.

Anaerobic soils are frequently phytotoxic or adverse to plant growth. McKee et al. (1988) showed that significantly lower concentrations of sulfide (a phytotoxic anion) correspond to the presence of aerial roots in *Rhizophora* and *Avicennia*. Oxygen release in the root zone raises sediment E_h and causes precipitation of ferric and manganese oxyhydroxides on or around plant roots (Wium-Anderson and Anderson 1972). The effects of root zone modification by vascular plant root systems remains important since solutes regulated by redox and pH include potentially toxic forms of aluminum, manganese, zinc, iron, and sulfur (Jaynes and Carpenter 1986).

Aerenchyma and the Formation of Cortical Air Space

Rhizosphere oxygenation depends on certain anatomical and physiological characteristics of wetland plants that facilitate gas transfer into the root zone. Many wetland plants contain aerenchyma, a cortical air tissue that provides a continuous system of interconnected gas-filled cavities or lacunae (Drew et al. 1981) in both stems and petioles (Dacey 1980, Kawase 1981). Rhizomes and roots possess an extensive system of air-filled cortical lacunae that are often more or less continuous with that of the stems, petioles and leaves (Sculthorpe 1967). Sculthorpe (1967) noted a lack of pit membranes in aerenchyma and suggested that gas may freely diffuse within these tissues.

Patterns of aerenchyma development are species-specific (Armstrong 1979, Smirnoff and Crawford 1983). Burdick (1989) studied the effect of root age as a factor in aerenchyma development as originally suggested by Yu et al. (1969). Luxmore et al. (1972) related aerenchyma development to light intensity. Sojka et al. (1972) also showed that air space development is sensitive to temperature.

In the early stages of root growth, aerenchyma forms through premature cell lysis about 10 mm behind in the root cortex about 10 mm behind the root apex (Campbell and Drew

1983). Aerenchyma formation in many wetland plants responds positively to poorly oxygenated environments (Justin and Armstrong 1987, Kawase 1981). Das and Jat (1977) indicated that reduced substrate aeration can promote breakdown of cortical tissues and increase internal porosity. Justin and Armstrong (1987) noted that some wetland plants do not form aerenchyma but survive in the wetland condition by forming shallow root systems. Aerenchyma formation is a relatively rapid process (Kawase and Whitmoyer 1980, Kawase 1981).

Although roots form aerenchyma in conditions of low O_2 concentration, Armstrong (1979) suggested that aerenchyma development is not solely a response to anoxia. Atwell et al. (1988) and Drew et al. (1979), however, showed that hypoxia stimulates the synthesis of ethylene and that an increase in internal ethylene concentration is closely related to induction of cell lysis. Both endogenous (Drew et al. 1981) and exogenous ethylene formation (He et al. 1992) have been shown to stimulate formation of aerenchyma. Further, Drew et al. (1981) and Van Noordwijk and Brouwer (1988) found that aerenchyma formation was inhibited when ethylene production was reduced in roots of some plant species.

The role of nutrient availability in aerenchyma formation has also been noted. Aerenchyma may form under fully anaerobic conditions with the temporary removal of NO_3^- and NH_4^+ (Drew et al. 1989, Konings and Verschuren 1980). Konings and Verschuren (1980) also suggested that cavity formation in aerated roots of maize was retarded by NO_3^- and NH_4^+ ions. They attributed their observed results to a relationship between nitrate reductase activity and NO_3^- uptake.

Although root porosities for terrestrial plants are lower than for aquatic or wetland plants or plants that have undergone a period of soil saturation, Jensen et al. (1969) reported root porosities of 3.6% for barley, 9.5% for corn, and 36.5% for rice. Armstrong (1979) found that internal air space in roots can be as high as 60% while Bristow (1975) reported 30 to 60% air space in wetland plant roots. In water lilies, lacunae constitute 20 to 40 % of volume in the root and rhizome, and 50% in the petiole (Dacey and Klug 1979).

Aerenchyma Function

The four primary functions of aerenchyma are:

1) as a plant adaptive response to seasonal flooding or long-term soil saturation

2) as a gas transport mechanism for oxygenation of root tissues and the rhizosphere

3) as a mechanism for providing O_2 to anaerobic soil substrates

4) as an anatomical and morphological property of roots providing structural resistance to saturated soils.

Although Kawase (1981) stated that aerenchyma contributes to survival of wetland species, differences in aerenchyma formation have been noted between flood-tolerant and flood-intolerant species (Pearson and Havill 1988b, Burdick and

Mendelssohn 1990). Crawford (1966) described the distribution of the genus *Senecio* in response to flood tolerance and anaerobic respiration. Laan et al. (1989) subsequently found aerenchyma development was the primary factor in determining flood tolerance of *Rumex* species. Smirnoff and Crawford (1983) and Justin and Armstrong (1987) also reported differences in effective root porosities between flood-tolerant and flood-intolerant species.

Laing (1940) described the continuity of air space in plants for the supply of O_2 and removal of carbon dioxide (CO_2) from root tissue. Lacunae supply O_2 for respiratory processes in anaerobic sediment (Sculthorpe 1967, Dacey and Klug 1979), O_2 storage (Armstrong 1967a, Sculthorpe 1967) and gas transport (Sculthorpe 1967). Sculthorpe (1967) stated that the measured linear gradients of O_2 concentration in plants support the hypothesis that underground organs derive their O_2 supply from aerial or floating portions of the plant. Lacunar gas space improves internal ventilation (Armstrong 1979, Drew et al. 1985), and reduces root respiratory demand (Armstrong 1979).

Van Raalte (1944) discussed the dependence of roots on shoots for O_2 supply and rhizosphere detoxification. Armstrong and Boatman (1967) stated that oxidized iron precipitates around the roots forming an effective barrier against sulfide. Oxygen diffusion helps mitigate the toxic effects of reduced soil near the root tip (Armstrong 1967b), and serves to immobilize phytotoxins (Armstrong 1972, 1979). The ability to withstand the phytotoxicity of sulfide is a function of a wetland plants' ability to withstand extreme anoxia (Pearson and Havill 1988a). Aerenchyma is not important in oxidation of endogenous sulfide (Pearson and Havill 1988a), but is important to flood tolerance (Pearson and Havill 1988b). Williams and Barber (1961) stated that aerenchyma functioned as a mechanical property in the protection of wetland plant roots embedded in saturated plastic (i.e. easily deformable) soils.

Plant Gas Exchange Mechanisms

As early as 1841, Raffeneau-Delile reported pressurization of gasses in the leaves of lotus. Laing (1940) observed concentrations of gasses in the internal atmosphere of the rhizomes and found that O_2 decreased from shoot to root with increased concentrations of CO_2. He also noted seasonal differences in the gas concentration between above and belowground portions of plants. Scholander et al. (1955) suggested that pressure changes in the roots of *Rhizophora* sp. and subsequent O_2 movement into roots through lenticels was driven by rise and fall of tides.

Plant O_2 transport modelling was pioneered by William Armstrong (1967b) who determined that the O_2 diffusion rate in plants is not affected by photosynthetic production of O_2. Others have noted that gas diffusion is a function of concentration gradient and resistance in lacunae (Sculthorpe 1967). Gas exchange rates are a function of internal gas partial pressure, route resistances, and cortical gas space. Fick's laws of diffusion were used to describe the rate of gas exchange in the roots of both wetland and non wetland plants (Armstrong 1979). Armstrong also attempted to explain the diffusive properties of O_2 in the roots of wetland plants, but recognized that some mass flow caused by temperature and pressure differentials between below and aboveground portions of plants must occur in aerenchyma.

Until 1980, models of gas transport were based on the assumption that the gas phase in the lacunae is stationary and that individual gasses diffuse along concentration gradients (Hutchinson 1957). In 1980, Dacey confirmed that the sun supplies the energy required for a thermo-osmotic pump that drives gas movement in lacunae of water lilies. He found that the flow is not static, and that gas exchange can occur at linear rates of up to 50 cm min^{-1}. He described gas flow from young leaves down petioles, through root tissue, and into older leaf petioles. With the use of tracer studies, gas flow within the petioles of leaves was found to be a linear function of the observed pressure gradient. This observation is in accordance with Darcy's law for flow through porous media. Temperature differences between leaf and atmosphere drive pressurization through two independent diffusive processes, thermal transpiration and hygrometric pressure (Dacey 1980). Both operate in sunlit conditions as temperatures and vapor pressure gradients (of all gasses including water vapor) are maintained. Variations in gas exchange mechanisms are based on structural differences among plant species, and historical perspectives on the operation of this thermo-osmotic phenomena are discussed by Mevi-Schutz and Grosse (1988).

Gas exchange within lacunar spaces of plants not only occurs between the shoot and root, but also from the root to shoot. Several researchers have measured gas transfer in aerenchyma from wetland soils. Dacey and Klug (1979) showed 37% of the methane generated in wetland sediments was removed through the aerenchyma of *Nuphar luteum*. Reddy et al. (1989) found that a significant portion of denitrified gas formed in the rhizosphere escaped through plant tissues. The role of aquatic plant lacunae has been recognized in promoting carbon cycling (Sebacher et al. 1985) during periods of active plant growth and dormancy (Brix 1989). Sand-Jensen et al. (1982) suggested that CO_2 transport is more important than O_2 transport. Constable et al. (1992) also stressed the importance of an internal CO_2 source in the roots of *Typha*.

One of the more interesting observations made about transport in the internal lacunae of plants is that root length is a function of effective root porosity and length of diffusive O_2 path per unit volume of respiring root (Armstrong 1979). Armstrong (1967b) stated that lateral roots are primary to O_2 diffusion and discussed the theoretical relationship between O_2 diffusion rate and root size.

Rhizosphere Oxygenation

An oxidized substrate is, in most cases, a favorable environment for root growth (van Raalte 1944, Armstrong and Boatman 1967, Teal and Kanwisher 1966, Wium-Anderson and Anderson 1972). Van Raalte (1944), and Teal and Kanwisher (1966) noted that O_2 release into a reducing medium occurred when the internal supply of O_2 exceeded respiratory demand. Teal and Kanwisher (1966) found comparable ferric hydroxide deposits on the roots of *Spartina* and suggested that these are produced by O_2 release from roots to sediment. Most wetland species modify the soil environment by transporting and releasing O_2 into the surrounding substrate (Armstrong 1964, Bristow 1975, Armstrong 1967a). Sand-Jensen et al. (1982) tested eight plants from the littoral zone of oligotrophic lakes and found evidence of O_2 exchange in these lake sediments. Submersed vascular plants characteristic of oligotrophic communities release O_2 while mosses lack the root and lacunar systems of vascular macrophytes that facilitate O_2 release to sediments (Jaynes and Carpenter 1986). Conversely, Bedford et al. (1991) argued that given microbial, soil oxidative, and root respiratory demands, plants are not capable of oxygenating sediments.

Armstrong (1964), used O_2 data and redox measurements to explain differences in plant O_2 transport in roots. Armstrong (1967a) and McKee et al. (1988) stated that diffusion rate in wetland plant species is species-specific and does not vary within a particular species. Morris and Dacey (1984) and Dacey (1980) also have done extensive work on O_2 transport rates in plant root systems. Rate of NH_4^+ uptake and root respiration are especially sensitive to O_2 concentration in the rhizosphere (Morris and Dacey 1984). Rates of gas transfer may depend on size or biomass of the plant and volume of the aerenchyma. Radial O_2 loss from roots increases with root porosity and decreases with root length (Armstrong 1972), varying both seasonally (Armstrong et al. 1990) and diurnally (Sand-Jensen et al. 1982, Sand-Jensen and Prahl 1982).

The effects of soils on plant adaptability and rhizosphere oxygenation appear to be minimal. Leakage of O_2 by *Typha* was found to be site dependant and varied by season. Rhizosphere oxidation did not appear to be related to E_h (Crowder and Macfie 1985) and pH regimes (Crowder and Macfie 1985, Macfie and Crowder 1987). McKee et al. (1988) explained that the occurrence of aerial roots of *Avicennia* and *Rhizophora*, not the reverse, influenced soil oxygenation.

Differences in the oxygenated rhizosphere in relation to root size supports arguments citing genetic variation in the O_2 transport mechanism operating in wetland soils. The oxygenated rhizosphere is a function of O_2 concentration in the root and the root radius, thus favoring large roots (Armstrong 1979). Armstrong (1979), however, showed that roots with a smaller radius had zones of oxygenation similar to those of larger roots and suggested that wetland plants with smaller roots may have some competitive advantage. Thin roots have a better O_2 supply to all root cells than thick roots and thus need less root porosity to provide a transport path for O_2. The highest O_2 transport rates from aerial plant tissue into the rhizosphere also are associated with plants having small root mass. Older plants have large root systems and higher O_2 transport rates (Moorhead and Reddy 1988). Armstrong et al. (1990) demonstrated that greater root numbers, not greater O_2 demand, account for sediment oxygenation.

Nitrogen Dynamics in Saturated Soils

Nitrogen additions to wetland soils consist of N fixation, atmospheric deposition, and biomass decay. Plant and microbial assimilation (mineralization), NH_3 volatilization, nitrification, and denitrification, are other transformation pathways that modify and remove N in wetland treatment systems. Nitrate, the thermodynamically stable form of N in terrestrial oxygenated aqueous systems (National Research Council 1972), may be biochemically assimilated from water by growing plants or may be converted to gaseous N in anoxic situations. In surface waters, main sinks for loss of inorganic N include NH_3 volatilization, and algal uptake (Wittgren and Sundblad 1990). Ammonia is the preferred form of N for algae (Richardson and Davies 1987). Natural concentrations of NO_3^- and NH_3 in soils vary greatly with season and soil aeration. Ammonium diffusion, however, proceeds more slowly than NO_3^- diffusion because of adsorption to soil particles (Lorenz and Biesboer 1987).

Removal of N in wetland wastewater treatment systems requires an understanding of NH_3 volatilization, plant assimilation, and nitrification-denitrification processes. Ammonia volatilization is seasonal and inconsistent (Stowell et al. 1981), varies diurnally, and is affected by temperature, pH (Beaucamp et al. 1982, Hoff et al. 1981) and wind speed (Hoff et al. 1981). At high pH values, bulk loss of NH_4^+-N occurs as NH_3 is volatilized from the water surface. This volatilization is a function of the partial pressure of NH_3 and the surface to volume ratio of the water body (Veen 1988). At a pH below 7.5 (Rogers et al. 1991, Wittgren and Sundblad 1990), volatilization becomes less important. Ammonium is stable at low pH values and at the normal pH of sewage (Davies and Hart 1990).

Stengel et al. (1987) and Lorenz and Biesboer (1987) inhibited microbial activity with acetylene and measured N_2O, as an indicator of denitrification. Additional reviews of nitrification-denitrification reactions in wetland systems are given by Reddy et al. (1989), Valiela and Teal (1979), and Veen (1988). The rates of these reactions are controlled by redox potential, pH, moisture, carbon source, and temperature (Denny 1972, Barnes and Bliss 1983). Denitrification rates are also related to microbially modified soil O_2 status (Obenhuber and Lowrance 1991) and carbon content of soil (Burford and Bremner 1975, Bowman and Focht 1974, Firestone 1982). Obenhuber and Lowrance (1991) stated that microbial denitrification can reduce NO_3^- concentrations provided adequate energy sources and appropriate environmental conditions exist. Adding carbon to an aquifer increases N_2O production or denitrification. Denitrification rates may be limited by carbon source, but not universally by soil type (Lorenz and Biesboer 1987).

Differences exist in the literature between the importance of denitrification in wetland systems and the role of plant assimilation as the primary N removal mechanism. Most researchers contend that the nitrification-denitrification reactions are key to N removal (Wood 1990, DeBusk et al. 1983, Swindell and Jackson 1990, Morris 1991, Wittgren and Sundblad 1990). Breen (1990), however, suggested that plant uptake accounted for 80% of N removal. Kadlec (1987b) stated that plants function primarily as uptake and transfer conduits unless there is harvest of biomass. Rogers et al. (1991) concurred; their study showed plant nutrient uptake as the dominant removal mechanism. Heterotrophic fixation of N_2, they argued, could also mask denitrification. However, they failed to note research by Oaks and Hirel (1985) and Buresh et al. (1980) that indicated nitrification and bacterial (N-fixing) nitrogenase was inhibited by NH_4^+. Reddy et al. (1982) found that 50% of N_2 loss in simulated wetland systems was through means other than plant uptake. Denitrification rates in natural wetlands can exceed natural N inputs by a factor of three (Zak and Grigal 1991). Weber and Tchobanoglous (1986) stated that when plant density is near a maximum, plant uptake of N is assumed to be minimal and nitrification-denitrification is thought to be the major pathway of N loss.

Veen (1988) evaluated the change in root activity with respect to a reduction of root respiration in terrestrial plants known to be unequally sensitive to O_2 stress. He found that lower root O_2 concentrations reduced NO_3^- uptake. These findings conflict with those of Lambers et al. (1978) who showed that nitrate reductase activity enhanced in roots under anaerobic conditions. Other work by Guyot and Prioul (1985) indicated that plant tolerance to O_2 deficiency improves with additional NO_{3-}.

Changes in soil structure, hydrology, plant community composition and microbial activity affect the speciation and dynamics of nutrients present in wetland soils. Nitrate will not adsorb in soils below pH 5.5 while NH_4^+ is strongly held on the cation exchange at this pH. In undisturbed wetlands with mineral soils the pH equilibrates toward a neutral value. The pH of more acidic soils increases in flooded conditions and enhances the reduction of Fe (III) to Fe (II). Conversely, the pH of alkaline soils decreases in flooded conditions with an accumulation of respiratory CO_2 (Ponnamperuma 1972). Further modification of pH is caused by microbial reactions in the sediments when nitrifying bacteria produce hydrogen ions that lower pH (Davies and Hart 1990). Acidification blocks the N cycle by inhibiting nitrification and leads to an accumulation of NH_4^+ (Rudd et al. 1988). Veen (1988) attributed an increase in nutrient solution alkalinity to reduced root O_2 concentration. Bicarbonate production of the root system is a reflection of the NO_3^- reduction rate of the root system (CO_2 generation). A study by Jaynes and Carpenter (1986), using pots planted with mosses and vascular plants, showed that E_h and pH did not vary by sediment type but rather by the influence of the plants. Chen and Patrick (1981) also found that additions of carbon additions reduced E_h and enhanced NO_3^- reduction rate (denitrification).

METHODS

Experimental Design

Forty-five microcosms (Figure 1) were planted with four wetland plant species - Nebraska sedge (*Carex nebraskensis* Dewey), beaked sedge (*Carex rostrata* Stokes), broad-leaved cattail (*Typha latifolia* L.), and panicled bullrush (*Scirpus microcarpus* Presl.), and placed in a greenhouse. These species are abbreviated throughout the tables as Cane, Caro, Tyla, and Scmi, respectively. Unplanted microcosms are abbreviated as Unpl. Placement of the microcosms within the greenhouse was randomized. The experiment was designed with two levels of repeated measures. The experimental units represent a five (four plants with unplanted controls) by three (control application plus two effluent concentrations) by three (replicate) factorial experiment. The units were treated with either of two concentrations of liquid dairy effluent or a control treatment of water. Each plant species, control, and treatment level (concentration) was replicated in triplicate. The first level of repeated measures consisted of the soil water at three soil depths within each microcosm. The second level of repeated measure occurred over time as each microcosm was measured at two-week intervals.

Statistical Analyses

The experimental design entailed two levels of repeated measure - depth in the microcosm and changes in effluent parameters over time. All statistical analyses were conducted at $p \leq 0.05$. Statistical contrasts of interactions and main effects between plant species, depth and effluent concentration within the treatment cycle were made for changes in TKN, COD, pH and solution NO_3^- using the PROC GLM procedure in SAS (SAS Inst 1987). Plant biomass, root porosity, and plant N content were statistically analyzed using the ANOVA procedure in NCSS (Hintze 1990). Comparison of treatment means included ranking of means and Duncan's multiple range test (Mize and Shultz 1985, Ott 1984).

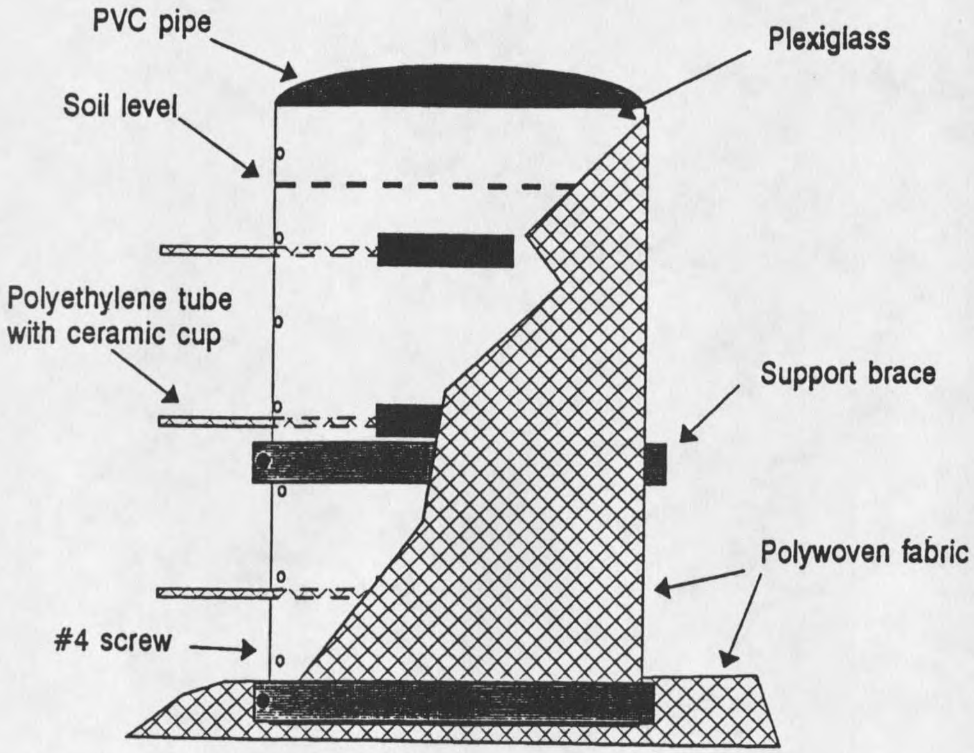

Figure 1. Side view schematic of microcosm experimental unit construction, including placement of porous ceramic cups.

Microcosms

The microcosms used in this study were constructed using PVC irrigation pipe (38 cm ID) cut to 60 cm lengths and split laterally to form half-round columns (Figure 1). The half-rounds were fitted with a 38 by 60 cm sheet of 0.32 cm plexiglass attached to the PVC pipe with an industrial silicon sealant and screws spaced along each edge.

Three holes (1.0 cm diameter) were drilled into the sides of each microcosm at 15 cm increments to allow for placement of lengths of 0.95 cm polypropylene tubing. Tubing was cut to 23 cm lengths and attached to round end ceramic cups (1 Bar, High Flow, Soil Moisture Equipment Corp.). The ceramic cups were aligned in the microcosms for center placement in the substrate. The base of each microcosm consisted of a piece of poly-woven ground cloth covering the open end and attached using silicon sealant. This base was not intended for structural support of microcosm contents, but served as a barrier that allowed for exchange of air and water from the microcosms. A standard greenhouse tray was placed to catch water beneath each microcosm.

The microcosm units were reinforced with two wood and wire braces (Figure 1) for structural support of the plexiglass. The braces were attached at the base and center of each microcosm and tightened using double-threaded rod and butterfly nuts. A double layer of ground cloth was attached to the plexiglass face of the microcosms to prevent light entry to the rhizosphere. This material could be removed for visual inspection of root growth in the soil column.

The ceramic cups were washed with 1 N HCl solution and then rinsed with distilled water until the pH and specific conductance of the rinse water equaled that of the distilled water influent (Creasey and Dreiss 1988). Cups were stored in a covered box to prevent dust accumulation prior to attachment of the polyethylene tubing. Tubing and ceramics were attached using an epoxy from Soil Moisture Equipment Corp. The epoxy was allowed to dry and cure for a period of 24 hours. Microcosms were assigned numbers and randomly located in the greenhouse (Figure 2).

Soil Collection and Preparation

Soils used in this study were collected from a natural wetland area located in Gallatin County, Montana (N.W. 1/4 Sec. 25 T.1 N R.3 E, P.M). This site was an open slough centered in a pasture receiving seasonal use by cattle. Vegetation in the slough included softstem bullrush (*Scirpus americanus* Pers.), broad-leaved cattail (*Typha latifolia* L.), plainleaf willow (*Salix planifolia* Pursh), Nebraska sedge (*Carex nebraskensis* Dewey), rabbitfoot grass (*Polypogon monspeliensis* L.), and smooth brome (*Bromus inermis* Leysser). Soil was collected on June 19 and July 28, 1992. The soil was kept damp in transit and placed in cold storage prior to placement into the microcosms.

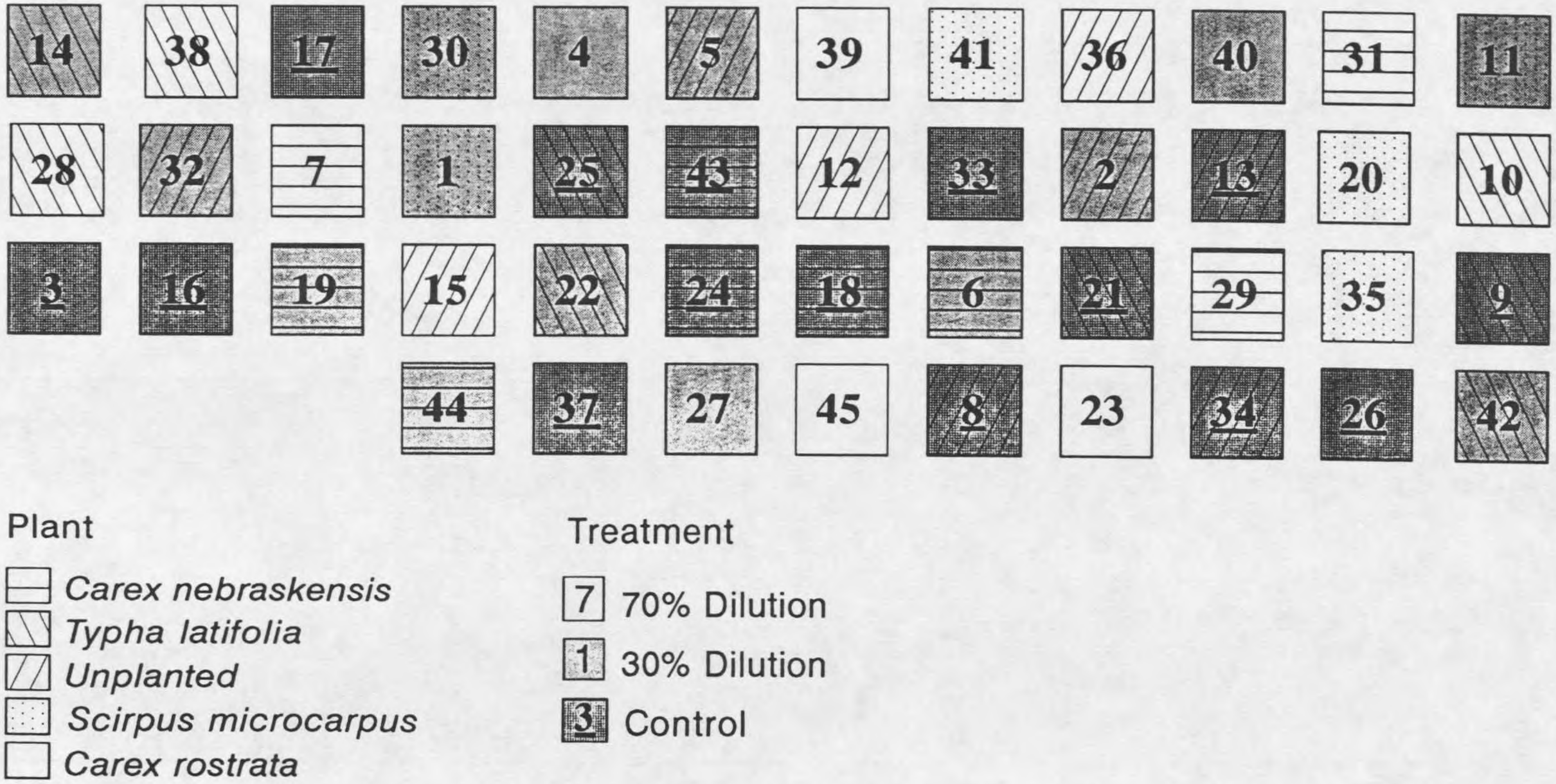

Figure 2. Placement and randomization of microcosms in greenhouse.

Sampling of the soil for baseline soil parameters was made during the first soil collection period (June 21, 1992). Five soil cores were taken at 0-15 cm and 15-30 cm soil depths. Samples were air dried ground, split and sieved to < 0.2 mm and analyzed for percent organic matter, cation exchange capacity (CEC), alkalinity, and N concentration. Additional unground samples were used in the analysis of particle size distribution. Organic matter was determined by combustion of a known weight of dry sample at 450°C for 16 hours (Ball 1964). Nitrogen analysis used methods specified by Bremner and Mulvaney (1982). Particle size analysis followed procedures outlined by Gee and Bauder (1986). Cation exchange capacity and alkalinity procedures followed Rhoades (1982).

Refrigerated soils were homogenated by hand to obtain uniform soil consistency and volume in each microcosm. Soils were manually placed in the wetland units on August 3. A small volume (64 cm^3) of autoclaved sand was placed around each ceramic pore cup to facilitate removal of soil water samples under negative applied pressure. Soil placement continued until the soil level was 10 cm below the top of the microcosms. A 5 cm head of distilled water was then applied to the top of the soil column to maintain saturation and a reduced state of the soil constituents. Saturation through continuous application of water was maintained throughout the experimental procedures.

Oxidation-Reduction Potential

Twenty-seven platinum electrodes were constructed and calibrated for measurement of soil E_h (Mueller et al. 1985, Faulkner et al. 1989). These electrodes were standardized against an Orion combination redox electrode in a standard ferrous-ferric solution (Light 1972). Electrodes were placed in several wetland microcosms to evaluate differences between planted (*C. rostrata*) and unplanted units at the three soil depths under control and high concentrate treatments (Table 1). Four measurements of millivolt potential were made at one week intervals beginning November 1992. Measurements were corrected to a standard hydrogen electrode (SHE) using the correction value of +200 mV (Orion 1992).

Table 1. Experimental design for redox measurement in greenhouse microcosms.

	Unplanted controls				*Carex rostrata*			
	Unit	Depth (cm)			Unit	Depth (cm)		
Treatment		15	30	45		15	30	45
Control	9	-	-	-	37	X	-	X
	25	X	X	X	26	X	X	-
	21	X	X	X	17	X	X	X
Low Conc.	22	X	X	X	40	-	X	X
	14	X	X	-	4	X	X	X
	42	-	X	X	27	X	X	-

X indicates placement of a platinum electrode at specified depth in unit
- indicates no electrode placement at specified depth in unit

Plant Collection and Preparation

The plant species used in this study comprise a major component of the wetland species composition in north-temperate regions of North America. Plant nomenclature is after Hitchcock and Cronquist (1981). A description of the plant species used, their distribution, and gross morphological characteristics is presented in Appendix A.

Whole, intact plants were collected from a wetland at the Nixon bridge of the Gallatin River in Gallatin County, Montana (NW 1/4 S.35 T.2 N R.3 E. P.M.). Collection of the plants was made on August 21, 1992. Plants were immediately pruned and refrigerated for 1 week prior to planting. Preparation for planting included tagging all plants with colored telephone wire, and recording fresh weight biomass of each plant. Plants were divided by species into groups with equivalent weights (100 ± 10 g). Plants were placed in randomly selected microcosms on August 28, 1992. Root crowns were set to their previous soil levels and roots were extended to full length in the microcosms. Roots were positioned close to the upper ceramic cup (15 cm depth). A constant head (5 cm) of water was maintained after planting.

Because the plants were collected in the fall, they were allowed to grow for a period of one month while temperatures in the greenhouse were increased in 5°C increments from 55°C night and day temperature to a 60°C night and 75°C day temperature. Supplemental lighting via metal halide lamps

(1000 N•m/s Sylvannia M1000\c\u) suspended 1.5 m above the plant canopy was increased in half hour increments each week to an 8 hour dark cycle over the same period.

Effluent Collection and Preparation

Fresh liquid manure from a herd of dairy cattle in Gallatin County, Montana was collected one day prior to each effluent application. The dairy cattle diet consisted of alfalfa and barley with some supplementation of trace nutrients and salts. No detergents, antibiotics, or iodinated teat washings were present in the effluent material. Manure (75.7 l) was collected from manure piles freshly scraped from feeding pens. Materials were collected from the entire manure pile at random locations. Manure effluents for the application were immediately prepared by adding 15.1 l of water to every 37.9 l of original manure material. The slurry was then placed in rinsed polyethylene bags, tied, and suspended from a brace for filtration. The filtrate was collected in 18.9 l buckets over a period of several hours, sealed and cooled overnight.

Two concentrations of the manures were made by diluting with distilled water. The two concentrations were 30% (low concentration) and 70% (high concentration) of the original wastewater filtrate. The dilutions were stirred, sub-sampled for laboratory analysis and then applied to the microcosms in 2 l volumes. These applications were made four times on a biweekly basis (Table 2). Placement of the liquid waste was made to avoid stirring of the soil surface by the liquid additions. No direct application of liquid manures was made on the plant surfaces. Any remaining volume in each microcosm was filled with distilled water and a constant water level was maintained between applications.

Table 2. Waste application schedule for wetland wastewater treatment study (1992).

	Soil Water Sampling	Effluent Application
Period	Date	Date
1	Sep. 18	Sep. 28
2	Oct. 3	Oct. 13
3	Oct. 18	Oct. 27
4	Nov. 1	Nov. 10
5	Nov. 15	

Sampling and Analysis

Effluents

Sampling of manure effluents followed the schedule outlined in Table 2. Table 3 is an outline of the chemical analyses performed on both influent and effluent samples. The first two effluents were sampled at the time of preparation, refrigerated, and sent to the analytical laboratory within 24 hours. Samples were analyzed for NH_3-N and NO_3^--N. Sub-samples also were analyzed separately for NH_3-N and total Kjeldahl nitrogen (TKN), COD, and pH. Analysis of the third and fourth effluent concentrates consisted of all parameters investigated in the first two sampling periods' effluents except for the analysis of NO_3^--N.

A companion study was utilized to determine the amount of N removal occurring independent of the wetland units. Open containers of effluent were placed in a ventilated hood for two weeks (the period between effluent applications in the

Table 3. Sample analyses conducted for influent and effluent water parameters.

	Parameter	Period(s)	Reference
Influent	TKN[1]	1-4	APHA 1992
	NH_3-N	1,2	EPA 1983 (350.2)
	NH_4^+-N	1-4	APHA 1992
	NO_3^--N	1,2	EPA 1983 (353.3)
	COD[2]	1-4	APHA, Hach 1992
	pH[3]	1-4	Orion 1992
Effluent	TKN	1-5	APHA 1992
	NO_3^--N	1,2	APHA 1992
	NO_3^--N	3-5	APHA 1992; Willis 1980
	NO_2^--N	1	APHA 1992
	COD	2,3,5	APHA, Hach 1992
	pH	1-5	Orion 1992

[1] - Keltec Auto Analyzer, Perstop Analytical, Inc., Model 1020
[2] - Open Reflux Method by block digestion and colorimetry, Hach Corporation
[3] - Orion Research pH meter 250A with pH combination electrode 91-57

microcosms) and subjected to similar temperatures and air flow environments found in the greenhouse. Evaporated volumes of water were replaced. Measurements of pH, E_h, total and NH_3-N were made before and after the two week period. Changes in pH, E_h, and total and NH_3-N concentration of the liquid dairy effluents were determined.

Microcosm Soil Water Sampling

Soil water from each microcosm at each of the three soil depths was sampled prior to the application of the manure effluents and two weeks after the final manure application (Table 2). Soil water was collected from the ceramic cups by applying a vacuum to the connective ports (Figure 1) and drawing aliquots of soil water into containers. Seven milliliters of each sample were preserved with 0.5 ml 6 N H_2SO_4. These samples were refrigerated until analyzed for TKN and COD. Five of the seven ml were used for TKN. The remaining 2 ml were used in the analysis of COD. The non-acidified remains were sent to the analytical laboratory (first two samplings) for measurement of NO_3^--N and NO_2^--N using anion chromatography. Soil pore water samples from the latter three sampling periods were analyzed for NO_3^--N using a colorimetric technique (Willis 1980). Nitrite was eliminated from the analytical program since it was not detected above 0.05 mg l^{-1} in any of the original 135 samples submitted in the first sampling period.

Plant Sampling

Plant characteristics measured in this study included fresh and dry weight of roots, rhizomes, and shoots; root porosity; and protein content of below and aboveground biomass. Root masses removed from the units were rinsed free of soil. A fresh sub-sample was removed from each microcosm root mass, sealed and refrigerated for root porosity evaluation. Root porosity determinations were made using a gravimetric test (Jensen et al. 1969, Burdick 1989, and Van Noordwijk and Brouwer 1988). All biomass was gravimetrically measured and corrected for percent moisture content. Protein content of ground plant samples was determined by TKN analyses and corrected for dry weight. Protein analyses were conducted on duplicate air dried samples.

Soil Sampling

The microcosms were disassembled at the termination of the experiments. At this time, soils were sampled at the surface of each unit and at the three soil depths adjacent to the ceramic cups. Samples were air dried, ground, and passed through a 2 mm screen. The samples were then analyzed for TKN and NO_{3-}-N.

Quality Control/Quality Assurance

To improve the precision and accuracy of data collected in this study, a quality control/quality assurance program was implemented. Measurements of TKN, nitrate, and COD in manures and soil water samples included a 1:20 field replicate sampling (blind field replicates for samples sent to the respective labs). A cross contamination blank and a bottle blank were included for each set of soil water samples. Accuracy of both the TKN and NO_3^--N concentration was determined using EPA nutrient standards in the final two sample sets (Periods 4 and 5). Data precision for COD analysis was based on regressions of known standards (Appendix B).

RESULTS

Interpretations of statistical outputs were based on the main effect and interaction differences observed initially in the microcosms compared to observed differences in the final measurement period. Results from repeated measures analyses were used to interpret the overall effects of pollutant input to the wetland microcosms. The statistical outputs from ANOVA are provided in Appendix C. Table grand means ($\bar{X}$) represent column or rowwise averages across concentration, depth, or plant treatment for the combined samples (n).

General Observations

Plants grew well in the greenhouse. Root growth was especially concentrated in the upper 20 cm of the soil column. *Typha latifolia* grew slower than the other plant species.

Initially, the microcosms showed high hydraulic conductivity that apparently decreased over the course of the experiment. Water continued to flow out of the sampling ports and from the bases of the microcosms throughout the study. Hydraulic conductivity of the media, overall volume of water applied, hydraulic residence time, and evapotranspiration were not quantified. *Carex rostrata* and *S. microcarpus* showed high vigor in all effluent treatments through the treatment cycle. The vigor of *C. nebraskensis* declined in the latter sampling periods as evidenced by chlorosis and some stem mortality.

Prior to wastewater application, all units contained a number of macroinvertebrates and benthic organisms. The origin of these organisms was presumed to be dormant life forms in the collected soil. Several species of aquatic annelids were observed to feed and move through the upper 10 cm of soil. Cladocera species were seen to feed on algae that grew in the water column. Several forms of algae were observed in both planted and unplanted units. Macroinvertebrates and benthic organisms in units receiving high concentrated wastes were killed following wastewater additions. The same organisms thrived in the control and the low concentrate microcosms.

A band of black coloring formed approximately 1 cm below the soil surface. This phenomenon was presumed to be a function of symbiotic microbial growth between sulfate-reducing bacteria and cyanobacteria under high nutrient concentrations (Hodges 1992).

System Parameters

The basic characteristics of the microcosms prior to applications of liquid dairy wastes are presented in Table 4. Baseline soil data with the exception of NO_3^--N analyses were determined from samples taken at the time of soil collection. The analyses were also performed on soil samples taken at the end of the experiment. Soil was classified as a Typic haplaquoll (Soil Survey Staff 1987) with a sandy clay texture (Gee and Bauder 1986). Analyses of soil water samples were conducted prior to pollutant application.

Microcosm Oxidation-Reduction Potential

Oxidation-reduction potentials (ORP) by depth, concentration level, and presence or absence of *C. rostrata* are given in Table 5. The millivolt potentials are corrected to the standard hydrogen electrode (+200 mV at 25°C). No significant differences were found within or among main effects by plant species, concentration, or depth.

Table 4. Microcosm characteristics and baseline soil and interstitial water parameters.

Characteristic	n	$\bar{x} \pm$ S.E.
EC (mS cm^{-1})	45	1.44 ± 0.20
pH	129	7.36 ± 0.20
CEC (meq 100 g^{-1})	2	29.65 ± 1.15
Soil TKN (%)[1]	20	0.527 ± 0.138
Soil TKN (%)[2]	60	0.476 ± 0.102
Soil NO_3^--N (mg kg^{-1})	60	15.29 ± 27.20
Soil OM (%)	10	9.90 ± 2.23
Soil water TKN (mg l^{-1})	131	1.961 ± 0.702
Soil water NO_3^--N (mg l^{-1})	133	0.436 ± 0.268
Soil alkalinity (mg l^{-1} as $CaCO_3$)	4	768.13 ± 68.13

[1] - Samples made from field collections
[2] - Samples taken following disassembly of microcosms

Plant Characteristics

Plant Biomass

The results of the analyses of plant biomass are presented in Tables 6 through 14. Plant root biomass of the four species by three effluent treatments is shown in Table 6.

Table 5. Microcosm oxidation-reduction potential (mV) by depth, concentration and presence or absence of Carex rostrata (Caro).

	Depth (cm) (n=4)			Concentration (n=6)	
	15	30	45	Control	High Conc.
Unpl[1]	-77.6 ± 51.2	-128.3 ± 54.0	-154.0 ± 69.5	-92.5 ± 53.5	-108.9 ± 48.6
Caro	-111.3 ± 53.0	-29.25 ± 89.4	-4.91 ± 35.0	-135.2 ± 21.0	-82.0 ± 40.7
$\bar{X}$	-96.3 ± 35.4	-128.7 ± 32.3	-79.4 ± 45.7	-111.8 ± 29.9	-95.4 ± 30.7

[1] - Unplanted microcosms

Table 6. Effect of effluent concentration on mean (± S.E.) root biomass (g).

	Concentration			
Plant	Control	Low	High	$\bar{X}$
Caro	17.76 ± 2.63 bx[1]	19.68 ± 0.81 ax	17.29 ± 4.87 ax	18.25 ± 1.66 b
Cane	9.31 ± 1.97 bx	8.53 ± 1.14 bx	10.19 ± 0.52 ax	9.34 ± 0.72 c
Tyla	13.75 ± 3.44 bx	14.35 ± 0.39 abx	14.86 ± 1.00 ax	14.32 ± 1.05 ab
Scmi	31.29 ± 5.67 ax	21.86 ± 4.48 ax	16.54 ± 2.18 ax	24.07 ± 3.35 a
$\bar{X}$	18.02 ± 2.94 x	16.11 ± 1.89 x	14.55 ± 1.50 x	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different ($n=3$, $p \leq 0.05$).

There were significant differences in root biomass production across concentrations. Plant root biomass varied by species in the control and low concentrate units. *Scirpus microcarpus* had the highest overall root mass.

Aboveground biomass varied by effluent concentration and species, however, no interactions between plant and concentration were noted (Table 7). Aboveground biomass of *C. rostrata* was higher than the other species across treatments. *Carex rostrata* and *S. microcarpus* had significantly higher aboveground biomass across the effluent treatments than *T. latifolia* and *C. nebraskensis* .

The interaction between plant biomass production by species and concentration was significant (Table 8). This differential response among species appeared in the analysis of variance across concentration for the combined model. *Scirpus microcarpus* showed a negative growth response to concentration. *Carex rostrata* and *S. microcarpus* produced significantly greater amounts of aboveground biomass in response to each of the three effluent concentrations.

Plant Nitrogen Concentration

Concentration of N varied by plant species and effluent concentration in units receiving the high concentration (Tables 9 and 10). *Scirpus microcarpus* was the only species showing a response to increased loading of nitrogenous wastes.

Table 7. Effect of effluent concentration on mean (± S.E.) aboveground biomass (g).

Plant	Concentration			
	Control	Low	High	$\bar{X}$
Caro	61.13 ± 13.27 ax[1]	55.37 ± 9.49 ax	41.87 ± 7.22 ax	52.79 ± 5.88 a
Cane	17.97 ± 6.86 bx	23.43 ± 6.25 cbx	15.55 ± 2.29 cbx	18.98 ± 3.00 b
Tyla	17.84 ± 8.70 bx	12.66 ± 4.11 cx	6.74 ± 1.90 cx	12.42 ± 3.55 b
Scmi	58.65 ± 0.65 ax	47.25 ± 6.18 bcx	28.94 ± 0.62 bcy	46.95 ± 4.80 a
$\bar{X}$	38.90 ± 7.33 x	34.68 ± 5.97 x	22.76 ± 4.73 x	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different ($n=3$, $p \leq 0.05$).

Table 8. Effect of effluent concentration on mean (± S.E.) total plant biomass (g).

Plant	Concentration			
	Control	Low	High	$\bar{X}$
Caro	78.89 ± 15.63 ax[1]	75.06 ± 9.34 ax	59.16 ± 11.64 ax	71.03 ± 6.93 a
Cane	27.28 ± 8.81 bx	31.96 ± 7.39 bx	25.74 ± 2.74 bx	28.33 ± 3.54 b
Tyla	31.59 ± 12.05 bx	27.02 ± 3.74 bx	21.60 ± 2.51 bx	26.74 ± 3.99 b
Scmi	89.94 ± 5.86 ax	69.11 ± 10.83 ay	45.48 ± 2.80 aby	71.01 ± 7.69 a
$\bar{X}$	56.93 ± 9.65 x	50.77 ± 7.37 x	37.31 ± 5.74 y	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different ($n=3$, $p \leq 0.05$).

Table 9. Effect of effluent concentration on mean (± S.E.) nitrogen concentration in root biomass (mg g^{-1}).

Plant	Concentration			
	Control	Low	High	$\bar{X}$
Caro	1.40 ± 0.17 ax[1]	1.39 ± 0.06 ax	1.82 ± 0.24 abx	1.54 ± 0.11 a
Cane	1.67 ± 0.36 ax	1.82 ± 0.32 ax	1.91 ± 0.10 abx	1.80 ± 0.15 a
Tyla	1.29 ± 0.19 ax	1.59 ± 0.06 ax	1.34 ± 0.20 ax	1.40 ± 0.09 a
Scmi	1.56 ± 0.17 ax	1.58 ± 0.15 ax	2.44 ± 0.19 by	1.64 ± 0.21 a
$\bar{X}$	1.38 ± 0.12 x	1.59 ± 0.09 xy	1.83 ± 0.14 y	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different (n=3, $p \leq 0.05$).

Table 10. Effect of effluent concentration on mean (± S.E.) nitrogen concentration in aboveground biomass (mg g^{-1}).

Plant	Concentration			
	Control	Low	High	$\bar{X}$
Caro	1.84 ± 0.07 ay[1]	2.04 ± 0.08 by	2.34 ± 0.08 ax	2.08 ± 0.08 b
Cane	2.33 ± 0.38 ax	2.37 ± 0.07 abx	2.92 ± 0.00 ax	2.54 ± 0.15 a
Tyla	1.80 ± 0.34 ax	2.71 ± 0.15 ax	2.66 ± 0.34 ax	2.39 ± 0.21 ab
Scmi	1.85 ± 0.19 ay	2.32 ± 0.15 aby	3.38 ± 0.18 ax	2.41 ± 0.24 ab
$\bar{X}$	1.96 ± 0.13 z	2.36 ± 0.09 y	2.77 ± 0.14 x	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different (n=3, $p \leq 0.05$).

Carex rostrata and *S. microcarpus* had higher concentrations of N in aboveground tissues than *C. nebraskensis* and *T. latifolia* (Table 10).

Plant Assimilated Nitrogen

Total assimilated N by effluent for the plant species is presented in Tables 11 to 13. Table 11 data represent root N assimilation. No significant differences were found with respect to effluent concentration but significant differences in N content were observed among the four plant species. These differences in plant N occurred chiefly in the control concentration.

The response relations between N assimilation and aboveground biomass and whole plant response to effluent concentration are presented in Tables 12 and 13, respectively. *Carex rostrata* and *S. microcarpus* had higher N uptake than *C. nebraskensis* and *T. latifolia* across all concentrations. These responses were independent of applied effluent concentration.

Plant Nitrogen Summary

Effluent concentration affected plant assimilatory response of the roots. Total plant biomass also was affected by effluent concentrations. There were, however, no significant interactions between effluent concentrations and plant species. *Scirpus microcarpus* and *C. rostrata* had consistently higher productivity and tissue N concentrations than *C.nebraskensis* and *T. latifolia*.

Root Porosity

Results of gravimetric measurements of root porosity are presented in Table 14 and are displayed graphically in Figure 3. Differences were noted across concentrations and among plant species, however, there was no significant interaction. Root porosity tended to decrease with increased concentration, but was only significant in *C. nebraskensis*. Despite similarities in plant N concentration between *S. microcarpus* and *C. rostrata* (Table 13), root porosity response of the two species was significantly different (Table 14). The large standard errors of mean root porosity attest to the inherently large variation in this plant characteristic.

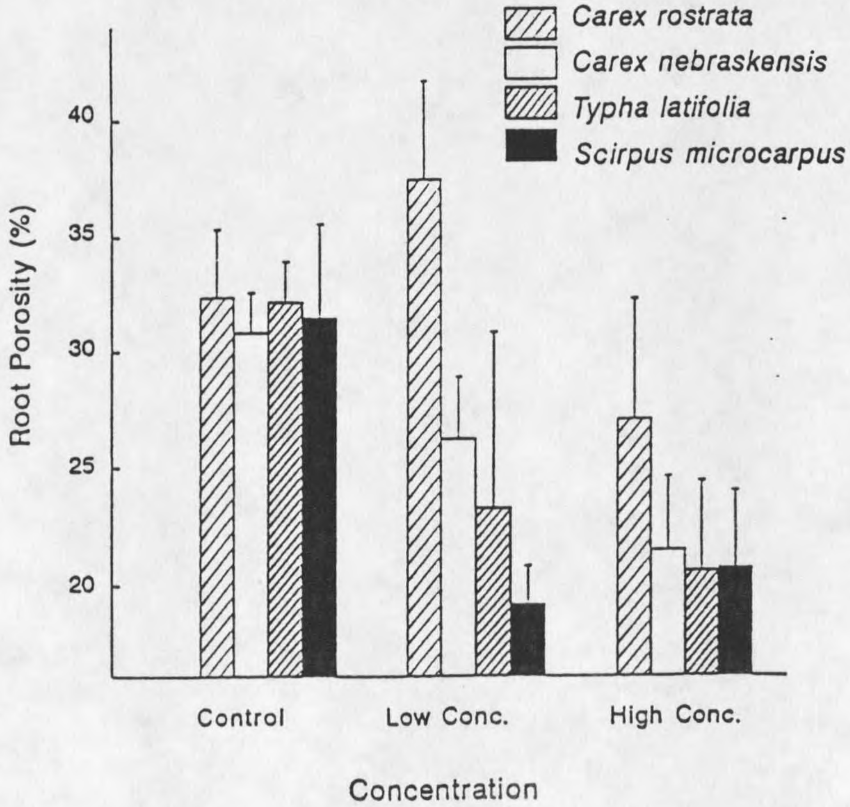

Figure 3. Root porosity (%) versus effluent concentration for four wetland plant species.

Table 11. Effect of effluent concentration on mean (± S.E.) nitrogen in root biomass (mg).

Plant	Concentration			
	Control	Low	High	$\bar{X}$
Caro	23.94 ± 1.06 abx[1]	27.40 ± 1.83 ax	30.77 ± 8.07 ax	27.37 ± 2.60 b
Cane	16.92 ± 7.03 bx	16.19 ± 4.86 ax	19.37 ± 0.36 ax	17.49 ± 2.52 c
Tyla	16.18 ± 1.38 bx	22.72 ± 0.48 ax	19.92 ± 3.22 ax	19.61 ± 1.39 c
Scmi	34.38 ± 0.51 ax	33.29 ± 5.42 ax	40.01 ± 2.16 ax	35.38 ± 2.10 a
$\bar{X}$	22.86 ± 2.69 x	24.90 ± 2.48 x	26.38 ± 3.25 x	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different ($n=3$, $p \leq 0.05$).

Table 12. Effect of effluent concentration on mean (± S.E.) nitrogen in aboveground biomass (mg).

Plant	Concentration			
	Control	Low	High	$\bar{X}$
Caro	111.31 ± 20.57 ax[1]	112.54 ± 19.96 ax	98.11 ± 19.06 ax	107.52 ± 10.18 a
Cane	46.64 ± 24.93 bx	54.81 ± 13.30 bx	45.45 ± 6.72 bx	48.97 ± 8.51 b
Tyla	33.65 ± 15.24 bx	35.42 ± 12.29 bx	18.53 ± 5.82 bx	29.20 ± 6.48 b
Scmi	108.38 ± 10.85 ax	108.08 ± 9.22 ax	97.67 ± 3.01 ax	105.56 ± 5.01 a
$\bar{X}$	75.00 ± 13.26 x	77.71 ± 11.75 x	62.11 ± 11.96 x	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different ($n=3$, $p \leq 0.05$).

Table 13. Effect of effluent concentration on mean (± S.E) total nitrogen uptake (mg).

Plant	Concentration			
	Control	Low	High	$\bar{X}$
Caro	135.26 ± 21.58 bx[1]	139.94 ± 21.14 ax	129.48 ± 27.07 ax	134.89 ± 11.81 a
Cane	63.57 ± 31.95 bx	71.00 ± 18.00 bx	64.82 ± 6.97 bx	66.46 ± 10.84 b
Tyla	49.83 ± 16.57 bx	58.14 ± 12.28 bx	38.45 ± 3.39 bx	48.81 ± 6.67 b
Scmi	142.77 ± 10.42 ax	141.37 ± 14.40 ax	137.57 ± 0.86 ax	140.95 ± 5.87 a
$\bar{X}$	97.86 ± 15.55 x	102.61 ± 13.59 x	88.49 ± 14.71 x	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different ($n=3$, $p \leq 0.05$).

Table 14. Mean (± S.E.) percent root porosity of four wetland plant species receiving biweekly applications of dairy effluent.

Plant	Concentration			
	Control	Low Conc.	High Conc.	$\bar{X}$
Caro	32.35 ± 3.01 ax	37.51 ± 4.17 ax	27.10 ± 5.17 ax	32.32 ± 2.59 a
Cane	30.81 ± 1.75 ax	26.25 ± 2.65 axy	21.47 ± 3.14 ay	26.17 ± 1.87 ab
Tyla	32.15 ± 1.78 ax	23.26 ± 7.59 ax	20.58 ± 3.98 ax	25.33 ± 3.07 ab
Scmi	31.49 ± 5.33 ax	19.68 ± 1.61 ax	20.73 ± 3.26 ax	23.96 ± 2.65 b
$\bar{X}$	31.70 ± 1.42 x	26.67 ± 2.81 xy	22.47 ± 1.88 z	

[1] - Means (± S.E.) in columns followed by the same letter (a,b,c) are not significantly different. Means (± S.E.) in rows followed by the same letter (x,y,z) are not significantly different ($n=3$, $p \leq 0.05$).

Liquid Dairy Effluents

The liquid dairy effluents were applied within 24 hours of preparation (Table 2). Wastes were analyzed for NH_3-N and TKN within 48 hours of preparation. Results of these analyses are given in Table 15. The companion experiment used to determine non-wetland-related N loss, change in pH, and effluent redox for low concentrate wastewater is summarized in Table 16. These same parameters are summarized for the high concentrate materials in Table 17.

Mean loading rate for high concentrates was 139.76 mg l^{-1} (± 43.17) N and 14605.6 mg O_2 l^{-1} (± 3375) COD. Mean loading rate for the low concentrates was 47.37 mg l^{-1} (± 29.75) N and 7320.60 mg O_2 l^{-1} (± 528.78) COD. These values are equal to a bi-weekly fertilization rate of 45.92 kg N ha^{-1} (high conc.) and 21.07 kg N ha^{-1} (low conc.). Ammonia nitrogen concentration in all wastewaters applied was 35% (± 0.04) of total nitrogen.

Tables 16 and 17 demonstrate the changes in manure constituents that occurred over a two week period in the two concentrate preparations. Sixty-four and sixty-one percent of the original NH_3-N present in the manures was lost in the low and high concentrations respectively over a two week period. This loss was reflected by the change in TKN, which fell 30% and 31% in the low and high concentrate preparations. Effluent pH increased an order of magnitude. The buffering

Table 15. Composition of liquid dairy effluents (n=2) applied to microcosms based on a biweekly basis.

	TKN ($mg\ l^{-1}$)		NH_4^+-N ($mg\ l^{-1}$)		EC ($mS\ cm^{-1}$)		COD ($mg\ O_2\ l^{-1}$)	
CONC.	L[1]	H[2]	L	H	L	H	L	H
App. 1	58.79	140.11	19.44	46.03	2.98	6.13	8090.4	18056.0
App. 2	40.92	86.24	12.55	31.37	2.73	5.86	6816.0	11054.0
App. 3	49.20	126.49	19.77	44.24	3.85	7.52	7531.9	11412.4
App. 4	81.47	206.19	29.35	72.11	4.08	8.48	6844.6	17899.0
$\bar{x}$	47.37	139.76	20.28	48.46	3.41	6.99	7320.7	14605.6
± S.E.	29.75	43.17	5.98	14.83	0.57	1.06	528.78	3375.0

[1] - Low concentration liquid dairy effluent, direct 70% dilution of prepared filtrate dairy effluent
[2] - High concentration liquid dairy effluent, direct 30% dilution of prepared filtrate dairy effluent

Table 16. Change in effluent characteristics in non-wetland condition. Data are for low concentrate preparation (n=2).

	TKN (mg l^{-1})		NH_4^+-N (mg l^{-1})		pH		ORP (mV)[1]	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
App. 1	58.79	31.30	19.44	5.54	7.00	8.15	-275.2	-388.3
App. 2	40.92	26.00	12.55	6.68	7.10	8.15	-328.3	-385.3
App. 3	49.20	47.59	19.77	4.79	7.05	8.28	-321.9	-372.0
App. 4	81.47	52.05	29.35	11.31	6.60	7.88	-300.3	-349.0
$\bar{x}$	47.37	32.74	20.28	7.08	6.94	8.11	-306.3	-373.6
± S.E.	29.75	20.42	5.98	2.54	0.20	0.15	20.7	15.4
% removed	31 ± 16		64 ± 11					

[1] - Values corrected to the Standard Hydrogen electrode value (+200 mV @ 25°C)

Table 17. Change in effluent characteristics in non-wetland condition. Data are for high concentrate preparation (n=2).

	TKN (mg l^{-1})		NH_4^+-N (mg l^{-1})		pH		ORP (mV)[1]	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
App. 1	140.11	75.06	46.03	18.48	7.05	8.20	-312.9	-402.0
App. 2	86.24	62.68	31.37	15.58	7.20	8.48	-309.7	-405.1
App. 3	126.49	113.06	44.24	13.27	6.85	8.28	-327.7	-389.7
App. 4	206.19	52.05	72.21	26.40	6.55	7.44	-291.1	-346.7
$\bar{x}$	139.76	96.82	48.46	18.43	6.91	8.10	-310.3	-385.9
± S.E.	43.17	29.48	14.83	4.96	0.24	0.40	13.02	23.3
% removed	30 ± 13		61 ± 7					

[1] - Values corrected to the Standard Hydrogen electrode value (+200 mV @ 25°C)

capacity of these effluents measured at 3150 and 7350 mg l^{-1} as $CaCO_3$ for high and low concentrates, respectively, and may have accounted for this increase. Oxidation-reduction potential of the wastewaters decreased (< -370.0 mV in both preparations).

Soil Water Samples

Soil Water pH

Concentrations and depth showed significant interaction within repeated measures main effects for microcosm pH. Concentration and plant species also showed significant interaction through time. The pH of soil waters in both the high and the low concentrations was different from the pH of the controls (Table 18). Differences between upper and lower soil depths in the initial sampling period were not evident across concentration in the final sampling period (Table 19). Plant influence on microcosm pH was observed in sample periods 2 and 5 (Table 20).

Soil Water COD

Results of repeated measures analysis of variance for COD data are shown in Tables 21, 22, and 23. The data from the COD analyses were highly variable. Concentration was the only main effect that affected COD. Differences in soil water COD concentration were noted during sampling periods 2 and 3, but did not occur in the final sampling period (Table 21). Over the length of the entire experiment, depth was the only

Table 18. Mean (± S.E.) microcosm pH by effluent concentration for five sampling periods (n=45).

	Sampling Period				
Conc.	1[1]	2	3	4	5
High	7.37 ± 0.19 a[2]	7.68 ± 0.21 ab	7.62 ± 0.24 a	7.84 ± 0.21 a	7.84 ± 0.25 a
Low	7.37 ± 0.16 a	7.70 ± 0.22 a	7.60 ± 0.28 a	7.79 ± 0.23 a	7.77 ± 0.25 a
Cont.	7.37 ± 0.20 a	7.60 ± 0.23 b	7.46 ± 0.23 b	7.59 ± 0.29 b	7.42 ± 0.30 b

[1] - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.
[2] - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 19. Mean (± S.E.) microcosm pH by soil depth for five sampling periods (n=45).

	Sampling Period				
Soil depth	1[1]	2	3	4	5
15 cm	7.26 ± 0.13 b[2]	7.70 ± 0.24 a	7.64 ± 0.29 a	7.77 ± 0.29 a	7.71 ± 0.39 a
30 cm	7.40 ± 0.19 a	7.60 ± 0.21 b	7.47 ± 0.23 b	7.68 ± 0.24 a	7.65 ± 0.28 a
45 cm	7.45 ± 0.16 a	7.67 ± 0.21 ab	7.58 ± 0.23 a	7.78 ± 0.25 a	7.68 ± 0.28 a

[1] - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.
[2] - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 20. Mean (± S.E.) microcosm pH by plant species for five sampling periods (n=36).

Plant	Sampling Period				
	1[1]	2	3	4	5
Caro	7.39 ± 0.16 a[2]	7.65 ± 0.17 ab	7.57 ± 0.28 a	7.77 ± 0.23 a	7.67 ± 0.27 ab
Cane	7.36 ± 0.18 a	7.69 ± 0.17 ab	7.60 ± 0.31 a	7.74 ± 0.22 a	7.71 ± 0.38 ab
Tyla	7.35 ± 0.19 a	7.51 ± 0.19 a	7.56 ± 0.22 a	7.76 ± 0.32 a	7.57 ± 0.36 b
Scmi	7.40 ± 0.21 a	7.72 ± 0.28 ab	7.59 ± 0.20 a	7.76 ± 0.23 a	7.70 ± 0.31 ab
Unpl	7.45 ± 0.15 a	7.65 ± 0.25 ab	7.50 ± 0.28 a	7.68 ± 0.31 a	7.74 ± 0.27 a

[1] - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.
[2] - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 21. Mean (± S.E.) microcosm COD (mg O_2 l^{-1}) by concentration for three sampling periods (n=45).

	Sampling Period		
Conc.	2[1]	3	5
High	288.67 ± 102.07 a[2]	375.06 ± 167.42 a	392.72 ± 248.98 a
Low	307.83 ± 93.25 a	349.58 ± 98.54 a	416.92 ± 331.56 a
Cont.	236.72 ± 78.80 b	288.61 ± 108.79 b	315.50 ± 116.82 a

1 - 2 = Oct. 3, 3 = Oct. 18, 5 = Nov. 15.
2 - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 22. Mean (± S.E.) microcosm COD (mg O_2 l^{-1}) by soil depth for three sampling periods (n=45).

	Sampling Period		
Depth	2[1]	3	5
15 cm	248.69 ± 134.90 a[2]	330.95 ± 186.17 ab	457.33 ± 399.84 a
30 cm	292.76 ± 65.30 b	229.30 ± 77.04 b	319.24 ± 95.67 b
45 cm	294.20 ± 60.77 b	373.09 ± 96.75 a	343.05 ± 140.14 ab

1 - 2 = Oct. 3, 3 = Ocy. 18, 5 = Nov. 15.
2 - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 23. Mean (± S.E.) microcosm COD (mg O_2 l^{-1}) by plant species for three sampling periods (n=36).

	Sampling Period		
Plant	2[1]	3	5
Caro	288.02 ± 104.29 ab[2]	355.28 ± 158.93 a	389.24 ± 351.92 a
Cane	307.50 ± 119.17 a	346.55 ± 176.81 a	378.66 ± 130.64 a
Tyla	240.87 ± 64.01 b	319.51 ± 74.42 a	305.63 ± 138.98 a
Scmi	286.97 ± 87.87 ab	336.57 ± 141.31 a	443.15 ± 379.87 a
Unpl	270.39 ± 93.68 ab	332.89 ± 101.12 a	363.17 ± 181.17 a

1 - 2 = Oct. 3, 3 = Oct. 18, 5 = Nov. 15.
2 - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

variable that showed significant differences between effects. Significant differences in COD level occurred among the three depths throughout the study period (Table 22). A significant interaction between concentration and depth was observed in the second and third sampling periods. Although the response of plant species to the treatments did not differ in sampling periods 3 and 5, the interaction among plants and depth was significant. Plant influences on soil water characteristics early in the experiment were most clearly evident during the second sampling period (Table 23).

Soil Water NO_3^--N

Concentration treatment had a significant effect on soil water NO_3^--N. This treatment effect was most apparent during the third sampling period (Table 24). The significant interaction between depth and concentration also affected NO_3^--N, with the highest concentrations occurring in the second sampling period 2 increasing with depth (Table 25). There was no interaction between plant species and soil water NO_3^--N level (Table 26).

Soil Water TKN

Results of the analyses of TKN are given in Tables 27 through 29. These data showed significant interaction between concentration and depth. The interaction between TKN level and effluent concentration is shown in Table 27. Units receiving the high concentrated wastes had progressively

Table 24. Mean (±S.E.) microcosm NO_3^--N (mg l^{-1}) by concentration for five sampling periods (n=45).

	Sampling Period				
Conc.	1[1]	2	3	4	5
High	0.45 ± 0.27 a[2]	0.70 ± 0.35 a	0.36 ± 0.22 ab	0.18 ± 0.11 a	0.25 ± 0.20 a
Low	0.46 ± 0.24 a	0.61 ± 0.33 a	0.51 ± 0.59 a	0.22 ± 0.20 a	0.30 ± 0.27 a
Cont.	0.44 ± 0.26 a	0.70 ± 0.21 a	0.33 ± 0.18 b	0.21 ± 0.12 a	0.34 ± 0.18 a

[1] - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.
[2] - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 25. Mean (± S.E.) NO_3^--N (mg l^{-1}) by soil depth for five sampling periods (n=45).

	Sampling Period				
Depth	1[1]	2	3	4	5
15 cm	0.45 ± 0.27 a[2]	0.55 ± 0.37 b	0.39 ± 0.23 a	0.20 ± 0.18 a	0.25 ± 0.20 a
30 cm	0.48 ± 0.23 a	0.73 ± 0.29 a	0.41 ± 0.57 a	0.21 ± 0.12 a	0.33 ± 0.19 a
45 cm	0.42 ± 0.26 a	0.74 ± 0.19 a	0.40 ± 0.26 a	0.20 ± 0.14 a	0.32 ± 0.27 a

[1] - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.
[2] - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 26. Mean (± S.E.) microcosm NO_3^--N (mg l^{-1}) by plant species for five sampling periods (n=36).

	Sampling Period				
Plant	1[1]	2	3	4	5
Unpl	0.36 ± 0.29 a[2]	0.72 ± 0.23 a	0.39 ± 0.25 a	0.26 ± 0.25 a	0.39 ± 0.32 a
Cane	0.40 ± 0.26 ab	0.70 ± 0.27 a	0.34 ± 0.18 a	0.18 ± 0.09 b	0.29 ± 0.19 ab
Scmi	0.46 ± 0.28 ab	0.60 ± 0.41 a	0.38 ± 0.22 a	0.17 ± 0.11 b	0.26 ± 0.15 b
Cane	0.49 ± 0.23 ab	0.65 ± 0.27 a	0.40 ± 0.28 a	0.18 ± 0.12 b	0.25 ± 0.19 b
Tyla	0.54 ± 0.19 b	0.69 ± 0.31 a	0.50 ± 0.72 a	0.22 ± 0.19 ab	0.30 ± 0.21 ab

1 - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.

2 - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 27. Mean (± S.E.) microcosm TKN (mg l^{-1}) by concentration for five sampling periods (n=36).

	Sampling Period				
Conc.	1[1]	2	3	4	5
High	1.97 ± 0.65 a[2]	2.33 ± 0.66 ab	3.47 ± 0.85 a	3.72 ± 1.95 a	4.34 ± 2.47 a
Low	1.98 ± 0.83 a	2.48 ± 0.48 a	3.25 ± 0.63 ab	3.16 ± 1.07 b	3.03 ± 0.83 b
Cont.	1.98 ± 0.67 a	2.14 ± 0.60 b	2.97 ± 0.67 b	2.57 ± 0.95 c	1.82 ± 0.90 c

1 - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.

2 - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

higher concentrations of total soil water N over time. Low concentrate units showed an initial increase in soil water N that leveled off in the final two sampling periods. Control units showed a slight decrease in TKN values at the fourth and fifth sampling periods. Table 28 is a presentation of TKN data against depth in the soil profile. Soil water TKN levels were significantly different in the upper soil zone from those at 30 and 45 cm. At lower soil depths, TKN levels did not differ from controls and those of the measurements made during the initial sampling period.

Total N level as a function of depth in the soil profile across plant and concentration treatments is presented in Figure 4. The control treatment showed a lower TKN level at the surface 15 cm, while the low concentrate TKN level was not significantly different among the three depths. High concentrate TKN levels were markedly higher at the 15 cm depth than the 30 and 45 cm depths. Total N levels in the interstitial water samples did not significantly differ at the 30 and 45 cm depths with respect to effluent treatments.

A graphic representation of the changes in TKN over time in the 15 cm soil zone is presented in Figure 5. The slope of the relationship between TKN and depth in low concentrate microcosms at the end of the experiment was negative. A positive slope was present in the corresponding curve for the units receiving high concentrate. Total N levels in unplanted units were only different from units planted with *T. latifolia*

Table 28. Mean (± S.E.) microcosm TKN (mg l^{-1}) by soil depth for five sampling periods (n=36).

Depth	Sampling Period 1[1]	2	3	4	5
15 cm	1.49 ± 0.47 a[2]	2.03 ± 0.54 a	3.23 ± 0.94 a	3.87 ± 2.10 a	3.71 ± 2.70 a
30 cm	2.16 ± 0.56 a	2.41 ± 0.51 a	3.44 ± 0.67 ab	2.93 ± 0.78 b	2.68 ± 1.27 b
45 cm	2.33 ± 0.81 b	2.54 ± 0.63 b	2.97 ± 0.44 b	2.58 ± 0.64 b	2.74 ± 0.95 b

[1] - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.
[2] - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 29. Mean (± S.E.) microcosm TKN (mg l^{-1}) by plant species for five sampling periods (n=36).

Plant	Sampling Period 1[1]	2	3	4	5
Unpl	2.21 ± 0.68 a[2]	2.46 ± 0.56 a	3.45 ± 0.48 a	3.40 ± 1.53 a	3.30 ± 1.53 a
Cane	2.01 ± 0.78 a	2.46 ± 0.52 a	3.30 ± 0.89 a	3.41 ± 1.82 a	3.40 ± 2.33 a
Scmi	1.95 ± 0.80 a	2.39 ± 0.58 a	3.18 ± 0.94 a	3.29 ± 1.51 ab	3.09 ± 2.03 ab
Cane	1.88 ± 0.76 a	2.22 ± 0.70 ab	3.11 ± 0.70 a	3.11 ± 1.44 ab	2.99 ± 2.22 ab
Tyla	1.84 ± 0.54 a	2.06 ± 0.56 b	3.10 ± 0.61 a	2.57 ± 0.78 b	2.51 ± 1.15 b

[1] - 1 = Sep. 18, 2 = Oct. 3, 3 = Oct. 18, 4 = Nov. 1, 5 = Nov. 15.
[2] - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

(Table 29) at the end of the study. Unplanted units and units planted with *C. nebraskensis* had higher relative concentrations of total N at each sampling period. Units planted with *C. nebraskensis* had significantly higher total N than the other three plant species.

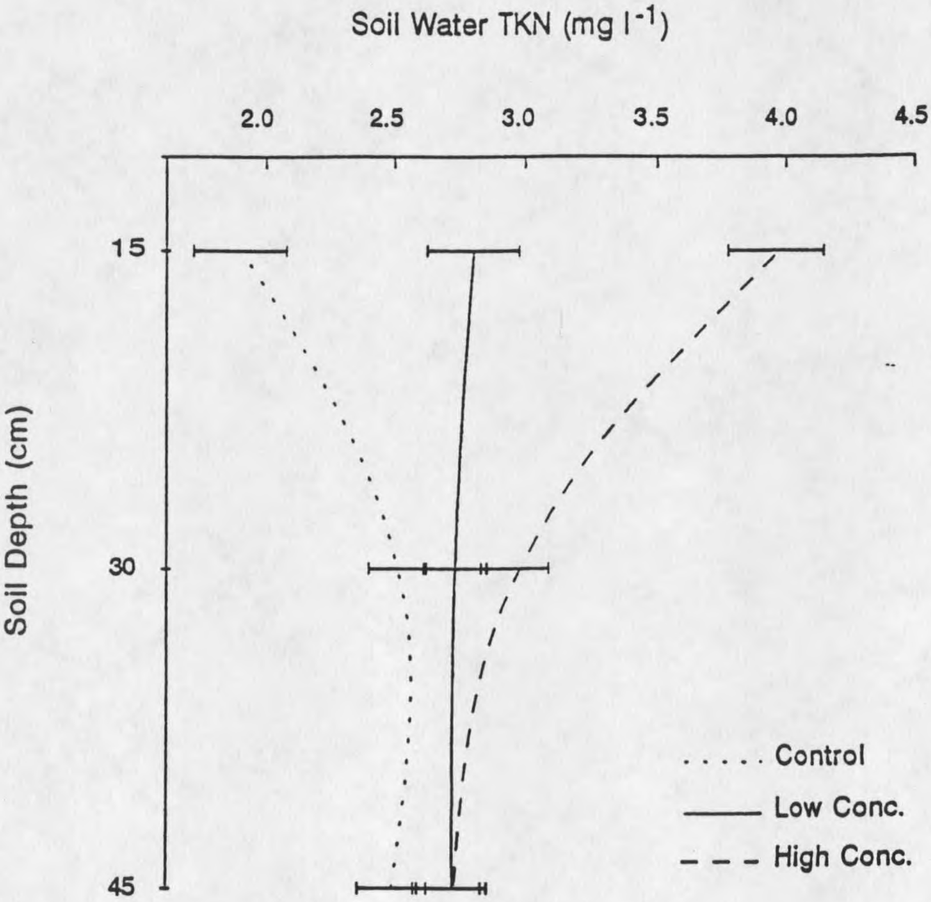

Figure 4. Soil water TKN level (mg l^{-1}) versus depth for three effluent concentrations.

Soil Nitrogen Levels

Soil samples were taken for NO_3^--N and TKN analysis upon disassembly of the microcosms. Nitrate analyses show unequal variance, thus an analysis of variance was not performed to compare plant and concentration treatments. Total soil N levels are shown in Tables 30 and 31. Significant differences occurred between concentrations and within depth by plant and concentration. Differences in total soil N by plant species (Table 30) were observed at the surface 2 cm. Soil N concentration was highest in units planted with *S. microcarpus* and lowest in microcosms containing *C. rostrata*. Table 31 shows that differences by depth across effluent concentration differed only in the surface 2 cm. Analysis of variance showed no interaction between plant and concentration by depth in the microcosm profile.

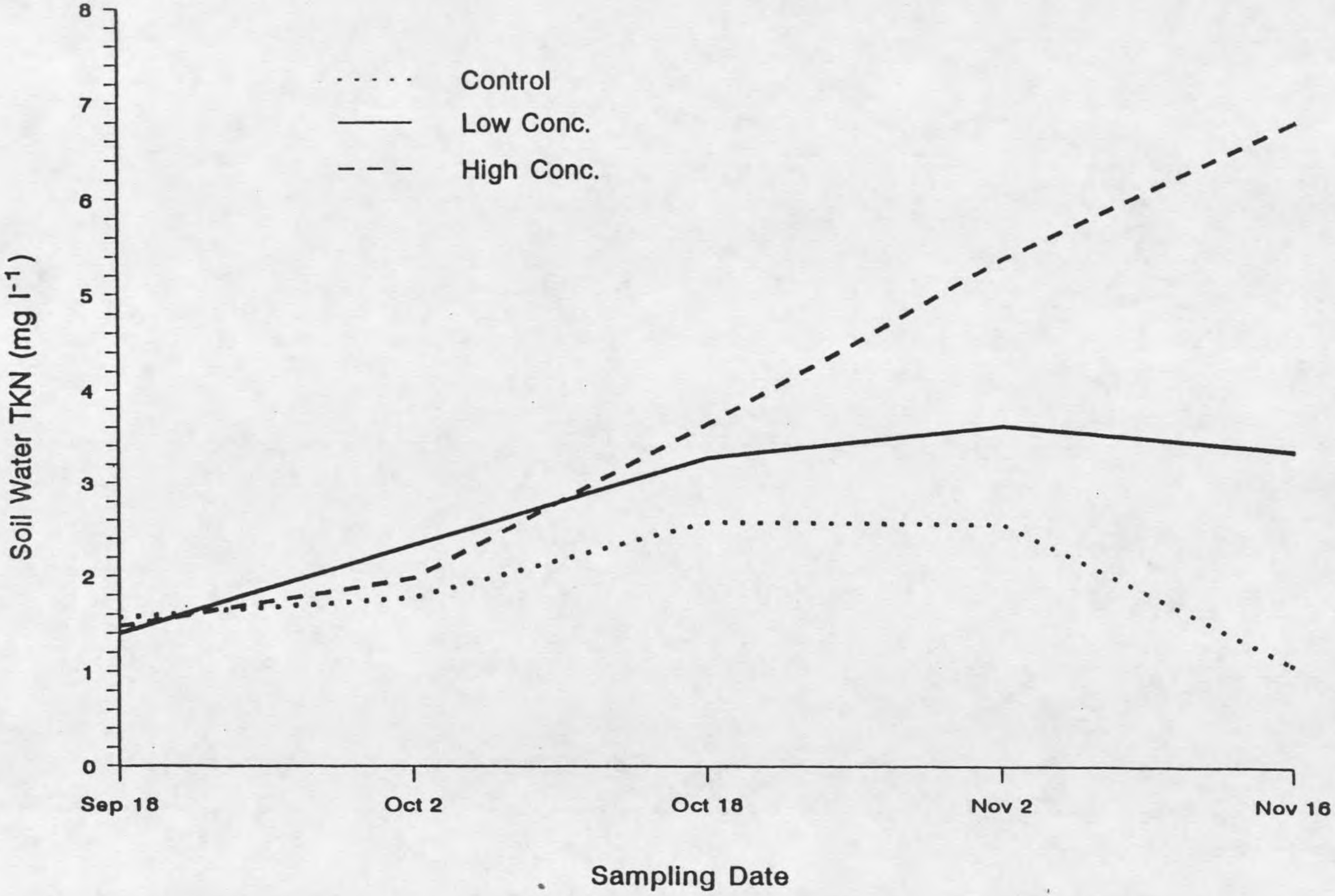

Figure 5. Soil water TKN level (mg l^{-1}) versus sampling date for three effluent concentrations.

Table 30. Mean (± S.E.) total nitrogen (%) in microcosm soil by plant species upon completion of effluent application (n=3).

	Depth (cm)			
	0-2	15	30	45
Caro	0.648 ± 0.115 c[1]	0.449 ± 0.082 a	0.452 ± 0.078 ab	0.469 ± 0.067 a
Cane	0.803 ± 0.237 ab	0.495 ± 0.090 a	0.511 ± 0.080 a	0.504 ± 0.076 a
Tyla	0.764 ± 0.174 abc	0.439 ± 0.082 a	0.422 ± 0.073 b	0.467 ± 0.084 a
Scmi	0.887 ± 0.326 a	0.440 ± 0.082 a	0.468 ± 0.070 ab	0.460 ± 0.089 a
Unpl	0.682 ± 0.154 bc	0.432 ± 0.071 a	0.459 ± 0.060 ab	0.471 ± 0.063 a

1 - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

Table 31. Mean (± S.E.) total nitrogen (%) in microcosm soil by effluent concentration upon completion of effluent application (n=3).

	Depth (cm)			
	0-2	15	30	45
Control	0.624 ± 0.166 c[1]	0.419 ± 0.091 a	0.448 ± 0.086 a	0.459 ± 0.083 a
Low	0.729 ± 0.148 b	0.461 ± 0.069 a	0.473 ± 0.068 a	0.484 ± 0.080 a
High	0.938 ± 0.230 a	0.472 ± 0.076 a	0.469 ± 0.070 a	0.484 ± 0.058 a

1 - Means in columns followed by the same letter are not significantly different ($p \leq 0.05$).

DISCUSSION

The microcosms used in this study represent a model system simulating the mineral soil response of northern temperate wetland ecosystems. Conditions of light and temperature were controlled to approximate climatic conditions typical of the month of June in Montana. Soil-borne invertebrate and zooplankton organisms were present in many of the microcosms and suggest the micro- and macrofauna component of north temperate wetlands were intact at the initiation of this study. Microcosm pH was consistent with soil pH values for wetlands with mineral soils (Ponnamperuma 1984). These microcosms had the capacity to buffer acidity given the soil alkalinity and cation exchange found in the native soils.

Oxidation-Reduction Potential

The lack of significance in redox data is perhaps best explained by the unbalanced design and highly variable readings. Equilibration of the platinum electrodes in the soil medium prior to reading, construction of the electrodes, and microenvironmental effects of electrode placement may have contributed to measurement variability (Cogger et al. 1992).

Perhaps the most appropriate interpretation of the ORP results lies in evaluating the trends with respect to the different treatments. Planted microcosms had larger mean millivolt potentials than unplanted microcosms. Given the capacity for plants to transport O_2 to their rhizospheres, a more oxidized state in planted microcosms was expected. Trends in ORP by depth are also consistent with expected oxidative states. The microcosms, open at the top and bottom and therefore exposed to the atmosphere at both ends, had higher ORP values at these locations.

The relative differences in redox by concentration in planted and unplanted units is interpreted using the Nernst equation, which relates the ORP to the activities of the reactants in the process reaction:

$$E_m = E^{\circ} + 2.3\frac{RT}{\eta F}\log\frac{A_{ox}}{A_{red}}$$

Where:

E_m = *ORP(mV)*,
E° = *Reference electrode constant(mV)*,
R = *gas constant*,
T = *absolute temperature*, $^{\circ}K$,
η = *number of reaction electrons*,
F = *Faraday constant*,
$A_{(\,)}$ = *activity of reactants*: *oxidizers*$_{(ox)}$, *reducers*$_{(red)}$

Systems containing greater activity of reduced species have more negative values than systems containing greater activity of oxidized species. In unplanted microcosms receiving concentrated wastes, the ORP approached a more reduced state than planted units receiving O_2 through plant roots. Further comparisons beyond theoretical evaluation may be made from these data given the absence of statistically significant differences between the treatments.

Plant Characteristics

Plant Nutrient Assimilation

Nitrogen concentration in belowground biomass of *S. microcarpus* was affected by effluent concentration. Nitrogen concentration in aboveground biomass of *S. microcarpus* and *C. rostrata* also changed under high concentrate treatment. A reduction in aboveground biomass accompanied the increased N uptake in *S. microcarpus*. Despite these changes, total N uptake was a function of species-specific nutrient uptake characteristics (Table 13) and not of effluent concentration.

The four plant species clearly differed in N assimilative capacity. *Scirpus microcarpus* and *C. rostrata* assimilated significantly greater amounts of N than *C. nebraskensis* and *T. latifolia*. Total plant weight (Table 8) was lower in *C. nebraskensis* and *T. latifolia* across all concentrations. These differences have been caused by factors related to species-specific characteristics and the growth of these plants in the microcosms. *Carex nebraskensis* grew with great vigor but declined over the latter half of the experiment. It is not known whether this decline was caused by nutrient loading or decreased plant survival under continuously saturated conditions. *Carex nebraskensis* is often located in slightly drier sites than the other three species. *Typha* *latifolia* lacked vigor which may be explained by the late planting date (August 1992) and slow initial response in the greenhouse. Edaphic influences were not thought to be important in plant performance because the plant community at the soil collection site contained all species used in this study except *S. microcarpus*. Differences in total N uptake at low and high concentrations, however, suggest nutrient loading may play a role in reduced N assimilation by *T. latifolia* and *C. nebraskensis* (Table 13). *Scirpus microcarpus*, the plant species with the largest root biomass in the control microcosms, also had greater root N concentration (Tables 6 and 11).

Plant Root Porosity

Root porosity in *C. rostrata*, *T. latifolia* and *S. microcarpus* was not significantly different across effluent concentration levels. *Carex nebraskensis* showed a significant decline in root porosity, a finding that mirrored the previously mentioned decline in plant vigor with increased concentration. *Carex rostrata* had the highest percentage root porosity while *S. microcarpus* had the lowest percent root porosity (Table 14). In general, aerenchyma formation decreased with increased effluent concentration. The negative response of aerenchyma to additions of nutrient was noted by Drew et al. (1989).

Basic differences in root size, number, and volume may explain the differences in root porosity observed between *C. rostrata* and *S. microcarpus*. The root masses of *S. microcarpus* largely consisted of numerous small diameter roots. *C. rostrata* had few larger diameter roots. The response in root porosity formation of these two species to applications of effluent did not affect plant biomass response to effluent application. Performance of both *C. rostrata* and *S. microcarpus*, given the differences in root porosity, suggest that plant survival under high nutrient loading may be a function of relative root size and number (Armstrong 1979), root mass (Chapin 1980), and carbon allocation (Watson and Casper 1984). Based on these results, the hypothesis that increased plant root aerenchyma formation results in increased N removal must be rejected. Total N levels in units containing *S. microcarpus* and *C. rostrata* did not differ over the study period (Table 29) despite differences noted in overall root porosity. Similarly, COD levels did not differ by plant species in a pattern consistent with ranked mean root porosity (Table 23). The suggestion that greater plant root porosity enhances pollutant removal capacity in wetland treatment systems is not supported by the data presented in this study.

Liquid Effluents in the Non-Wetland Condition

More than 60% of NH_3-N in the liquid dairy effluents was lost from open containers placed under a fume hood in a two week period (Tables 16 and 17). Total N fell by 30% during this time. This N loss suggests that NH_3 volatilization is a major N removal pathway in open water application of dairy wastes to wetlands. Denitrification also may occur under these same conditions. The absence of oxidation in the manure samples implied low diffusion of O_2 into the effluents. Similarly, crusting of the surface by solids could contribute to slower O_2 diffusion rates in a column of liquid effluent. The decrease in oxidation-reduction potential provides additional evidence for nitrification in the effluent, as nitrification consumes O_2 in the conversion of NH_4^+ to NO_3^-.

The observed increase in pH of effluents may be explained in two ways. Carbon in the effluent materials imparts CO_2 to bicarbonate buffering capability. Denitrification and the production of OH^- would also increase pH. The pH and low ORP of the effluents were favorable for nitrifying and denitrifying bacteria. Carbon was readily available for microbial carbon assimilation. Nitrification and denitrification yield 1 mole of H^+ for every mole of NH_4^+ nitrified (Barnes and Bliss 1983). Alkalinity of the effluent material buffered H^+ liberated by nitrification and NH_3 volatilization.

Soil Water Characteristics

Soil Water pH

Statistical analysis of the soil water samples taken prior to effluent application showed significant differences in pH by soil depth. The pH in the upper 15 cm was significantly lower than at the two lower depths (Table 19). This result indicated production of H^+ or reduction in buffering capability in the rhizosphere. Plant modification of soil water pH was supported by the significant two way interaction of plant and depth.

Significant differences in microcosm pH at the fifth sampling period were governed by effluent application (Table 18). All microcosms receiving effluent had pH values that were significantly higher than the controls. These data further indicated that microbial nitrification and denitrification occurred in the microcosms. The results of repeated measures analysis showed differences in pH that were influenced by the main effects of concentration and depth. Significant changes in pH at 15 cm soil depth, in microcosms receiving liquid dairy wastes, indicated that this layer was the most biologically active.

Soil Water COD

Soil depth and effluent treatment controlled the differences observed in COD. Concentration was the only significant main effect, and COD level changed over time by soil depth. At the end of the experiment the upper 15 cm soil depth of both concentration treatments generally had greater COD values (Table 22) but was not significantly different than the control concentration (Table 21). This result may indicate that the upper 15 cm layer of soil in the microcosms played the greater role in reducing chemically degradable organic compounds in the dairy effluent.

The significant effects of concentration during the second and third sampling periods, followed by a lack of significant difference in the final sampling period, is explained as follows (Table 21). Given the two week increment between effluent application, it is unlikely that soil chemical equilibria were established. A trend toward increased COD levels over time was also evident, yet this phenomena occurred in both control and treated microcosms. Although chemical equilibria may not have been established by the end of the treatment cycle, microbial growth rate may have attained a maximum associated value with effluent concentration (Barnes and Bliss 1983).

The large amount of variation in COD measurements may have been caused by non-uniform organic matter distribution in the soil media. Soils from the collection site contained undecomposed plant matter that could have contributed to variation in the COD of microcosm aliquots. Root channels and pores formed by the activity of macrofauna also may have created avenues for movement of organics within the profile. Control microcosms (Table 21) consistently had higher baseline COD values than treated microcosms.

Comparison of the dairy effluent COD with soil water COD indicated a substantial reduction in oxidizable organic materials in those microcosms receiving effluent concentrates.

Under these conditions removal pathways may include microbial and faunal assimilation, respiratory oxidation of organic matter to CO_2 and H_2O, and filtration of solid phase organics at the soil-water interface. However, O_2 requirements for these transformations must have been met in both unplanted and planted microcosms since there were no differences in COD among the four plant species' treatment (Table 23). This result follows the evidence that the 30 and 45 cm soil depths were not important in COD removal. Macrofauna and algal growths at or near the soil surface were probably more effective in the decomposition than plant oxygenation of the root zone.

Soil Water NO_3^--N

Analysis of soil water NO_3^- did not provide additional evidence for evaluating the relationship between root zone oxygenation and N removal. However, the low NO_3^- levels observed were consistent with the reduced soil conditions (Table 5). Although rhizosphere oxygenation may have enhanced transformation of effluent NH_3-N to NO_3^- there was no clear relation between NO_3^- and ranked root porosity. Plant assimilation and denitrification at aerobic-anaerobic soil interfaces, however, could quickly consume any NO_3^- produced.

The consistently low NO_3^- levels in soil water samples indicate that wetlands may be used to prevent NO_3^- contamination of receiving streams. Nitrate concentrations were well below EPA limits (EPA 1976). The lack of significance at the 30 and 45 cm soil depth also suggests little if any NO_3^- was removed from the microcosm through soil leaching.

Soil Water TKN

The effects of plant growth on soil water TKN was significant. *Typha latifolia* affected greater total N removal than *C. nebraskensis* and unplanted units (Table 29). This removal, however, was not the result of plant uptake because *T. latifolia* assimilated less N than *S. microcarpus* and *C. rostrata* (Table 13). Further, rooting characteristics and aerenchyma formation in the units planted with *T. latifolia* could not be used to explain this difference (Table 14). Differences in microbial and macrofaunal associations between plant species serves as a possible explanation that may warrant further investigation.

The interaction of soil depth and effluent concentration was also evident in the analysis of TKN (Tables 27 and 28, Figure 4). Differences between concentrations at the soil surface clearly demonstrated that N transformation, soil adsorption, and assimilation occurred in the plant rooting zone. Control unit soil water TKN levels at the 15 cm soil depth indicated translocation or plant uptake to the degree that N was significantly lower in this portion of the soil. High concentration effluent had higher TKN values compared to lower depths of the soil profile (Figure 4). Although the presence of plants reduced soil water TKN levels (Table 29), unplanted units also remained effective in removing N from liquid dairy wastes.

Inspection of soil water TKN values by sampling period (Figure 5) reveals further system specific information in the dynamics of nutrient removal. As mentioned previously in the general description of the microcosms, macrofauna and water column zooplankton were killed under high concentrate wastewater applications. In Figure 5, the curve for high concentrate effluents shows an increase in TKN with each effluent application at the 15 cm soil depth. Two explanations are plausible. The faunal component may have been necessary to effect nutrient cycling and N removal, or the concentration destroyed the removal capability of the system. Low concentrate applications did not significantly increase soil water TKN between the third and fifth sampling periods. The depletion of total N in control units shown in Figure 4 is also evident in Figure 5. Control concentration of soil water TKN declined during the latter half of the study.

Soil Nitrogen Levels

Nitrogen in the surface 2 cm of soil was significantly greater than percent TKN at lower sampling depths (Table 30 and 31). This relationship held across both plant and concentration treatments. Soil N at 15, 30, and 45 cm depths were not significantly different from initial field soil samples (Table 4). Plant species had a significant effect on surface soil N (Table 30) as did concentration of effluent (Table 31). Thus, application of concentrated dairy effluents affects soil surface adsorbed N in saturated soils and does not influence the total N content at depth. Observed differences in plant response are likely the result of differences in rooting characteristics or microbial and faunal plant-host relationships. The similarity between planted and unplanted units with respect to depth suggested that the faunal component was independent of plant activity.

CONCLUSIONS

The reduction of N and organic constituents in constructed wetland microcosms is sufficient to meet EPA water quality requirements. Consistent significance in the interaction of depth and concentration on nitrogen, COD, and soil pH indicated that the upper 15 cm of soil is the zone of active pollutant modification. The results of this study indicate that constructed wetlands may be used for treatment of high organic livestock wastes.

Although the presence of healthy plant growth in wetland microcosms showed higher nutrient removal, the absence of significant statistical difference between planted and unplanted microcosms leaves the role of aquatic macrophytes in wetland treatment systems in question. Nutrient uptake varied by species and did not clearly influence total nutrient removal efficiency. Wetland plants, however, vary in their N assimilative capacity and species-specific characteristics not associated with the nutrient availability may constrain plant response (Chapin 1980).

Root porosity response to additions of high organic wastes also varied according to species-specific characteristics. Survival of the plant species under high nutrient and organic loading was not dependant on aerenchyma volume. Changes in aerenchyma volume were not related to the plant assimilatory capacity of N or the level of wastewater treatment in simulated wetland microcosms.

Given the observed plant performance in this experiment, both planted and unplanted constructed microcosms sufficiently reduce pollutants from livestock waste. *Carex rostrata* and *S. microcarpus* grow well in wetland treatment systems. *Typha latifolia* performance warrants further investigation using plants collected and grown shortly after vernalization. Use of *C. nebraskensis* is not recommended as it appears the tolerance of this plant to dairy effluent loading is limited.

Nitrogen removal does not appear to depend on plant uptake capacity. Other mechanisms, such as NH_3 volatilization, nitrification and denitrification, and the adsorption of reduced NH_4^+ in the solid phase of the soil system may be important. Nitrification and denitrification required an O_2 source, a requirement presumed to have been met by plant root zone oxygenation and the activity of faunal organisms.

Continuity of reduced conditions in the wetland microcosms facilitated nutrient removal in low concentrate effluents. Applications of high concentrate liquid dairy effluent appeared to overload the removal capacity in this wetland treatment system. This loss of capacity may be attributed to washout of the microbial populations, mortality of fauna, decrease in plant-soil oxygenation and nutrient assimilation. Clearly, some optimum levels of dilution of the liquid dairy effluents exist between the two dilution ratios used in this experiment.

Mortality of faunal organisms under high concentrate applications associated with increasing N level suggest that wetland fauna play an important role in nutrient cycling and surface soil oxygenation. Maintenance of these wetland organisms could serve as a basis for further investigation. The implication that the faunal component is an important factor in the treatment of wastewater has been granted little attention in the literature.

In north temperate climates the use of wetlands in wastewater treatment is limited by long winters and cold temperatures. Further, the effect of continual application of wastes over long periods (beyond the two month cycle used in this study) is unknown. These questions should be investigated prior to wide-scale application of high organic wastes to natural wetlands.

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APPENDICES

APPENDIX A

Plant Descriptions

Carex nebraskensis Dewey. Nebraska Sedge.

An important forage plant found in wet meadows, seeps, swamps, and along ditches at elevations from 1000 to 3300 m throughout the Western United States. Plant is tolerant of some alkali. Stout horizonal rootstocks emanate from basally reddish tinged leaves extending to distinctly blue-green bi-dentate leaves standing 2-12 dm tall. Rhizomes long, creeping scaly from which culms rise singly. Leave sessile, 3-11 mm wide borne on lower quarter of the culm, persistent and yellow brown with age (Welsh et al. 1987, Hitchcock and Cronquist 1981, USDA 1970).

Carex rostrata Stokes. Beaded Sedge, Beaked Sedge.

Plants 6-14 dm tall on spongy, bluntly triangular culms rising in groups from robust, scaly, long-creeping rhizomes, often spongy thickened and to one cm thick near base. Roots consisting of both long, deeply extending primary roots and short roots densely situated in the upper rooting zone (15 cm). Plants are circumboreal in ponds, lakes, along streams and swampy meadows generally in quiet water. Elevation range is 1200 to 3500 m (Welsh et al. 1987, Hitchcock and Cronquist 1981, USDA 1970). Bernard and Hankinson (1979) found *C. rostrata* nutrient levels in roots and shots to vary by season and age. Percent N in roots ranged from 0.7 to 1.5% and 2.3 to 5.5 % in shoots. Underground portions of *C.*

rostrata obtain an abundant supply of O_2 via the parent plant (Armstrong and Boatman 1967)

Scirpus microcarpus Presl. Panicled Bulrush.

Plants 6-15 dm tall from curved robust rhizomes. Culms are obtusely triangular thick and rigid toward base and have a red to purple tinge on base of culms. Roots are small diametered, plastic, with large amount of adventitious growth. Prefers streambanks and wet meadows 1300-2800 m in elevation (Welsh et al. 1987, Hitchcock and Cronquist 1981). Rooting is dense at surface with thin roots, the large diametered roots extending deep into substrate.

Typha latifolia L. Broad-leaved Cattail.

Plant 1 to 3 m tall with 12 to 16 7-16 mm wide leaves. A plant of circum global distribution found in slow moving often brackish waters at elevations from 500-3000 m. Roots are from long 4-16 mm diametered rhizomes in upper 20 cm soil. Roots form thick rootstocks (Welsh et al. 1987, Hitchcock and Cronquist 1981). This plant often forms pure stands (Kadlec 1987) in which the plant biomass of the roots comprise some 45-60% of total (as opposed to an average 1-10% in most aquatic plants) biomass. Nutrient levels in cattail are influenced by soil type (Boyd and Hess 1970) with tissue concentrations of N ranging from 5-24 g kg^{-1} (Reddy and Debusk, 1987).

APPENDIX B

COD Regression

Regression Information for COD Standards

Standard solution curve based on serial dilutions of potassium hydrogen phthalate standard with a theoretical COD of 500 g O_2 ml^{-1}. Standard solutions were digested by open reflux method (APHA 1992) with zero standard of distilled water. Absorbance (620 nm) was read on Milton Roy Spectronic 1201.

Table 32. Regression output for COD standards

Constant	0
Std Err of Y Est	102.8893
R Squared	0.959936
No. of Observations	9
Degrees of Freedom	8
X Coefficient(s)	2863.845
Std Err of Coef.	133.6214

APPENDIX C

ANOVA Output

Table 33. Analysis of variance output for redox measurements in planted and unplanted wetland microcosms.

Source	DF	SS	MS	F Value	P > F
DEPTH	2	3574.46	1787.23	0.09	0.9115
PLANT	1	29804.93	29804.93	1.56	0.2314
DEPTH*PLANT	2	43153.77	21576.88	1.13	0.3502
CONC	1	2727.48	2727.48	0.14	0.7112
DEPTH*CONC	2	5760.27	2880.13	0.15	0.8617
PLANT*CONC	1	18428.12	18428.12	0.96	0.3423
DEPTH*PLANT*CONC	2	19529.25	9764.57	0.51	0.6108
Error	15	287399.00	19159.93		

Table 34. Analysis of variance output for biomass of plant roots.

Source	DF	SS	MS	F-Ratio	P > F
CONC	2	63.61	31.80	1.14	0.3371
PLANT	3	899.90	299.90	10.76	0.0001
CONC*PLANT	6	272.95	45.49	1.63	0.1838
ERROR	23	641.47	27.89		

Table 35. Analysis of variance output for aboveground biomass.

Source	DF	SS	MS	F-Ratio	P > F
CONC	2	1504.72	752.36	5.30	0.0128
PLANT	3	9955.71	3318.57	23.39	0.0000
CONC*PLANT	6	623.40	103.90	0.73	0.6286
ERROR	23	3263.22	141.87		

Table 36. Analysis of variance output for total plant biomass.

Source	DF	SS	MS	F-Ratio	P > F
CONC	2	2149.22	1074.61	4.45	0.0233
PLANT	3	15340.03	5113.34	21.15	0.0000
CONC*PLANT	6	1536.17	256.02	1.06	0.4147
ERROR	23	5560.00	241.73		

Table 37. Analysis of variance output for nitrogen concentration in root biomass.

Source	DF	SS	MS	F-Ratio	P > F
CONC	2	1.46	.734	5.93	0.0084
PLANT	3	.86	.289	2.34	0.1001
CONC*PLANT	6	1.59	.266	2.15	0.0861
ERROR	23	2.84	.123		

Table 38. Analysis of variance output for nitrogen concentration in aboveground plant biomass.

Source	DF	SS	MS	F-Ratio	P > F
CONC	2	4.35	2.17	17.24	0.0000
PLANT	3	1.17	0.39	3.11	0.0463
CONC*PLANT	6	1.66	0.27	2.20	0.0798
ERROR	23	2.90	0.12		

Table 39. Analysis of variance output for total nitrogen content of root biomass.

Source	DF	SS	MS	F-Ratio	P > F
CONC	2	125.64	62.82	1.29	0.2940
PLANT	3	1812.14	604.04	12.42	0.0000
CONC*PLANT	6	94.37	15.72	0.32	0.9179
ERROR	23	1118.57	48.63		

Table 40. Analysis of variance output for total nitrogen content of aboveground biomass.

Source	DF	SS	MS	F-Ratio	P > F
CONC	2	1021.49	510.74	0.74	0.4888
PLANT	3	40526.29	13508.76	19.53	0.0000
CONC*PLANT	6	180.39	30.06	0.04	0.9995
ERROR	23	15907.56	691.63		

Table 41. Analysis of variance output for total plant nitrogen uptake.

Source	DF	SS	MS	F-Ratio	P > F
CONC	2	580.19	290.09	0.29	0.7485
PLANT	3	56919.22	18973.07	19.18	0.0000
CONC*PLANT	6	273.56	45.59	0.05	0.9995
ERROR	23	22749.41	989.10		

Table 42. Analysis of variance output for root porosity of four wetland plant species.

Source	DF	SS	MS	F Value	Pr > F
PLANT	3	354.29	118.09	2.93	0.0542
CONC	2	437.49	218.74	5.42	0.0114
PLANT*CONC	6	158.91	26.48	0.66	0.6847
Error	24	967.91	40.32		

Table 43. Repeated measures analysis of variance among subject effects on pH of soil water samples.

Source	DF	SS	MS	F Value	Pr > F
CONC	2	3.92	1.96	23.05	0.0001
PLANT	4	0.46	0.11	1.36	0.2535
CONC*PLANT	8	0.75	0.09	1.11	0.3629
DEPTH	2	0.54	0.27	3.18	0.0464
CONC*DEPTH	4	1.03	0.25	3.05	0.0212
PLANT*DEPTH	8	0.40	0.05	0.59	0.7833
CONC*PLANT*DEPTH	16	1.80	0.11	1.33	0.2006
Error	85	7.23	0.08		

Table 44. Repeated measures analysis of variance within subject effects on pH of soil water samples.

Source	DF	SS	MS	F Value	Pr > F
TIME	4	10.67	2.66	64.68	0.0001
TIME*CONC	8	2.63	0.32	7.99	0.0001
TIME*PLANT	16	0.64	0.04	0.98	0.4808
TIME*CONC*PLANT	32	2.38	0.07	1.80	0.0059
TIME*DEPTH	8	1.52	0.19	4.63	0.0001
TIME*CONC*DEPTH	16	0.59	0.03	0.90	0.5642
TIME*PLANT*DEPTH	32	1.22	0.03	0.92	0.5890
TIME*CONC*PLANT*DEPTH	64	2.04	0.03	0.78	0.8914
Error(TIME)	340	14.03	0.04		

Table 45. Repeated measures analysis of variance among subject effects on COD of soil water samples.

Source	DF	SS	MS	F Value	Pr > F
CONC	2	443732.56	221866.28	7.27	0.0013
PLANT	4	199256.08	49814.02	1.63	0.1741
CONC*PLANT	8	210574.34	26321.79	0.86	0.5511
DEPTH	2	95110.01	47555.00	1.56	0.2167
CONC*DEPTH	4	249570.55	62392.63	2.05	0.0959
PLANT*DEPTH	8	410530.51	51316.31	1.68	0.1157
CONC*PLANT*DEPTH	16	579361.55	36210.09	1.19	0.2967
Error	79	2409447.44	30499.33		

Table 46. Repeated measures analysis of variance within subject effects on COD of soil water samples.

Source	DF	SS	MS	F Value	Pr > F
TIME	2	555874.95	277937.47	8.90	0.0002
TIME*CONC	4	31707.21	7926.80	0.25	0.9070
TIME*PLANT	8	109133.91	13641.73	0.44	0.8974
TIME*CONC*PLANT	16	302888.46	18930.52	0.61	0.8756
TIME*DEPTH	4	492312.64	123078.16	3.94	0.0045
TIME*CONC*DEPTH	8	280860.47	35107.55	1.12	0.3497
TIME*PLANT*DEPTH	16	197442.30	122340.14	0.40	0.9822
TIME*CONC*PLANT*DEPTH	32	553556.36	17298.63	0.55	0.9743
Error(TIME)	158	4933128.41	31222.33		

Table 47. Repeated measures analysis of variance among subject effects on nitrates in soil water samples.

Source	DF	SS	MS	F Value	Pr > F
CONC	2	0.12	0.06	0.83	0.4401
PLANT	4	0.52	0.13	1.77	0.1420
CONC*PLANT	8	0.61	0.07	1.02	0.4236
DEPTH	2	0.51	0.25	3.43	0.0368
CONC*DEPTH	4	0.74	0.18	2.50	0.0483
PLANT*DEPTH	8	0.52	0.06	0.88	0.5386
CONC*PLANT*DEPTH	16	1.07	0.06	0.90	0.5764
Error	88	6.57	0.07		

Table 48. Repeated measures analysis of variance within subject effects on nitrates in soil water samples.

Source	DF	SS	MS	F Value	Pr > F
TIME	4	16.56	4.14	57.06	0.0001
TIME*CONC	8	1.15	0.14	1.99	0.0472
TIME*PLANT	16	1.08	0.06	0.93	0.5345
TIME*CONC*PLANT	32	2.26	0.07	0.97	0.5107
TIME*DEPTH	8	0.86	0.10	1.49	0.1608
TIME*CONC*DEPTH	16	1.00	0.06	0.86	0.6133
TIME*PLANT*DEPTH	32	1.76	0.05	0.76	0.8254
TIME*CONC*PLANT*DEPTH	64	5.15	0.08	1.11	0.2754
Error(TIME)	352	25.54	0.07		

Table 49. Repeated measures analysis of variance among subject effects on TKN of soil water samples.

Source	DF	SS	MS	F Value	Pr > F
CONC	2	70.23	35.11	23.15	0.0001
PLANT	4	20.79	5.19	3.43	0.0125
CONC*PLANT	8	22.69	2.83	1.87	0.0772
DEPTH	2	5.64	2.82	1.86	0.1628
CONC*DEPTH	4	63.22	15.80	10.42	0.0001
PLANT*DEPTH	8	4.67	0.58	0.39	0.9252
CONC*PLANT*DEPTH	16	15.37	0.96	0.63	0.8467
Error	76	115.29	1.51		

Table 50. Repeated measures analysis of variance within subject effects on TKN of soil water samples.

Source	DF	SS	MS	F Value	Pr > F
TIME	4	137.49	34.37	54.91	0.0001
TIME*PLANT	16	81.86	10.23	16.35	0.0001
TIME*CONC*PLANT	32	7.38	0.46	0.74	0.7552
TIME*DEPTH	8	23.60	0.73	1.18	0.2397
TIME*CONC*DEPTH	16	85.50	5.34	8.54	0.0001
TIME*PLANT*DEPTH	32	20.23	0.63	1.01	0.4565
TIME*CONC*PLANT*DEPTH	64	29.09	0.45	0.73	0.9384
ERROR (TIME)	304	190.29	0.62		

Table 51. Repeated measures analysis of variance between subject effects on soil nitrate concentration.

Source	DF	SS	MS	F Value	Pr > F
PLANT	4	378.80	94.70	0.08	0.9872
CONC	2	6163.12	3081.56	2.68	0.0853
CONC*PLANT	8	5121.08	640.13	0.56	0.8035
Error	29	33316.56	1148.84		

Table 52. Repeated measures analysis of variance within subject effects on soil nitrate concentration.

Source	DF	SS	MS	F Value	Pr > F
TIME	3	8075.45	2691.81	3.21	0.0268
TIME*PLANT	12	14077.36	1173.11	1.40	0.1810
TIME*CONC	6	9621.02	1603.50	1.91	0.0873
TIME*CONC*PLANT	24	12101.04	504.21	0.60	0.9208
Error(TIME)	87	72854.31	837.40		

Table 53. Repeated measures analysis of variance between subject effects on soil TKN levels.

Source	DF	SS	MS	F Value	Pr > F
PLANT	4	0.16	0.04	2.35	0.0764
CONC	2	0.41	0.20	12.07	0.0001
CONC*PLANT	8	0.26	0.03	1.90	0.0971
Error	30	0.51	0.01		

Table 54. Repeated measures analysis of variance within subject effects on TKN levels.

Source	DF	SS	MS	F Value	Pr > F
TIME	3	2.94	0.98	167.45	0.0001
TIME*PLANT	12	0.24	0.02	3.44	0.0003
TIME*CONC	6	0.66	0.11	19.00	0.0001
TIME*CONC*PLANT	24	0.15	0.01	1.12	0.3360
Error(TIME)	90	0.52	0.005		