IMPACTS OF MUNICIPAL WASTEWATER EFFLUENT ON SEED BANKS, CHEMISTRY, AND MICROORGANISMS OF SOILS EXCAVATED FROM WETLAND IMPOUNDMENTS DESIGNATED FOR WILDLIFE

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

IMPACT OF MUNICIPAL WASTEWATER EFFLUENT ON SEED BANKS, CHEMISTRY, AND MICROORGANISMS OF SOILS EXCAVTED FROM WETLAND IMPOUNDMENTS DESIGNATED FOR WILDLIFE

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DISSERTATION ABSTRACT

The use of treated municipal wastewater effluent (WWE) has become a common alternative irrigation source for wetlands designated for wildlife. Several workers have reported on the nutrient and pollutant retention capabilities of vegetation and soils in constructed wetlands designed for water treatment. However, reports as to the effects of such high nutrient concentrations and salinity on vegetation and soil properties in wetlands designated for wildlife have received less attention. To evaluate the effects of WWE and Missouri River water (MOR) as irrigation sources on soil chemistry, seed banks, and microorganisms, a field study was conducted in conjunction with a set of greenhouse studies and laboratory microbial assays. Samples of soils collected from WWE-irrigated and MOR-irrigated impoundments at Eagle Bluffs Conservation Area were evaluated for spatial and temporal changes in soil electrical conductivity and pH. The greater soil electrical conductivity and lower pH in the WWE-irrigated impoundment was attributed to greater concentration of electrolytes, organic matter, and ammonium in the WWE. However, soil properties that influence ion exchange and drainage in addition to water-management may have contributed to the significant differences in these soil parameters within and between impoundments. Soil electrical conductivity increased by more than 59% in the WWE-irrigated impoundment from 1998 to 2004 and is likely to increase with prolonged use of WWE.

Results of greenhouse studies show that irrigation with WWE decreased vegetative taxa richness, stem densities, and biomass relative to other irrigation sources. Increases in electrical conductivity and exchangeable sodium resulted from irrigation

with WWE, which altered edaphic conditions and inhibited germination of the seed banks. Additionally, microbial activity was decreased in soil materials irrigated with WWE although microbial abundance was similar among treatments. Increased salinity and sodicity in the soil materials irrigated with WWE were concluded to be responsible for the depressed soil microbial activity.

Wastewater irrigated wetland impoundments may develop elevated levels of salinity and sodicity that alters edaphic conditions and ecological processes. However, heterogeneity of soils in impoundments may promote or prohibit areas of salt accumulation. Soil properties such as drainage, texture, and exchange capacity, as well as hydrologic connectivity to ground-water or surface-water of better quality water (e.g., lower EC), which could flush salts from the soils, should be a consideration for wetland impoundments that receive WWE. This may be particularly pertinent to wetland managers that employ moist-soil practices to stimulate germination of selective taxa from freshwater seed banks. Additional monitoring and research are needed to assess the use of WWE in wetlands in order to prudently use this inexpensive, alternative irrigation source.

Chapter 1

WETLANDS AND WASTEWATER

INTRODUCTION

Vegetation is the most conspicuous sign of the functionality of a wetland. The vegetation of a wetland reflects current and past conditions of climate, water quality, hydrology, and soils. Several studies have linked the use of wetlands by wildlife, notably birds, to specific vegetation composition, form, and density (Weller and Spatcher 1965, Kaminski and Prince 1981, Weller 1981 and 1999, Fredrickson and Taylor 1982, Kantrud 1986). Of the myriad of factors that influence vegetation in a wetland, water has a pivotal role. Water quality and quantity, as well as flood timing, duration, and rate influence vegetation and subsequently the wildlife of wetlands (Swanson et al. 1984, Kantrud et al. 1989, LaBaugh 1989, Swanson and Duebbert 1989, Merendino and Smith 1991, Goslee et al. 1997). According to Swanson and Duebbert (1989), water chemistry of prairie lakes and wetlands not only controls abundance and distribution of key foods and quality of drinking water, but other habitat components such as over-water nesting cover and predator escape cover, which influence use of these habitats by waterfowl and several other trophic levels.

As water sources become more segmented by political forces and are used to satisfy multiple users (Pollice et al. 2004, Brooks et al. 2006), the protection of water sources has become a critical area of concern for those involved in wetlands (Brennan et al. 1985, Reed and Kubiak 1985). One strategy is to use alternative water sources to meet the needs of existing, restored, and created wetlands. The use of wastewater

effluent from various sources (e.g., agriculture, mines, and municipalities) as a water source for the creation or restoration of wetlands has almost become commonplace. However, the use of wastewater on wetlands needs additional investigation to understand how these economical water sources may affect the ecology of these critical habitats.

Because most wastewater effluents contain compounds that are nutrients (i.e., N, P) that promote plant growth (Kadlec 1985), one generally would expect an increase of wildlife as a result of increased primary vegetation production and subsequent detritus material, which plays a major role in wetland energy exchange systems. However, constituents in effluents may alter edaphic conditions that shift vegetation community composition, altering habitat quality and may cause corresponding shifts in other biotic systems such as soil fauna and flora, aquatic invertebrates, and vertebrates.

Constituents of municipal wastewater effluent typically contain large levels of salts, organic matter, soaps, detergents, oils, and grease (Kadlec and Knight 1996, Toze 2005) that can stress ecological processes and organisms (Choules et al 1978, Swanson et al. 1984, Leighton and Wobeser 1994). Salinity can impair seed germination, seedling development, and vegetation richness and productivity (Tisdale and Nelson 1975, Balba 1995, Gough and Grace 1998, Porter et al. 2007). Sodium in particular can disperse soil colloids, which may lead to altering soil pore size distribution, clogging, and decreased hydraulic conductivity of the soil (Szabolc 1989, Ben-hur et al. 1992, Ghassemi et al. 1995, Morshedi and Sameni 2000). Viability of seeds can be compromised by exposure to saline and sodic conditions (Galinato and Van Der Valk 1986). Seed banks may become irreversibly impaired by exposure to salinity and sodicity or response of some species may improve on return to fresher conditions after exposure (Baskin and Baskin

1998). Additionally, large levels of soil salinity and sodicity have been reported to alter soil microbial abundance, biomass, diversity, and activity (Balba 1995, Batra and Manna 1997, Malkawi and Mohammad 2003), which may alter decomposition and nutrient cycling. Some workers have reported that application of wastewater to soils increases microbial biomass, which in turn impedes hydraulic conductivity by clogging soil pores (Magesan et al. 2000). Reports however, on response of fresh-water seed banks to prolonged saline-sodic conditions in managed wetlands that use shallow-flooding or other intensive water manipulations (e.g., moist-soil) to develop targeted vegetation communities, which may enhance salinity, are limited (Galinato and Van Der Valk 1986).

The recent establishment and development of the Eagle Bluffs Conservation Area (EBCA) offers a unique opportunity to examine the effects of municipal wastewater effluent (WWE) on native vegetation and soils. Commissioned by the Missouri Department of Conservation (MDC) in 1997, the EBCA wetland complex is one of a growing number of restored wetland areas among the mid-western states to use WWE as a supplemental irrigation source on seasonal and semi-permanent wetlands. Municipal wastewater is used to supplement water pumped from the Missouri River for management purposes. Although WWE is released onto the site, EBCA is not intended to treat or remove nutrients, pollutants, or other substances from the water. The prime objectives of EBCA are to provide wildlife habitat and public use.

Eagle Bluffs Conservation Area

Eagle Bluffs Conservation Area is an intensely managed riparian wetland complex located along the Missouri River (38° 53'N, 92° 27' W), near the town of McBaine, Missouri. The 1794-ha area site uses a combination of WWE and Missouri River water (MOR) to irrigate 17 managed wetland impoundments. Management controls the irrigation amount and to a lesser extent, the irrigation source (WWE, MOR, or both) for each impoundment. Prior to entering EBCA, the WWE undergoes primary and secondary treatment at the Columbia Regional Wastewater Treatment Plant. Effluent from this facility is directed to the Columbia Constructed Wetland Wastewater Treatment Units (WTUs) for further treatment. The WTUs are three engineered surface-flow wetland impoundments in series with a total land surface area of 36.8 ha, that are designed to treat wastewater. Cattails (*Typha* species) are planted in the WTUs to filter nutrients and other substances from the water and to slow the flow of water across the impoundment. After a retention time of several days, the WWE is gravity fed from the last WTU through an underground piping system to EBCA. Missouri River water is pumped into a main distribution channel that carries a mixture of MOR and WWE to other wetland impoundments. Pumping of MOR is contingent on a sufficient water level in the Missouri River. Costs associated with the pumping of water from the Missouri River (i.e., electricity, maintenance) strain limited financial sources, and costs will likely continue to increase in the future. Wastewater effluent, on the other hand, is virtually a cost-free water source and is available all year. Concentrations of N, P, and Na salts in the WWE are excessive compared to Missouri River water. The WWE, on average, contains approximately 10.71 ± 4.56 mg L^{-1} of total nitrogen (TN) and 2.21 ± 0.64 mg L^{-}

[1]total phosphorous (TP). Average concentration of TN and TP in MOR is 2.14 ± 0.90 mg L^{-1} and 0.39 ± 0.31 mg L^{-1}, respectively (Knowlton and Jones 2003). Both water sources contribute to the estimated 200 t of nitrogen and 40 t of phosphorus that are loaded into the EBCA system annually. Furthermore, the WWE typically contains approximately 4 times more sodium (161.1 ± 36 mg Na l^{-1}), than MOR (42.9 ± 13.9 mg Na l^{-1}) and has a chloride concentration that is approximately twelve times greater (215 ± 46 mg l^{-1}) than MOR (17.9 ± 5.2 mg l^{-1}). Other monitored properties such as trace metals, suspended solids, and micro-nutrients also are inherently different in the WWE compared to MOR.

Research Objectives

Wastewater effluent is an inexpensive and reliable water source for EBCA. However, the effects of the constituents in the WWE on biotic and abiotic systems at EBCA are unknown. Since its conception, surface- and ground-water at EBCA has been monitored by the U.S. Geological Survey and others for signs of pollutants. The fresh-water supply for the city of Columbia is taken from an aquifer beneath EBCA. Hence, water contamination is of the highest concern. Furthermore, MDC has additional concerns as to the effects of WWE on vegetation and soils at EBCA. In the Research and Monitoring Plan for EBCA, MDC states:

> "the overall goal (of the plan) is to monitor the condition of the wetlands resources at Eagle Bluffs Wildlife Area, to evaluate the ecological effects of using wastewater as a water source for management of restored natural wetlands, and to identify emerging problems before they become widespread or irreversible".

Research questions stated in the Plan ask: i) what is the fate of nutrients and contaminants entering EBCA; and ii) which wetland basins are improving or degrading and what are

the likely causes of the changes in those basins (Missouri Department of Conservation 1991).

From these questions proposed by MDC, a field study, a set of greenhouse studies, and laboratory assays were conducted to quantify the effects of WWE and MOR on seed banks, soil chemistry, and soil microorganisms. The goal of these studies was to provide information on the prudent use of this potentially valuable resource for the restoration and creation of wetlands. The objective of the field study was to evaluate WWE-irrigated and MOR-irrigated impoundments at EBCA for spatial and temporal changes in soil EC and pH. The objective of the first greenhouse study was to quantify the response of the seed banks to irrigation with WWE and MOR (Appendix 1, Figure 1). Soil chemistry was assessed to relate seed bank response to changes in edaphic conditions.

Based on the results obtained from the first study, a second study was conducted to evaluate the response of the seed banks previously irrigated with WWE to a lesser saline water source (i.e., MOR). It was hypothesized that changing the irrigation source from WWE to MOR, which had a smaller electrical conductivity (EC) and Na content, would increase vegetation productivity by alleviating the inhibitory effects on germination and seedling development induced by WWE (Appendix 1, Figure 2). The final trial of the second study was conducted to test the hypothesis that the seed banks remained viable after prolonged exposure to WWE-irrigation. The aim was to remove excessive Na concentrations and decrease the soil EC to establish suitable conditions for germination and seedling development in order to assess the resilience (i.e., viability) of the seed banks (Appendix 1, Figure 3).

Because seed germination and seedling development have been shown to be associated with the soil microbial community, abundance and activity assays were conducted on soil samples collected from the greenhouse microcosms to evaluate if irrigation sources had altered these properties. Through these studies and assays, this dissertation provides information on the effects of WWE on seed banks, vegetation, soil chemistry, and soil microorganisms that may be beneficial for the management of wetlands receiving municipal wastewater effluent.

Explanation of Dissertation Format

Chapters two through six are written and formatted as individual manuscripts for publication in preselected peer-reviewed journals. Chapter two is formatted for the journal *Wetlands* and is planned to be submitted to that journal or the journal *Wetlands Management*. Chapter three is formatted for the journal *Wetlands* and has been accepted for publication in 2009. Chapters four and five are formatted for the journal *Wetlands* and have not been submitted. Chapter five may be submitted to the journal *Bulletin of Environmental Contamination & Toxicology*. Chapter six is formatted for the journal *Communications in Soil Science and Plant Analysis* and has been accepted for publication in 2009. Chapters one and seven (Introduction and Summary, respectively) are not intended to be submitted for journal publication and are formatted in the *Wetlands* journal format.

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Chapter 2

EFFECT OF MUNICIPAL WASTEWATER EFFLUENT IRRIGATION ON SOIL ELECTRICAL CONDUCTIVITY AND pH IN WETLAND IMPOUNDMENTS AT EAGLE BLUFFS CONSERVATION AREA

Abstract: After seven years of irrigation with either municipal wastewater effluent (WWE) or Missouri River water (MOR), wetland impoundments were examined for changes in and distribution of electrical conductivity (EC) and pH in soils. These commonly used soil quality indicators reveal conditions important for several ecological processes. The normal pool elevation of the WWE-irrigated impoundment showed significantly greater EC and lower pH than the flood elevation and the impoundment irrigated with MOR. The significant changes in EC and pH in the WWE-irrigated impoundment are attributed to greater electrolyte, organic matter, and ammonium concentrations in the WWE compared to MOR. However, soil properties and water-management of the impoundments may have contributed to changes in these soil parameters. Soil properties such as exchange capacity, texture, and drainage in concurrence with the hydrology of the impoundment can affect distribution of soil salinity and pH. Continuous irrigation with WWE may increase salinity and decrease pH to a point that alters soils, seed banks, vegetation, and other biotic communities of wetlands.

INTRODUCTION

In response to demand and mitigation, the use of various wastewater effluents (e.g., municipal, industry, and mining) has become a common alternative water source for wetlands managed for wildlife. Effluents typically have greater concentrations of nutrients, salts, and metals than natural water sources (Kadlec 1981, Toze 2005). The continuous impoundment of these elevated concentrations may adversely affect wetland habitat quality by altering the chemistry of soils and associated mechanisms and processes.

Salinity is typically a large constituent of municipal wastewater effluent (Toze 2005) and has been shown to impair seed germination (Ayers 1951, Bliss et al. 1984), plant growth (Brock et al. 2005), soil microbial activity (Rietz and Haynes 2001, Finocchiaro and Kremer 2009), and aquatic invertebrates in freshwater wetlands (Swanson et al. 1988, Pinder et al. 2005). Other constituents in the WWE such as organic materials and nitrogen compounds may affect soil pH which controls several ecological processes including availability of nutrients and metals. Soil EC and pH are commonly used to determine the concentration of salts (i.e., electrolytes) and the hydrogen ion activity in the soil solution, respectively. Some workers reported that soil pH and sometimes EC increased after being irrigated with wastewater effluent from a variety of sources (Brockway et al. 1980, Cromer et al. 1984, Neilsen et al. 1991, Qian and Mecham 2005). Schipper et al. (1996) examined the impact of wastewater applied to a forest site and attributed the increase in soil pH to a larger rate of denitrification that produced hydroxyl ions. In contrast, Taha and Malik (2000) reported decreases of 0.2 to 1.1 units in soil pH and a 1.3 mS cm^{-1}decrease in EC in soils that were irrigated with

saline municipal wastewater effluent for 10 to 30 years. Irrigation with alkaline and saline municipal wastewater effluent for two years on agricultural fields resulted in an increase in soil EC with a corresponding decrease in soil pH (Mohammad and Mazahreh 2003). The decrease in soil pH was associated with nitrification of the larger concentration of ammonium that accumulated in the soil due to wastewater irrigation. Oxidation of the ammonium was a source of hydrogen ions, which decreased pH in the soil. Increased soil EC was attributed to the transfer of salts from the wastewater to the soil's exchange complex. Aziz et al. (1996) however, conducted a long-term study of the effects of a petrochemical wastewater effluent on agricultural crops and reported soil pH and EC showed no significant change over an eight year period. Changes in soil pH and EC are related to the chemical composition of the irrigation source; however, soil properties such as exchange capacity and hydrology of the wetland can influence the effect of the irrigation source on these soil parameters (Kelley 1940, Richards 1954, Arndt and Richardson 1989).

To evaluate the impact of WWE on the distribution of soil EC and pH, a field study was conducted on a managed wetland complex that augments a river water source with WWE to irrigate wetland impoundments designated for wildlife. Objectives of the study were to quantify soil EC and pH in impoundments based on irrigation source and flooding regime. The distribution of EC and pH were evaluated in relation to soil properties and water-management. Because of the chemical composition of the WWE, water-management, and properties of the alluvial soils in the impoundments, it is hypothesized that over time soil EC will increase and pH decrease more extensively in the WWE-irrigated impoundment than the impoundment irrigated with river water.

METHODS AND MATERIALS

Study Site

The study was conducted at the Eagle Bluffs Conservation Area (EBCA) located in the Missouri River floodplain near the city of Columbia, Missouri (Figure 1). Acquired by the Missouri Department of Conservation as a wildlife refuge and public use area, the majority of impoundments at EBCA are managed as seasonal wetlands for waterfowl. Water sources for irrigating impoundments at EBCA are the nearby Missouri River (MOR) and WWE from the City of Columbia, Missouri Wastewater Treatment Facilities. The WWE, which has undergone conventional primary and secondary treatment at the city's facilities, is piped directly to Pools 2 and 3, and mixed with MOR in the Distribution Channel where it is used to irrigate the remaining impoundments. Eagle Bluffs Conservation Area is not designated or managed as a tertiary treatment for the WWE. Wastewater was first used as an irrigation source on EBCA's wetlands in 1996.

Water Management of Impoundments

Pool 2 and the River Supply Channel (RSC) wetland impoundments at EBCA were selected on the basis of irrigation source. Pool 2, which is approximately 30 ha, is irrigated with WWE and the approximately 22 ha RSC is irrigated with MOR (Figure 2). Average chemical composition of the water used to irrigate these impoundments is given in Table 1. Pool 2 is managed as a semipermanent wetland. Starting in early summer

(typically June) and throughout the growing season, Pool 2 contains ponded WWE at the normal pool elevation, which is approximately 171.9 m (565.5 ft.) above mean sea level (MSL). The water level is maintained by frequent additions of WWE. In early spring (typically April) and again in late fall (typically October), Pool 2 is irrigated with WWE to flood elevation (172.2–172.9 m (566.5–569 ft.) above MSL) and water level decreases to, and sometimes below, the normal pool elevation over winter. Occasionally, Pool 2 is completely drained of ponded water in mid- to late-summer and re-flooded to the flood elevation in the fall. The RSC is managed as a seasonal wetland with water levels reaching flood elevation in early spring and late fall (approximately 172.6–173.2 m (568–570.2 ft.) above MSL). In early summer, water is at normal pool or below (approximately 171.9 m (565.5 ft.) above MSL). Throughout the summer, water levels decrease to minimal levels (water remains only in lowest elevations as small puddles or in drainage channels). The RSC typically does not contain ponded water over winter. However, water elevations are occasionally altered in both impoundments by the EBCA management in order to control undesirable vegetation, perform maintenance, and provide specific habitats.

Soils of the Impoundments

The upper northwest portion of Pool 2 is mapped as Leta silty clay (clayey over loamy, smectitic, mesic Fluvaquentic Hapludolls) and a small area is mapped as Haynie loam (coarse-silty, mixed, superactive, calcareous, mesic, Mollic Udifluvents) (Figure 3). The small area along the northwest levee of Pool 2 mapped as Haynie was not sampled. Leta soils are slightly effervescent and slightly alkaline (pH 7.4 to 7.8) from the surface

to a depth of 76 cm. Below 76 cm to approximately 100 cm, soils are strongly effervescent and moderately alkaline (pH 7.9 to 8.4). The remaining portion of Pool 2 (northeast and the entire southern portion) is mapped as Darwin silty clay loam (fine, smectitic, mesic, Fluaquentic Vertic Endoaquolls), and has neutral pH (6.6 to 7.3) to a depth greater than 100 cm. Much of the RSC impoundment is mapped as Blake silt loam (fine-silty, mixed, superactive, calcareous, mesic, Aquic Udifluvents) except for small portions of the northwest and southwest, which are mapped as Sarpy fine sand (mixed, mesic, Typic, Udipsamments). Haynie is mapped adjacent to Sarpy in the southwest of the RSC and was sampled (Figure 4). From the surface to a depth of 100 cm, Blake soils are slightly alkaline (pH 7.4 to 7.8) and at depths greater than 100 cm, moderately alkaline. Blake soils are slightly effervescent throughout. Soils mapped as Sarpy are slightly alkaline at the surface to greater than 150 cm depth. Haynie soils are slightly alkaline from the surface to a depth of 33 cm and moderately alkaline to a depth greater than 160 cm.

Parent materials of Leta and Haynie are alluvium, clayey alluvium for Darwin, silty alluvium for Blake, and sandy alluvium for Sarpy. Leta is somewhat poorly drained with slow (upper part) and moderate (lower part) permeability. Darwin is very poorly drained with very slow permeability and Blake is somewhat poorly drained with moderate permeability. Sarpy is excessively drained with rapid permeability and Haynie is well drained with moderate permeability (Young et al. 2003).

Sampling Strategy

To sampling the soils in 2004, we stratified the impoundments by elevation and randomly selected sampling points within each elevation. Stratification of surface elevation was based on normal pool and flood elevations of each impoundment. This stratification was used to compare affects of ponded water duration (i.e., hydroperiod) and water source on soil EC and pH within and between impoundments. Normal pool elevations had longer hydroperiods than flood elevations. For this study, the upper northwest to northeast section of Pool 2, which much is mapped as Leta, served as the flood elevation and the center to southern section, mapped as Darwin, the normal pool elevation (Figure 3). Flood elevation of the RSC encompassed the northwest and southwest sections (mapped as Sarpy and Haynie) and parts of the center section (mapped as Blake) that had visually higher elevations relative to adjacent areas and having sandy surface textures. The lower-lying areas of the center and most of the southern section mapped as Blake, served as the normal pool elevation (Figure 4).

Eight sample points within each elevation of both impoundments were selected. Five of the 16 points in each impoundment corresponded to locations of sampling points used in 1998. The remaining points were randomly selected within an elevation. Location of sampling points for each elevation and impoundment are shown in Figures 3 and 4. A hydraulic soil coring tool (Giddings Machine Company) was used to take one soil core at each sample point. Each soil core was 4.12 cm in diameter and excavated to approximately 1 m in depth. Soil cores were wrapped in plastic film and stored at room temperature. Soil materials of cores were described using guidelines set by Schoeneberger et al., 1998. Soil texture was determined by the hydrometer method (Gee and Bauder 1986). Soil cores were divided into 5-cm depth intervals from 0 to 30 cm and

the last 10 cm of each core (approximately 90–100 cm) for determination of EC and pH. Soil pH was determined using a glass electrode in a 1:1 soil to dionized water saturated paste (Soil Survey Staff 1996). Soil EC was determined using a platinum electrode in a 1:1 soil to dionized water saturated paste (North Dakota Agricultural Experiment Station 1988).

In 1998, five samples of soil were hand-collected from the surface to a depth of 15 cm from both impoundments at locations that included both normal pool and flood elevations used in 2004. Surface soils (0–15 cm) are of particular interest because that is where soil salinity and pH are likely to have the greatest influence on soil organisms, vegetation, root absorption, and the seed bank (i.e., germination) (Froud-Williams et al. 1984, Bonis and Lepart 1994, Benvenuti et al. 2001). Soil pH and EC of the 1998 samples were determined using the previous methods. Soil cation exchange capacity (CEC) of the 1998 soil samples were based on displacement after washing with ammonium acetate at pH 7.0 (procedure 5A8c; Soil Survey Staff 1996).

Statistical Analyses

Soil EC and pH of soil core samples taken from impoundments at different elevations in 2004 were subjected to analysis of variance (ANOVA). A repeated measures ANOVA was applied using SAS 9.1 (SAS institute 2002–2003) with the MIXED procedure (mixed linear model) with soil EC and pH as dependent variables. Because the design included both fixed effects (impoundment, elevation, and depth) and random effects (cores) the mixed model was selected. A significance level of $P = 0.05$ detected differences among depths, elevations, and impoundments. All mean separation

analyses for ANOVA used Least Squares Means comparison testing. Paired student's t-test was used to determine significant differences in mean soil EC and pH of samples collected in impoundments in 1998 and 2004 with a significance level of $P = 0.05$.

Figure 1. Location of Eagle Bluffs Conservation Area near Columbia, Missouri, and the City of Columbia's Wastewater Treatment Facilities.

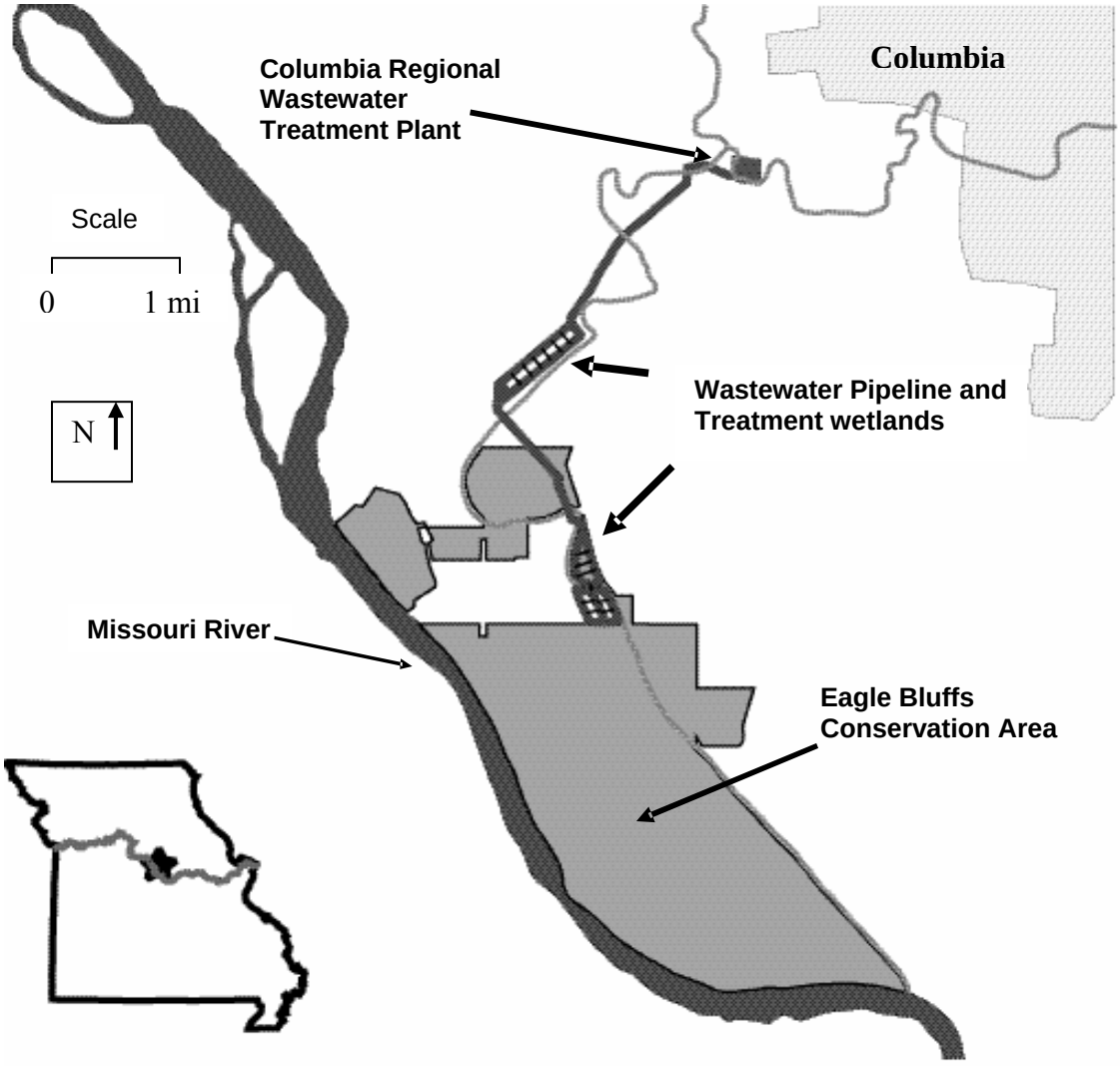

Figure 2. Locations of study impoundments Pool 2 and River Supply Channel at Eagle Bluffs Conservation Area.

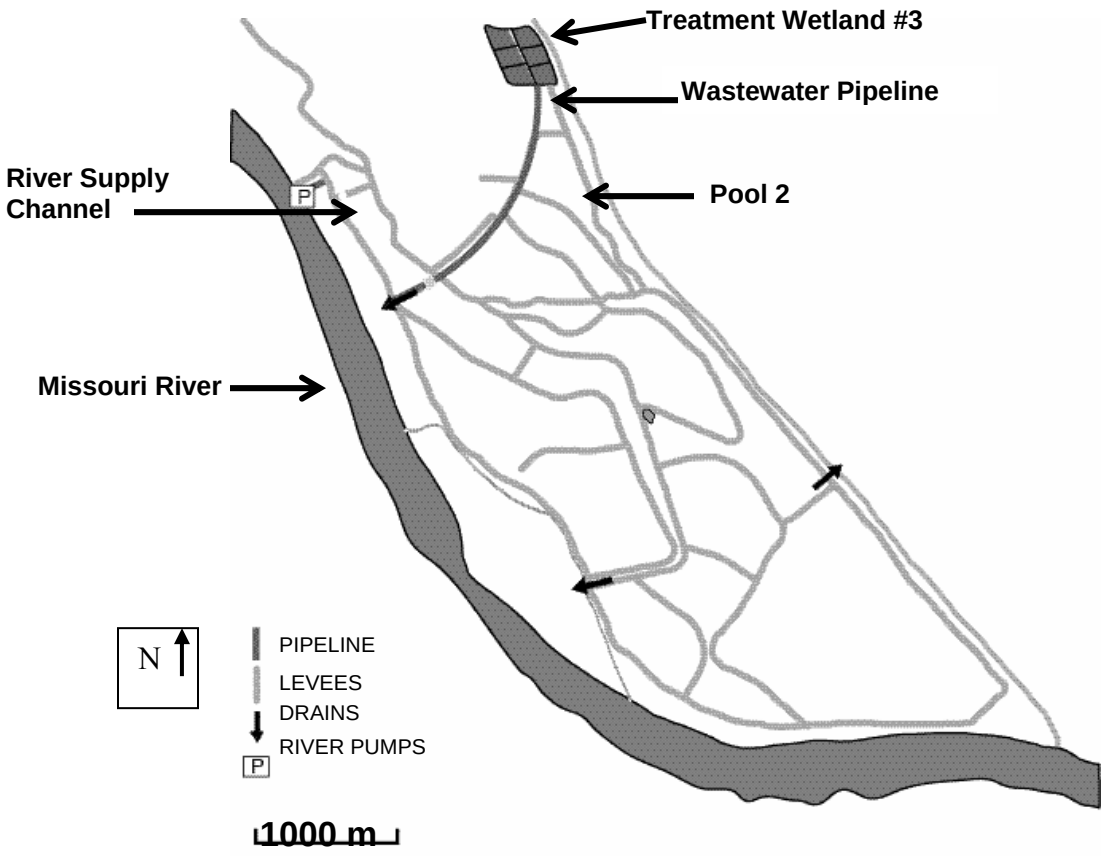

Figure 3. Approximate delineations of soil map units and sampling points in Pool 2 impoundment at Eagle Bluffs Conservation Area (Photo modified from Young et al. 2003). Dashed lines indicate approximate boundaries of the impoundment. Dotted lines indicate approximate soil map units.

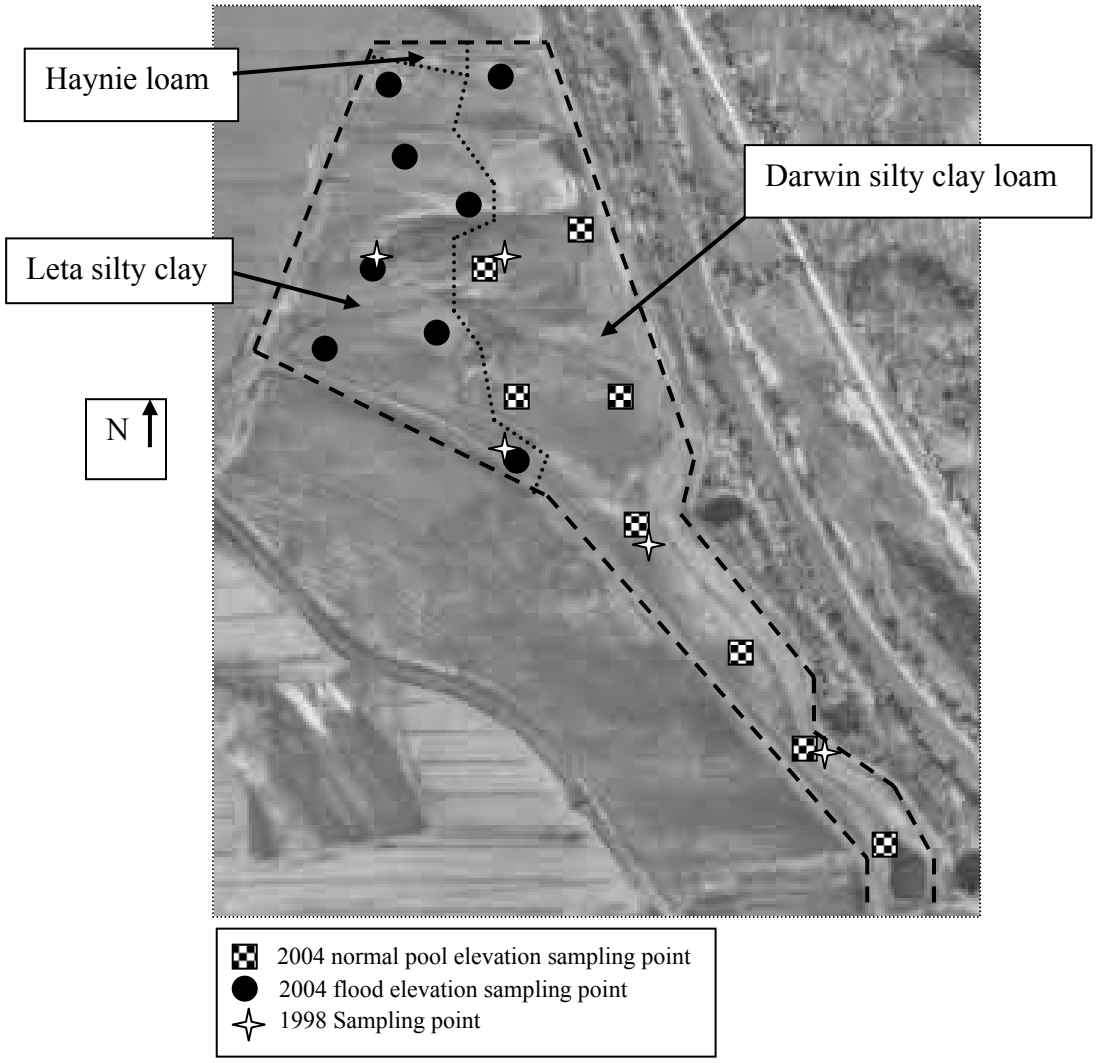

Figure 4. Approximate delineations of soil map units and sampling points in the River Supply Channel impoundment at Eagle Bluffs Conservation Area (Photo modified from Young et al. 2003). Dashed lines indicate approximate boundaries of the impoundment. Dotted lines indicate approximate soil map units.

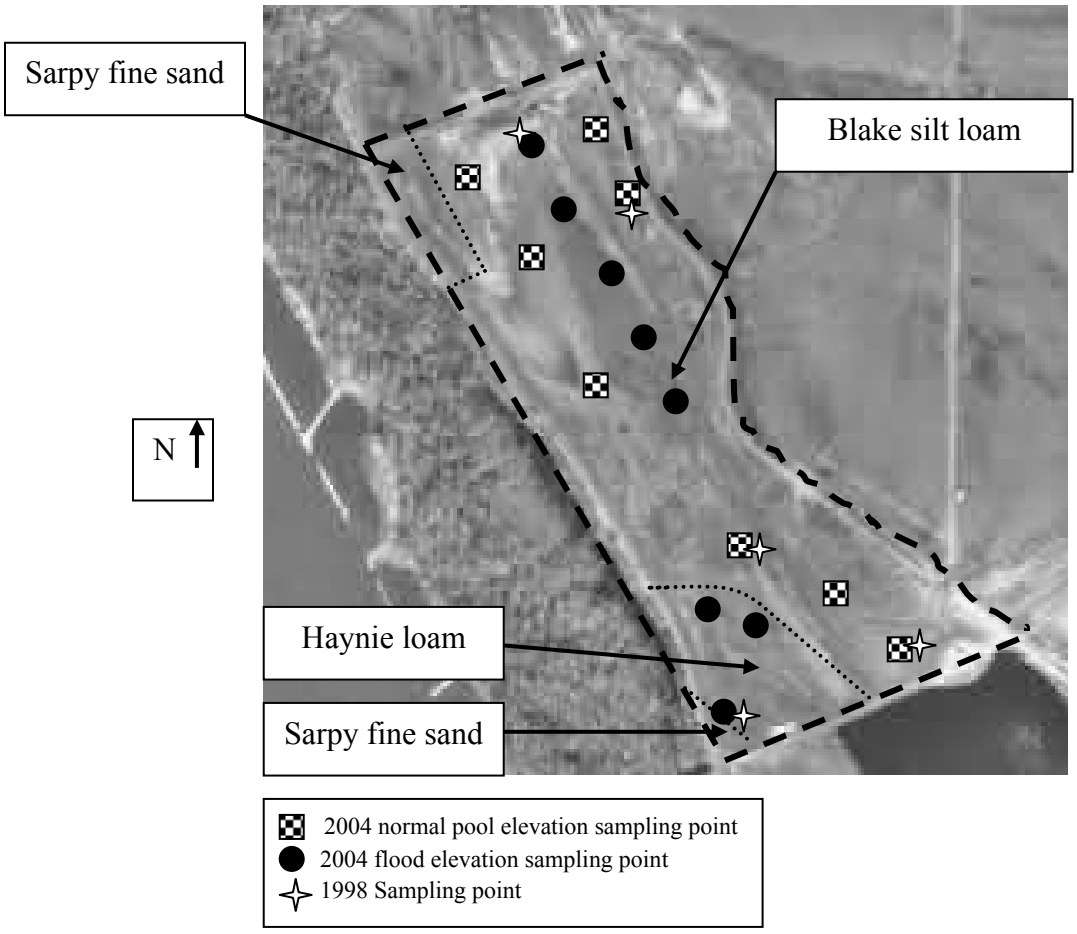

Table 1. Means and standard error (± SE) of chemical concentrations, pH, and electrical conductivity (EC) of Missouri River water (MOR) and municipal wastewater effluent (WWE) used to irrigate the study impoundments at Eagle Bluffs Conservation Area. Values taken from Knowlton and Jones (2003).

Parameter	Irrigation source	
(mg L^{-1})	MOR	WWE
Total N	2.2 ± 0.9	10.7 ± 4.5
NO_3	1.3 ± 0.6	2.0 ± 2.3
NH_4	0.06 ± 0.08	7.36 ± 4.3
Total P	0.4 ± 0.3	2.2 ± 0.6
Alkalinity	164 ± 23	222 ± 25
Cl	17.9 ± 5.2	215 ± 46
pH (units)	8.2 ± 0.22	8.2 ± 0.11
EC (mS cm^{-1})	0.68 ± 0.13	1.34 ± 0.19

RESULTS

Soil EC was significantly different between impoundments but dependent on elevation and soil depth ($F_{18,168} = 2.39$, $P = 0.0021$) (Appendix 2.1). The normal pool elevation of Pool 2 had significantly greater soil EC at all depth intervals than the flood elevation and both elevations of the RSC, except the 90–100 cm interval (Table 2). Among the 90–100 depth intervals, EC was significantly greater in the RSC normal pool elevation than the Pool 2 flood elevation, but similar to the other locations. In the RSC, EC was similar between the elevations for all depth intervals. With all depth intervals combined, the normal pool elevation of Pool 2 had significantly greater mean soil EC (1.231 mS cm^{-1}) than the flood elevation (0.685 mS cm^{-1}) and both the normal pool and flood elevations of the RSC (0.744, and 0.665 mS cm^{-1}, respectively), ($F_{3,28} = 8.15$, $P = 0.0005$).

The normal pool elevations of both impoundments had a smaller change in EC with depth than flood stage elevations (Figure 5). The change in EC from the surface to 5 cm depth of the normal pool elevation was slightly greater in Pool 2 (0.107 mS cm^{-1}) than the RSC (0.89 mS cm^{-1}). However, mean change in EC from the depth of 5 cm to 20 cm was smaller in Pool 2 (0.026 mS cm^{-1}) showing little change compared with the RSC (0.107 mS cm^{-1}). Beyond 20 cm, EC decreased in Pool 2, but increased at the 25–30 cm and the 90–100 cm intervals in the RSC. At the flood stage elevations, changes in EC were more similar between impoundments for all depth intervals.

Soil pH

In general, soil pH increased with depth for both elevations in both impoundments and showed significant differences at the interaction of impoundment, elevation, and soil

depth ($F_{18,168} = 5.07$, $P < 0.0001$). Soils sampled in Pool 2 at the normal pool elevation had significantly smaller pH than all other locations for depth intervals 0–5 through 15–20 cm (Table 2). Soil pH at depth intervals 20–25 and 25–30 cm of the normal pool elevation in Pool 2 were significantly less than both locations of the RSC, but similar to the flood elevation of Pool 2. Soil pH was similar among all locations at the 90–100 cm depth. With all depth intervals combined, the normal pool elevation of Pool 2 had significantly ($P = 0.0005$) smaller mean pH (6.93 ± 0.06) than the mean pH of both elevations in the RSC (7.27 ± 0.06, 7.29 ± 0.06, respectively) and the flood elevation of Pool 2 (7.14 ± 0.06). Mean change in pH of all depth intervals at the normal pool and flood elevations were greater in Pool 2 (0.13, 0.11 units, respectively) than the RSC (0.07, 0.04 units, respectively). Also, the range of pH was greater in Pool 2 than the RSC for either elevation, and greatest at the normal pool elevation of Pool 2 (Figure 6).

Comparison of 1998 and 2004 Samples of Surface Soils

Surface soil samples (0–15 cm) taken from approximately the same positions sampled in 1998 indicate mean soil EC significantly increased and pH significantly decreased in the WWE-irrigated Pool 2 in 2004 compared with 1998 measurements (Table 3; $P = 0.0127$, $P = 0.0087$, respectively). Soil EC in Pool 2 increased from 1998 levels by 59% and pH decreased by 0.6 units in 2004. Soil EC of the RSC in 2004 increased by 25% and pH decreased by 0.2 units, but these changes were not significantly different from the 1998 measurements.

Table 2. Mean soil electrical conductivity (mS cm^{-1}) and pH by depth for soils sampled from normal pool (NP) and flood (FL) elevations in Pool 2 and River Supply Channel (RSC) at Eagle Bluffs Conservation Area in 2004. Means within a column with the same letter are not significantly different (P = 0.05). Standard errors for EC and pH means are ± 0.126, ± 0.08, respectively.

	Depth (cm)						
				Soil EC			
Sample Location	0–5	5–10	10–15	15–20	20–25	25–30	90–100
Pool 2 NP	1.510[a]	1.403[a]	1.373[a]	1.396[a]	1.191[a]	1.139[a]	0.601[ab]
Pool 2 FL	0.981[b]	0.856[b]	0.755[b]	0.721[b]	0.530[b]	0.529[b]	0.424[b]
RSC NP	0.968[b]	0.879[b]	0.780[b]	0.665[b]	0.502[b]	0.612[b]	0.801[a]
RSC FL	1.001[b]	0.799[b]	0.650[b]	0.542[b]	0.510[b]	0.567[b]	0.582[ab]
				Soil pH ($CaCl_2$)			
Pool 2 NP	6.61[b]	6.75[b]	6.77[b]	6.84[c]	7.05[b]	7.12[b]	7.37[a]
Pool 2 FL	6.99[a]	7.19[a]	7.09[a]	7.07[b]	7.15[ab]	7.08[b]	7.38[a]
RSC NP	7.13[a]	7.18[a]	7.31[a]	7.34[a]	7.34[a]	7.38[a]	7.20[a]
RSC FL	7.15[a]	7.27[a]	7.29[a]	7.29[ab]	7.34[a]	7.34[a]	7.37[a]

Figure 5. Mean soil electrical conductivity (EC) by depth of cores collected in 2004 from the normal pool (NORMAL POOL) and flood (FLOOD STAGE) elevations in wastewater irrigated Pool 2 and Missouri River water irrigated River Supply Channel (RSC) at Eagle Bluffs Conservation Area.

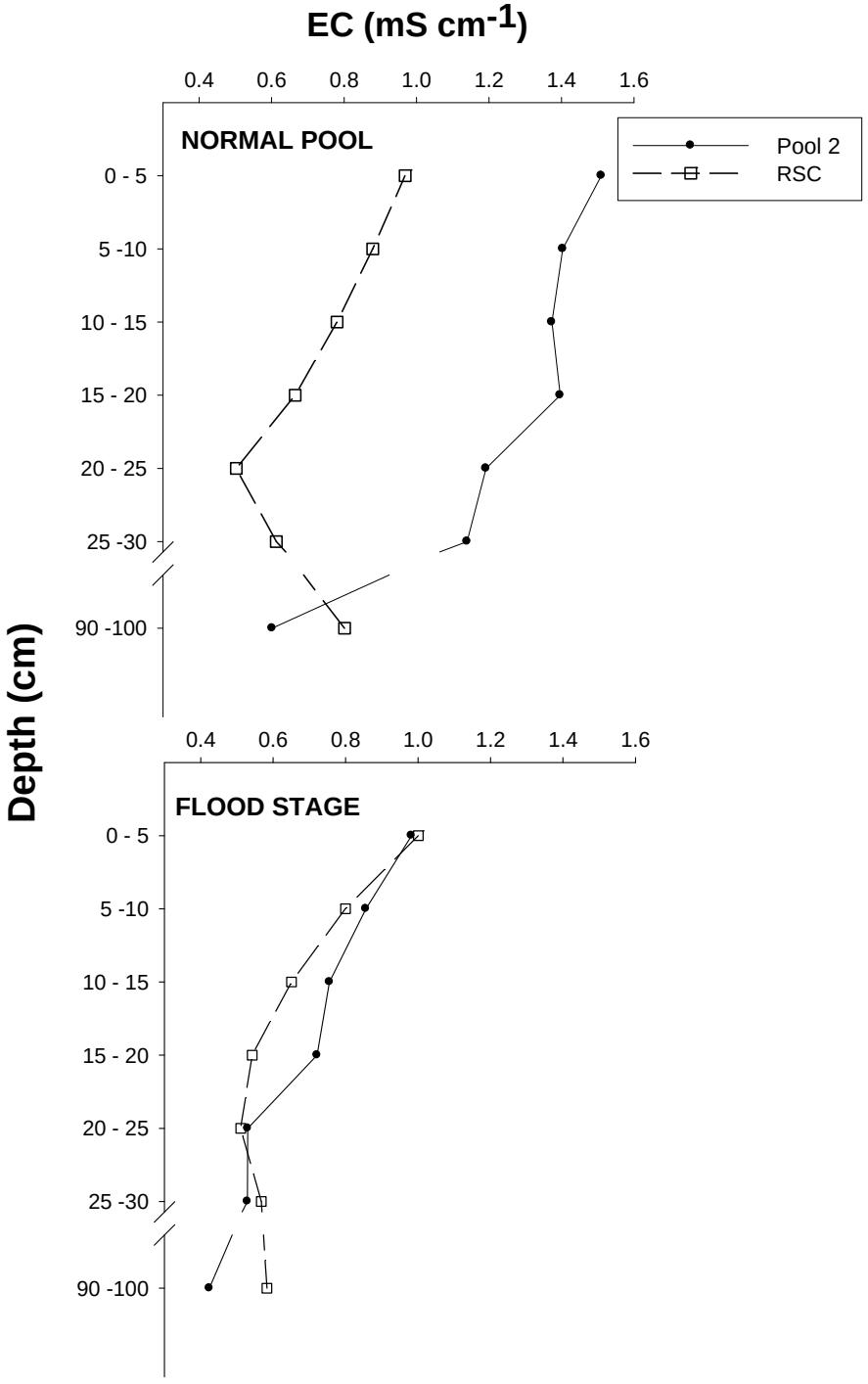

Figure 6. Mean soil pH ($CaCl^{-2}$) by depth of cores collected in 2004 from the normal pool (NORMAL POOL) and flood (FLOOD STAGE) elevations in wastewater irrigated Pool 2 and Missouri River water irrigated River Supply Channel (RSC) at Eagle Bluffs Conservation Area.

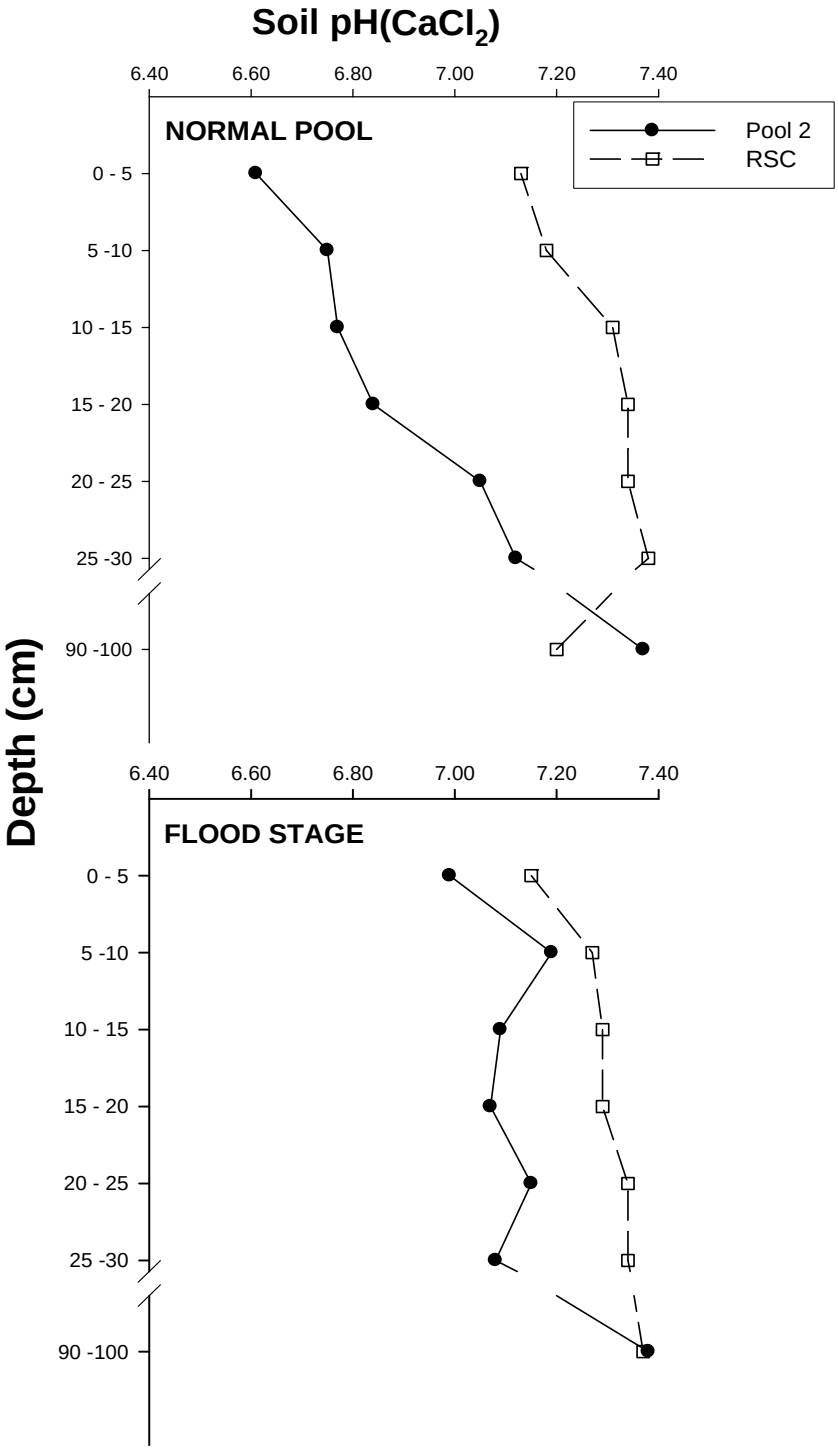

Table 3. Mean electrical conductivity (EC) and pH ($CaCl_2$) of surface soil samples (0–15 cm) taken from the River Supply Channel (RSC) and Pool 2 (regardless of elevation) at eagle Bluffs Conservation Area in 1998 and 2004. Means for 2004 values were calculated from the 0–5, 5–10, and 10–15 cm depth intervals combined. Means within columns followed by the same letter are not significantly different (P > 0.05).

Sample		RSC		Pool 2	
Year	N	EC ($mS\ cm^{-1}$)	pH_{CaCl2}	EC ($mS\ cm^{-1}$)	pH_{CaCl2}
1998[§]	5	0.681 ± 0.206 [A]	7.42 ± 0.03 [A]	0.761 ± 0.166 [A]	7.52 ± 0.03 [A]
2004	5	0.851 ± 0.08 [A]	7.22 ± 0.04 [A]	1.209 ± 0.232 [B]	6.91 ± 0.13 [B]
Difference		0.17 ± 0.398	-0.19 ± 0.21	0.448 ± 0.233	-0.6 ± 0.28

[§] Values taken from Finocchiaro 2002.

DISCUSSION

The greater soil EC in Pool 2 compared with the RSC was probably due to the EC of the irrigation source and promoted by the combination of soil properties in the impoundment and water-management. The WWE used to irrigate Pool 2 contains approximately twice the concentration of electrolytes as the MOR water (Knowlton and Jones, 2003). Because of equilibrium reactions between ions in the soil solution and the exchange complex of the soil, soil EC tends to change in accordance with the EC of the irrigation source (Richards 1954). However, the capacity of the soil to exchange ions often varies with the exchangeable ion, concentrations, and pH (Bohn et al. 2001). Most of these neutral pH soils have a medium range in ion exchange capacity. Mean CEC of surface soil samples collected in 1998 from the normal pool (N = 11) and flood (N = 9) elevations of Pool 2 were 34.6 ± 8.63 cmol kg^{-1} and 25.6 ± 5.17 cmol kg^{-1}, respectively. The mean CEC values from the normal pool (N = 10) and flood elevations (N = 3) soils in the RSC were less than Pool 2 (22.5 ± 5.71 and 5.1 ± 0.01 cmol kg^{-1}, respectively) (Finocchiaro, 2002). These values are within the reported CEC ranges for Darwin and Leta soils (i.e., Pool 2) and for Blake and Sarpy soils (i.e., RSC), respectively (Young et al. 2003) and contributed to the exchange of ions in solution with the soil's exchange complex.

Cation exchange capacity correlates positively to the clay content and organic matter in several soils (Helling et al. 1964, Wright and Foss 1972). However, coarse textures (Syers, et al. 1970), salts (Chapman 1965), and mineralogy (Miller 1970) among other materials, reduces accuracy of these correlations (Seybold et al. 2005). Fine-textured materials (i.e., clayey) were consistently detected in the normal pool elevation

cores of Pool 2 from the surface to an average depth of 51 ± 16 cm. Average depth of fine-textured materials in the flood elevation cores of Pool 2 was 24 ± 15 cm. In contrast, cores in the normal pool and flood elevations of the RSC did not have fine-textured materials and had mostly moderately fine-textured and medium-textured materials (i.e., loams) (soil core descriptions are in Appendix 2.2). The depth of fine-textured materials of the normal pool elevation in Pool 2 may have contributed to a greater retention of cations in the upper portion of the profile and therefore aided in the significantly greater EC in that impoundment. This effect may be reflective of the small changes in EC over the 0 to 20 cm depths in Pool 2 compared to the RSC. In addition, the poor drainage and slow permeability of the soils in Pool 2 in concurrence with the semi-permanent water-management probably aided in accumulation of salts by providing a greater time for exposure of the soil exchange complex to the electrolytes in the WWE.

Comparison of Elevations in Pool 2

In Pool 2, soils of the normal pool elevation had significantly greater EC than the flood elevation from the surface to a depth of 30 cm. The greater soil EC of the normal pool elevation relative to the flood elevation may be attributed to the management of the impoundment as a semi-permanent wetland. Frequent irrigation with WWE to maintain water levels at the normal pool elevation in concurrence with the extended hydroperiod probably allowed a greater accumulation of electrolytes in the soils. In contrast, the flood elevation is infrequently inundated for relatively short periods during early spring and late fall when more precipitation occurs and evaporation is usually less.

Ground-Water Influence on EC

The large increase in soil EC in the normal pool elevation of the RSC at the 90–100 cm interval may be the result of saline ground-water. The ground-water flow of the McBaine Bottoms area, where EBCA is located, has been described as radial at a location southwest of Pool 2 and southeast of the RSC (Smith 2003). Ground-water flows from the southern portion of EBCA and passes beneath the RSC. Smith (2003) reported the altitude of the ground-water reaches an apex of 566 to 567 ft (National Geodetic Vertical Datum of 1929; NGVD29) in the fall and drops to 558 ft (NGVD29) in winter. Altitude of the ground-water apex suggests that the flow gradients are close to the normal pool elevation of the RSC (568–570 ft MSL). It has been demonstrated that surface-water in several of the impoundments at EBCA is readily transported to other areas through subsurface paths (M. F. Knowlton, University of Missouri-Columbia, unpublished data) and to the ground-water through the unconsolidated sediments forming the Missouri River alluvium (Smith 2003). Richards (2002) examined ground-water and surface-water basins in and surrounding EBCA prior to and after the release of WWE onto impoundments and reported increases in Ca, Na, K, Cl, and S after WWE application compared to pre-effluent values.

Ground-water samples from well sites south of the RSC and near Treatment Wetland Units 1 and 2 had a significant increase in specific conductance values between pre- and post-effluent samples (Richards 2002). Average specific conductance of these samples from sites south of the RSC and near the Treatment Wetlands Units are 0.975 and 0.892 ± 0.184 mS cm^{-1}, respectively (average based on values of Figure 4, Richards 2002). These specific conductance estimates of the ground-water that flows toward the RSC are similar to the mean soil EC of the normal pool elevation cores taken from the

RSC at the 90–100 cm depth. Therefore, if the ground-water gradient flow is toward the RSC and at the altitudes indicated by Smith (2003), then the possibility exists that slightly saline ground-water may be responsible for the greater soil EC at the 90–100 cm depth in the RSC.

Soil pH

The smaller soil pH in Pool 2 also reflected the interaction of irrigation source, soils, and water-management. Although pH of the irrigation sources was similar (Table 1), two particular characteristics of the WWE may have contributed to lowering pH; the loading of organic matter and concentration of ammonium nitrogen (NH_4-N). The subsequent decomposition of organic matter and nitrification of NH_4-N can decrease pH. An estimate of the conditions for decomposition and amount of organic material in the WWE can be made with measurements of oxygen demand. Knowlton et al. (2002) reported mean biological oxygen demand (BOD_5) and chemical oxygen demand (COD) concentrations of 8.2 mg L^{-1} and 36.4 mg L^{-1}, respectively, in WWE that entered EBCA (for the years 1994–2000). Estimated BOD_5 of the Missouri River water was unavailable for comparative time periods however, the Missouri Department of Natural Resources estimates BOD of the Missouri River typically is well below 3 mg L^{-1} (J. Ford, Missouri Department of Natural Resources. personal communication 2006). Mean COD concentration was approximately 27 mg L^{-1} in the Missouri River water (M. F. Knowlton unpublished data). The BOD and COD estimates indicate the WWE has greater concentrations of organic matter loading than the MOR and the organic matter is actively being decomposed.

The WWE contains more than 122 times the NH_4-N concentration of the MOR, and most of NH_4-N is converted to NO_3-N while in the impoundments at EBCA (Knowlton and Jones 2003). Hence, the smaller soil pH in Pool 2 may be partly reflective of the decomposition of organics and nitrification of the greater concentration of NH_4-N in the WWE. Additionally, the smaller pH in Pool 2 compared with the RSC may be related to the concentration of calcium carbonates in the soils. Blake and Haynie soils, which are mapped in the RSC normal pool elevation, are slightly calcareous. Leta and Darwin soils mapped in Pool 2 have less concentrations of calcium carbonates (0–2% in the upper portion; Young et al. 2003). Soils with a large percentage of calcium carbonates typically are above neutral pH and well buffered. Laboratory measurements of calcareous soils typically have a pH around 8.3, if exchangeable sodium is small. However, field pH values of calcareous soils are usually less than 8.3 (Bohn et al. 2001). The small concentration of carbonates in the soils of Pool 2 may indicate that buffering capacity was small and the soils' ability to buffer the pH produced by decomposition and nitrification processes was limited.

The significantly smaller pH from the surface to a depth of 20 cm of the normal pool elevation in Pool 2 compared with the flood elevation may also be associated with the semi-permanent water-management of the impoundment. Greater quantities of WWE in the normal pool elevation combined with the longer hydroperiod would provide more organic matter for decomposition and ammonium for conversion than at the flood elevation.

Comparison of 1998 to 2004 sample

The significant increase in soil EC and decrease in pH in Pool 2 compared with the RSC for the same time period indicate that continuous irrigation with WWE has altered these soil parameters more than irrigation with MOR. It is probably the salinity, organic matter content, and ammonium concentration of the WWE, in combination with the soil properties and extended hydroperiod of the impoundment have helped promote these changes.

CONCLUSIONS

The greater soil EC and smaller pH in the WWE-irrigated Pool 2 compared with the MOR-irrigated RSC are attributed to the chemical composition of the WWE in conjunction with the soil properties and water-management of the impoundment. Samples of soils from the impoundment collected in 1998 compared with those collected in 2004 suggest that repeated irrigation with WWE has increased EC and decreased pH. Continued irrigation with WWE in wetland impoundments may increase soil salinity to a point that negatively alters the ecology of soil seed banks (Finocchiaro et al. 2009), soil microorganisms (Pankhurst et al. 2001), and invertebrates (Lovvorn 1999). Soil properties such as drainage, texture, and exchange capacity, as well as hydrologic connectivity to ground-water or surface-water of better quality water (e.g., smaller EC), which could flush salts from the soils, should be a consideration for wetland impoundments that receive wastewater effluent.

Periodic measurement of soil EC and pH may be advisable to monitor these important soil quality parameters. Measurement of soil EC and pH is relatively easy, inexpensive to perform, and provides essential information regarding soil chemistry and nutrient cycling. Additionally, nutrient loading of WWE-irrigated impoundments should be determined periodically to provide a better understanding of its influence on soil pH.

ACKNOWLEDGMENTS

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Chapter 3

IMPACT OF MUNICIPAL WASTEWATER EFFLUENT ON SEED BANK RESPONSE AND SOILS EXCAVATED FROM A WETLAND IMPOUNDMENT

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IMPACT OF MUNICIPAL WASTEWATER EFFLUENT ON SEED BANK RESPONSE AND SOILS EXCAVATED FROM A WETLAND IMPOUNDMENT

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Abstract: Intensive management of wetlands to improve wildlife habitat typically includes the manipulation of water depth, duration, and timing to promote desired vegetation communities. Increased societal, industrial, and agricultural demands for water may encourage the use of alternative sources such as wastewater effluent in managed wetlands. However, water quality is commonly overlooked as an influence on wetland soil seed banks and soils. In four separate greenhouse trials conducted over a 2-yr period, we examined the effects of municipal wastewater effluent (WWE) on

vegetation of wetland seed banks and soils excavated from a wildlife management area in Missouri, USA. We used microcosms filled with one of two soil materials and irrigated with WWE, Missouri River water, or deionized water to simulate moist-soil conditions. Vegetation that germinated from the soil seed bank was allowed to grow in microcosms for approximately 100 d. Vegetative taxa richness, plant density, and biomass were significantly reduced in WWE-irrigated soil materials compared with other water sources. Salinity and sodicity rapidly increased in WWE-irrigated microcosms and probably was responsible for inhibiting germination or interfering with seedling development. Our results indicate that irrigation with WWE promoted saline-sodic soil conditions, which alters the vegetation community by inhibiting germination or seedling development.

Key Words: Eagle Buffs Conservation Area, salinity, sodicity, wetland vegetation

INTRODUCTION

Intensive management of seasonal wetlands for wildlife habitat typically involves flooding and dewatering impoundments seasonally to stimulate development of desirable plant communities (e.g., seed-producing annuals) from soil seed banks. This practice, referred to as moist-soil management (Fredrickson and Taylor 1982), has been used to provide wildlife habitats on more than 80% of the national wildlife refuges (Havera et al. 1996). Traditional sources of water used for intensely managed wetlands are commonly diverted or pumped from rivers, reservoirs, or aquifers. However, the allocation burden on these sources continues to increase as demands from the public, industrial, and agricultural sectors intensifies (EPA 2002). Hence, alternative water sources are constantly being sought to augment traditional sources.

One alternative that has received considerable attention is wastewater effluent (WWE) from municipal treatment facilities. Nitrogen (N) and phosphorous (P) concentrations tend to be greater in WWE compared to other wetland water sources (Kadlec 1981), which can benefit plant biomass and seed production (Mudroch and Capobianco 1979, Kadlec 1981, Finlayson et al. 1986). However, the use of WWE also poses potential risk because some constituents in wastewater may detrimentally affect ecological processes. Salts are of particular concern because WWE often contains elevated concentrations relative to traditional water sources used to manage wetlands (Toze 2005). Large concentrations of Na (sodicity) can damage soil structure (Sumner and Naidu 1998), resulting in altered soil pore size distribution, aeration, infiltration, and hydraulic conductivity, and may dissolve potentially toxic heavy metals from primary and secondary minerals. In addition, salinity also can influence plant community

dynamics (Ayers 1951, Kantrud et al. 1989, Baskin and Baskin 1998). For many freshwater species, excess salinity can impair seed imbibition, germination, and reduce plant growth (Bliss et al. 1984, Pearce-Pinto et al. 1990, Mamo et al. 1996, Gul and Weber 1998, Khan and Ungar 1999). Although the inhibitory effects of salinity on seeds of some species (e.g., glycophytes, halophytes) can be reversed by exposure to less salinity (Ungar 1995, Khan and Ungar 1999), this is not true for all species. Ionic toxicity from salts (i.e., NaCl) may irreversibly impair seed viability (Macharia et al. 1995). Consequently, the repeated use of salt-laden water sources, such as WWE, in freshwater wetlands may ultimately contribute to less diverse vegetation communities (Brock et al. 2005).

The potential risks associated with WWE may be particularly relevant when wetlands are managed to promote moist-soil vegetation due to intensive water level manipulations. Given the popularity of this technique, we designed a greenhouse experiment to more fully evaluate the effect of WWE on soil chemistry and plant community dynamics. Soil materials for this experiment were obtained from a managed wetland complex that has augmented a traditional water source with WWE since the complex's inception in 1996. Our objective was to quantify the soil seed bank response in terms of vegetation richness, density, and biomass to irrigation with WWE. We hypothesize that irrigation with WWE will alter soil chemistry and induce stress to the soil seed bank thereby changing the plant community composition.

METHODS

Collection and Processing of Soil Seed Banks

Soil seed banks and soil materials for greenhouse microcosms were collected during August 1997 from Eagle Bluffs Conservation Area located 9.7 km southwest of Columbia, Missouri. Adjacent to the Missouri River, the 1,794-ha Eagle Bluffs Conservation Area includes 15 wetland impoundments (526 ha) managed for wildlife by the Missouri Department of Conservation. A primary water source for the wetland complex is WWE pumped through a pipeline originating from wastewater treatment wetlands operated by the city of Columbia. Input of this water onto Eagle Bluffs Conservation Area is achieved through a series of control structures. Water from the Missouri River serves as an additional source and can be pumped into Eagle Bluffs Conservation Area through the River Supply Channel that also functions as an impoundment. Water from both sources can be mixed to irrigate most impoundments. The River Supply Channel was selected for collection of soil materials because it has never received WWE. Soil materials were collected when the River Supply Channel was dewatered and soil materials were accessible.

Based on a survey of the River Supply Channel, two surface soil materials (0–15 cm) were selected because they represented the most common surface textures in that impoundment. The soil textures of the materials as determined by the pipet method for particle size analysis (Gee and Bauder 1986) were loamy fine sand (Sarpy; mixed, mesic Typic Udipsamments) and silt loam (Blake; fine-silty, mixed, superactive, calcareous, mesic Aquic Udifluvents) (Young et al. 2003). Approximately 2,000 kg of each soil material was excavated from the surface to an approximate depth of 15 cm and stored

under tarps for 2 to 3 days. Each soil material was mixed and screened through a 1.27-cm^2 hardware cloth to remove rocks and vegetative debris. Three 100-g samples of each soil material were collected for analyses before distribution into microcosms. Samples were air-dried, sieved, and stored at 4ºC until processed for determination of electrical conductivity (EC), pH, and exchangeable bases. Electrical conductivity was determined using the 1:1 soil to water method at 25°C (Whitney 1998) and pH was measured using a combination pH-reference electrode in a 1:1 soil to water and salt solution (0.01 M $CaCl_2$) (SSL Methods 2004). Exchangeable Ca and Mg were determined by atomic absorption and exchangeable Na and K by flame-photometric (emission) methods (Perkin Elmer 560 AA Spectrophotometer). Exchangeable sodium percentage (ESP) was determined following methods described by Bohn et al. (2001).

For each soil material, we constructed 27 microcosms using plastic containers (91-cm length, 61-cm width, 20-cm height). Each microcosm consisted of a 5-cm base layer of clean gravel (5–20 mm dia.) followed by 15 cm (approximately 72 kg) of air-dried soil material. A fine-mesh nursery cloth was placed between the gravel and soil to prevent mixing of materials.

Water Sources

Microcosms for both soil materials were irrigated with one of three water sources: WWE, Missouri River water (MOR), or de-ionized water (DI). Based on water quality data collected from 1994–2001 (Richards 1999, Knowlton and Jones 2003, USGS Missouri River Water Quality Data Base 2006), WWE contained an average of five times more total N and total P, four times more sodium (Na), 12 times more chloride (Cl), and

six times more potassium (K) than MOR water (Table 1). Electrical conductivity and mean turbidity of WWE were approximately two and 13 times greater than MOR (respectively), whereas calcium (Ca) and magnesium (Mg) concentrations in WWE were slightly less than in MOR. Wastewater effluent was collected from the Columbia Wastewater Treatment Unit 3 and MOR water was collected from the river's channel at a location on Eagle Bluffs Conservation Area. De-ionized water was supplied in the greenhouse (Culligan Water Company, Unibed system). To decrease the likelihood of including seeds in water sources during collection, water was collected with a pump fitted with small-aperture screen (approximately 1.3 mm) and water was visually examined for plant material and other debris. However, the addition of seeds to the microcosms through the water sources during irrigation was possible.

Greenhouse Experiment

The experiment consisted of four trials that lasted approximately 100 d each, which allowed most species that germinated from the seed bank to mature (Table 2). Spring and summer trial start dates were used to simulate schedules of water manipulation (flooding and dewatering) commonly used on impoundments managed for wildlife. Microcosms filled with one of two soil materials were randomly assigned to be irrigated with one of the three water sources, which yielded six soil-water combinations (i.e., treatments). Nine microcosm replicates of each treatment were established. Water sources assigned to microcosms were not changed for the duration of the experiment. A randomized complete block design was used to assign microcosms a position in one of nine rows of six mutually exclusive treatments in the greenhouse. Rows and microcosms

within a row were equally spaced apart. This process was repeated for each trial. The greenhouse was temperature-controlled and located in Columbia, Missouri. Air temperature ranged from 20 to 28°C during spring trials (April–July) and 25 to 38°C during summer trials (August–November). During non-trial periods (December–March), temperature ranged from 4.4 to 15°C. Artificial light sources were not used in the greenhouse and natural photoperiod ranged from 12 to 15 hrs of light during spring trials and 11 to 14 hrs during summer trials.

For all trials, microcosms were initially irrigated with assigned water source (i.e., WWE, MOR, or DI) to saturate soil and pond water approximately 5 cm above the soil surface. During each trial, subsequent irrigations were applied to maintain soil water content of microcosms at approximately 80% field capacity. To monitor soil water content, tensiometers connected to mercury manometers were installed in microcosms filled with loamy fine sand and electrical resistance sensors were installed in microcosms filled with silt loam. Based on soil water retention curves developed for each soil material using the pressure chamber method (Klute 1986), we irrigated the loamy fine sand and silt loam when soil water content was below 17% (15 kPa soil water tension) and 30% (20 kPa soil water tension), respectively. For all trials and periods in between trials, microcosms were not drained. The closed design of the microcosms allowed us to simulate conditions that commonly develop from moist-soil practices, shallowly ponded soil, mud flats, and moist-soil, and evaluate irrigation of wetland soils that have a subsoil of slow permeability or may contain a restrictive layer (e.g., claypan), which impedes hydraulic conductivity and leaching. Water movement in the microcosms was primarily

influenced by evaporation and transpiration, which permitted soluble and insoluble constituents in the water sources to accumulate.

Seed Bank Response to Water Sources. During trials, maturing seed heads of plants were covered with fine-mesh cloth or seeds were removed by hand to prevent seed rain onto the soil material. All removed seeds were included in biomass measurements. At the completion of each trial, mean density (plants m^{-2}) and biomass of both alive and senesced plants were recorded by species for each microcosm. Biomass was determined by harvesting all above- and below-ground vegetative parts, rinsing material to remove soil, oven-drying material at 60º C for three days, and weighing ($\pm$ 0.1 g) (Cain and Castro 1959). To facilitate collection of belowground plant biomass and apply equal disturbance to all microcosms, soil material was turned-over and mixed with hand trowels during harvest. The soil surface was leveled after harvest and microcosms were not disturbed between trials. During trial one, plant density was only recorded for a few species (i.e., *Amaranthus tamariscinus, Ammannia coccinea, Echinochloa crus-galli, Polygonum lapathifolium, and Xanthium strumarium*). In subsequent trials, plant density was determined for all species. Vegetation was identified to genus, and species if possible, using Steyermark (1963) and Yatskievych (1999). During non-trial periods, germination in microcosms was minimized by ceasing all irrigation and during non-trial winter months, temperature in the greenhouse was lowered to approximately match the outside ambient temperature. Few plants germinated in between trials (< 10 plants/trial) and these were discarded and excluded from measurements.

Following completion of trial four, a 5-cm diameter core sample of soil material was extracted from each microcosm. Three randomly selected replicates of each treatment were combined to create a composite sample of approximately 100 g; therefore, each treatment was represented by three composite soil samples. Composite samples formed after trial four were analyzed with methods previously described.

Statistical Analysis

Analysis of variance (ANOVA) was performed with soil material and water source as fixed factors blocked within rows of the greenhouse array. A repeated measures ANOVA model was applied using SAS 9.1 (SAS Institute 2002-03) procedure MIXED (mixed linear model) with taxa richness, plant density, and biomass as dependent variables. Because the design included both fixed effects (soil, water, and trial) and random effects (rows), the mixed linear model was selected. Exchangeable bases, EC, and pH of soil materials were analyzed with soil and water factors fixed and blocked within assigned rows of the greenhouse array. Because soil samples were composites of three replicates, each composite sample for a treatment was assigned a different row in order to block by row. In all analyses, $P \leq 0.05$ was considered significant. Fisher's protected Least Squares Means comparison tests were used to separate means following ANOVA results when main effects were significant (Milliken and Johnson 1984).

Principle component analysis (PCA, based on a covariance matrix) was used to examine general relations among microcosms based on the composition of the plants that germinated (CANOCO version 4.5, ter Braak and Smilauer 2002). Biplots were created

for each trial showing the microcosms for each water source as well as plant species vectors.

Table 1. Chemical properties of water sources used to irrigate microcosms during trials. Means are shown with one standard deviation[†] (if available).

	Missouri River Water	Municipal Wastewater Effluent
Total N (mg L^{-1})	2.2 ± 0.9	10.7 ± 4.5
NO_3 (mg L^{-1})	1.3 ± 0.6	2.0 ± 2.3
NH_4 (mg L^{-1})	0.04 ± 0.06	5.0 ± 3.4
Total P (mg L^{-1})	0.4 ± 0.3	2.2 ± 0.6
Alkalinity (mg L^{-1})	164 ± 23	222 ± 25
Cl (mg L^{-1})	17.9 ± 5.2	215 ± 46
SO_4 (mg L^{-1})	166 ± 42	109 ± 17
Ca (mg L^{-1})	64	55
Mg (mg L^{-1})	23	19
K (mg L^{-1})	6	35
Na (mg L^{-1})	42.9 ± 14	161 ± 36
pH	8.2 ± 0.22	8.19 ± 0.11
EC (mS cm^{-1})	0.67 ± 0.13	1.33 ± 0.19
Turbidity (NTU)[‡]	11.1	151.8

[†] Data sources: USGS 1998, Knowlton and Jones 2003, USGS Water Quality Data Base 2006.

[‡] Mean turbidity (M. F. Knowlton, University of Missouri-Columbia, unpublished data 2005).

Table 2. Start and end dates of each trial.

Trial	Start date	End date
1	August 1997	November 1997
2	April 1998	July 1998
3	August 1998	November 1998
4	April 1999	July 1999

RESULTS

Seed Bank Response to Water Sources

We identified 51 plant taxa over all four trials (35 dicots and 16 monocots) of which several taxa are important waterfowl food sources and are intolerant to salinity (Table 3). Of the 51 taxa recorded, 48 occurred in DI, 48 in MOR, and 39 in WWE irrigated soil materials. Total taxa recorded during individual trials ranged from 30 to 38. Average taxa richness differed significantly among water sources ($F_{6,144} = 2.82$, $P = 0.0127$), but differences varied by trial and soil material. During trial one, taxa richness in WWE-irrigated silt loam microcosms was significantly less ($P < 0.0001$) than DI-irrigated silt loam, but similar in richness to MOR-irrigated silt loam (Figure 1). Taxa richness in loamy fine sand microcosms was similar among water sources. In all subsequent trials taxa richness of WWE-irrigated microcosms was significantly less ($P < 0.0001$) than other water sources for either soil material.

Using presence and absence data, ordination results indicated that water source is related to plant species composition over time (Figure 2). In trial one, there were no grouping of microcosms based on plant communities that related to particular water source. All microcosms (water sources) were represented in all directions, indicating that plant communities were relatively similar among water sources. Composition of plant species occurring in WWE-irrigated microcosms started to differ from plant composition in DI- and MOR-irrigated microcosms in trial two and became more pronounced in subsequent trials. By trials three and four, WWE-irrigated microcosms were negatively correlated with most species vectors. In contrast, most species vectors were positively correlated with DI- and MOR-irrigated microcosms.

Irrigation with WWE also significantly reduced plant density compared with both DI- and MOR-irrigated microcosms in the last three trials ($F_{4,96} = 4.65$, $P = 0.0018$; Figure 3A). Similarly, plant biomass was significantly less in WWE-irrigated microcosms than DI- or MOR- irrigated microcosms in trials three and four ($F_{6,144} = 8.50$, $P < 0.0001$; Figure 3B).

At the end of the last trial, concentrations of soil exchangeable Mg in microcosms differed among water sources ($F_{2,10} = 32.29$ $P < 0.0001$; Table 4) and was significantly less in the DI-irrigated microcosms than microcosms irrigated with either MOR or WWE ($P < 0.0001$). Exchangeable K in microcosms also differed among water sources ($F_{2,10} = 44.79$, $P < 0.0001$). Exchangeable K was significantly greater in the WWE-irrigated microcosms than either DI- or MOR-irrigated microcosms (both $P < 0.0001$). Microcosms of all water sources differed in exchangeable Na, EC, and ESP from each other ($F_{2,10} = 131.59$, $P < 0.0001$; $F_{2,10} = 159.51$, $P < 0.0001$; $F_{2,10} = 80.21$, $P < 0.0001$; respectively). WWE-irrigated microcosms had significantly greater exchangeable Na, EC, and ESP than microcosms irrigated with other water sources ($P < 0.0001$), and MOR-irrigated microcosms had significantly less of these three soil attributes ($P < 0.0103$, < 0.0060, < 0.0017, respectively). Exchangeable Ca and pH were similar among microcosms of all water sources.

Table 3. Number of plants germinated from the soil seed bank by water source and trial regardless of soil material. Species known to be waterfowl food sources are in bold face type (Laubhan et al 1996, Stader and Stinson 2005). Salinity tolerance is assigned (0) = none, (1) = low, (2) = moderate, (3) = high tolerance to salinity. Trials two and four started in April of 1998 and 1999 (respectively), and trial three started in August 1998. Taxa occurring in trial one (August 1997) are indicated by an asterisks (*) next to species name if present in all water sources or a letter that represents the water source (D for deionized water, M for Missouri River water, W for wastewater).

			Deionized			Missouri River			Wastewater		
Species (abbreviation)	Wetland Indicator[§]	Salinity tolerance[‡]	Trial 2	Trial 3	Trial 4	Trial 2	Trial 3	Trial 4	Trial 2	Trial 3	Trial 4
Abutilon theophrasti Medic. (abuthe)*	FACU	--	2	9	0	0	19	0	1	5	0
Amaranthus tamariscinus Nutt. (amarud)*	OBL	0	297	130	57	287	138	98	59	115	22
Ammannia coccinea Rothb. (ammcoc)*	OBL	1	423	447	26	161	259	9	19	10	0
Apocynum sp. (aratha)[M]	--	--	0	0	0	0	0	0	0	0	0
Arabidopsis thaliana (L.) Heyn. (aratha)*	--	0	519	1898	311	53	847	73	10	19	0
Bidens aristosa (Michx.) Britt. (bidari)	FACW	0	5	0	0	3	0	0	0	0	0
Bidens frondosa L. (bidfro)*	FACW	0	0	76	0	0	10	0	0	15	0
Callitriche sp. (calspp)[DM]	--	0	0	0	0	0	0	0	0	0	0
Cenchrus longispinus (Hack.) Fern. (cenlon)[D]	--	--	0	0	0	0	0	0	0	0	0
Chaerophyllum procumbens (L.) Crantz (chapro)[W]	FAC+	--	33	13	22	36	12	33	1	2	0
Chenopodium album L. (chealb)*	FAC-	0	13	25	19	29	38	21	13	8	6
Conyza canadensis L. (concan)*	FAC-	0	3	2	1	1	4	0	0	0	0
***Cyperus* sp**. (cypspp)*	--	1-2	941	158	147	740	59	236	196	14	63
Dichanthelium acuminatum (Sw.) Gould & C. A. Clark (dicaca)	FAC	--	0	0	344	0	9	306	0	0	0

***Digitaria* sp**. (digspp)*	--	--	3995	1810	2652	5091	3423	6607	874	310	144
Echinochloa crus-galli (L.) Beauv. (echcru)*	FACW	1-2	3200	142	1478	4699	128	2209	3240	270	507
Eclipta alba (L.) Hassk. (eclalb) ¶	FACW	0	451	223	285	304	182	195	167	148	19
***Eragrostis* sp**. (eraspp)*	--	0	90	72	32	101	87	112	19	17	3
Eupatorium cuneifolium var. *Semiserratum* (DC.) Fern. & Grisc. (eupcus) *	FACW	--	1	0	0	1	0	2	0	0	0
Euphorbia sp. (eupspp)[W]	--	--	4	6	1	5	11	4	0	0	0
Forb (unknown A)	--	--	1	0	0	1	1	0	0	0	0
Fragaria vesca L. (fraves)*	--	--	2	8	0	0	6	2	0	0	0
Grass (unknown A)	--	--	0	0	27	0	0	29	0	0	0
Helianthus maximiliani Schrad. (helmax)	UPL	1	5	0	2	2	0	3	1	0	0
Hemicarpha micrantha (Vahl) Pax. (hemmic)	OBL	--	8	0	0	2	0	0	0	0	0
Ipomoea lacunose L. (ipolac)	FACW	--	2	0	4	5	0	6	2	0	0
Leptochloa filiformis Beauv. (lepfil)	--	--	0	114	0	0	105	0	0	3	0
Leptochloa fusca (L.) Kunth (lepfus)	--	3	13	2	27	30	0	68	26	4	15
Lindernia anagallidea (Michx.) Pennell (linana)	OBL	0	730	0	407	611	0	526	3	0	0
Lippia lanceolata Michx. (phylan)[D]	OBL	--	0	0	0	2	0	0	0	0	0
Lolium sp. (lolspp)[DW]	--	2	0	0	0	0	0	0	0	0	0
Lysimachia nummularia L. (lysnum)[D]	FACW	--	0	0	0	0	0	0	0	0	0
Mollugo verticillata L. (molver)*	FAC	--	73	947	201	12	314	32	1	29	11
Panicum capillare L. (pancap)	FAC	1-2	0	183	0	0	138	0	0	54	0

Panicum dichotomiflorum Michx. (pandic)	FAC-	2	970	0	343	528	0	281	293	0	52
Pancium virgatum L. (panvir)*	FAC+	2	81	23	4	125	74	13	44	37	0
***Paspalum* sp**. (passpp)	--	--	0	0	0	1	0	0	0	0	0
Phytolaccaceae americana L. (phyame)*	FAC-	--	0	0	0	0	0	0	0	0	0
Polygonum lapathifolium L. (pollap)[DW]	FACW+	0	88	0	29	61	0	31	28	0	2
Ranunculus sceleratus L. (ransce)*	FACW+	1	17	2	24	16	0	40	11	0	55
***Ranunculus* sp**. (ranspp)[W]	--	--	1	0	0	2	0	0	0	0	0
Rorippa sessiliflora (Nutt.) Hitchc. (rorses)*	OBL	0	0	0	0	0	0	0	0	0	0
Rotala ramosior (L.) Koehne (rotram)*[¶]	OBL	0	660	129	12	192	29	62	25	0	0
Rumex orbiculatus Gray (rumorb)	OBL	--	6	6	14	1	3	1	2	1	1
Setaria fabreri Herm. (setfab)	FACU+	--	0	0	0	0	0	0	0	1	0
Setaria virdis (L.) Beauv. (setvir)*	--	--	5	0	0	6	0	0	12	0	1
Solanum americanum Mill. (solame)	FACU-	--	6	0	43	17	10	65	11	0	10
Sorghum halpense (L.) Pers. (sorhal)[D]	FACU	1	0	1	0	6	0	0	1	0	0
Veronica peregrine L. (verper)	FACW+	0	579	10	658	234	26	219	0	8	3
Wisteria frutescens L. (wisfru)[D]	NI	0	1	2	2	14	0	0	0	0	0
Xanthium strumarium L. (xanstr)*	FAC	--	0	0	1	3	0	0	0	0	0
Total number of dicots / taxa		35/51	26/34	17/26	20/29	26/36	17/25	19/28	16/25	11/20	9/16

[§]Wetland indicator status based on USDA, NRCS. 1998.

[‡]Salinity tolerance for many species may vary by salt type, life stage, population, duration of exposure, temperature, and other factors (Lieffers and Shay 1983). Ratings are based on soil electrical conductivity (solution extract): none = 0-2 dS/m; low = 2.1-4.0 dS/m; medium = 4.1-8.0 dS/m; high > 8.0 dS/m (USDA, NRCS. 2008, Stewart and Kantrud 1972, Xiong and Zhu 2002)

[¶] Salinity tolerance estimated (Flynn et al. 1995)

Table 4. Mean Exchangeable bases, electrical conductivity (EC), exchangeable sodium percentage (ESP), and pH of both soil materials before start of trials (none) and after trial four that were irrigated with deionized water (DI), Missouri River water (MOR) or

Water Source	Exchangeable Bases (cmol kg^{-1})				EC ($mS\ cm^{-1}$)	ESP (%)	pH(salt)
	Ca	Mg	K	Na			
none	25.9 ± 8.8	3.2 ± 1.6	0.3 ± 0.14	0.2 ± 0.1	0.2 ± 0.0	0.64 ± 0.3	7.2 ± 0.0
DI	23.3 ± 1.0 [a]	2.7 ± 0.1 [a]	0.4 ± 0.03 [a]	3.1 ± 0.2 [a]	4.1 ± 0.2 [a]	11.9 ± 0.8 [a]	7.3 ± 0.05 [a]
MOR	25.8 ± 1.0 [a]	4.0 ± 0.1 [b]	0.4 ± 0.03 [a]	2.3 ± 0.2 [b]	3.3 ± 0.2 [b]	7.5 ± 0.8 [b]	7.3 ± 0.05 [a]
WWE	24.5 ± 1.0 [a]	3.9 ± 0.1 [b]	0.7 ± 0.03 [b]	6.8 ± 0.2 [c]	7.9 ± 0.2 [c]	20.3 ± 0.8 [c]	7.4 ± 0.05 [a]

municipal wastewater effluent (WWE). Means (LS means ± 1 SE) within columns followed by the same letter are not significantly different ($P > 0.05$).

Figure 1. Number of plant taxa (Least Square Means) by water source and trial for silt loam and loamy fine sand. Water sources were deionized (DI), Missouri River (MOR), and municipal wastewater effluent (WWE). Different letters above columns indicates significant differences ($P < 0.05$) among water sources in that trial. Vertical bars within a column represent one standard error.

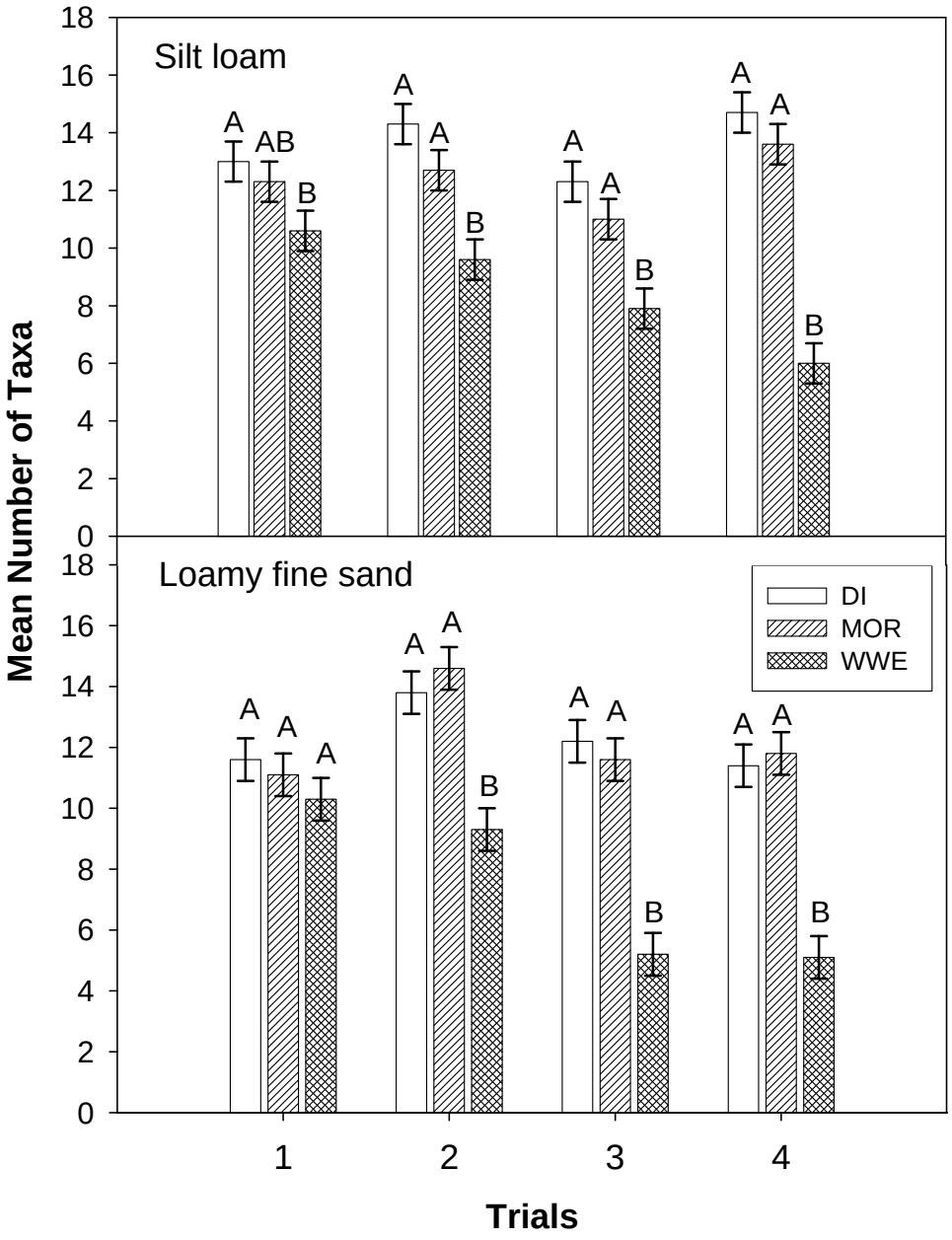


Figure 2. Biplots produced by principal components analysis of presence/absence data for species that germinated in microcosms during each trial. Microcosms were irrigated with deionized water (circles), Missouri River water (squares), or wastewater effluent (diamonds). Species vectors (arrows) point towards the corresponding species acronym, which are defined in Table 3. Species with a fit range of less than 10% were excluded from the biplots. Data are from 18 replicates of each water source regardless of soil material. (next page)

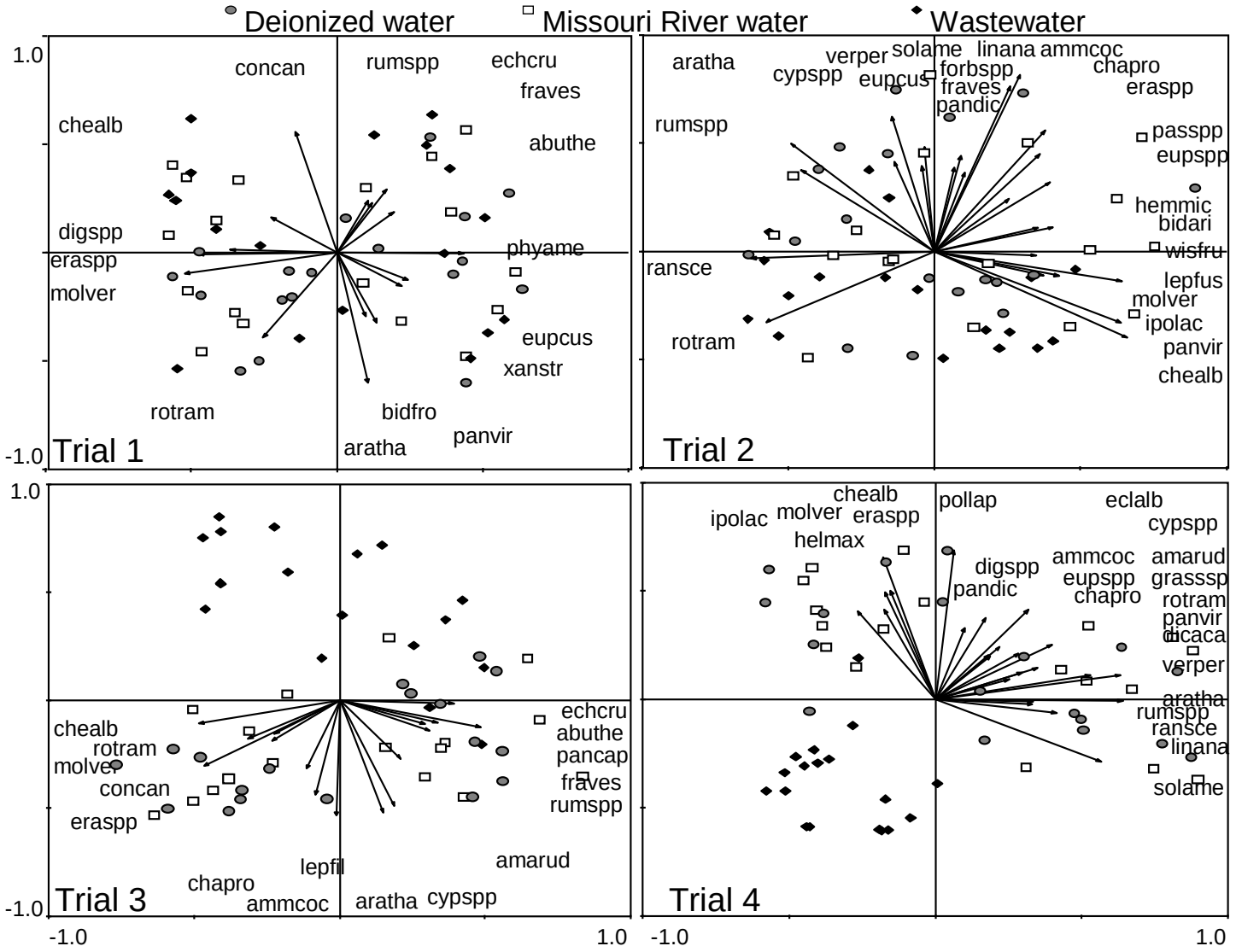
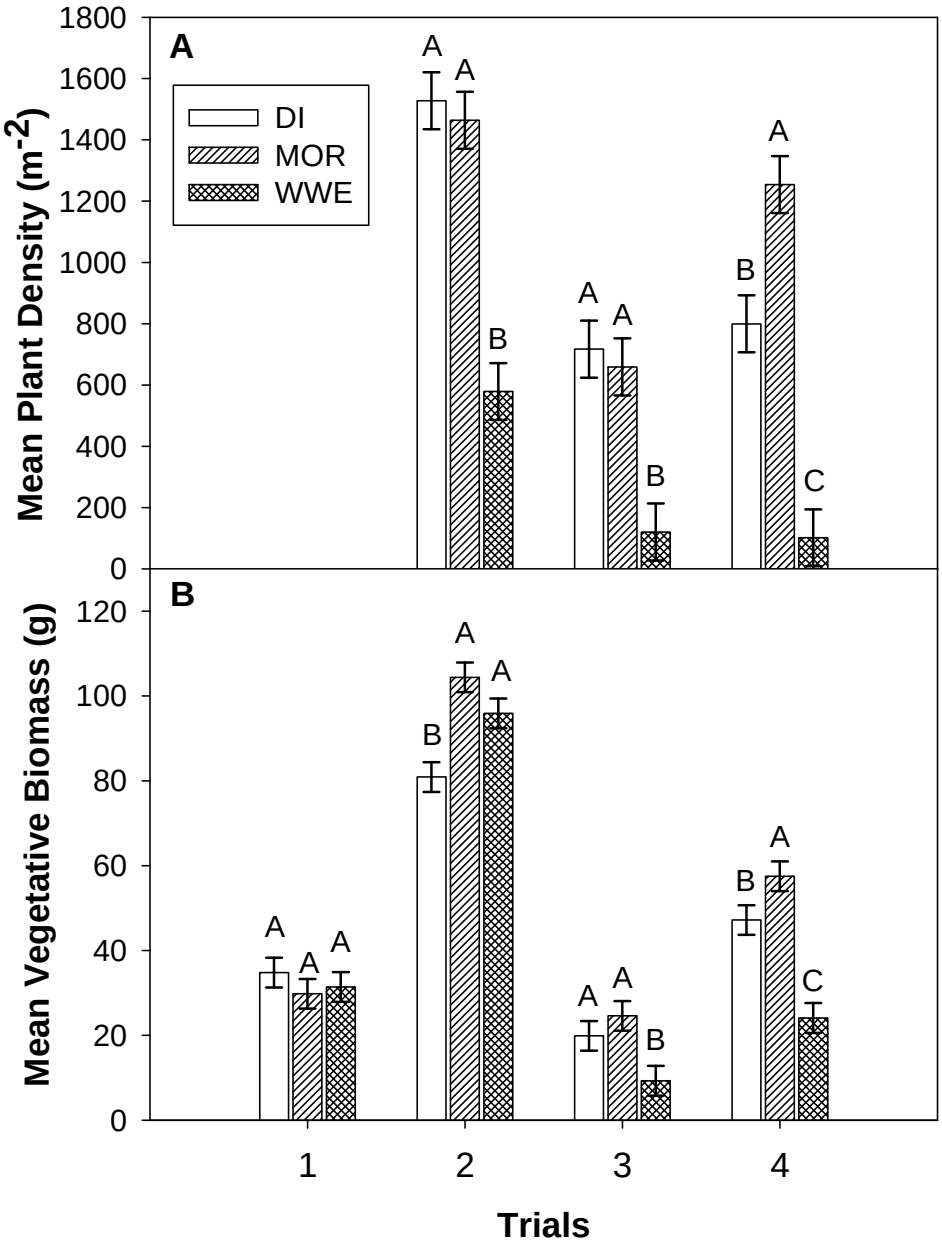

Deionized water
Missouri River water
Wastewater
Trial 1
concan
rumspp
echchru
fraves
chealb
abuthe
digspp
phyame
eraspp
molver
eupcus
xanstr
rotram
bidfro
panvir
aratha
Trial 2
aratha
verper
solame
linana
ammcoc
cypspp
eupcus
forbspp
fraves
chapro
eraspp
pandic
rumspp
passpp
eupspp
hemmic
bidari
wisfru
ransce
lepfus
molver
ipolac
rotram
panvir
chealb
Trial 3
chealb
rotram
molver
concan
eraspp
echcru
abuthe
pancap
fraves
rumspp
lepfil
amarud
chapro
ammcoc
aratha
cypspp
Trial 4
chealb
ipolac
molver
eraspp
helmax
pollap
eclalb
cypspp
digspp
ammcoc
amarud
pandic
eupspp
grasssp
chapro
rotram
panvir
verper
aratha
rumspp
ransce
linana
solame
1.0
-1.0

Figure 3. Least-squares means of plant density (A) and vegetative biomass (B) by water source and trial regardless of soil material. Water sources were deionized (DI), Missouri River (MOR), and municipal wastewater effluent (WWE). Plant density not record for all species in trial one therefore it was omitted. Different letters above columns indicates significant differences ($P < 0.05$) among water sources in that trial. Vertical bars within a column represent one standard error.



DISCUSSION

Our study suggests that irrigation with WWE may not initially affect plant recruitment from the seed bank (i.e., trial one), but repeated exposure may eventually decrease the diversity of seeds that germinate and also affect plant density and biomass. Collectively, our results also suggest that changes in taxa richness, density, and biomass probably were the result of germination inhibition caused by increases in EC and ESP in the WWE-irrigated soil materials. Compared to MOR-irrigated microcosms, EC and ESP more than doubled in the soil materials irrigated with WWE. In fact, by the end of the experiment, WWE-irrigated materials had such substantial increases in EC and ESP that they were classified as saline-sodic (Havlin et al. 1999). Significant increases in soil salinity and sodicity can inhibit seed germination by restricting imbibition and causing Na and Cl ion toxicity (Bewely and Black 1982, Mansour 1994, Al-Karaki 2001). Sodium, in particular can be detrimental because, under certain conditions, Na can alter soil structure, thus impeding hydraulic conductivity, leaching, and root penetration (Oster 1982, Qadir et al., 1996).

We compared EC of soils at Eagle Bluffs Conservation Area impoundments receiving WWE or MOR between 1998 and 2004. Over the six-year period, EC increased 59% (0.76 ± 0.17 mS cm^{-1} to 1.21 ± 0.23 mS cm^{-1}) in a WWE-irrigated impoundment and increased 25% (0.68 ± 0.41 mS cm^{-1} to 0.85 ± 0.13 mS cm^{-1}) in a MOR-irrigated impoundment. These measurements are substantially less than those measured in microcosms at the end of trial four. However, measurements of EC in impoundments were based on a general sampling scheme and were not focused on specific areas that may have subsoil stratigraphy and hydrology that could be more conducive to

accumulation of salts. Additionally, several factors are involved such as precipitation and natural flood events that could affect the soils of these impoundments.

The inhibitory effect on germination induced by irrigation with WWE appeared to gradually affect a wide variety of species in the seed banks regardless of their salinity tolerance. For example, *Chenopodium album, E. crus-galli,* and *Pancium virgatum*; which are intolerant to moderately-tolerant to salinity, respectively, (0 - 8 dS m^{-1}; USDA-NRCS 2008, Rahman and Unger 1990), showed declines in abundances in almost all trials. Moreover, the highly salinity tolerant *Leptochloa fusca* (USDA-NRCS 2008) declined in WWE-irrigated microcosms, while abundance of this species increased in microcosm irrigated with DI or MOR. This wide-spread effect may impair the value of waterfowl habitat because approximately 50% of the germinated taxa from these seed banks are known waterfowl food sources.

From the biplots of each trial, and Table 3 it is possible to see that irrigation with WWE inhibited a wide variety of species regardless of salt tolerance or habitat preference (i.e., wetland indicator status). In trial two, a few taxa (i.e., *C. album, Cyperus* sp., *Ipomoea lacunose, L. fusca, Mollugo verticillata, P. virgatum, Ranunculus sceleratus,* and *Rotala ramosior*) were still positively correlated with WWE-irrigated microcosms. By trial four, no taxa were positively correlated with the majority of WWE-irrigated microcosms.

If the WWE-irrigated microcosms were leached of salts, it is likely that more seeds and species would germinate once the induced inhibitory effects diminished. Several other workers have reported increased seed bank response after leaching; indicating that exposure to saline-sodic soil conditions did not irreversibly impair the

viability of the seed banks (Walsh et al. 1991, Foderaro and Ungar 1997, DiTommaso 2004). Leaching also may influence germination and seedling development by removing germination-inhibiting compounds, such as abscisic acid from the seed coats (Wareing and Foda 1957, as cited in Baskin and Baskin 1998).

Some studies have reported greater salinity sensitivity in dicotyledous species than monocotyledous (Blanchar 2000, Davies et al. 2004). Results from these trials also suggest dicots in these soil seed banks may be more sensitive to soil salinity-sodicity than monocots. The WWE-irrigated microcosms had a greater overall net loss in the number of dicotyledous species (20%) across all trials than DI- (3.4%) or MOR-irrigated (6.1%) microcosms. In contrast, the number of monocotyledous species had an overall net gain for any water source.

Almost all species regardless of water source, decreased in species abundance as trials progressed. This was probably related to the use of the seed bank reserves without recruitment. Because seeds of maturing plants were kept from replenishing the seed banks, seed reserves declined over time. However, plant densities of more than 800 to 1300 (plants m^{-2}) in DI- and MOR-irrigated microcosms (respectively) imply that abundance of viable seeds still persisted by the end of trial four, indicating that reserves were not depleted. Species diversity in the microcosms may have been restricted because collection of seed bank materials in August may have excluded species (i.e., transients) from the seed bank materials and therefore would not be represented in the vegetation composition.

CONCLUSIONS

Repeated irrigation with WWE on seed banks of soil materials excavated from wetlands at Eagle Bluffs Conservation Area decreased vegetative taxa richness, plant density and biomass. Seed germination and perhaps seedling development in WWE-irrigated soil materials was probably inhibited by the substantial increase in soil salinity and sodicity. Although these experiments were conducted in closed microcosms, which probably accelerated salt accumulation as water evaporated and vegetation transpired; irrigation with WWE escalated development of saline-sodic conditions relative to MOR as a water source and these soil conditions significantly impaired the vegetation community.

Wetland managers that employ moist-soil practices and use saline or sodic water sources on impoundments that contain seed banks comprised primarily of freshwater species may experience similar results. Irrigation with WWE on wetland impoundments that contain soils of slow permeability or with restrictive layers (e.g., pans) and not hydrologically connected to a freshwater source (e.g., ground water, flood water) may develop concentrations of salinity and sodicity that can alter composition of the vegetation communities. Elevated salinity and sodicity may adversely affect other wetland biotic systems such as the microbial community (Finocchiaro and Kremer 2009). Connection to freshwater and adequate drainage (especially when evaporation and transpiration is high) will likely prevent accumulation of Na and other salts from reaching detrimental concentrations in the soil.

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Chapter 4

INFLUENCE OF REMEDIATION OF WASTEWATER EFFLUENT-IRRIGATED SOILS ON SEED BANK RESPONSE AND SOIL PROPERTIES

Abstract: Recovery of seed banks from induced germination inhibition by irrigation with a lesser saline-sodic water source and leaching was examined in greenhouse microcosms. Microcosms were designed to allow simulation of moist-soil conditions with restricted drainage and were previously subjected to irrigation with either municipal wastewater effluent (WWE) or Missouri River water (MOR). Irrigation with a lesser saline-sodic water source (i.e., MOR) did not improve seed bank response in terms of taxa richness, plant density, and biomass in microcosms previously irrigated with WWE. Germination inhibition was not alleviated because restricted drainage prohibited removal of salts from the germinating cohort of the seed banks. This may have implications for wetland impoundments irrigated with saline waters with soils of restricted drainage or hydrologically isolated from other water sources that could remove salts from the system. Leaching accompanied by adequate drainage, significantly decreased soil exchangeable bases, electrical conductivity (EC), and the exchangeable sodium percentage (ESP). The lower saline-sodic conditions are thought to be responsible for the 3-fold increase in taxa richness and plant density, and the 85% increase in biomass. Results also provide evidence of the resiliency of these fresh-water seed banks to extended saline-sodic edaphic conditions.

Key Words: Eagle Buffs Conservation Area, salinity, sodicity, wastewater, wetland vegetation, wetland seed bank.

INTRODUCTION

In a previous study, it was demonstrated that germination of seed banks became inhibited in microcosms irrigated with WWE compared with irrigation with MOR (Chapter 3). The inhibition of the seed bank and concurrent decrease in taxa richness, plant density, and vegetative biomass were attributed to repeated WWE-irrigation which increased the average salinity (as measured by electrical conductivity (EC)) of the soils from 0.2 mS m^{-1} to 7.9 ± 0.2 mS m^{-1}. Reduction of soil salts is thought to improve response of these fresh water seed banks. Previous investigators have shown that salinity and sodicty can induce germination inhibition of several halophytic species and inhibition can be alleviated by reducing salt concentrations (Ahmad et al. 1990, Keiffer and Ungar, 1997, Katembe et al. 1998). Common amelioration practices typically include the use of water sources of lesser salinity or a progression of decreasing salt concentrations to remove soil salts without adversely impacting soil aggregates and hydraulic conductivity (Reeve and Bower 1960, Sumner and Nadui 1999). Other methods employ the use of calcium-containing materials such as gypsum that exchanges with monovalent ions (i.e., sodium) on the soil's exchange complex and aids in leaching exchanged ions by improving soil hydraulic conductivity by flocculation of soil particles into aggregates (Richards 1954, Bohn et al. 2001, Ghafoor et al. 2001).

Germination of some halophytic species is enhanced by exposure to salinity and this trait may be an edaphic advantage in systems of variable salinities (Heydecker et al. 1973, Baskin and Baskin 1998, Rubio-Casal et al. 2003). However, exposure of fresh water wetland seed banks to saline and sodic conditions may alter dormancy or viability

of seeds thereby altering plant communities that may impact the quality of wetland habitats. Several fresh water seed bank species that are important food and structure for wetland biota were demonstrated to be impaired by saline-sodic conditions (Chapter 3). The objectives of this study were to 1) evaluate recovery of fresh-water seed banks that exhibited saline-sodic induced germination inhibition by irrigation with a lower saline-sodic water source (i.e., MOR) and 2) evaluate seed bank response after leaching and gypsum addition. To perform these evaluations, a series of greenhouse trials were conducted using the microcosms of the previous study. Response of seed banks were quantified in terms of vegetation richness, density, and biomass. Soils also were examined to evaluate changes in exchangeable bases, EC, and pH to seed bank response.

This greenhouse study indicates that seed bank response was not improved with use of lower saline-sodic water source without removal of salts from the germinating cohort of the seed bank. This may have implications in wetland impoundments that contain soils that impede salt removal. Seed bank response was enhanced after leaching and draining, and demonstrated evidence of the resiliency of these fresh water seed banks to prolonged saline-sodic conditions.

METHODS

Experiment Design

In a greenhouse, microcosms were used to grow plants that germinated from the natural soil seed bank of two soil materials. Microcosms contained one of two soil materials and were used in a previous study (Chapter 3). The design, water sources,

irrigation, monitoring soil water content, and seed-rain prevention procedures of the microcosms followed methods previously described in Chapter 3. For this study, four trials were conducted that lasted approximately 100 d each, which allowed most species that germinated from the seed bank to mature (Table 1). Spring and summer trial start dates were used to simulate schedules of water manipulations (flooding and dewatering) commonly used on wetland impoundments managed for wildlife. The last two trials were conducted with summer start dates in order to compare before and after leaching effects on the seed bank and soils without the effects of multiple seasons. Temperatures and photoperiods in the greenhouse during trials were previously described in Chapter 3.

Seed Bank Response to Lesser Salinity Water Source. To evaluate the WWE-irrigated soil seed bank response to a less saline and sodic water source, four of the nine replicate microcosms of each soil material irrigated with WWE in the previous study (Chapter 3) were reassigned to be irrigated with MOR. These microcosms are hereafter referred to as previously irrigated with wastewater (PWW). The remaining five microcosms of each soil material originally irrigated with WWE were again irrigated with WWE and served as controls. For comparison, three of the nine replicate microcosms of each soil material irrigated with MOR in the previous study were randomly selected and again irrigated with MOR. Exchangeable bases, EC, ESP, and pH of soil materials prior to irrigation trials are presented in Table 2. Microcosms were randomly placed in rows and equally spaced apart at the start of each trial. At the end of each trial, all vegetation from each microcosm was harvested, identified, and processed using methods described in Chapter

3. Taxa richness, plant density (plants m^{-2}) and vegetative biomass by species were recorded for each microcosm. Following completion of the third trial, approximately 100 g of soil material were sampled from the entire depth of each microcosm. Samples were air-dried, sieved, and stored at 4º C until processed for determination of exchangeable bases, EC, and pH. Determination of exchangeable bases, EC, ESP, and pH followed methods described in Chapter 3.

Seed Bank Response to Leached Soil Materials. To evaluate seed bank response to reduced salinity after prolonged exposure to high saline-sodic conditions and assess changes in soil chemistry, the three microcosms of each soil material irrigated with only MOR and three randomly selected microcosms of each soil material irrigated with only WWE in trials one through three were subjected to a leaching treatment. Two wood panels, the width of the microcosm, were placed on edge into the soil material across the width of each microcosm at 30-cm intervals to retard the flow of water across the soil surface and promote uniform infiltration. Subsurface drainage was unimpaired because panels did not intersect the gravel layer at the bottom of microcosms. Leaching was accomplished by saturating soils with deionized water (mean EC = 0.92 $\mu S\ cm^{-1}$) until approximately 2 cm of water ponded on the soil surface. Water was allowed to pond for a minimum of 2 hrs and then drained through a 2-cm diameter opening located near the bottom of each microcosm. The EC of the leachate was monitored with a portable EC meter. To facilitate Na^+ (sodicity) removal from the exchange complex of the soil materials (by substitution with Ca^{2+}), powdered gypsum ($CaSO_4 \cdot 2\ H_2O$) was

incorporated into the soil material using a hand-trowel when the EC of the leachate was ≤ 2.0 mS cm^{-1}. The quantity of gypsum applied followed guidelines established by Richards (1954). Pore volumes and amount of gypsum applied to microcosms are presented in Appendix 4. Leaching continued until the soil EC was < 3.5 mS cm^{-1}. Microcosms were allowed to drain for approximately 2 d, and then plugs were placed in drains of microcosms. One trial was then conducted following previous methods. At the end of the trial, vegetation in each microcosm was harvested, identified, and processed using methods described in Chapter 3. Approximately 100 g of soil material from each microcosm was collected immediately after the leaching treatment and at the end of the trial. Soil samples from both sampling times were submitted for determination of exchangeable bases, EC, ESP, and pH following methods described in Chapter 3.

Statistical Analysis

Analysis of variance (ANOVA) was performed with soil material and water source as fixed factors blocked within rows of the greenhouse array. A repeated measures ANOVA model was applied using SAS 9.1 (SAS Institute 2002-03) using the MIXED procedure (mixed linear model) with taxa richness, plant density, and biomass as dependent variables. Because the design included both fixed effects (soil, water, and trial) and random effects (rows), the mixed linear model was selected. Because PWW treatments were not included in trial four, a separate ANOVA was applied to trial four and for the comparison of preleached (trial three) with after leaching (trial four) conditions. Exchangeable bases, EC, and pH of soil materials were analyzed with soil

and water factors fixed and blocked within assigned rows of the greenhouse array. In all analyses, $P \leq 0.05$ was considered significant. Fisher's protected Least Squares Means comparison tests were used to separate means following ANOVA results when main effects were significant (Milliken and Johnson 1984).

Table 1. Start and end dates of each trial.

Trial	Start date	End date
1	August 2001	November 2001
2	April 2002	July 2002
3	August 2002	November 2002
4	August 2003	November 2003

Table 2. Mean (LS means ± SE) exchangeable bases, electrical conductivity (EC), exchange sodium percentage (ESP), and pH of soil materials in microcosms prior to irrigation trials. Water sources used to irrigate microcosms were Missouri River water (MOR) and municipal wastewater effluent (WWE).

		Exchangeable Bases (cmol kg^{-1})				EC	ESP	pH
Water	Soil	Ca	Mg	K	Na	(ms cm^{-1})	(%)	(1M $CaCl_2$)
MOR	Loamy fine sand	16.8 ± 2.0	2.4 ± 1.2	0.3 ± 0.1	1.8 ± 1.8	2.8	8.3	7.3
	Silt loam	34.7 ± 2.0	5.7 ± 1.2	0.5 ± 0.1	2.7 ± 1.8	3.8	7	7.3
WWE	Loamy fine sand	17.2 ± 2.0	2.4 ± 1.2	0.6 ± 0.1	6.0 ±1.8	6.7	23	7.4
	Silt loam	31.8 ± 2.0	5.4 ± 1.2	0.8 ± 0.1	7.6 ± 1.8	9.0	19	7.3

RESULTS

Seed Bank Response to Lesser Saline Water Source

Twenty five plant taxa were recorded (16 dicots and 9 monocots) during the first three trials (Table 3). Total taxa recorded during each trial ranged from 15 to 18. Of the 25 taxa recorded, 24 occurred in MOR-, 14 in WWE-, and 11 in PWW-irrigated microcosms. Mean taxa richness of trials 1 through 3 was significantly greater ($F_{2, 14}$= 161.19, $P < 0.0001$) in MOR-irrigated microcosms (9.2 ± 0.3) than either WWE- (2.2 ± 0.3) or PWW-irrigated microcosms (2.5 ± 0.3) regardless of soil material. Abundance of moderately salt tolerant *Echinochloa crus-galli* and greatly tolerant *Leptochloa fusca* were greater in PWW-irrigated microcosms relative to ones irrigated with WWE. Whereas *Eclipta alba* (no salt tolerance) and a grass (unknown A) in the WWE-irrigated microcosms had greater abundance than PWW-irrigated microcosms. However, a majority of the taxa showed only small or no changes in abundance in the PWW- and WWE-irrigated microcosms in trials one through three. In each trial, MOR-irrigated microcosms had greater number of taxa than PWW- or WWE-irrigated microcosms, although means were not significantly different at the trial and water source interaction ($P = 0.2$, Figure 1A). Number of taxa was similar between PWW- and WWE-irrigated microcosms.

Plant density was significantly different ($F_{4,36} = 7.02$, $P = 0.0003$) among water sources, although relationships varied by trial. In the first three trials, MOR-irrigated microcosms had significantly greater plant density than the PWW- and WWE- irrigated microcosms ($P < 0.0001 – 0.0029$). Plant density was similar between PWW- and

WWE-irrigated microcosms in trials one and three, but PWW-irrigated microcosms had significantly ($P = 0.0244$) greater density in trial two (Figure 1B). Vegetation biomass also differed significantly ($F_{4,36} = 5.27$, $P = 0.0019$) among water sources and trials. Biomass from trial one was similar between PWW and MOR, and both had significantly greater biomass than WWE-irrigated microcosms ($P < 0.0086$) (Figure 1C). In the second trial, the PWW- and WWE-irrigated microcosms had significantly less biomass than the MOR-irrigated microcosms ($P < 0.0001$). By the third trial, biomass was similar among treatments. Although biomass was less in the WWE-irrigated microcosms, biomass on a per plant basis (mean biomass/mean abundance) was typically greatest in WWE-irrigated microcosm compared with other treatments (Table 3).

Soil exchangeable K and Na, and EC were significantly different among treatments at the end of the third trial ($F_{2,18} = 57.55, 10.91, 51.19$, $P < 0.0001, 0.0008, < 0.0001$, respectively) (Table 4). MOR-irrigated microcosms had significantly less exchangeable K and Na, and EC than WWE- or PWW-irrigated microcosms ($P < 0.0001–0.05$). Microcosms irrigated with WWE had significantly greater K and Na than those irrigated with PWW ($P < 0.0001–0.01$), but similar EC. Exchangeable Mg and ESP were similar among treatments. Exchangeable Ca and pH were also significantly different among water sources, however these were dependent on soil materials ($F_{2,18} = 3.64, 3.54$, $P = 0.0469, 0.0506$, loamy fine sand, silt loam, respectively). PWW- and WWE-irrigated silt loam had significantly greater exchangeable Ca than the other treatment ($P < 0.0001–0.003$). WWE-irrigated loamy fine sand had greater soil pH than other treatments ($P < 0.0001–0.03$), except PWW-irrigated loamy fine sand.

Seed Bank Response to Leached Soil Materials

After leaching, 17 taxa (12 dicots and 5 monocots) were identified at the end of the fourth trial. All 17 taxa germinated in MOR-irrigated microcosms and 13 were found in WWE-irrigated microcosms (Table 3). Mean taxa richness was still significantly greater ($F_{1,6} = 8.68$, $P = 0.0257$) in MOR-irrigated microcosms than WWE-irrigated microcosms (Figure 1A). Mean plant density also was significantly greater ($F_{1,6} = 28.01$, $P = 0.0018$) in microcosms irrigated with MOR than WWE (Figure 1B). In contrast, mean vegetative biomass was similar between MOR-irrigated and WWE-irrigated microcosms (Figure 1C). Mean plant biomass on a per plant basis was greater in the WWE-irrigated microcosms than ones irrigated with MOR (Table 3). Mean taxa richness and plant density significantly increased after leaching in trial four compared with the third trial, but differences varied by water source ($F_{1,8} = 20.48, 13.20$, $P = 0.0019, 0.0067$, respectively). Taxa richness was significantly greater after leaching in the WWE-irrigated microcosms (8.1 ± 0.7 species) compared with preleached values (2.2 ± 0.61 species, $P < 0.0001$). Taxa richness was similar between these two trials in MOR-irrigated microcosms. Plant density after leaching significantly increased in the microcosms irrigated with MOR (2,564.6 ± 180 plants m^{-2}) compared with preleached values (836 ± 180 plants m^{-2}, $P = 0.0001$). Although density increased after leaching in the WWE-irrigated microcosms, a statistical difference was not detected between preleached (72.4 ± 139.4) and post-leached (559 ± 180.2) values ($P = 0.06$).

Immediately after leaching soil materials, the sum of exchangeable bases decreased by 86%, EC by 93%, ESP by 47%, and pH by 0.2 units in the WWE-irrigated microcosms compared with preleached conditions of trial three (Table 5). Exchangeable Ca, Na, and K significantly decreased after leaching, but differences varied by water source ($F_{1,8}$ = 9.88, 11.55, 36.69, P = 0.0137, 0.0094, 0.0003, respectively). Immediately after leaching, soil exchangeable Ca and Na in MOR-irrigated microcosms were significantly less than preleached materials (P < 0.0002, 0.0032, respectively). In WWE-irrigated microcosms, exchangeable Ca, Na, and K significantly decreased immediately after leaching compared with preleached materials (P < 0.0001). Electrical conductivity in both WWE- and MOR-irrigated microcosms significantly decreased immediately after leaching compared with preleached levels (P < 0.0001, 0.0205, respectively). After leaching, WWE-irrigated microcosms still had significantly greater exchangeable K, ESP, and pH than MOR-irrigated microcosms ($F_{1,8}$ = 66.04, 10.58, 10.08, P < 0.0001, = 0.0116, = 0.0131, respectively). Concentration of exchangeable Na was significantly greater in the WWE-irrigated silt loam microcosms ($F_{1,8}$ = 6.25, P = 0.0370), but concentrations were similar among the other treatments.

At the conclusion of the fourth trial, soil exchangeable K, EC, ESP, and pH were significantly greater ($F_{1,8}$ = 24.76–344.09, P < 0.0001–0.0011) in the WWE-irrigated microcosms than MOR-irrigated microcosms. Exchangeable Ca and Mg were similar between MOR- and WWE-irrigated soil microcosms. A significant ($F_{1,8}$= 18.74, P = 0.0025) interaction for water source and soil material remained for exchangeable Na after the last trial. WWE-irrigated silt loam had significantly greater exchangeable Na than

other treatments ($P < 0.0001$). MOR–irrigated microcosms and WWE-irrigated loamy fine sand had similar exchangeable Na concentrations.

Table 3. Vegetation abundance germinated from the soil seed bank before (trials 1-3) and after leaching (trial 4) by water source and trial, regardless of soil material. Previous wastewater irrigated treatments were not leached. Species known to be waterfowl food sources are in bold face type (Laubhan et al. 1996, Stader and Stinson 2005). Salinity tolerance is assigned (0) = none, (1) =low, (2) = moderate, (3) = high tolerance to salinity. Trial 2 started in April of 2002, and trials 1, 3, and 4 started in August 2001, 2002, and 2003 (respectively).

		Previous Wastewater			Missouri River				Wastewater			
Species	Salinity tolerance‡	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 4	Trial 1	Trial 2	Trial 3	Trial 4
Amaranthus tamariscinus Nutt.	0	0	0	0	8	0	0	19	1	1	0	23
Ammannia coccinea Rothb.	1	247	0	1	717	357	334	266	34	20	0	40
Arabidopsis thaliana (L.) Heyn.	0	0	0	0	151	0	0	1	0	0	0	0
Capsella bursa Medic.	--	0	0	1	0	288	165	733	0	0	0	29
Chaerophyllum procumbens (L.) Crantz	--	0	0	0	22	2	5	2	0	0	0	0
Chenopodium album L.	0	0	0	0	1	6	7	3	0	0	1	2
Cyperus spp L.	1-2	3	0	0	82	16	15	54	3	2	0	45
Digitaria spp. Heist.	--	2	1	0	96	267	517	356	0	0	2	0
Echinochloa crus-galli (L.) Beauv.	1-2	660	2274	560	777	4719	862	4385	551	1053	305	1242
Eclipta alba (L.) Hassk.¶	0	6	9	20	73	166	167	890	18	23	34	122
Eragrostis spp. Beauv.	0	0	0	0	141	2	23	77	0	0	1	4
Euphorbia spp. L.	--	0	0	0	0	0	1	0	0	1	0	0
Fragaria vesca L.	--	0	0	0	0	0	1	0	0	0	0	0

Grass (unknown A)	--	4	0	0	2	0	0	0	11	0	0	0
Grass (unknown B)	--	0	16	0	0	1	10	19	0	12	8	11
Leptochloa fusca (L.) Kunth	3	0	97	14	43	0	0	0	0	5	10	0
Lindernia anagallidea (Michx.) Pennell	0	0	0	0	0	287	113	767	0	0	0	131
Melilotus alba Desr.	2	0	0	0	0	0	16	25	0	0	0	0
Mollugo verticillata L.	--	2	0	0	60	1	93	62	0	1	1	13
Pancium virgatum L.	2	0	0	0	0	0	29	0	0	0	0	0
Poa spp. L.	--	0	1	0	0	0	0	0	0	0	0	0
Ranunculus sceleratus L.	1	0	0	0	0	13	4	13	0	0	0	7
Rumex spp.	--	0	0	0	0	1	0	0	1	0	0	0
Solanum americanum Mill.	--	0	0	0	196	68	146	22	0	0	0	8
Veronica peregrina L.	-0	0	0	0	78	0	0	0	0	0	0	0
Biomass per plant (g plant^{-1})		0.276	0.202	0.137	0.111	0.123	0.05	0.01	0.28	0.517	0.082	0.173
Total number of dicots / taxa	16/25	3/7	1/6	3/5	9/15	10/15	12/18	12/17	4/7	5/9	3/8	9/13

‡Salinity tolerance for many species may vary by salt type, life stage, population, duration of exposure, temperature, and other factors (Lieffers and Shay 1983). Ratings are based on soil electrical conductivity (solution extract): none = 0-2 dS/m; low = 2.1-4.0 dS/m; medium = 4.1-8.0 dS/m; high > 8.0 dS/m (USDA, NRCS. 2008, Stewart and Kantrud 1972, Xiong and Zhu 2002)

¶ Salinity tolerance estimated (Flynn et al. 1995)

Figure 1. Least-squares means of number taxa (A), plant density (B) and vegetative biomass (C) by water source and trial regardless of soil material. Water sources were Missouri River (MOR), municipal wastewater effluent (WWE) and previously irrigated with WWE (PWW). Different letters above columns indicates significant differences among water sources in that trial. Vertical bar within a column represent one standard error.

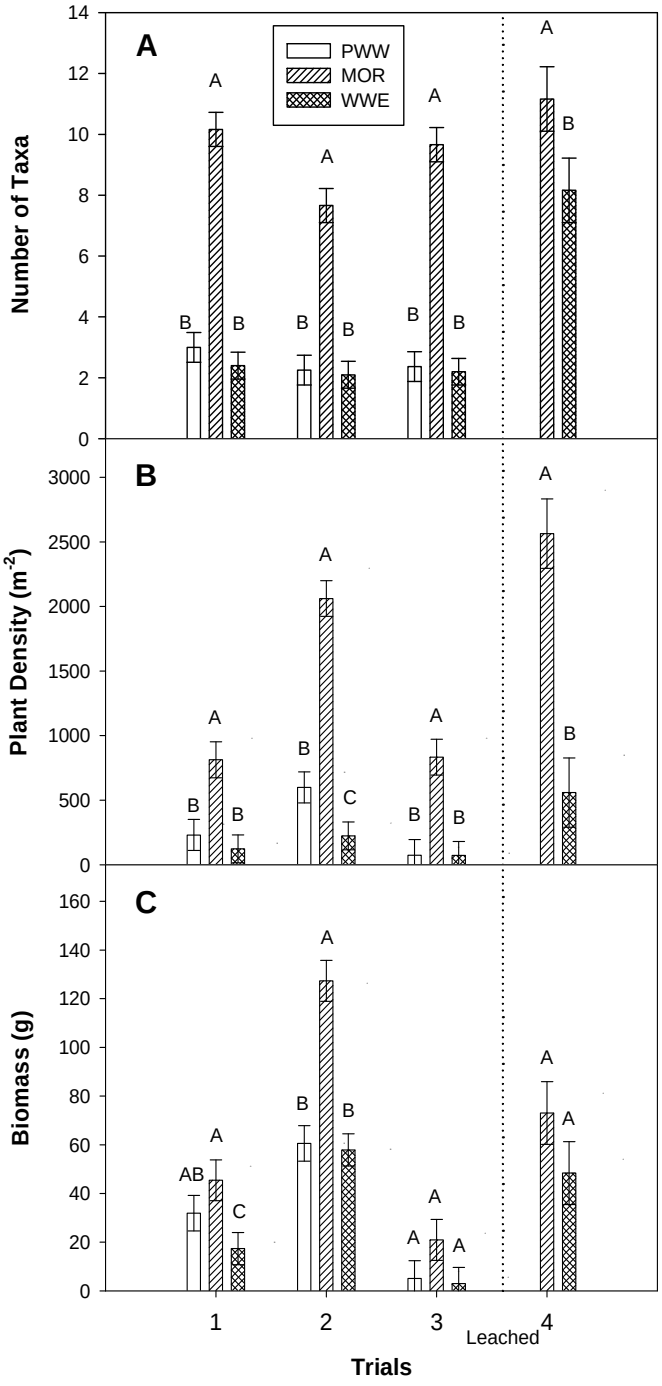

Table 4. Mean Exchangeable bases, electrical conductivity (EC), exchangeable sodium percentage (ESP), and pH of both soil materials irrigated with Missouri River water (MOR), municipal wastewater effluent (WWE), or MOR on previously WWE irrigated (PWW) at the end of the third trial. Means (LS means ± SE) within columns followed by the same letter are not significantly different ($P > 0.05$).

	Water Level Effects					Water and Soil Level Effects			
	Exchangeable Bases ($cmol\ kg^{-1}$)			EC	ESP	Exchangeable Ca ($cmol\ kg^{-1}$)		pH (1M $CaCl_2$)	
Water	Mg	K	Na	($mS\ cm^{-1}$) †	(%) ‡	Loamy fine sand	Silt loam	Loamy fine sand	Silt Loam
MOR	4.9 ± 1.4 [a]	0.4 ± 0.06 [a]	10.1 ± 1.9 [a]	8.9 ± 2.7 [a]	29.7 ± 3.6 [a]	15.7 ± 4.3 [a]	21.6 ± 4.3 [a]	7.8 ± 0.1 [b]	7.6 ± 0.1 [a]
PWW	8.0 ± 1.2 [a]	0.8 ± 0.06 [b]	15.4 ± 1.7 [b]	36.7 ± 2.4 [b]	34.3 ± 3.1 [a]	15.2 ± 3.7 [a]	40.5 ± 3.7 [b]	7.9 ± 0.1 [bc]	7.5 ± 0.1 [a]
WWE	6.6 ± 1.1 [a]	1.2 ± 0.05 [c]	21.3 ± 1.5 [c]	43.0 ± 2.1 [b]	40.7 ± 2.8 [a]	16.1 ± 3.3 [a]	40.4 ± 3.3 [b]	8.0 ± 0.0 [c]	7.5 ± 0.0 [a]

† Electrical conductivity determined by the 1:1 soil to water method (Whitney 1998).

‡ Exchangeable sodium percentage as determined by: ESP = {(100 • ESR) / 1+ ESR} where ESR (exchange sodium ratio) = exchangeable Na^{+} / exchangeable (Ca^{2+} + Mg^{2+}) (Bohn et al. 1998).

Table 5. Mean Exchangeable bases, electrical conductivity (EC), exchangeable sodium percentage (ESP), and pH of soil materials irrigated with Missouri River water (MOR) or municipal wastewater effluent (WWE) immediately after leaching (IAL) and after the fourth trial. Means (LS means ± SE) within columns for a sample period followed by the same letter are not significantly different ($P > 0.05$).

Sample Period and Water Source	Water Level Effects						Water and Soil Level Effects	
	Exchangeable Bases (cmol kg^{-1})			EC	ESP	pH	Exchangeable Na (cmol kg^{-1})	
	Ca	Mg	K	(mS cm^{-1}) †	(%) ‡	(1M $CaCl_2$)	Loamy fine sand	Silt loam
IAL								
MOR	4.1 ± 0.2 [a]	2.1 ± 0.1 [a]	0.3 ± 0.02 [a]	2.8 ± 0.4 [a]	11.6 ± 2.2 [a]	7.3 ± 0.4 [a]	0.5 ± 0.3 [a]	1.2 ± 0.3 [a]
WWE	4.0 ± 0.2 [a]	1.9 ± 0.1 [a]	0.5 ± 0.02 [b]	3.0 ± 0.4 [a]	21.6 ± 2.2 [b]	7.5 ± 0.4 [b]	0.8 ± 0.3 [a]	2.8 ± 0.3 [b]
Trial 4								
MOR	19.6 ± 1.1 [a]	2.4 ± 0.2 [a]	0.3 ± 0.01 [a]	2.7 ± 0.2 [a]	6.7 ± 0.6 [a]	7.5 ± 0.3 [a]	1.2 ± 0.3 [a]	2.0 ± 0.3 [a]
WWE	18.9 ± 1.1 [a]	2.3 ± 0.2 [a]	0.6 ± 0.01 [b]	4.1 ± 0.2 [b]	12.6 ± 0.6 [b]	7.7 ± 0.3 [b]	1.7 ± 0.3 [a]	4.7 ± 0.3 [c]

† Electrical conductivity determined by the 1:1 soil to water method (Whitney 1998).

‡ Exchangeable sodium percentage as determined by: ESP = {(100 • ESR) / 1+ ESR} where ESR (exchange sodium ratio) = exchangeable Na^{+} / exchangeable (Ca^{2+} + Mg^{2+}) (Bohn et al. 1998).

DISCUSSION

Switching water sources in the PWW microcosms from WWE to MOR, which had lesser EC and Na concentrations, did not increase taxa richness, plant density, or biomass for either soil material relative to WWE-irrigated microcosms. Over the course of three trials, exchangeable bases, EC, and ESP remained great in the PPW-irrigated microcosms compared with MOR-irrigated microcosms. This suggests that under conditions which prohibit the removal of salts from the germination cohort of the seed bank, inhibition may not be alleviated by irrigation with a better quality water source (Richards 1954). Removal of salts through drainage is required otherwise evapotranspiration processes sustain salt concentrations in the soil (Qadir et al. 1996). This may have application to wetland impoundments irrigated with large saline or sodic water and complemented with other sources that are relatively small in salinity and sodicity. Irrigating with a less saline water source to reduce salinity in impoundments previously irrigated with a high saline water sources, such as WWE, may be unsuccessful because of soil properties or hydrologic connectivity. Impoundments underlain with slowly permeable soil or a restrictive layer may impede leaching of salts away from the seed bank. Impoundments that are hydrologically isolated from ground water of lower salinity may also be unable to reduce salinity.

Although no new species germinated after reducing soil salts by leaching, abundance increased for several species compared with the third trial. Among the largest increases were the abundance of *Echinochloa crus-galli* and *Eclipta alba*, which increased by more than 80% in the MOR-irrigated soils and by more than 55% and 80% (respectively) in the WWE-irrigated soil materials. Germination of both species has been

reported to be reduced in saline conditions (Rahman and Ungar 1990, Tripathi et al. 2004 and references within) however, the exposure of the seed bank to saline and sodic edaphic conditions followed by lesser salinity and sodicity after leaching may have enhanced germination of these species (Heydecker et al.1973, Baskin and Baskin 1998), which may be beneficial in wetlands as both species are considered waterfowl foods.

Results also suggest dicots in the soil seed bank may be more sensitive to soil salinity-sodicity than monocots. After leaching, the number of dicotylous species that germinated in the WWE-irrigated microcosms increased to nine (average of 5.67 ± 0.6 species per treatment replicate) compared to three species (average of 0.67 ± 0.6 species per treatment replicate) before leaching in trial three. Number of germinated monocots decreased from five species (average of 1.5 ± 0.4 species per treatment replicate) in the third trial to four species (average of 2.5 ± 0.4 species per treatment replicate) after the last trial. Dicot sensitivity to irrigation with WWE was also noted in the previous study (Chapter 3).

The reduction in soil salts probably aided in the 3-fold increase in number of taxa and plant density, and the more than 85% increase in biomass in the WWE-irrigated microcosms compared with the average number of taxa, density, and biomass before leaching. Microcosms irrigated with MOR had less of an increase in taxa richness and biomass (21% and 12%, respectively) after leaching, however plant density also increased by 3-fold over the average density before leaching. The increased seed bank response after leaching indicates that exposure to saline-sodic soil conditions did not irreversibly impair the viability of these freshwater seed banks, which is consistent with other studies (Walsh et al. 1991, Foderaro and Ungar 1997, DiTommaso 2004).

Resuming irrigation with water sources during trial four increased exchangeable Ca by more than 3-fold and Na by > 70% in both WWE- and MOR-irrigated microcosms. This large increase in exchangeable Ca is assumed to be associated with the Ca concentrations of the irrigation sources (MOR, 55 mg Ca l^{-1}; WWE, 64 mg Ca l^{-1}, Knowlton and Jones 2003) and the gypsum added during the leaching treatment. The solubility of gypsum and $CaCO_3$ present in the soils may have been enhanced by sodium chloride concentrations in the water sources (Reeve and Bower 1960). The large increase in Ca resulted in decreasing the ESP of the soils after trial 4.

Decreased soil salts may not have been the only factor that contributed to increased seed bank response following the leaching treatment. Although for all previous trials soil material was mixed at harvest, the incorporation of gypsum during the leaching treatment may have disrupted conditional dormancy of seeds (e.g., exposure to surface temperature and light) and stimulated germination of some species. The addition of gypsum may have promoted germination by improving leaching efficiency and by neutralizing toxic effects of salts that remained after leaching (Tobe et al. 2003). Alleviation of salt toxicity may involve the ability of Ca^{2+} to induce and maintain soil colloid flocculation. Improved soil structure may decrease salinity near seeds by increasing hydraulic conductivity and availability of exchange sites on soil colloids that can sequester salts within microstructures (Rengasamy et al. 1986, Silvertooth and Norton 2000). Leaching also may have influenced germination and seedling development by removing germination-inhibiting compounds, such as abscisic acid from the seed coat (Wareing and Foda 1957, as cited in Baskin and Baskin 1998).

Greater biomass on a per plant basis followed the order WWE> PWW> MOR for nearly all trials. This may be attributed to the greater concentration of N and P in the WWE compared with the MOR and suggests nutrient uptake was not impaired by the associated salinity-sodicity induced by the WWE. Possibly, concentrations of N and P in the WWE may be beneficial to plant productivity after germination has completed. Germination and seedling stages are generally more sensitive to saline-sodic conditions than later stages (Ungar 2001 and references within, Brock et al. 2005). A potential shallow-water (e.g., moist-soil) irrigation strategy for wetland impoundments that could utilize nutrient concentrations in the WWE, but avoid salinity induced germination inhibition, may start with irrigation with MOR until seedlings are established followed by irrigation with WWE. In this manner, established plants may aid in attenuating accumulation of salts by excretion and secretion of root exudates, aggregation of soil particles, increased porosity, and addition of organic materials to the soils (Ahmad et al. 1990). However, this strategy would be susceptible to salt accumulation unless salts could be removed from the soils.

CONCLUSIONS

Reducing the salinity and sodicity of the irrigation source in microcosms previously irrigated with WWE failed to improve seed bank response. The restrictive soil depth and drainage of the microcosms in conjunction with moist-soil conditions, which is conducive to salinity development through evaporative processes, prohibited the removal of sufficient salts from the germinating cohort of the seed bank. This may have implications for WWE-irrigated wetland impoundments with limited leachable soil

depths. The use of saline-sodic irrigation water on impoundments underlain with slowly permeable soils or restrictive layers may promote concentration of salts that alter ecological processes in the soil and vegetation communities. However, decreasing soil salinity and sodicity through unrestrictive leaching did increase taxa richness, plant density, and biomass. Seed bank response after leaching indicate that these fresh-water seed banks were still viable after prolonged exposure to saline-sodic soil conditions and that decreasing soil salts alleviated for some species the germination inhibition induced by irrigation with WWE.

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Chapter 5

EFFECTS OF MUNICIPAL WASTEWATER EFFLUENT AS A WETLAND WATER SOURCE ON SOIL MICROBIAL ABUNDANCE

Abstract: Soil microorganisms are often involved in promoting or inhibiting some aspect of seed physiology and these associations may be affected by water source. The use of wastewater effluent on wetlands may induce changes to soil salinity, sodicity, or microbial communities that alter interactions with soil seed banks. This study evaluated cultured microbial abundances obtained from samples of bulk soil and the rhizosphere of barnyard grass (*Echinochloa crus-galli*) that were irrigated with either municipal wastewater effluent (WWE), Missouri River (MOR) or deionized (DI) water. Abundances were not statistically different among treatments; however abundances were significantly different between soil materials which were dependent on sampling location and media. Soil salinity and sodicity in the MOR and DI treatments may have accumulated to levels that impaired microbial abundances and therefore produced abundances similar to WWE treatments. Wider contrasting levels of soil salinity and sodicity should be used to ascertain differences among microbial abundances in soils irrigated with these water sources.

INTRODUCTION

Studies have shown seeds retrieved from soil often possess strong microbial associations (Curl and Truelove 1986, Kremer 1993). These associations are often responsible for inducing or breaking dormancy, seed protection, decay, and affect seed bank ecology. Studies of weed seed germination and seedling development indicate microorganisms can contribute significantly to seed mortality or inhibit seedling growth (Kremer 1993). A study of four annual weed species showed several strains of associated seedborne fungi affected seed germination and seedling development (Kirkpatrick and Bazzazz 1979). Another study utilizing different soil textures at water capacities in excess of 50% found soil and seedborne microorganisms enhanced suppression of wild oat seed germination (Kiewnick 1964). Seeds may possess mechanisms that protect them from microbial impairment. Kennedy (1998) reported diffusion of antimicrobial substances secreted from the seed coat into the surrounding spermosphere, which may protect the seed from microbial decay. Phenolic compounds, which are toxic to a large variety of microbes (McKey 1979), were found in the palisade cell layers and chalazal end of velvetleaf seed (Kremer et al. 1984). These exuded seed coat compounds inhibited 58% of the bacteria and all fungi tested in a subsequent study (Kremer 1986).

Water treatment facilities commonly use chemical additives (i.e., chlorine) and rely on microbial degradation of organic substances to reclaim wastewater. These wastewater effluents are being used as water sources for wetlands designated for wildlife. Such is the case at the Eagle Bluffs Conservation Area (EBCA) in central Missouri. Municipal wastewater effluent (WWE) is used to irrigate wetland impoundments designated as wildlife habitat. However, the effects of effluent from these facilities on

ecological systems such as wetlands have not been thoroughly investigated. Several studies indicate soil microbial biomass, enzymatic activity, and denitrification rates were enhanced by application of wastewater (Kannan and Oblisami 1990, Goyal et al. 1995, Monnett et al. 1995, Filip et al. 1999). Filip et al. (2000) reported that irrigation of a sandy Haplic Luvisol with municipal wastewater for almost 100 years resulted in a reduced C:N ratio, increased microbial counts (> 200%), and increases in total microbial biomass and soil enzyme activities, compared to irrigation treatments free of wastewater. However, Ghinogeanu et al. (1984) reported effluent from a paper mill reduced soil bacterial abundance and induced a 10-fold reduction in enzyme activity.

Results from other studies (Finocchiaro et al. 2009, Chapter 4) indicate a detrimental interaction between nutrient rich WWE and seed germination or seedling emergence of native vegetation from soil seed banks. Irrigation with WWE decreased vegetation diversity, density, and biomass by inhibiting germination of the seed bank of two soil materials compared to irrigation with water from the Missouri River (MOR) or deionized water (DI). Germination inhibition in the WWE-irrigated microcosm was attributed to high soil salinity and sodicity induced by the WWE. However, the high salinity and sodicity may have altered microbial abundances and subsequently inhibited germination by disrupting interactions with seeds and associated dormancy mechanisms. Additionally, it is possible WWE contains populations or concentrations of microorganisms that alter abundance of indigenous soil microorganisms associated with germination or seedling development, or alter interactions and dormancy mechanisms of soil seed banks. Kannan and Oblisami (1990) suggested the increase in microbial populations and activity in soil receiving pulp and paper mill effluent may have been due

to addition of microbes from the effluent. I hypothesize that soils irrigated with WWE have altered microbial abundances compared to soils irrigated with MOR water. In this study, cultures obtained from bulk soil and rhizosphere samples were developed to evaluate abundances of microorganisms in microcosms irrigated with WWE or MOR water.

METHODS

Previous Treatment of Soil Materials

Soil materials were collected from existing microcosms that were used in a previous seed bank study (Chapter 3). Soil materials were previously irrigated with MOR, DI, or WWE during growth trials that lasted approximately 100 d each. Soil materials sampled from the microcosms were loamy fine sand (Sarpy, mixed, mesic, Typic Udipsamments) and silt loam (Blake, fine-silty mixed superactive, calcareous, mesic, Aquic Udifluvents). A treatment-microcosm consisted of one soil material irrigated with one water source yielding six treatments. For the present study, abundance of microorganisms was determined from plated cultures obtained from bulk soil materials and rhizosphere soil collected from the microcosms. Microcosm design, irrigation scheme, and greenhouse conditions were previously described in Chapter 3.

Bulk Soil Materials Used for Dilution Plating

Soil materials from nine replicates of each treatment were randomly grouped by threes to create a composite sample. This produced three composite, replicate soil material samples per treatment and was repeated for all six treatments yielding a total of 18

samples. Samples were collected between visible seedlings in the microcosms with a 4.5-cm diameter core sampler at the beginning of the trial. Soil material from the entire soil depth of the microcosm (approximately 10-15 cm) was sampled in April 1999. In addition, surface samples of both soil materials were collected from the field in April 1999 at the original soil-collection sites on EBCA. Samples at field-collected moisture, were passed through a 2-mm sieve and stored at 4° C in plastic sealed bags until plated. Microcosm samples were stored at 4° C for 21 d and field samples for 3 d. Electrical conductivity was determined using the 1:1 soil to water method at 25°C (Whitney 1998) and pH was measured using a combination pH-reference electrode in a 1:1 soil to water and salt solution (0.01 M $CaCl_2$) (SSL Methods 2004). Exchangeable sodium percentage (ESP) was determined following methods described by Bohn et al. (2001). Soil organic matter was determined by loss on ignition method (Combs and Nathan 1998). Soil total organic carbon (TOC) was estimated from the organic matter content and multiplied by 1.78 to obtain the organic carbon fraction. Total nitrogen (TN) was determined by the total Kjeldahl procedure (Bremner 1996) (Table 1).

Enumeration of Microbes from the Bulk Soil Material

Soil materials of one treatment were thoroughly mixed and sieved again through a 2-mm screen after removal from cold-storage. A 10-g (moist weight) sub-sample from each soil material was weighed into dilution bottles containing 95 ml of sterile phosphate buffered saline (PBS; 0.1 M KH_2PO_4 / K_2HPO_4, 0.14 M NaCl; pH 7.0) plus 0.01% Tween 20. Diluted soil samples were shaken for 30 min on a rotary shaker at 200 rpm. Immediately after shaking, 1.0 ml of suspended soil was withdrawn, serially diluted (10-

fold steps) in sterile PBS, transferred aseptically onto duplicate petri plates containing agar culture media, and distributed over the agar surface by spread-plating. Culture media used for bacterial enumeration were King's B (King-B, Sands and Rovira 1970) and Trypic Soy Agar (TSA, Difco Laboratories Detroit Michigan, USA). Culture media for fungi was plated in duplicate onto Martin's Rose Bengal medium (Martin, Martin 1950). Plates were incubated aerobically in the dark at room temperature (24-26° C). After 5 to 7 days of incubation, visible colonies were enumerated. Two replicate samples of approximately 10 g of the remaining collected soil material was dried at 60° C for 3 d and enumeration of colony forming units (CFU) was adjusted to oven-dried weight of soil (Zuberer 1994).

Enumeration of Rhizosphere and Rhizoplane Microbes

Barnyard grass (*Echinochloa crus-galli)* plants were carefully removed from soil material of microcosms using hand trowels. Plant samples were taken from all treatments. Roots were gently shaken to remove large aggregates of loosely adhering soil material and stored at 4° C for 20 d. Soil remaining on roots was considered rhizosphere soil (Ames 1984). Because of the low mass of barnyard grass roots, three replicates per treatment were randomly grouped to obtain adequate root mass for assays. A total of 18 root samples were used in the assays. Whole roots were added to 95 ml sterile PBS, and shaken on a rotary shaker at 200 rpm for 30 min. Ten-fold dilutions of root washings were prepared and spread-plated on culture media following the procedures previously described. Roots were then oven-dried at 60° C for 3 d and enumeration of rhizosphere extracted CFUs were adjusted to root oven-dry weight.

Data Analyses

Analysis of variance (ANOVA) was performed with soil material and water source as fixed factors blocked within rows of the greenhouse array. Because samples of soil materials and roots for rhizosphere assays were composites of three replicates, each composite sample for a treatment was assigned a different row in order to block by row. Plate duplicates of each sample replicate for a treatment was averaged to obtain the mean enumeration (i.e., abundance) per treatment replicate sample and this was used in all analyses. Analyses were performed on microbial abundances of bulk soil samples obtained at the 10^{-6} dilution on King-B and TSA media, and 10^{-4} on Martin. For rhizosphere samples, the 10^{-5} dilution on King-B and TSA, and 10^{-3} on Martin were used in the analyses. Abundance data were diagnosed with the W-test (Shapiro and Wilk 1965) and were lognormal. A repeated measures ANOVA model was applied using SAS 9.1 (SAS Institute 2002-03) with the MIXED procedure (mixed linear model) with $\log_{10}$ transformed abundance as the dependent variable. Separate ANOVA models were applied to microbial abundances of soil material and rhizosphere samples and abundances were analyzed by growth media. In all analyses, $P \leq 0.05$ was considered significant. Fisher's protected Least Squares Means comparison tests were used to separate means following ANOVA results when main effects were significant (Milliken and Johnson 1984).

RESULTS

Microbial Abundance of Bulk Soil Materials

Numbers of culturable, viable microbial colonies were not significantly different among treatments or between water sources for any media (Appendix 5.1). Field-collected samples of soil materials typically had the least bacterial abundance and the greatest fungal abundance compared with other treatments (Appendix 5.2). However, microbial abundance was significantly different between soil materials for all three media ($F_{1,10}$ = 10.47, 9.11, 7.62, P = 0.0089, 0.0129, 0.0201, King-B, TSA, Martin, respectively). On Martin media, significantly greater numbers of fungi occurred in silt loam than loamy fine sand (Table 2). On King-B and TSA, significantly greater numbers of bacterial colonies occurred in silt loam than in loamy fine sand.

Microbial Abundance of the Rhizosphere

Rhizosphere microbial abundances also were not significantly different among treatments or water sources on all three media (Appendix 5.2). A significant difference in rhizosphere bacterial abundance between soil materials cultured on TSA was present ($F_{1,10}$ = 5.27, P=0.0446). Abundance of rhizosphere bacteria was significantly greater in loamy fine sand than in silt loam (Table 2). Microbial abundances cultured on King-B and Martin media were not significantly different between soil materials.

In general, for both bulk soil material and rhizosphere samples, culturable, viable bacterial colonies were more abundant than fungal colonies. Furthermore, abundances of CFUs were greater in rhizosphere soils than bulk soil materials on all growth media.

Rhizosphere soil bacterial and fungal abundances were two orders of magnitudes greater than in the bulk soil materials.

Table 1. Means (LS means ± SE) electrical conductivity (EC), pH, total organic carbon (TOC), total nitrogen (TN), and the carbon to nitrogen ratio (C:N) of soil materials used to obtained bacterial and fungal colony counts. Soil materials were collected from greenhouse microcosms and irrigated with either Missouri River water (MOR), wastewater effluent (WWE) or deionized water (DI). Field soil samples (Field) were collected from Eagle Bluffs Conservation Area.

Soil	Silt loam				Loamy fine sand			
Property	MOR	WWE	DI	Field	MOR	WWE	DI	Field
EC	3.8	9	4.6	0.9 †	2.8	6.7	3.8	0.3 †
($mS\ cm^{-1}$)	± 0.5	± 1.2	± 0.1	± 0.4	± 0.9	± 0.2	± 0.1	± 0.1
ESP	6.5	18	9.1	0.9 †	8.3	23	14	0.9 †
(%)	± 0.9	± 3.3				± 1.1	± 0.4	
pH	7.3	7.4	7.4	7.5	7.3	7.4	7.3	7.5
($CaCl_2$)	± 0.1	± 0.2	± 0.2	± 0.1	± 0.1	± 0.1	± 0.2	
TOC ‡	11685	13483	12921	15393	6741	7303	6741	6179
($mg\ kg^{-1}$)								
TN	1043	1197	1177	1370	587	647	623	530
($mg\ kg^{-1}$)	± 110	± 126	± 105		± 87	± 110	± 15	
C:N	11:1	11:1	11:1	10:1	11:1	11:1	11:1	12:1

‡ TOC estimated from organic matter and divided by 1.78.
† Estimated from samples collected in June 1998.

Table 2. Means (LS means) and standard errors of microbial abundances obtained from bulk soil material and Rhizosphere of *Echinochloa crus-galli* collected from microcosms. Bacteria enumerations were determined from TSA and King-B growth media and fungal enumeration from Martin media. Values are $\log_{10}$ transformed. Soil materials were sampled from greenhouse microcosms after four 100-d trials of irrigation with deionized water, Missouri River water, or wastewater effluent. Means in rows followed by a different letter are significantly different ($P < 0.05$).

Sampled Material	Growth Media	Dilution for Enumeration	Loamy fine sand (g oven-dry soil^{-1})	Silt loam (g oven-dry soil^{-1})	Standard Error
Bulk Soil					
	TSA	10^{-6}	5.56a	5.93b	± 0.11
	King-B	10^{-6}	6.04a	6.31b	± 0.59
	Martin	10^{-4}	3.32a	3.83b	± 0.13
Rhizosphere					
	TSA	10^{-5}	8.03a	7.65b	± 0.11
	King-B	10^{-5}	8.00a	7.71a	± 0.19
	Martin	10^{-3}	4.93a	5.31a	± 0.31

DISCUSSION

Microbial Abundances of Bulk Soil Materials

Contrary to this work, which found microbial abundance similar among higher saline (WWE), and lower saline (MOR and DI) irrigation sources, some studies have reported depressed abundance (i.e., biomass) in soils irrigated with high saline water sources (Batra and Manna 1997, Pankhurst et al. 2001), while others have reported increases in microbial biomass (Friedel et al. 2000). Greater soil microbial abundance in silt loam than loamy fine sand (regardless of irrigation source) probably reflects the differences in TOC and TN content between the two soil materials. Large concentrations of these nutrients have been associated with favorable environments for microorganisms (Hassink 1994). Average TOC and TN were approximately 2 times greater in the silt loam than in loamy fine sand. Greater concentrations of TOC and TN in the silt loam probably supported greater abundance of microorganisms than the loamy fine sand (Lynch and Whipps 1990; Semnov et al. 1998). Textures of the materials also may have affected microbial abundances in the bulk soil samples. Kabir et al. (1994) and van Gestel et al. (1996) reported greater abundance of bacteria in fine-textured soils than in coarse-textured soils. Some workers have attributed greater microbial numbers and biomass in fine-soil fractions to hospitable microsites within microaggreates (2-50 μm) (Chotte et al. 1998, Monreal and Kodama 1997). Another possible explanation may be related to greater soil particle charge that typically accompanies finer soil fractions. Inorganic and organic compounds attracted and held in place by oppositely charged fine soil particles may act as bridges that attract and aid adherence of microbes to the finer fraction of soil particles (Hartel 1998).

Bacterial colonies ranged from 10^5 to 10^6 g dry soil^{-1} and were more abundant than fungal colonies for all soil and water treatments. Fungal abundance in these treatments ranged from 10^3 to 10^4 g dry soil^{-1}. The greater number of bacteria colonies than fungi agree with that reported for typical soils, but colony counts were below the typical soil range for bacteria (10^6 to 10^8 g dry soil^{-1}) and for typical fungi abundance in surface soil (10^4 to10^6 g dry soil^{-1}) (Brady 1995, Alexander 1998, Wollum 1998). The lesser abundance of culturable colonies may be attributed to the relatively high EC and ESP of the treatments. The repeated irrigation and design of the microcosms promoted accumulation of salts and by the fourth trial EC and ESP possibly had reached levels that affected microbial abundances regardless of treatment.

Although not statistically different, average bacterial abundance in the field soil samples (soils averaged) was typically less than the soil materials of the greenhouse treatments, however, average fungal abundance was greater in the field soil samples than the greenhouse treatments. It was expected that fungal abundance to be less in the field soil samples than the greenhouse treatments because bacteria abundance was less in the field soil samples than the greenhouse treatments. This suggests that microcosms may have depressed abundance of fungi relative to field soils. A possible explanation is that constant fluctuations in soil water content (i.e., saturated at trial initiation, maintained just below field capacity) of irrigated treatments throughout the course of a trial may have repressed fungal abundance. Fungi are suggested to be more sensitive to drying and wetting cycles because they are often located on outer surfaces of aggregates and in large pores (Denf et al. 2001). Additionally, the soil materials used in this work contained high concentrations of free-carbonates (i.e., calcium and magnesium carbonates), and are

slightly alkaline (pH range 7.2-7.4). Generally, most species of fungi prefer slightly acidic to neutral pH soils (Morton 1998). The slightly alkaline pH and low TOC in these soil materials may have depressed fungal abundance in the bulk soil samples.

Microbial Abundances of the Rhizosphere

Rhizosphere bacteria and fungi abundances ranged from 10^7 to 10^8 g of dry root^{-1} and 10^4 to 10^6 g of dry root^{-1}, respectively and were consistent with reported ranges of 10^6 to 10^9 g of dry root^{-1} for bacteria and 10^5 to 10^6 g of dry root^{-1} for fungi (Kennedy 1998). Also, abundances obtained from the rhizosphere were greater than bulk soil samples. These results are consistent with others that reported greater abundance of microbes, as well as biomass and diversity, in the rhizosphere than the bulk soil (Lynch and Whipps 1990, Semenov et al. 1999, Smalla et al. 2001). The rhizosphere and related rhizoplane tend to have greater abundance of microorganisms than bulk soil material because the rhizosphere provides readily accessible substrates released by roots and cells (Campbell and Greaves 1990, Gilbert et al. 1996, Papavizas and Davey 1961). However, microbial diversity may not be greater. The lesser volume of soil in the rhizoplane and surrounding root web may effectively hinder colonization (Marilley et al. 1997).

In contrast to the greater abundance obtained from the silt loam of the bulk soil, the loamy fine sand had greater rhizosphere bacterial abundance than silt loam, but fungal abundance was greater in the silt loam. As mentioned previously, fine-textured materials have been reported to provide more favorable environments for microorganisms. However, the greater abundance of bacteria in the loamy fine sand may be related to development of a stronger rhizosphere effect. Mean EC and ESP of the silt loam

(excluding field samples) were higher than the loamy fine sand and may have stressed roots (Keiffers and Ungar 2002); decreasing the rhizosphere effect. Salt stress to plants may have inhibited production or release of root compounds (e.g. exudates, secretions, mucilages) and consequently impaired rhizosphere microbial abundances. The rhizosphere effect of the loamy fine sand also may have been enhanced by the pore sizes. Larger and more stable pores of the loamy fine sand may have sustained greater rhizosphere abundance by extending spatial distribution of microbes along roots (Kennedy 1998) and providing greater access to root compounds and sloughed cells than the smaller, dynamic pores in silt loam.

Although Wollum (1994) suggested that rhizosphere assays should be performed immediately after collection and samples should not be cold-stored to avoid recovery loss; rhizosphere cultures of the present study had abundances well within reported ranges after 20 d in cold-storage. However, this observation cannot conclude any effect on abundances from cold storage because samples were not cultured immediately after collection to be compared with cold-stored samples.

CONCLUSIONS

Despite having two to three times greater soil EC and ESP in the WWE-irrigated microcosms, abundances of microorganisms obtained from either bulk soil or rhizosphere samples were not statistically different among treatments. Soil EC and ESP in the MOR and DI microcosms may have accumulated to levels that impaired microbial abundances and therefore produced abundances similar to WWE treatments. Wider contrasting levels of soil EC and ESP should be used to ascertain differences among these water sources.

Another possibility is that abundances of culturable, viable colonies were similar among the treatments and the germination inhibition observed in the microcosm is due to other factors that are not discernable with culture plates.

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Chapter 6

EFFECT OF MUNICIPAL WASTEWATER AS A WETLAND WATER SOURCE ON SOIL MICROBIAL ACTIVITY

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EFFECT OF MUNICIPAL WASTEWATER AS A WETLAND WATER SOURCE ON SOIL MICROBIAL ACTIVITY

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ABSTRACT

Microbial activity was compared between two soil materials excavated from a wetland and irrigated with municipal wastewater effluent or Missouri River water. The wastewater had twice the electrical conductivity and four times the sodium concentration as River water. We performed activity assays on the soils before leaching, immediately after leaching, and after harvesting plants. Gas chromatography was used to measure CO_2 evolved in soil samples incubated for 7 d. Activity was significantly reduced in preleached wastewater-irrigated soils compared with river-water irrigation. Immediately after leaching, activity significantly increased and was similar to river-water irrigated soils. Activity decreased slightly after plant harvest in post-leached treatments. Increased activity after leaching may be related to decreased salinity and sodicity, which probably lowered osmotic pressure in the soil. Our study demonstrated that soil salinity and sodicity induced by wastewater irrigation decreased microbial activity, which may impact nutrient cycling and glycophytic vegetation communities in wetlands

Key Words: salinity, soil water, microbiology, sewage.

INTRODUCTION

Soil microbial activity is influenced by soil water content, illustrated by decreased aerobic activity when water-filled pore space of soil exceeds 60% (Linn and Doran, 1984). However, microbial activity may be detrimentally affected before this soil water content is reached if water contains high salt concentrations (Pankhurst et al., 2001). Municipal wastewater effluent (WWE) containing high salt contents or Missouri River water (MOR) are used to irrigate wetland impoundments at the Eagle Bluffs Conservation Area (EBCA) located near McBain, Missouri (38° 53'N, 92° 27' W). We previously used soil materials collected from EBCA in a greenhouse study that examined seed bank response to repeated irrigation with WWE (Finocchiaro et al., 2009). In that study, salinity and sodicity rapidly increased in soil materials irrigated with WWE, which were responsible for inhibiting germination of the soil seed bank. Studies have shown seeds retrieved from soil often possess characteristic microbial associations (Kiewnick, 1964; Kirkpatrick and Bazzazz, 1979; Curl and Truelove, 1986; Kremer, 1993). Composition of the microbial associations may be affected by diffusion of antimicrobial substances secreted from the seed coat into the soil surrounding the spermosphere (McKey 1979; Kremer et al., 1984; Kremer, 1986; Kennedy, 1998). These substances are thought to protect the seed from microbial decay. However, these microbial associations or physiological processes of the seed may become altered by soil salinity and sodicity (Baskin and Baskin, 1998).

Microbial processes important for sustaining nutrient cycling also can be altered by salinity and sodicity. Several studies indicated soil microbial biomass, activities of various enzymes, and denitrification rates were enhanced by application of wastewater

(Kannan and Oblisami, 1990; Goyal et al., 1995; Monnett et al., 1995; Filip et al., 1999; Friedel et al., 2000). Filip et al. (2000) reported that irrigation of a sandy Haplic Luvisol with municipal wastewater for almost 100 years resulted in increased microbial counts, total biomass, enzyme activity, and reduced C:N ratios compared with irrigation treatments free of wastewater. In contrast, other studies report that application of saline and sodic wastewater reduced microbial biomass, diversity, respiration, enzyme activity, as well as decreased nutrient cycling (Ghinogeanu et al., 1984; Mahasneh et al., 1984; Pankhurst et al., 2001).

Alterations to the plant community and nutrient cycles from irrigation with WWE may have prolonged and undesirable effects on the ecology of freshwater wetlands. Therefore, the effects of WWE as an irrigation source for wetlands should be understood in order to sustain these sensitive habitats. In this study, soil microbial activity determined by carbon dioxide evolution (CO_2) was compared in soil materials irrigated with either WWE or MOR. Because of the salinity and sodic concentration of the WWE, we hypothesized that soil materials irrigated with WWE will have less microbial activity compared with soils irrigated with MOR and decreasing salinity and sodicity should increase activity.

MATERIALS AND METHODS

Previous Treatment of Soil Materials

Soil materials were collected from an existing greenhouse study that consisted of large plastic microcosms (61 cm x 91 cm x 20 cm) filled with one of two soil materials and irrigated with either WWE or MOR to stimulate germination and vegetative growth

of the soil seed bank (Finocchiaro et al., 2009). A greenhouse treatment-microcosm consisted of one soil material and irrigated with one water source. For the present study, respiration assays were conducted on soil materials used in two sequential greenhouse trials separated by a leaching treatment. Each trial lasted approximately 100 d. At the beginning of each trial, microcosms were initially flooded with a water source until the volume of soil material was completely saturated and water ponding was evident (~5 cm). During trials, microcosms were not drained and subsequent irrigations were applied to maintain soil water content of microcosms at approximately 80% field capacity for both soil materials. Water movement in microcosms was primarily influenced by evaporation and transpiration, which permitted soluble and insoluble constituents in the water sources to accumulate. At the end of each trial, all above and belowground vegetation in the microcosms was harvested. In between trials, microcosms were leached to reduce the salinity and sodicity in the soil materials. Soil materials were flushed with deionized water and powdered gypsum ($CaSO_4 \cdot 2H_2O$) was incorporated into the soil materials. Leaching was discontinued when the electrical conductivity (EC; 1:1 method; Whitney, 1998) of the soil materials was less than 3.5 mS cm^{-1}. Microcosms were not irrigated nor disturbed in between trials except during leaching treatment.

The soils used in the greenhouse microcosms were Sarpy loamy fine sand (mixed, mesic, Typic Udipsamments) and Blake silt loam (fine-silty mixed superactive, calcareous, mesic, Aquic Udifluvents), excavated from the 0-15 cm depth of the River Supply Channel at EBCA in June 1997. Soils of the River Supply Channel are inundated periodically throughout the year with MOR water and are not irrigated with WWE.

Soil Material Collection

Samples of soil materials were collected from microcosms at three different times. The first followed the first trial after vegetation was harvested from the microcosms (mid-November 2002), but before leaching (hereafter referred to as preleached). The second sampling was taken immediately after leaching concluded in early-August 2003 (hereafter referred to as IAL). During the second sampling seedling emergence was not apparent. The third was taken after the harvest of the subsequent trial in mid-November 2003 (hereafter referred to as post-leached). At each sampling, a 5-cm diameter core sample of soil material was collected from the entire depth (0-15 cm), excluding the underlying gravel layer, of the same three replicates of each soil material irrigated with MOR or WWE. In addition, samples of the soil materials were collected from the River Supply Channel in mid-June 2003 and assayed separately from the greenhouse samples (field-collected). Samples were passed through a 2-mm sieve and assayed within 72 hours after collection with the exception of the field samples, which were stored at 0° C for 9 d. Chemical analysis of the soil materials at the sampling times are listed in Table 1.

Microbial Activity

Microbial activity was estimated by measuring CO_2 evolution using the substrate-induced respiration assay (Horwath and Paul, 1994). Five grams of soil material (dry weight) was placed in glass tubes (10 cm x 1.5 cm) with screw-caps fitted with septa. Soil material was adjusted to 18% moisture by weight with deionized water. Two hundred µl of 5% glucose solution was added to the moistened soil material in the tubes.

Tubes containing the glucose-amended soil materials were immediately incubated at 27° C in the dark for 24 h. Three tube replicates were prepared for each treatment replicate and for each field-collected soil material sample. Tube headspace contents were sampled for CO_2 at 24 h, 48 h, 72 h, and at 7 d. A 1-ml sample was withdrawn from the tubes using a 1-ml syringe after aspirating the tubes 5 times. The 1-ml headspace volume from each tube was analyzed by gas chromatography (Buck Scientific model 910; PEAKNT software operating system) with a thermoconductivity detector (TCD), He carrier gas at a flow rate of 14 ml/L using a silica gel column at 50° C. After each sampling the caps of tubes were removed for approximately 5 minutes to allow ambient air to enter the tubes. Tubes were re-capped and incubated after each sampling. Total CO_2 evolved over the seven-day periods was determined from known calibration standards (Zibilske, 1994).

Data Analyses

All values reported are expressed on a dry weight basis after moisture content of assayed samples were determined from loss of weight after drying at 105° C for 24 h. Treatment means were analyzed using repeated measures of samples and sampling time in an ANOVA model using SAS (SAS Institute 2002-2003) procedure MIXED. Microbial activity (CO_2 evolution) data were analyzed by sampling period (i.e., preleached, IAL, post-leached) with the total of seven days accumulation as the dependent variable. Additionally, evolved CO_2 by incubation time was analyzed using a similar ANOVA model to possibly provide information on microbial groups (e.g. copiotrophs, oligotrophs). For all ANOVA models a significance level of $P = 0.05$ was

set to detect differences among treatment means. All mean separation analyses for ANOVA models used Least Squares Means comparison testing.

RESULTS

Total CO_2 Evolution by Sampling Period

Prior to leaching, when soil EC was relatively high in WWE-irrigated materials (Table 1), CO_2 evolution was significantly greater in MOR-irrigated soil materials than WWE-irrigated ones with respect to soil material ($F_{2,39}$= *5.17, P = 0.0102*; Figure 1A). MOR-irrigated loamy fine sand had significantly greater CO_2 evolution than all other treatments ($P < 0.0004$); WWE-irrigated silt loam showed the lowest CO_2 evolution. Silt loam irrigated with MOR had similar CO_2 evolution as the WWE-irrigated loamy fine sand and field-collected, non-irrigated samples. Immediately after leaching, CO_2 evolution for MOR- and WWE-irrigated soil materials increased significantly (*P = 0.0021, P <0.0001*, respectively) compared with preleached concentrations (Figure 2). MOR- and WWE-irrigated soil materials sampled at IAL showed ~ 25% and ~ 45% increases in evolved CO_2, respectively, compared with preleached amounts (Figure 1B). At IAL, evolved CO_2 was similar within each soil regardless of water source, but both had significantly more than field-collected samples. After vegetative biomass harvest (post-leached), evolved CO_2 from MOR-irrigated soil materials significantly declined by ~ 22% (*P = 0.0008*) relative to IAL amounts. Post-leached CO_2 evolution of WWE-irrigated soil materials declined slightly (~ 6%) compared with IAL amounts. At this sampling, CO_2 evolution was similar in both WWE-irrigated soil materials and the MOR-irrigated loamy fine sand (Figure 1C). CO_2 evolution from MOR-irrigated silt loam was

significantly less than WWE-irrigated silt loam (*P = 0.0216*) and similar to Field-collected soil materials.

CO_2 Evolution by Incubation Time

CO_2 evolution of preleached soil materials differed significantly for the combination of soil, water source, and incubation time ($F_{6,27} = 2.90$, $P = 0.0259$). No treatment differences were detected at 24 hr incubation, however, after 48 h, MOR-irrigated materials had greater CO_2 evolution than WWE-irrigated or field-collected soil materials (Figure. 3A). In fact, MOR-irrigated loamy fine sand had significantly greater CO_2 evolution than all other treatments except MOR-irrigated silt loam (*P < 0.002*). After 72 h, MOR-irrigated loamy fine sand had significantly greater CO_2 evolution than all other treatments (*P < 0.001*), and CO_2 evolution of all other treatments were similar. After seven days of incubation, the field-collected silt loam and loamy fine sand had the greatest CO_2 evolution and along with the WWE-irrigated loamy fine sand had significantly greater CO_2 evolution than other treatments (*P < 0.03*). Maximum evolution of CO_2 occurred at 48 hrs for MOR- and WWE-irrigated silt loam and at 72 hrs for MOR-irrigated loamy fine sand. Maximum CO2 evolution occurred at 7 d for WWE-irrigated loamy fine and field-collected samples.

CO_2 evolution of the treatments collected at IAL and post-leached sampling periods were significantly different among water source and incubation time ($F = {}_{6,27,}7.36$, $P < 0.0001$; $F = {}_{6,27,}39.78$, $P < 0.0001$, respectively), but not between soil materials. Immediately after leaching, CO_2 evolution between MOR-irrigated and WWE-irrigated soil materials were more similar than before leaching (Figure 3B). At 24

h, 72 h, and 7d incubation times, evolved CO_2 concentrations were not significantly different between the two water sources. Only after the 48 h sampling was CO_2 from WWE-irrigated soil materials significantly less than MOR-irrigated (*P = 0.024*). Maximum CO_2 evolution for MOR- and WWE-irrigated soil materials occurred after 48 h and declined for each sampling thereafter. The exception was WWE-irrigated loamy fine sand which had maximum CO_2 occurrence at 72 hrs. Post-leached CO_2 evolution from WWE-irrigated soil materials was similar to MOR-irrigated materials for nearly all sampling times (Figure 3C). At 48 h, however, WWE-irrigated soil materials had significantly greater CO_2 than MOR-irrigated materials (*P = 0.004*) and maximum CO_2 evolution occurred at this time for both water sources and soil materials.

Table 1. Mean (SD) of total organic carbon (TOC), total nitrogen (TN), carbon to nitrogen ratio (C:N), electrical conductivity (EC), exchangeable sodium percentage (ESP) and pH (0.01M $CaCl_2$) of soil materials irrigated with either Missouri River water (MOR) or municipal wastewater effluent (WWE). Soil materials were collected from Eagle Bluffs Conservation Area in June 2003 (Field) and from greenhouse microcosms before leaching with deionized water (Preleached), immediately after leaching (IAL), and after harvesting vegetation that germinated from the soil seed bank (Post-leached).

Sample period	N	Silt loam						Loamy fine sand					
		TOC %	TN %	C:N	EC mS/cm	ESP %	pH	TOC %	TN %	C:N	EC mS/cm	ESP %	pH
Preleached													
MOR	3	0.80 (0.01)	0.073 (0.003)	11:1	5.3 (3.8)	19.1 (5.1)	7.6 (0.06)	0.26 (0.01)	0.029 (0.009)	9:1	12.4 (3.8)	40.2 (5.1)	7.8 (0.06)
WWE	5	0.72 (0.05)	0.074 (0.006)	10:1	40.4 (2.9)	27.8 (3.9)	7.5 (0.04)	0.28 (0.03)	0.022 (0.001)	13:1	45.6 (2.9)	53.4 (3.9)	8.0 (0.04)
IAL													
MOR	3	0.76 (0.02)	0.065 (0.001)	12:1	2.5 (0.5)	13.1 (3.0)	7.3 (0.05)	0.27 (0.06)	0.010 (0.002)	27:1	2.9 (0.5)	10.1 (3.0)	7.3 (0.05)
WWE	3	0.73 (0.03)	0.062 (0.001)	12:1	2.6 (0.5)	26.5 (3.0)	7.5 (0.05)	0.20 (0.04)	0.011 (0.003)	19:1	3.2 (0.5)	16.6 (3.0)	7.4 (0.05)
Post-leached													
MOR	3	0.80 (0.04)	0.061 (0.002)	13:1	2.6 (0.2)	6.5 (0.8)	7.3 (0.05)	0.30 (0.05)	0.009 (0.001)	34:1	2.7 (0.2)	6.8 (0.8)	7.3 (0.05)
WWE	3	0.77 (0.02)	0.061 (0.004)	13:1	4.2 (0.2)	14.1 (0.8)	7.5 (0.05)	0.24 (0.01)	0.008 (0.002)	29:1	3.8 (0.2)	11.1 (0.8)	7.4 (0.05)
Field	2	0.92 (0.13)	0.083 (0.018)	11:1	0.23 (0.03)	> 1	7.27 (0.06)	0.70 (0.02)	0.071	10:1	0.22 (0.01)	> 1	7.29 (0.06)

Figure 1. Mean total microbial CO_2 evolution of soil materials collected from greenhouse microcosms before leaching (Preleached), immediately after leaching (IAL), and after leaching and harvesting vegetation (Post-leached) that were irrigated with Missouri River (MOR) or municipal wastewater effluent (WWE). Field-collected soils (Field) were collected from a wetland impoundment at Eagle Bluffs Conservation Area. Vertical bars within column indicated SE of the mean. Different letters among columns indicate means are significant different ($P < 0.05$, LSM).

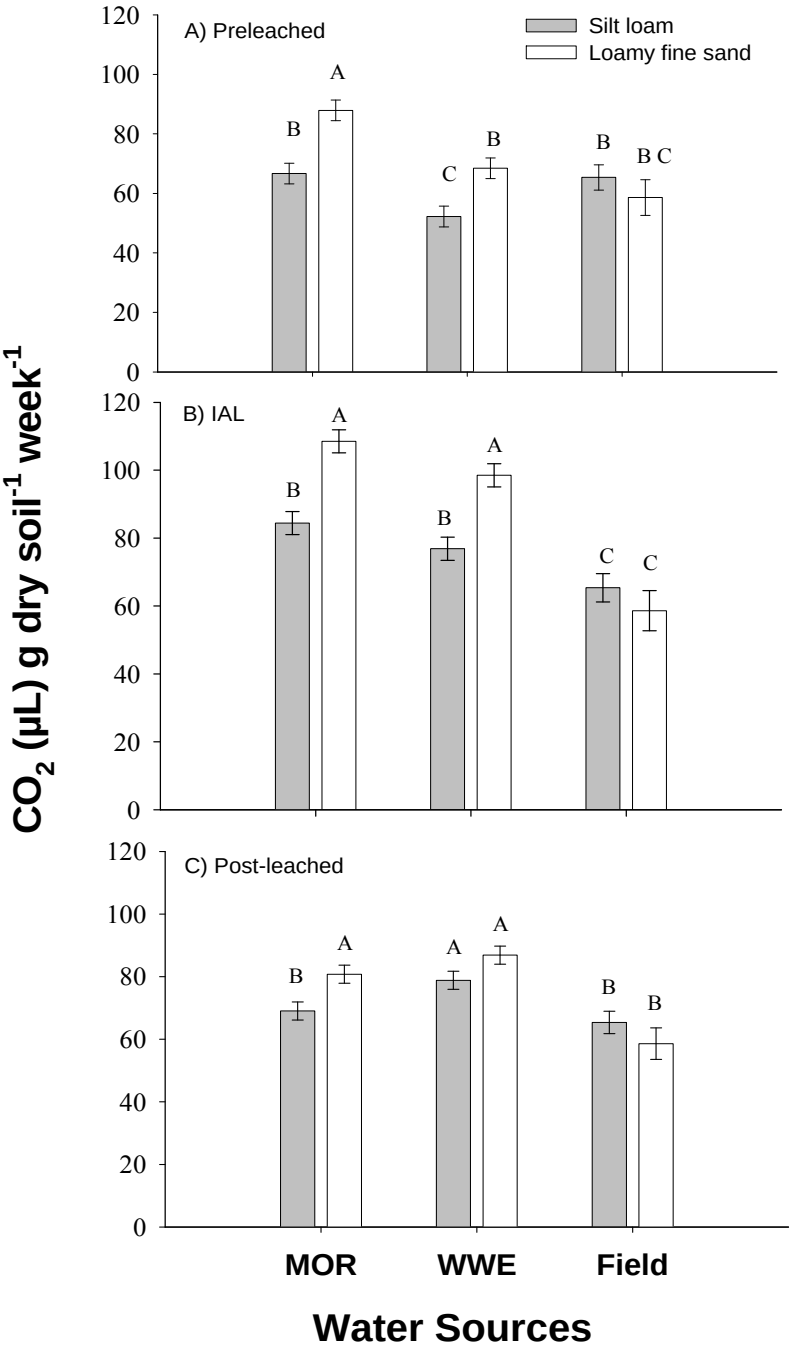


Figure 2. Mean total microbial CO_2 evolution for Missouri River-irrigated (MOR) and municipal wastewater effluent-irrigated (WWE) greenhouse microcosms regardless of soil material for all sample periods. Vertical bars indicate SE of the mean.

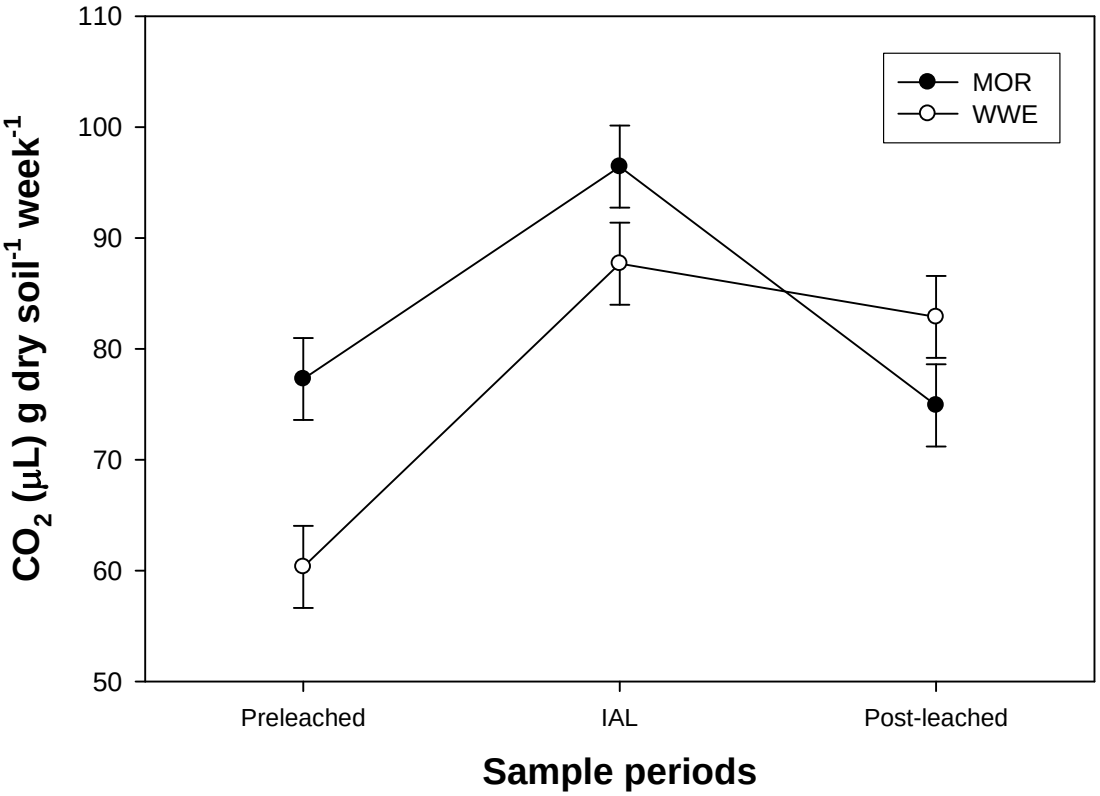
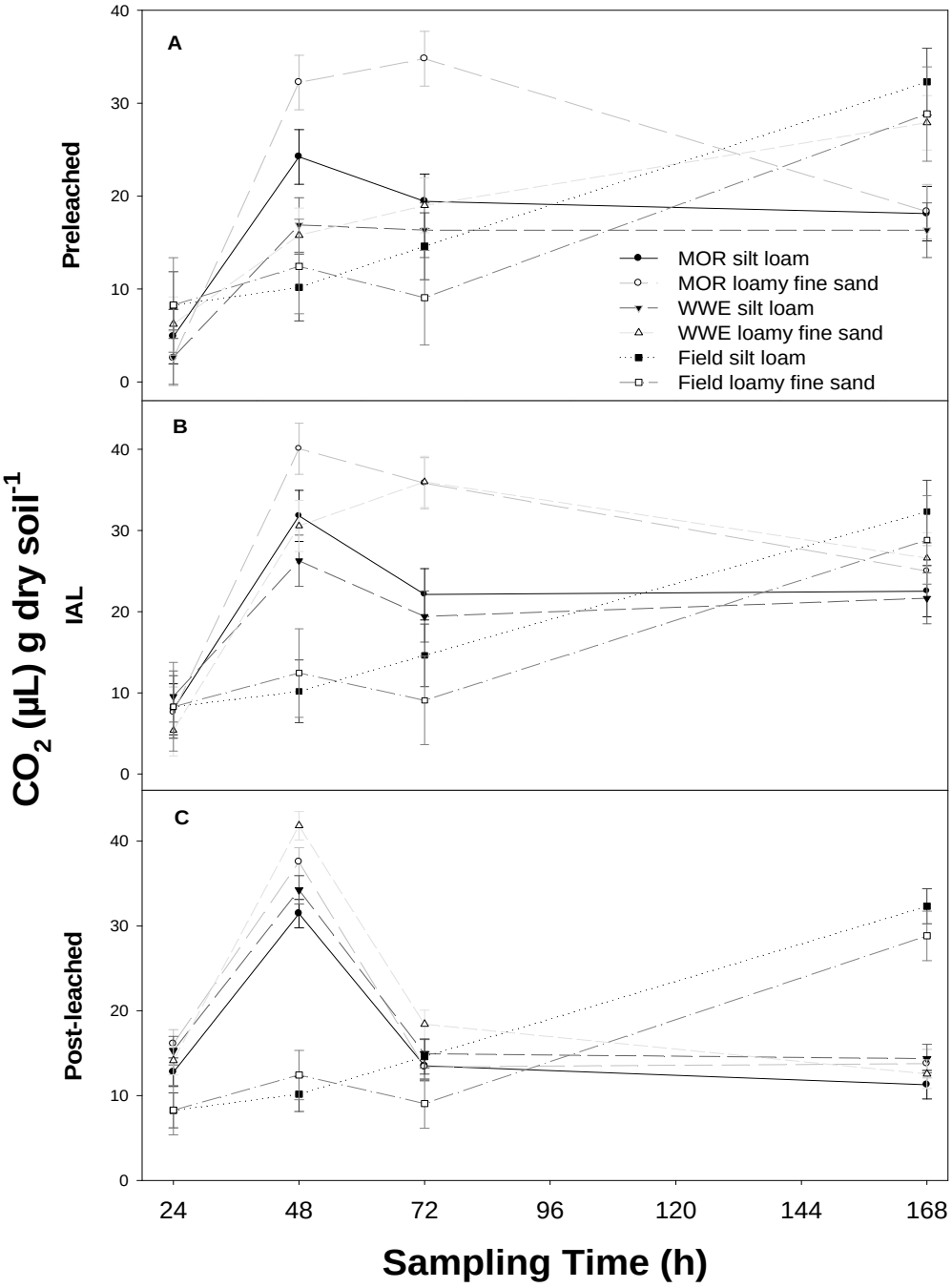


Figure 3. Mean total microbial CO_2 evolution by incubation time of soil materials collected from greenhouse microcosms before leaching (Preleached), immediately after leaching (IAL), and after leaching and harvesting vegetation (Post-leached) that were irrigated with Missouri River (MOR) or municipal wastewater effluent (WWE). Field-collected soils (Field) were collected from a wetland impoundment at Eagle Bluffs Conservation Area. Vertical bars indicated SE of the mean.



DISCUSSION

Microbial Activity among Soil Materials and Water Sources

Total CO_2 evolution of preleached soil materials indicated that microbial activity was repressed in soil materials irrigated with WWE compared with MOR. The relatively greater soil salinity and sodicity, as measured by EC and ESP, respectively (Table 1), resulting from repeated irrigation with WWE was probably responsible for suppressing soil microbial activity despite the favorable conditions of narrow C:N ratios and soil water content. Other studies also reported reduced microbial activity in saline soils or soils irrigated with wastewater effluents (Ghinogeanu et al., 1984, Mahasneh et al., 1984, Garcia and Hernandez, 1996, Pankhurst et al., 2001, Rietz and Haynes, 2003).

The significant increase in CO_2 evolution immediately after leaching suggested that microbial activity responded to the leaching treatment, which decreased soil EC by 69% and 93% and ESP by 61% and 47% in the MOR- and WWE-irrigated soil materials, respectively. Decreasing salinity and ESP of soil materials produced a lower osmotic gradient between soil and microorganisms, which may have reduced osmotic stress on microbial activity (Schimel et al., 1989). Reduced osmotic stress may decrease immobilization of C and N in microbial biomass thereby increasing C and N mineralization (Sarig et al., 1993). Additionally, the decrease in soil salts may allow development of a greater functionally diverse microbial community (Pankhurst et al., 2001).

The decline in CO_2 evolution in post-leached treatments indicated lower microbial activity in nearly all treatments probably due to increases in EC and ESP resulting from resumption of irrigation. However, net changes in CO_2 relative to preleached conditions

favored WWE-irrigated treatments. Immediately after leaching, soil materials irrigated with WWE had a greater net increase in evolved CO_2 (average of both soil materials) than with MOR. WWE-irrigated soil materials had an average increase of 27.4 μL CO_2 g dry soil^{-1} at IAL compared with 19.3 μL CO_2 g dry soil^{-1} in MOR-irrigated soil materials. Furthermore, post-leached WWE-irrigated soil materials had a smaller net decrease in CO_2 evolution (4.9 μL CO_2 g dry soil^{-1}) than MOR-irrigated soil materials (20.2 μL CO_2 g dry soil^{-1}). The greater net gain and smaller net loss in evolved CO_2 at these sampling periods in the WWE-irrigated soil materials may be related to a combination of decreased salinity, utilization of residual labile soil carbon, and greater plant density and biomass that established after leaching. Sodicity has been reported to solublilize labile and recalcitrant organic materials; however, its effect can be hindered by salinity and anaerobisis (Abdou, 1975; Nelson et al., 1996). Labile carbon sources may have remained prior to and during the preleached trial when high salinity impaired the ability of Na to solublilize carbon sources and repressed microbial activity. After leaching, carbon sources could be subject to mineralization under decreased salinity and re-activation of microbial communities as osmotic pressure decreased.

Labile carbon may also be derived from release of intracellular contents (i.e., amino acids, sugars) of lysed microbial cells, resulting from wetting (i.e., flushing) and drying (i.e., draining) cycles during the leaching treatment (Lund and Goksøyr, 1980; Fierer et al., 2002). Even though wet / dry cycles (resulting from periods in between irrigation applications) occurred during all trials, salinity was considerably greater during the preleached trial and continued to suppress microbial activity. In addition, root

exudates, which increased as plants were established during the post-leached trial, probably contributed to liable carbon sources.

CO_2 Evolution by Incubation Time

Peak respiration tends to occur at 48 to 72 hrs during laboratory incubations (Lund and Goksøyr, 1980). In this study, maximum CO_2 evolution from MOR- and WWE-irrigated silt loam occurred at 48 hrs for each sampling period. Maximum CO_2 evolution from loamy fine sand of both water sources shifted to 48 hrs after leaching. In contrast, maximum CO_2 evolution of field-collected soil materials occurred at the 7 d sampling.

The CO_2 evolution in the loamy fine sand microcosms occurring at 48 hrs may indicate a change in the microbial community from slow-responding, oligotrophic microorganisms to fast-responding copiotrophic microorganisms as more readily metabolizable carbon became available (Fierer et al., 2007). This shift could also be in response to reduced salinity due to leaching and the wet / dry cycles, which may favor copiotrophic microbial groups capable of rapid growth (Fierer et al., 2002). Field-collected soil materials, on the other hand, which were not subjected to leaching, may have contained more recalcitrant carbon sources or perhaps, more oligotrophic microorganisms. Field-collected soils contained greater concentration of exchangeable Ca than soil materials in microcosms at the time of these samplings (Finocchiaro, unpublished data, 2009). Greater exchangeable Ca in the field-collected soil materials may have hindered microbial activity during the initial incubation (24 h) and activity increased after Ca linkages were disintegrated (Nelson et al., 1996). Another possibility

is oligotrophic microbial groups, which respond slowly to substrate additions (i.e., glucose; Fierer et al., 2007) were dominant in the field-collected soil materials. This suggests that a shift in microorganism groups occurred in microcosms from oligotrophic to copiotrophic strategy. Hirsch et al. (1979) and Gottschal (1985) have reported that soil bacteria can switch from one strategy to another depending on environmental conditions and life stage.

CONCLUSIONS

Microbial activity was significantly impaired in soil materials irrigated with WWE compared with MOR as a water source. The greater EC and sodium concentration of the WWE increased soil salinity and sodicity, which is thought to have inhibited microbial activity. After reducing soil EC and ESP by leaching, activity increased and was similar between water sources. A shift from oligotrophic to copiotrophic microbial groups may occur in the loamy fine sand for both water sources in response to leaching of soil salts. Because soil microorganisms are critical to many soil processes such as nutrient cycling, aeration, aggregate development and stability, decreased microbial activity may affect the plant community and other biotic systems, thereby negatively impacting these processes. Our results agree with Pankhurst et al. (2001) and Zahran (1997) who concluded that microbial community function may be affected by salinity only when the salt is actually present in soil; yet recovers when salts are leached from the soil.

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Chapter 7

Summary and Monitoring of Wastewater Effluent Effects on Wetland Impoundments

This dissertation addresses some ecological and edaphic aspects of the use of municipal wastewater effluent (WWE) on wetland impoundments designated for wildlife at Eagle Bluffs Conservation Area (EBCA). However, the information gained from these studies can be applied to other areas that use or intend to use high saline and sodic water sources on freshwater wetlands.

To address the questions put forth by the Missouri Department of Conservation for EBCA (Missouri Department of Conservation 1991), a combination of field, greenhouse, and laboratory studies were used to evaluate the effects of WWE on wetland seed banks, vegetation, soil chemistry, and soil microorganisms.

In a comparison between impoundments irrigated with different water sources at EBCA (Chapter 2), the WWE-irrigated impoundment had greater soil EC and lower pH than the impoundment irrigated with Missouri River water (MOR). The significantly different EC and pH in the WWE-irrigated impoundment is attributed to the greater concentration of electrolytes, organic matter, and ammonium in the WWE than the MOR water. However, soil properties that influence ion exchange and drainage in addition to water-management may have contributed to the significant differences in these soil parameters within and between impoundments.

Electrical conductivity of soils in the WWE-irrigated impoundment was below the 4 mS cm^{-1} threshold, and therefore not considered saline (Havlin et al. 1999). However,

soil EC has increased by more than 59% in the WWE-irrigated impoundment over a seven year period (comparison of 1998 to 2004) and is likely to increase with prolonged use of WWE. A current estimate of EC in Pool 2 (WWE irrigated) is 1209 ± 232 μS cm^{-1}. Some research on the affects of salinity on vegetation have demonstrated that an EC of 2000 μS cm^{-1} induces plant stress thereby decreasing productivity and altering the plant community (Summer and Nadui 1998).

Results from greenhouse trials (Chapters 3 and 4) indicate that irrigation with wastewater effluent significantly decreased vegetation taxa richness, plant density, and biomass. Salinity and sodicity rapidly increased in WWE-irrigated microcosms relative to irrigation with other water sources and probably was responsible for inhibiting germination or interfering with seedling development of the soil seed bank. Soil salinity can cause stress in vegetation by interfering with the uptake of water and plant essential nutrients. High concentrations of sodium can disperse soil particles thereby destroying soil aggregation (structure). Loss of soil structure impacts soil pore size distribution, infiltration, hydraulic conductivity and rooting depth. Leaching of soil salts in the microcosms with a high quality water source and application of gypsum to the soils previously irrigated with wastewater effluent resulted in a 3-fold increase in taxa richness and plant density, and a 85% increase in biomass (Chapter 4). Seed bank response after leaching indicates that these freshwater seed banks were still viable after prolonged exposure to saline-sodic soil conditions. Results also suggest that dicotylous species of these soil seed banks may be more susceptible to germination inhibition induce by high salinity and sodicity than monocots.

Soil microbial abundances obtained from cultures of the bulk soil and rhizosphere of the microcosms and samples of soils from an impoundment at EBCA were not statistically different among treatments (Chapter 5). Abundances of bulk soil microbes were significantly different between soil materials regardless of water source and attributed to soil properties that influence the exchange complex and nutrients. Furthermore, saline and sodic conditions may have stressed plants and in conjunction with repeated dry–wet cycles in the microcosms may have impaired overall abundance of microbes. Additionally, microbial activity determined by CO_2 respiration, showed soil microbes were significantly less active in the high saline-sodic soils (Chapter 6). Immediately after leaching, activity significantly increased and was similar to the MOR-irrigated soils. Increased activity after leaching may be related to decreased salinity and sodicity, which probably lowered osmotic potential in the soil. Decreased microbial activity may reduce soil organic matter decomposition, affect soil pH, and impact nutrient cycling and glycophytic vegetation communities in wetlands.

Wastewater irrigated wetland impoundments may develop elevated levels of salinity and sodicity that alter edaphic conditions and ecological processes. However, heterogeneity of soils in impoundments may promote or prohibit areas of salt accumulation. Soil properties such as drainage, texture, and exchange capacity, as well as hydrologic connectivity to ground-water or surface-water of better quality (e.g., lower EC), which could flush salts from the soils, should be a consideration for wetland impoundments that receive WWE. This may be particularly pertinent to wetland managers that employ moist-soil practices to stimulate germination of selective taxa from freshwater seed banks. Several wetland species that are known to be waterfowl foods

were inhibited by salinity and sodicity conditions (Chapter 3). Alternating or mixing water sources and alternating periods of inundation (e.g., seasonal instead of semi-permanent), frequency of flooding, and drawdown to decrease salt loading and increase flushing of soil salts may be an inexpensive strategy to reduce the likelihood of developing saline or sodic conditions.

The amount and chemical composition of the WWE typically changes within and among seasons. Periodic measurement of soil EC, ESP, and pH may be advisable to monitor these important soil quality parameters to determine seasonal and longer temporal trends. Areas in impoundments that may be more susceptible to salinity-sodicity alterations such as depressional areas underlain by fine textured materials should be emphasized. Measurement of soil EC and pH is relatively easy, inexpensive to perform, and provides essential information regarding soil chemistry and nutrient cycling. Additionally, nutrient loading of WWE-irrigated impoundments should be determined periodically to provide a better understanding of its influence on soil pH.

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Appendix 1

Figure 1. Generalized arrangement of greenhouse microcosms used to grow vegetation during trials 1–4 of Experiment I. A microcosm was filled with one of two soil materials and irrigated with one of three water sources. The combination of soil and water was considered a treatment. There were six different treatments with nine replicates of each treatment. One of each treatment replicate was randomly assigned a column in each row (letters A–I). Each row contained six mutually exclusive treatments. All treatment replicates were re-randomized within the array prior to each trial.

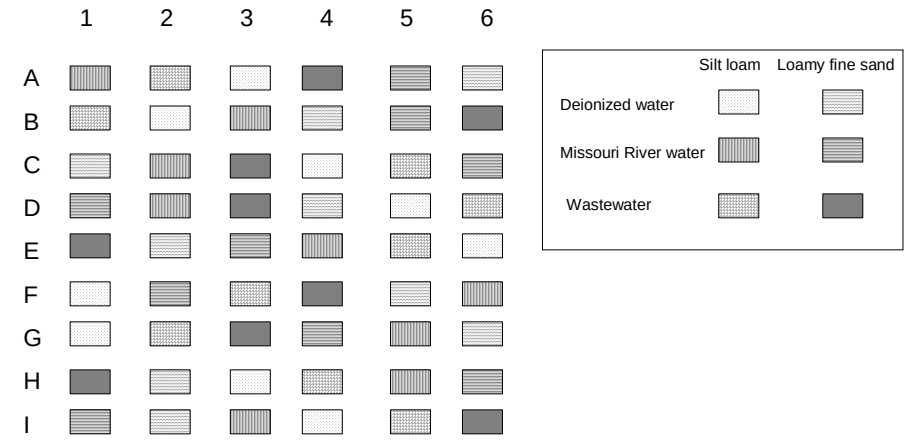


Figure 2. Generalized arrangement of greenhouse microcosms used to grow vegetation for the second study. There were six different treatments (2 soils x 3 water sources). There were three replicates of the treatments irrigated with Missouri River water and four replicates of the treatments previously irrigated with wastewater effluent that were switched to Missouri River water. Wastewater effluent irrigated treatments had five replicates. Treatments were randomly assigned a column in a row (letters A–D). Each row contained at least one of each treatment. All treatment replicates were re-randomized within the array prior to each trial.

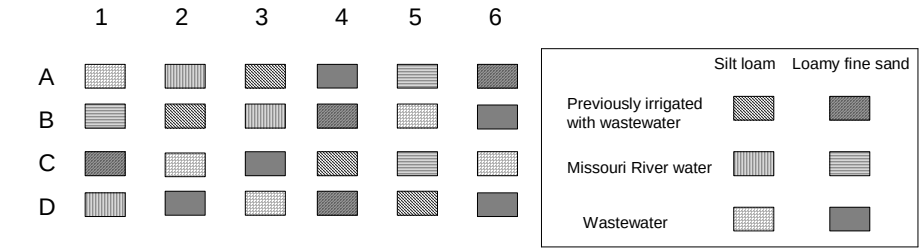
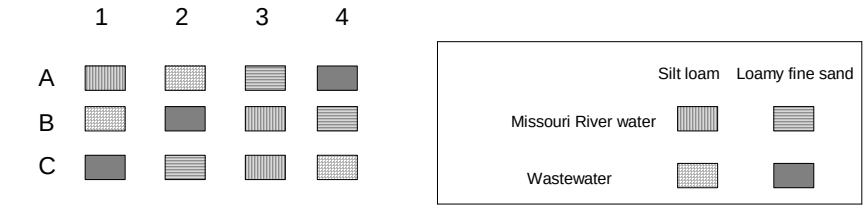


Figure 3. Generalized arrangement of greenhouse microcosms after leaching during the last trial of the second study. There were four different treatments (2 soils x 2 water sources) with three replicates of each treatment. Treatments were randomly assigned a column in a row (letters A–C). Each row contained four mutually exclusive treatments.



Appendix 2.1. Analysis of Variance tables (Mixed procedure) of main factors and interactions for soil electrical conductivity and salt derived pH. Soil cores were taken from the River Supply Channel and Pool 2 impoundments at Eagle Bluffs Conservation Area in 2004. Elevation refers to normal pool or flood stage. Soil cores were separated into 5-cm increments by depth for 0–30 cm and 10 cm for 90–100cm. Degrees of freedom for each source are given for the numerator (DF) and denominator (Den DF).

Electrical conductivity**.**

Source	DF	Den DF	F value	Pr > F
Impoundment * Elevation	3	28	8.15	0.0005
Depth	6	168	15.65	< 0.0001
Impoundment * Elevation * Depth	18	168	2.39	0.0021

Soil pH ($CaCl_2$)

Source	DF	Den DF	F value	Pr > F
Impoundment * Elevation	3	28	7.42	0.0008
Depth	6	168	17.68	< 0.0001
Impoundment * Elevation * Depth	18	168	5.07	< 0.0001

Appendix 2.2. Description of soil core materials taken from both normal and flood elevations of the River supply Channel (RSC) and Pool 2 at Eagle Bluffs Conservation Area in 2004.

Abbreviations and codes used for describing soil core material (Schoeneberger et al., 1998):

Structure

sbk	= subangular blocky
ab	= angular blocky
pl	= platy
gr	= granular
sg	= single grain
m	= massive

Grade

0	= structureless; no discrete units
1	= weak
2	= moderate
3	= strong

Root Quantity (assessed based on root size)

VF	= very few (< 0.2 per area)
F	= few (< 1 per area)
C	= common (1 to < 5 per area)
M	= many (5 or more per area)

Root size

VF	= very fine
F	= fine
M	= medium
C	= coarse

Root Location

T	= throughout matrix
P	= between peds (ped faces)

Appendix 2.2. Soil core descriptions by Pool location and water elevation. Cores were excavated from Eagle Bluffs Conservation Area in 2004.

Pool location	core #	elevation	horizon	start depth (cm)	end depth	texture	structure grade	color	root quantity	root size	root location	effer-vescence
2	1	normal	A	0	6	clay	sbk 3	10YR 2/1	F	F	T	no
			Bw1	6	30	clay	sbk 3	10YR 3/1	F	C	T	no
			Bw2	30	42	clay	ab 3	2.5Y 5/3	none			yes
			2C	42	84	sandy loam	sbk 1	2.5 Y 6/3	F	C	T	yes
2	2	normal	A1	0	3	clay	sbk 3	10 YR 2/1	F	VF	T	no
			A2	3	17	clay	sbk 3	10 YR 2/1	F	VF	T	yes
			Bw	17	35	clay	sbk 3	10 YR 3/2	none			yes
			2Bg	35	57	silty clay	sbk 3	2.5 Y 4/2	F	F	T	yes
			3C	57	112	sandy loam	sbk 1	2.5 Y 6/3	none			yes
2	3	normal	A	0	9	clay	sbk 3	10 YR 2/1	M	F	T	no
			Bw	9	20	clay	sbk 3	10 YR 2/1	F	VF	T	no
			2C1	20	73	sandy clay	sbk 3	2.5 Y 4/2	F	VF	T	yes
			3C2	73	87	loam	sbk 1	2.5 Y 4/2	none			yes
			3C3	87	100	loam	sbk 3	2.5 Y 4/2	none			yes
2	4	normal	A	0	3	silty clay	sbk 3	10YR 2/1	C	VF	T	no
			Bw	3	20	silty clay	sbk 3	10YR 2/1	F	VF	T	no
			2Bg	20	28	sandy clay loam	sbk 3	2.5 Y 4/2	F	VF	T	yes
			3C1	28	49	sandy loam	sbk3	2.5 Y 4/3	F	VF	T	yes
			4C2	49	64	sandy clay loam	sbk 3	2.5 Y 4/2	F	VF	T	yes
			4C3	64	80	sandy clay loam	sbk 3	2.5 Y 4/2	F	VF	T	yes
			5C4	80	112	sandy loam	sbk 2	2.5 Y 4/2	none			yes

Appendix 2.2. continued.

Pool location	core #	elevation	horizon	start depth (cm)	end depth	texture	structure grade	color	root quantity	root size	root location	effer-vescence
2	5	normal	A1	0	14	clay (vf sand)*	sbk 2	2.5 Y 3/1	C	F	T	no
			A2	14	57.5	clay	ab 3	2.5 Y2.5/1	F	VF	T	no
			2C1	57.5	71.5	sandy clay loam (vf sand)*	ab 2	2.5 Y 4/3	none			no
			2C2	71.5	91.5	sandy clay loam	ab 2	2.5 Y 4/2	none			yes
			3C3	91.5	99.5	sandy loam	ab 1	2.5 Y 4/3	none			yes
2	6	normal	A	0	29	clay	sbk 3	10YR 3/1	F	F	P	no
			Bg	29	53	clay	ab 3	2.5 Y 3/1	none			no
			2C	53	96	silt loam	sbk 3	2.5 Y 6/2	F	VF	T	no
2	7	normal	A	0	58	clay	ab 3	10YR 3/1	F	VF	T	no
			2Bw	58	77	silt loam	ab 3	2.5 Y 5/3	F	VF	T	yes
			2C	77	119	silt loam	ab 3	2.5 Y 5/3	F	VF	T	yes
2	8	normal	A1	0	2	clay	sbk 2	2.5 Y2.5/1	C	F	T	no
			A2	2	44	clay	ab 3	2.5 Y2.5/1	F	VF	P	no
			2C	44	103	silt loam	ab 3	2.5 Y 3/2	none			yes

Appendix 2.2. continued.

Pool location	core #	elevation	horizon	start depth (cm)	end depth	texture	structure grade	color	root quantity	root size	root location	effer-vescence
2	1	flood	A	0	12	clay	sbk 2	10 YR 3/1	F	F	T	no
			Bg	12	23	clay	sbk 3	2.5 Y 3/1	F	F	T	no
			2C	23	100	loam	sbk 2	2.5 Y 4/3	F	VF	T	yes
2	2	flood	A	0	9	clay	sbk 2	10 YR 3/1	F	F	T	no
			2Bw1	9	22	loam	sbk 2	2.5 Y 5/3	F	F	T	no
			3Bw2	22	77	sandy loam	sbk 1	2.5 Y 5/3	F	F	T	no
			4C	77	112	silt loam	sbk 3	2.5 Y 4/2	F	F	T	yes
2	3	flood	Ap	0	20	clay	sbk 3	2.5 Y 3/1	F	VF	T	no
			2Bw1	20	30	loam	sbk 3	2.5 Y 3/2	F	VF	T	no
			2Bw2	30	38	loam	sbk 2	2.5 Y 4/3	F	VF	T	yes
			3C	38	60	sandy loam	sbk 1	2.5 Y 6/4	F	VF	T	yes
2	4	flood	A	0	4	silty clay loam	sbk 3	2.5 Y 3/1	F	F	T	no
			Bg1	4	21	silty clay loam	sbk 3	2.5 Y2.5/1	F	F	T	no
			Bg2	21	42	silty clay loam	ab 3	2.5 Y2.5/1	none			no
			Bg3	42	56	silty clay loam	ab 3	2.5 Y 4/2	F	VF	P	no
			2C	56	116	silt loam	sbk 2	10YR 5/4	F	VF	T	yes

Appendix 2.2. continued

Pool location	core #	elevation	horizon	start depth (cm)	end depth	texture	structure grade	color	root quantity	root size	root location	effer-vescence
2	5	flood	A1	0	3	clay	sbk 2	2.5 Y 3/1	VF	F	T	no
			A2	3	19	clay	sbk 3	2.5 Y 3/1	VF	F	T	no
			2C1	19	31	sandy clay loam	pl 3	2.5 Y 4/3	none			no
			3C2	31	45	sandy loam	sbk 1	2.5 Y 5/3	none			no
			4C3	45	60	sandy clay loam	pl 3	2.5 Y 4/3	none			yes
			5C4	60	73	silty clay	sbk 3	2.5 Y 4/2	VF	F	T	yes
			6C5	73	88	sandy loam	sbk 2	2.5 Y 4/3	none			yes
2	6	flood	A1	0	13	clay	sbk 3	2.5 Y 3/1	F	F	T	no
			A2	13	20	clay	sbk 3	2.5 Y 3/2	F	F	T	no
			Bg	20	38	clay	ab 3	2.5 Y2.5/1	F	F	P	no
			C1	38	48	clay	pl 3	2.5 Y 4/1	F	VF	P	no
			2C2	48	63	sandy loam	sbk 2	2.5 Y 5/3	none			yes
			3C3	63	76	sandy clay loam	sbk 2	2.5 Y 4/3	none			yes
			4C4	76	88	clay	sbk 3	2.5 Y 4/2	none			yes
			5C5	88	112	sandy loam	sbk 2	2.5 Y 4/3	none			yes
2	7	flood	A1	0	20	clay loam	sbk 3	10 YR 3/1	none			no
			A2	20	43	clay	ab 3	10 YR2.5/1	none			no
			Bg	43	62	clay	sbk 3	10 YR 4/2	none			yes
			C1	62	68	clay	sbk 1	2.5 Y 6/3	none			yes
			C2	68	84	clay	sbk 1	2.5 Y 4/4	none			yes
2	8	flood	A	0	53	clay loam	sbk 3	2.5 Y 3/1	VF	F	P	no
			Bw	53	93	sandy clay loam	sbk 3	2.5 Y 4/4	VF	F	T	yes
			C1	93	111	sandy clay loam	sbk 1	2.5 Y 4/2	VF	F	T	yes
			C2	111	116	sandy loam	sbk 2	2.5 Y 4/2	VF	F	T	yes

Appendix 2.2. continued

Pool location	core #	elevation	horizon	start depth	end depth	texture	structure grade	color	root quantity	root size	root location	effer-vescence
RSC	1	normal	A1	0	8	silty clay loam	sbk 2	10 YR 4/1	F	VF	T	no
			2Bg1	8	38	loam	sbk 2	10 YR 4/1	F	VF	T	yes
			2Bg2	38	52	loam	sbk 2	10 YR 4/2	F	VF	T	no
			3C1	52	90	sandy loam	sbk 2	10 YR 4/3	none			no
			4C2	90	102	silty clay loam	sbk 2	10 YR 4/3	F	VF	T	yes
RSC	2	normal	A1	0	18	clay loam	sbk 2	10 YR 2/1	F	VF	T	yes
			C1	18	24	loam	sbk 2	2.5 Y 6/4	F	VF	T	yes
			2Ab	24	31	clay loam	sbk 2	10 YR 5/3	F	VF	T	yes
			2Bgb1	31	58	silty clay loam	sbk 2	10 YR 4/2	F	VF	T	yes
			2Bgb2	58	100	silty clay	m 2	10 YR 3/2	F	VF	T	no
			3Cb1	100	112	sandy loam	sg 1	2.5 Y 4/4	none			yes
			4Cb2	112	117	silty clay	sbk 3	10 YR 4/3	none			no
RSC	3	normal	A	0	22	loam	sbk 2	10 YR 3/2	C	VF	T	yes
			Bw1	22	36	loam	sbk 2	2.5 Y 5/4	F	VF	T	yes
			2Bw2	36	61	silt clay loam	sbk 3	10 YR 3/2	F	VF	T	no
			3Bw3	61	79	silty clay	sbk 3	10 YR 3/2	F	VF	T	no
			4Bw4	79	106	silty clay loam	sbk 3	10 YR 3/3	F	VF	T	no
			5C1	106	110	sandy loam	sg 1	2.5 Y 4/4	F	VF	T	yes
			5C2	110	121	silty clay loam	sbk 3	10 YR 4/2	F	VF	T	yes
RSC	4	normal	A	0	5	silty clay loam	sbk 1	10 YR 2/1	F	VF	T	yes
			Bg1	5	30	silty clay loam	sbk 3	10 YR 3/1	F	VF	T	yes
			Bg2	30	38	silty clay loam	sbk 3	2.5 Y 2/1	F	VF	T	no
			2C1	38	44	sandy loam	sg 1	2.5 Y 5/3	none			yes
			2C2	44	51	clay loam	sbk 3	2.5 Y 4/1	none			no
			3C3	51	70	sandy loam	sg 1	2.5 Y 5/3	none			yes

Appendix 2.2. continued

Pool location	core #	elevation	horizon	start depth	end depth	texture	structure grade	color	root quantity	root size	root location	effer-vescence
RSC	5	normal	A1	0	8	silty clay loam	sbk 3	10YR 2/2	F	VF	T	yes
			A2	8	17	silty clay loam	sbk 3	10 YR 2/1	F	VF	T	yes
			Bw1	17	28	silty clay loam	sbk 3	10 YR 3/2	none			yes
			2Bw2	28	85	sandy clay loam	sbk 3	2.5 Y 3/3	F	VF	T	yes
			2C1	85	90	sandy loam	sg 1	2.5 Y 4/4	none			yes
			2C2	90	120	sandy clay loam	sbk 3	2.5 Y 4/4	none			yes
RSC	6	normal	A1	0	4	loam	gr 3	10 YR 3/1	F	VF	T	yes
			A2	4	10	loam	sbk 3	10 YR 3/1	F	VF	T	yes
			Bw1	10	26	loam	sbk 3	2.5 Y 4/3	F	VF	T	yes
			Ab	26	42	clay loam	sbk 3	10 YR 3/1	F	VF	T	no
			2Cb1	42	58	sandy loam	sbk 3	2.5 Y 5/4	F	VF	T	yes
			3Cb2	58	90	silty clay loam	sbk 3	2.5 Y 4/2	none			yes
			3Cb3	90	103	silty clay loam	sbk 3	2.5 Y 4/3	none			yes
			3Cb4	103	112	silty clay loam	sbk 3	2.5 Y 5/2	none			yes
RSC	7	normal	A	0	27	sandy loam	sbk 3	2.5 Y 4/3	F	VF	T	no
			Ab	27	34	clay loam	sbk 3	10 YR 3/1	F	VF	T	no
			Cb1	34	56	sandy loam	sbk 2	2.5 Y 5/3	F	VF	T	yes
			Ab`	56	110	clay loam	sbk 3	2.5 Y 3/1	F	VF	T	no
RSC	8	normal	A	0	16	clay loam	sbk 3	10YR 3/2	F	F	T	no
			Bg	16	41	clay loam	m 3	10 YR 4/2	F	F	T	yes
			2C1	41	43	sandy loam	pl 3	2.5 Y 6/3	none			yes
			3C2	43	64	clay loam	m 3	2.5 Y 3/2	F	VF	T	no
			4C3	64	72	sandy clay loam	sbk 3	2.5 Y 4/2	none			no
			5C4	72	120	clay loam	ab 3	2.5 Y 3/1	none			no

Appendix 2.2. continued

Pool location	core #	elevation	horizon	start depth	end depth	texture	structure grade	color	root quantity	root size	root location	effer-vescence
RSC	1	flood	A	0	21	silt loam	sbk 3	10 YR 3/2	F	VF	P	no
			2Bw1	21	48	loam	sbk 3	10 YR 4/2	F	VF	T	no
			3Ab	48	70	silt clay	sbk 3	10 YR 3/1	none			no
			4Bwb1	70	87	loam	sbk 2	10 YR 4/3	none			no
			5Bwb2	87	109	sandy loam	sbk 2	2.5 Y 5/4	none			no
RSC	2	flood	A	0	11	clay loam	sbk 2	10 YR 3/1	F	VF	T	no
			2Bw1	11	31	loam	sbk 3	10 YR 3/3	F	VF	T	no
			3Bw2	31	35	silty clay	sbk 3	10 YR 3/1	none			no
			4C1	35	96	silt clay loam	sbk 2	10 YR 4/1	none			no
			4C2	96	117	silt clay loam	sbk 3	10 YR 4/3	none			no
RSC	3	flood	A	0	10	clay loam	sbk 3	10YR 3/1	F	VF	T	no
			2Bg1	10	23	loam	sbk 3	10 YR 4/1	F	VF	T	none
			3Bg2	23	34	silty clay	sbk 3	2.5 Y 4/1	F	VF	T	no
			4Bw	34	50	sandy loam	sbk 3	2.5 Y 5/4	F	VF	T	no
			5Bg`3	50	87	silty clay loam	sbk 3	10 YR 4/2	F	VF	T	yes
			6C	87	117	silt clay	sbk 3	10 YR 4/2	F	VF	T	yes
RSC	4	flood	A	0	20	loamy sand	sbk 3	10 YR 4/4	F	VF	T	no
			2A2	20	34	silty clay	sbk 3	10 YR 3/1	F	VF	T	no
			3Bw	34	50	sandy clay loam	sbk 2	10 YR 3/3	F	VF	T	no
			4Bg1	50	58	silty clay	sbk 2	10 YR 3/2	F	VF	T	yes
			4Bg2	58	70	silty clay	sbk 2	10YR 3/2	F	VF	T	yes
			5C	70	80	sandy loam	sbk 1	10 YR 4/2	none			yes

Appendix 2.2. continued

Pool location	core #	elevation	horizon	start depth	end depth	texture	structure grade	color	root quantity	root size	root location	effer-vescence
RSC	5	flood	A	0	3	sandy loam	gr 2	10 YR 3/1	F	VF	T	no
			2A2	3	13	clay loam	sbk 2	10 YR 3/1	F	VF	T	no
			3Bw1	13	34	sandy loam	sbk 3	10 YR 5/4	F	VF	T	no
			4Bw2	34	43	silty clay loam	sbk 3	2.5 Y 6/3	F	VF	T	yes
			5Ab1	43	60	silty clay	sbk 2	10 YR 3/3	F	VF	T	no
			5Ab2	60	88	silty clay	sbk 2	10 YR 3/2	F	VF	T	no
			5Bgb	88	98	silty clay	sbk 3	10 YR 4/2	F	VF	T	yes
			6Cb	98	113	silty clay loam	sbk 3	2.5 Y 6/3	F	VF	T	no
RSC	6	flood	A	0	19	loam	sbk 1	10 YR 4/3	F	VF	T	yes
			2C	19	25	silt loam	sbk 2	10 YR 4/2	F	VF	T	yes
			3Ab1	25	37	silty clay	sbk 3	10 YR 2/1	F	VF	P	no
			4Cb1	37	66	loamy sand	sg 1	2.5 Y 5/4	F	VF	T	yes
			5Cb2	66	95	silty clay loam	sbk 2	10 YR 4/3	F	VF	T	yes
			6Cb3	95	97	loamy sand	sg 1	10 YR 4/3	F	VF	T	yes
RSC	7	flood	A	0	14	loam	sbk 2	10 YR 2/1	C	VF	T	yes
			Bw	14	23	loam	sbk 2	2.5 Y 5/4	F	VF	T	yes
			2C	23	32	loamy sand	sg 1	2.5 Y 5/4	F	VF	T	yes
			3Bgb	32	45	silty clay loam	sbk 3	10 YR 4/2	F	VF	T	yes
			4Bwb1	45	114	sandy loam	sbk 3	10 YR 4/3	F	VF	T	no
			5Bwb2	114	117	silty clay	sbk 2	10 YR 4/1	C	VF	T	yes
RSC	8	flood	A	0	24	sandy loam	sbk 2	10 YR 4/3	F	VF	T	yes
			2A	24	31	loam	sbk 3	10 YR 3/2	F	VF	T	no
			3C	31	36	silty clay loam	pl 3	2.5 Y 5/4	C	VF	T	yes
			4Ab	36	55	sandy clay	sbk 3	10 YR3/3	F	VF	T	no
			5Bgb1	55	78	silty clay	m 3	10 YR 4/2	F	VF	T	no
			5Bgb2	78	88	silty clay	sbk 3	10 YR 2/1	F	VF	T	no
			5Bgb3	88	113	silty clay	sbk 3	10 YR 4/1	F	VF	T	no

Appendix 4. Amount of gypsum ($CaSO_4 \cdot 2\ H_2O$) applied to microcosms irrigated with either Missouri River water (MOR) or Wastewater effluent (WWE) during leaching treatment and mean number of pore volumes in which deionized water was used to flush soil salts from soil material before and after gypsum application.

Treatments		Pore volumes (L)		Gypsum
Soil Material	Water	Before	After	applied (g)
Silt loam	MOR	7.8	3.5	215
	WWE	8.2	4.8	429
Loamy fine sand	MOR	9.6	5.5	344
	WWE	11.9	6.3	561

Appendix 5.1. ANOVA table (proc MIXED) of mean abundances of culturable, viable bacterial and fungal extracted from bulk soil materials and the rhizosphere of barnyard grass (*Echinochloa crus-galli*). Degrees of freedom of each source are given for the numerator (Num) and denominator (Den).

Extracted from	Media and source	DF (Num, Den)	F value	Pr >F
Soil	TSA (bacteria)			
	Soil	1,10	9.11	0.0129
	Water	3, 10	01.45	0.2866
	soil*water	3, 10	01.56	0.2593
	Kings (bacteria)			
	soil	1,10	10.47	0.0089
	water	3, 10	1.45	0.2852
	soil*water	3, 10	1.39	0.3028
	Martins (fungi)			
	soil	1, 10	7.62	0.0201
	water	3, 10	1.54	0.2645
	soil*water	3, 10	0.63	0.6109
Rhizosphere	TSA (bacteria)			
	soil	1,10	5.27	0.0446
	water	2, 10	0.17	0.8445
	soil*water	2, 10	0.15	0.8601
	Kings (bacteria)			
	soil	1,10	1.12	0.3158
	water	2, 10	0.01	0.9860
	soil*water	2, 10	0.64	0.5490
	Martins (fungi)			
	soil	1, 10	0.88	0.3712
	water	2, 10	0.29	0.7528
	Soil*water	2, 10	0.15	0.8589

Appendix 5.2. Mean colony abundances and standard errors by soil and water treatment. Soil materials (silt loam, loamy fine sand (loamy fs)) were collected from greenhouse treatments irrigated with Missouri River water (MOR), wastewater effluent (WWE), or deionized water (DI). Field collected soil materials were not irrigated with water sources and did not include rhizosphere material for assays. Values are log 10 transformed. Bacteria assays from bulk soil samples were enumerated from King-B and TSA growth media at10^{-6} serial dilution and fungi assay were enumerated from Martin's Rose Bengal growth medium at10^{-4} serial dilution. For rhizosphere samples, bacteria assays were enumerated from King-B and TSA growth media at10^{-5} serial dilution. Fungi assay were enumerated from Martin's Rose Bengal growth medium at10^{-3} serial dilution.

Table 1. Mean colonies by soil material and water source.

Soil and water treatment	Kings (g oven dry soil^{-1})	TSA (g oven dry soil^{-1})	Martins (g oven dry soil^{-1})
MOR- silt loam	6.42 ± 0.096	6.08 ± 0.168	3.88 ± 0.212
MOR – loamy fs	6.05± 0.096	5.71 ± 0.168	3.03 ± 0.212
WWE – silt loam	6.42 ± 0.096	6.13 ± 0.168	3.65 ± 0.212
WWE – loamy fs	5.95 ± 0.096	5.30 ± 0.168	3.01 ± 0.212
DI – silt loam	6.34 ± 0.096	6.02 ± 0.168	3.45 ± 0.212
DI- loamy fs	6.22 ± 0.096	5.79 ± 0.168	3.89 ± 0.212
Field –silt loam	6.06 ± 0.166	5.60 ± 0.281	3.91 ± 0.367
Field -- loamy fs	5.94 ± 0.166	5.42 ± 0.281	3.81 ± 0.367

Table 2. Mean colonies by water source (regardless of soil material).

Water source	Kings (g oven dry soil^{-1})	TSA (g oven dry soil^{-1})	Martins (g oven dry soil^{-1})
MOR	6.24 ± 0.068	5.90 ± 0.128	3.45 ± 0.150
WWE	6.19 ± 0.068	5.72 ± 0.128	3.33 ± 0.150
DI	6.28 ± 0.068	5.90 ± 0.128	3.67 ± 0.150
Field	6.00 ± 0.118	5.51 ± 0.210	3.86 ± 0.260

Table 3. Mean rhizosphere soil extracted colony abundances and standard errors by soil material and water source.

Soil and water treatment	Kings (g oven dry soil^{-1})	TSA (g oven dry soil^{-1})	Martins (g oven dry soil^{-1})
MOR - silt loam	7.60 ± 0.331	7.65 ± 0.205	5.60 ± 0.520
MOR - loamy fs	8.08 ± 0.331	8.16 ± 0.205	4.92 ± 0.520
WWE - silt loam	7.96 ± 0.331	7.67 ± 0.205	5.27 ± 0.520
WWE - loamy fs	7.82 ± 0.331	7.98 ± 0.205	5.14 ± 0.520
DI - silt loam	7.59 ± 0.331	7.62 ± 0.205	5.07 ± 0.520
DI - loamy fs	8.10 ± 0.331	7.95 ± 0.205	4.73 ± 0.520

Plate 1. General layout of greenhouse microcosms in column by row array. Microcosms are 90 L X 60 W X 20 D (cm).

Plate 2. Vegetative growth in greenhouse microcosms irrigated with either Missouri River water (Plate A) or municipal wastewater effluent (Plate B) from trial 4, in 1999.

A)

B)

Vita

Raymond Finocchiaro was born in St. Louis, Missouri to the parents of John and Dorothy Finocchiaro. He obtained a background in computer science from Meramec Community College in St. Louis. Attended and received a Bachelors of Arts degree from Webster University, St. Louis in biology while employed full-time as computer programmer. In 1996, Ray started a masters program in wetland ecology with Leigh Fredrickson at the University of Missouri – Columbia, which was completed in 2000. In 2001, he enrolled in the Soils, Environmental and Atmospheric Sciences at the University of Missouri under the advisement of Robert Kremer. In 2004, he accepted employment with United States Geological Survey in Jamestown, North Dakota as an ecologist researching wetland ecology. Currently, Ray and his spouse Diane live with their two children in Jamestown, North Dakota and Ray is employed at the Northern Prairie Wildlife Research Center.