SELECTING PLANT SPECIES TO OPTIMIZE WASTEWATER TREATMENT IN

CONSTRUCTED WETLANDS

by

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

of

Master of Science

in

Land Rehabilitation

MONTANA STATE UNIVERSITY
Bozeman, Montana

March 2009

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of a thesis submitted by

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

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March 2009

ACKNOWLEDGEMENTS

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ABSTRACT

Constructed wetlands are used around the world for treating domestic, agricultural, and industrial wastewater, stormwater runoff, and acid mine drainage. Plants may affect efficacy of wastewater treatment through their influence on microbial activity by creating attachment sites and releasing carbon exudates and oxygen. My research investigated seasonal plant effects on wastewater treatment by monitoring water chemistry in model subsurface wetlands planted with monocultures of 19 plant species and unplanted controls. Chemical oxygen demand (COD) removal, an indicator of water quality, declined during colder temperatures in the unplanted control, likely caused by a decrease in microbial activity. In contrast, wetlands with most plant species had constant COD removal across seasons. Redox potential and sulfate concentrations were measured as indirect measurements of the oxygenation of the wastewater. Wetlands that had a decline in COD removal during cold temperatures had constant low redox potential and sulfate concentrations throughout the seasons. Wetlands with high COD removal across seasons had elevated redox potentials and sulfate concentrations during the winter, indicating elevated oxygen availability, which may offset the negative temperature effect on microbial processes. I measured root oxygen loss (ROL) in the summer and the winter to determine whether oxygen release was sufficient to influence wastewater treatment and cause seasonal and species-specific effects on water chemistry. COD removal and ROL were positively correlated at 4˚C but not at 24˚C; however, the amount of root oxygen release only accounted for a portion of the required oxygen to facilitate plant's influence on COD removal. Flooding tolerance was quantified for each species by comparing plants' biomass between flooded and drained conditions. Plants' botanical grouping, Wetland Indicator Status, and flooding tolerance were compared to plants' influences on wastewater treatment to determine whether easily measured plant traits can be used to identify plants that will optimize wastewater treatment. All the sedges and rushes, obligate wetlands species, and 8 of 9 flood-tolerant plants had greater COD removal than the control at 4˚C, the coldest temperature incubation. These results can be applied for wetland design by selecting plant species to optimize wastewater treatment, especially in cold climates.

INTRODUCTION

Background

Natural Wetlands

Wetlands are diverse ecosystems teeming with life and activity which can activate all of our senses. After the disappearance of more than 50% of the wetlands in the U.S., these habitats have been increasingly recognized for their unique ecological functions and are protected by the federal government. Wetlands are defined by the US Army Corps of Engineers (Federal Register 1982) and the Environmental Protection Agency (Federal Register 1980) as "areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions." A great diversity of ecosystems around the earth are considered wetlands, including salt marshes, tidal freshwater marshes, inland marshes, riverine marshes, forested wetlands, mangrove swamps, and peatlands.

These diverse ecosystems perform similar landscape functions, which can be divided into three main categories: hydrology, habitat, and biogeochemistry (Cronk and Fennessy 2001). Hydrologic functions include regulating flows within watersheds and reducing flood and storm damage since wetlands act as water storage basins. Wetlands are among the most productive ecosystems in the world and provide habitat to a diversity of microbes, plants, insects, amphibians, reptiles, birds, fish, and mammals. A number of biogeochemical processes occur in wetlands due to saturation and high primary

productivity. Biogeochemical processes transport and transform chemicals through settling, absorption, microbial processes, plant uptake, and other processes, are part of global water and nutrient cycles and can remove many common pollutants that enter wetlands from surface and groundwater.

Water saturation in wetlands largely determines how the soil develops and the types of plant communities present. When saturated, soil pore spaces are filled with water rather than gas, reducing the rate of oxygen diffusion by a factor of 10,000 (Cronk and Fennessy 2001). Slow oxygen diffusion coupled with the respiration demands of plant roots and soil microorganisms result in anaerobic conditions. Because of this, reduced inorganic and organic compounds accumulate in the soil. Low oxygen levels and reduced compounds create a stressful environment for plant growth. Only those plants that are physiologically and structurally adapted to these anoxic conditions are capable of growing in wetlands.

Wetland plants have various traits and mechanisms for avoiding or tolerating anoxia. Flood-tolerant species' metabolic response to anaerobic conditions differs from less flood-tolerant plants (McManmon and Crawford 1971, Drew 1997, Schluter and Crawford 2001, Crawford 2003). Flooding-sensitive plants are severely damaged by 24 hours of anoxia. Some plants have strategies to withstand temporary anoxia by accelerating anaerobic metabolism, while other plants are well adapted to survive and grow for extended periods in anaerobic conditions. Wetland plants which are flood tolerant may down-regulate metabolism and have adapted structurally to create an aerobic environment within anoxic conditions. Aerenchyma tissue, which has gas-filled

spaces, called lacunae, separating cells, provides a low-resistance internal pathway for the exchange of gases between the plant parts above the water and the submerged tissues (Armstrong 1964, Armstrong 1979, Justin and Armstrong 1987).   Oxygen is transported to roots, and is either used in respiration or released to the surrounding soil or water matrix (Armstrong 1971a). Many wetland plants prevent excessive oxygen loss from the basal root zones by forming roots with a complete or partial 'barrier' to radial oxygen loss in their epidermis, exodermis or subepidermal layers with lignified or suberized cells (Armstrong 1979, Jackson and Drew 1984, Armstrong and Armstrong 1988, Jackson and Armstrong 1999, Visser et al. 2000b).

Root oxygen loss to the surrounding soil or water matrix results in the oxidation and detoxification of potentially harmful reducing substances in the rhizosphere thus benefitting plants (Armstrong 1979, Barko and Smart 1983, Tolley et al. 1986, Ernst 1990, Koncalova 1990). Reduced manganese, iron, and sulfur interfere with enzyme structure, chlorophyll formation, and metabolism, respectively (Cronk and Fennessy 2001). The reduced substances are products of microorganisms' anaerobic metabolism. Under saturated soil conditions, anaerobic soil microorganisms use terminal electron acceptors other than oxygen, reducing nitrate (NO_3^-), manganese ions (Mn^{4+}), ferric ions (Fe^{3+}), sulfate (SO_4^{2-}), and carbon dioxide (CO_2). Root oxygen loss provides an energetically favorable terminal electron acceptor for microbial respiration resulting in oxidation of organic carbon, sulfide (S^{2-}), and reduced iron and manganese.

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Wetlands as Treatment Systems

Constructed wetlands designed specifically for wastewater treatment take

advantage of purifying processes found in natural wetlands. They can provide an energy-

efficient, cost-effective, and low maintenance alternative to conventional wastewater

treatment technologies (Kadlec and Knight 1996). They also have ancillary benefits that

include creating habitat for plants and wildlife and an aesthetic greenspace in

communities. Constructed wetlands are used around the world to treat domestic,

agricultural, and industrial wastewater, stormwater runoff, and acid mine drainage. They

are designed either as free water surface wetlands with standing water or as subsurface

flow wetlands with water below the soil or gravel surface. Wastewater is delivered in

batches or continuously and flows either horizontally or vertically through the substrate.

Wastewater in constructed wetlands is treated by physical, chemical, and

biological interactions between wastewater, wetland media, microorganisms, and plants.

As in natural wetlands, basic treatment mechanisms include sedimentation, chemical

precipitation, adsorption, uptake by vegetation, and microbial processes. Microbial

activity has been considered to be the primary mechanism for removing most pollutants

including organic carbon and nitrogen (Hatano et al. 1993, Kadlec and Knight 1996).

Constructed wetlands are designed to increase the predictability and efficiency of

treatment, enabling use of wetlands that are less land-intensive.

Plants are major components of wetlands, yet relatively little is known about their

effects on wastewater and microbial processes or seasonal responses to temperature. A

greater understanding of plants' seasonal influences would allow designers to manipulate

another variable to increase the efficiency of wastewater treatment. In currant practice, relatively few plant species are used in constructed wetlands. Of the 'first generation' of constructed wetlands, one-third of the free water surface systems used only *Typha* spp. and about 40% of the subsurface systems used only *Scirpus* spp. (Reed and Brown 1992). *Phragmites australis* is the most widely used plant species in European systems (USEPA 1993). The low number of species used is likely due to the lack of knowledge regarding plant influences on wastewater treatment and a shortage of evidence for species-specific effects.

Past studies that compared the inflow and outflow chemistry in constructed wetlands either with or without plants have revealed contradictory results. Some publications report that plants have little direct impact on wastewater treatment (Brix and Schierup 1990, Haberl and Perfler 1990, Kadlec and Knight 1996, USEPA 2000, Vacca et al. 2005), while others studies have found improved treatment with plants present (Coleman et al. 2001, Hunter et al. 2001, Lin et al. 2002, Picard et al. 2005, Iamchaturapatr et al. 2007, Yang et al. 2007). These contradictions may reflect the plants selected to test against unplanted wetlands or the season in which studies have been conducted.

In addition to determining that the presence of plants improved treatment over unplanted wetlands, a few studies have documented species-specific effects. However, the ranking of species performance differed. Gersberg et al. (1986), Allen et al. (2002), Fraser et al. (2004), and Stein and Hook (2005) found that *Scirpus* spp. and *Carex* spp. had greater nutrient removal than *Typha* spp., while Akratos and Tsihrintizis (2007)

found that *Typha* spp. had the greatest removal. No direct measurements were taken to determine the mechanisms behind species' influences in these studies, but researchers conjectured that differences were due to either root oxygen loss or direct plant uptake.

Plants can improve treatment via the direct uptake of nutrients, but plant nutrient content cannot account for the enhanced nutrient removal from wastewater (Gersberg et al. 1986, Bachand and Horne 2000, Lin et al. 2002, Akratos and Tsihrintizis 2007). Rather, plant's influences on microbial activity, which include providing attachment sites and releasing carbon exudates and oxygen, are believed to be their primary role in treatment wetlands (Reed and Brown 1992, Brix 1997, Tanner 2001).

Research Objectives

The purpose of my research was to determine whether plants affect treatment efficacy in constructed wetlands, and if so, by what means. Additionally, my research evaluated species-specific effects and investigated relationships between plant traits and influences on wastewater treatment, so that plant traits can guide species selection to help optimize wastewater treatment. The objectives of the experiment in Chapter 2 were to investigate effects of plants and season on wastewater treatment. Chapter 2 describes an experiment which measured and compared water chemistry (COD removal, sulfate concentrations, and redox potential) across seasons and compared unplanted columns and columns planted with monocultures of 19 plant species.

The third chapter addresses the mechanisms by which plants increase treatment efficacy. The objectives of the experiments included in this chapter were to determine

whether root oxygen loss is a mechanism driving plants' seasonal effects, and whether relationships exist between plant traits and their influences on wastewater. This could be valuable for increasing a designer's ability to select species that maximize wetland efficacy. I measured root oxygen loss in the summer and the winter to determine whether oxygen release was sufficient to influence wastewater treatment and cause the seasonal and species-specific effects on water chemistry documented in Chapter 2. To determine whether there is a relationship between plant traits and treatment efficacy, COD removal data reported in Chapter 2 were compared to the plants' Wetland Indicator Status, flooding tolerance, root morphology, and root anatomy.

Together, these studies create a bridge between the biology and ecology of wetland plants and applied interests in engineering wastewater treatment systems. Increasing our knowledge of the interactions in the 'black box' of the constructed wetland substrate could help provide a more rational basis for plant selection and increase wastewater treatment efficacy. In addition, ancillary functions of artificial wetlands, such as providing a diverse habitat would benefit from the increased use of native plants are shown to provide good water treatment (USEPA 2000).

SEASONAL PLANT EFFECTS ON WASTEWATER TREATMENT

Introduction

Constructed wetlands can provide an energy-efficient and cost-effective alternative to conventional wastewater treatment technologies, but their efficacy in cold climates was uncertain for many years. Numerous case studies during the last decade have now demonstrated effective wastewater treatment in subsurface flow wetlands in cold regions (Mander and Jenssen 2003). Nonetheless, understanding of the factors that allow effective treatment year-round, and how they can be optimized, remains poor. Allen et al. (2002) reported evidence that plants can contribute to effective COD removal at low temperatures.

The role of plants in constructed wetlands has long been debated. Some studies have found that plants improve wastewater treatment (Gersberg et al. 1986, Reddy et al. 1989, Lin et al. 2002, Fraser et al. 2004, Picard et al. 2005, Stein and Hook 2005, Akratos et al. 2007, Yang et al. 2007), while others have found little or no benefit (Brix and Schierup 1990, Haberl and Perfler 1990, Vacca et al. 2005). In practice, only a handful of plants, including *Phragmites australis* and *Typha*, *Scirpus* and *Schoenoplectus* species, are widely used in constructed wetlands. Most research has focused on these species and their effects in mild climates.

Plant effects during cold periods have received little attention. Plants' primary role in treatment wetlands is thought to be their influence on microbial activity through creating attachment sites and releasing carbon exudates and oxygen (Reed and Brown

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1992, Tanner 2001). While variation in temperature affects microbial activity directly, seasonal cycles of plant growth and physiology may also influence ROL, other root-zone processes and, consequently, seasonal wastewater treatment. Because morphological, physiological, and phenological characteristics vary widely among wetland plants, different species' effects on seasonal CW performance may also vary. Hook et al. (2003) found that seasonal patterns of COD removal differed among three species and hypothesized that this resulted from differences in ROL, particularly at low temperatures.

The purpose of this study was to compare the effects of a large number of species on seasonal removal of organic carbon from wastewater. Variation in wastewater treatment was evaluated at temperatures from 4˚C to 24˚C by measuring COD, sulfate, and redox potential in batch-loaded model wetlands planted with monocultures of 19 species as well as unplanted controls. Specific questions addressed were:

1. Does the presence of plants affect seasonal wastewater chemistry and are those effects common for many species or species- specific?

2. Is variation in COD removal with different plant species and at different temperatures related to two indicators of root-zone oxidation status, sulfate and redox potential?

<div align="center">Materials and Methods</div>

Plant Materials

A diverse selection of sedges, rushes, grasses, and forbs was chosen for the study (Table 1). Plants ranged from Facultative to Obligate Wetlands species (U.S. Fish and

Wildlife Service 1988), represented several families, and included plants in common or

limited use in constructed wetlands or wetland restoration. Selection was also based on

their availability and use in past constructed wetland research. The plants were either

purchased from nurseries or transplanted from the field.

Table 1: Plant species studied and their Wetland Indicator Status. Obligate Wetland
species occur in wetlands >99% of the time, Facultative Wetland species 67-99% of the
time, and Facultative species 34-66% of the time.

Family	Species	Common name
	Obligate Wetland Species	
Cyperaceae	*Carex aquatilis* Wahlenb.	water sedge
	Carex bebbii Olney ex Fernald	Bebb's sedge
	Carex nebrascensis Dewey	Nebraska sedge
	Carex utriculata Boott	Northwest Territory sedge
	Schoenoplectus acutus (Muhl. ex Bigelow) A. Love & D. Love	hardstem bulrush
Typhaceae	*Typha latifolia* L.	broadleaf cattail
	Facultative Wetland Species	
Cyperaceae	*Carex praegracilis* W. Boott	clustered field sedge
Juncaceae	*Juncus arcticus* Willd.	arctic rush
	Juncus torreyi Coville	Torrey's rush
Poaceae	*Calamagrostis canadensis* (Michx.) P. Beauv.	bluejoint
	Deschampsia cespitosa (L.) P. Beauv.	tufted hairgrass
	Phalaris arundinacea L.	reed canarygrass
	Phragmites australis (Cav.) Trin. ex Steud.	common reed
Iridaceae	*Iris missouriensis* Nutt.	Rocky Mountain iris
	Facultative Species	
Cyperaceae	*Carex microptera* Mack.	smallwing sedge
Poaceae	*Hordeum jubatum* L.	foxtail barley
	Leymus cinereus (Scribn. & Merr.) A. Love	basin wildrye
	Panicum virgatum L.	switchgrass
Lamiaceae	*Prunella vulgaris* L.	common selfheal

Experimental Design and Operation

The greenhouse experiment was conducted in the Plant Growth Center at Montana State University in Bozeman, MT (46˚N, 111˚W). Six replicates of each species and controls were planted in model subsurface wetlands ("columns") in a randomized block design. Each block contained 20 model subsurface wetlands consisting of 15 cm diameter by 30 cm tall polyvinyl chloride (PVC) columns planted with one of the 19 plant species in monoculture or left unplanted as a control. Plant material was either nursery grown or collected from the field. Roots were washed free of soil and cut to approximately 15 cm in length and planted in 1-5 mm gravel.

During an establishment period of six months, plants were grown at 16˚C with supplemental lighting (GE Multi-Vapor MVR1000/C/U) to maintain a 16-hour day length. From June 20, 2006, through February 12, 2008, greenhouse temperature was changed every 60 days to mimic natural seasonal temperature cycles. The temperature sequence was 24, 16, 8, 4, 8, 16, 24, 16, 8, and 4˚C. Supplemental lighting was not used so that patterns of natural light and controlled temperature would induce seasonal cycles of plant dormancy and growth.

A synthetic wastewater simulating secondary domestic effluent was used throughout the experiment. The wastewater was made with 0.58 mM sucrose ($C_{12}H_{22}O_{11}$), 0.73 mM Primatone (hydrolyzed meat protein, Sigma Chemical Company), 10.7 mM NH_4Cl, 0.25 K_2HPO_4, 0.25 mM $MgSO_4$, 0.16 mM H_3BO_3, 0.05 mM $MnSO_4$, 0.03 mM $ZnSO_4$, 0.02 mM Na_2MoO_4, 0.01 mM $CaCl_2$, 0.01 mM KI, 3.2 μM $CuSO_4$, and 1.0 μM $FeCl_3$. Throughout the experiment, the wastewater influent was sampled

immediately before being added to the experimental columns and had mean and standard

error of 490 ± 4.3 mg/L COD, 0.8 ± 0.1 mg/L NO_3, 8 ± 0.3 mg/L PO_4, and 14 ± 0.5

mg/L SO_4. To simulate batch operation in subsurface flow wetlands, columns were

drained and filled with synthetic wastewater every 20 days during the plants'

establishment period and throughout the experiment. Three wastewater batches occurred

within each 60-day temperature period. Columns had drains at the base and were

connected to a standpipe to maintain the water level just below the gravel surface.

Water Sampling and Chemical Analysis

Chemical oxygen demand (COD), sulfate (SO_4) concentration, and redox

potential (Eh) were measured to determine whether plant species or seasons affected

wastewater treatment (COD removal) and aspects of water chemistry related to oxidation

(SO_4 and Eh). Sampling began in July 2006 and ended in February 2008.

In the center of each column, a sampling tube (0.3 cm inner-diameter vinyl

tubing) with an intake 15 cm below the gravel surface was attached to a vertical 13-cm

ID CPVC pipe. Water samples were collected with a 20 mL syringe during the third 20-

day wastewater batch of each temperature period. Collection from 3 replicates of each

species and unplanted control occurred on days 0, 1, 3, 6, 9 and 20 during 24°C and 4°C

batches, and on days 1 and 6 during the 16°C and 8°C batches. Influent wastewater was

collected immediately after wastewater was made and prior to filling the experimental

columns. Samples of the influent wastewater and bulk solution from the columns were

analyzed immediately for COD. COD was measured colorimetrically using HACH

reagents and spectrophotometer (0-1500 mg/L COD range test using dichromate

reagents, DR 2010 Spectrophotometer, HACH Company, Loveland, CO). Additional

samples were filtered (0.2 μm filter) to sterilize, stored in sterile test tubes at 2˚C, and

then analyzed for SO_4 using ion chromatography (Dionex Corporation, Sunnyvale, CA).

Platinum redox electrodes (Faulkner et al. 1989) were permanently installed at 15

cm below the gravel surface in two replicates of each plant species. Columns with redox

probes were connected by a salt bridge with a centrally located saturated calomel

reference electrode (Veneman and Pickering 1983). Redox potential was measured

automatically every 4 hours with a computer and multiplexer. Redox potential was

calculated from measured electrode potential by adding a 250 mV as the correction factor

(Vepraskas et al. 2002).

Statistical Analysis

To establish whether plant species and seasons had an effect on wastewater

treatment, COD removal, SO_4 concentration, and Eh were compared among planted and

control columns across seasons. Values measured on day 6 of each wastewater batch

were used in all statistical analysis since this is a reasonable duration for batch operations

in constructed wetlands. Additionally, patterns among plant species on day 6 of the batch

were typically similar to those found on other days of the incubation.

Analysis of variance (ANOVA, SPSS, Inc., Chicago, IL Version 15.0) was used

to compare plant species effects on COD and SO_4 within wastewater batches. Planned

contrasts were conducted to test differences between each species and the unplanted

control. Post hoc Tukey HSD (honestly significant difference) tests were used to identify

differences among plant species. The significance level was set at $\alpha = 0.05$. Redox

potentials were not compared statistically because only two replicates were monitored.

Seasonal temperature effects on COD and SO_4 were analyzed in two ways.

Repeated measures analysis of variance (ANOVAR, SPSS, Inc., Chicago, IL Version

15.0) was used to compare 4 and 24°C batches, which represent the extremes of

temperatures tested. Plant species and the control were analyzed individually. The

significance level was set at $\alpha = 0.05$. Additionally, Pearson's correlation (SPSS, Inc.,

Chicago, IL Version 15.0), using data from across the seasons, was used to determine

COD removal and SO_4 concentration response to temperature for each plant treatment

and the unplanted control. Measurements from the first sampling period (24°C in 2006)

were not used in ANOVARs and correlations so as to exclude effects associated with the

start-up of the experiment; COD removal was markedly lower in the first batch, and

patterns of plant effects were not consistent with those seen throughout the rest of the

study. Regression (SPSS, Inc., Chicago, IL Version 15.0) was used to determine

relationships between COD removal and SO_4 during each temperature period.

<div align="center">Results</div>

COD Removal

COD removal throughout wastewater batches was influenced by plant species but

showed limited differences between summer and winter. Rapid COD removal occurred

within 24 hours in both planted and unplanted columns during 20-day wastewater batches

at all temperatures (Figure 1). In some cases the column's maximum removal was

reached within 24 hours, and in others removal continued for up to 20 days; maximum

removal was normally achieved by day 6. COD removal on day 6 ranged from

45% to 100%, with a median value of 89% for planted columns and 66% for unplanted

columns. When present, differences among plant species emerged by day 3 and normally

persisted throughout the batch.

Figure 1 shows controls and a subset of the 19 species that represent the range of

removal patterns seen over the course of the warmest and coldest batches. The unplanted

control was relatively effective at COD removal during warm temperatures, reaching

85% and 93% removal by day 20 of the 24˚C 2006 and 2007 batches, respectively;

removal in controls was less effective during cold temperatures, with 62% and 64%

removal during the 4˚C 2007 and 2008 batches. In columns planted with many of the

species, COD removal was nearly complete within 3 days in both summer and winter. *C.

bebbii*, *S. acutus*, and *D. cespitosa* are examples of this pattern. *P. virgatum* is an

example of a species with lower removal at 4˚C than 24˚C, and *T. latifolia* is

representative of the plants that had intermediate patterns of COD removal.

Seasonal differences in removal measured on day 6 were statistically significant

for a minority of species. Repeated measures ANOVA comparing results from 4˚C and

24˚C indicated that controls and columns with *I. missouriensis*, *L. cinereus* and *P.

virgatum* had significantly lower COD removal at 4˚C, while *C. utriculata* and *P.

arundinacea* had significantly higher removal at 4˚C (Table 2). Other species did not

differ significantly between 4 and 24˚C or showed inconsistent differences. Only the

controls and three plant species showed significant correlations between COD removal

4˚C 2007   **24˚C 2007**
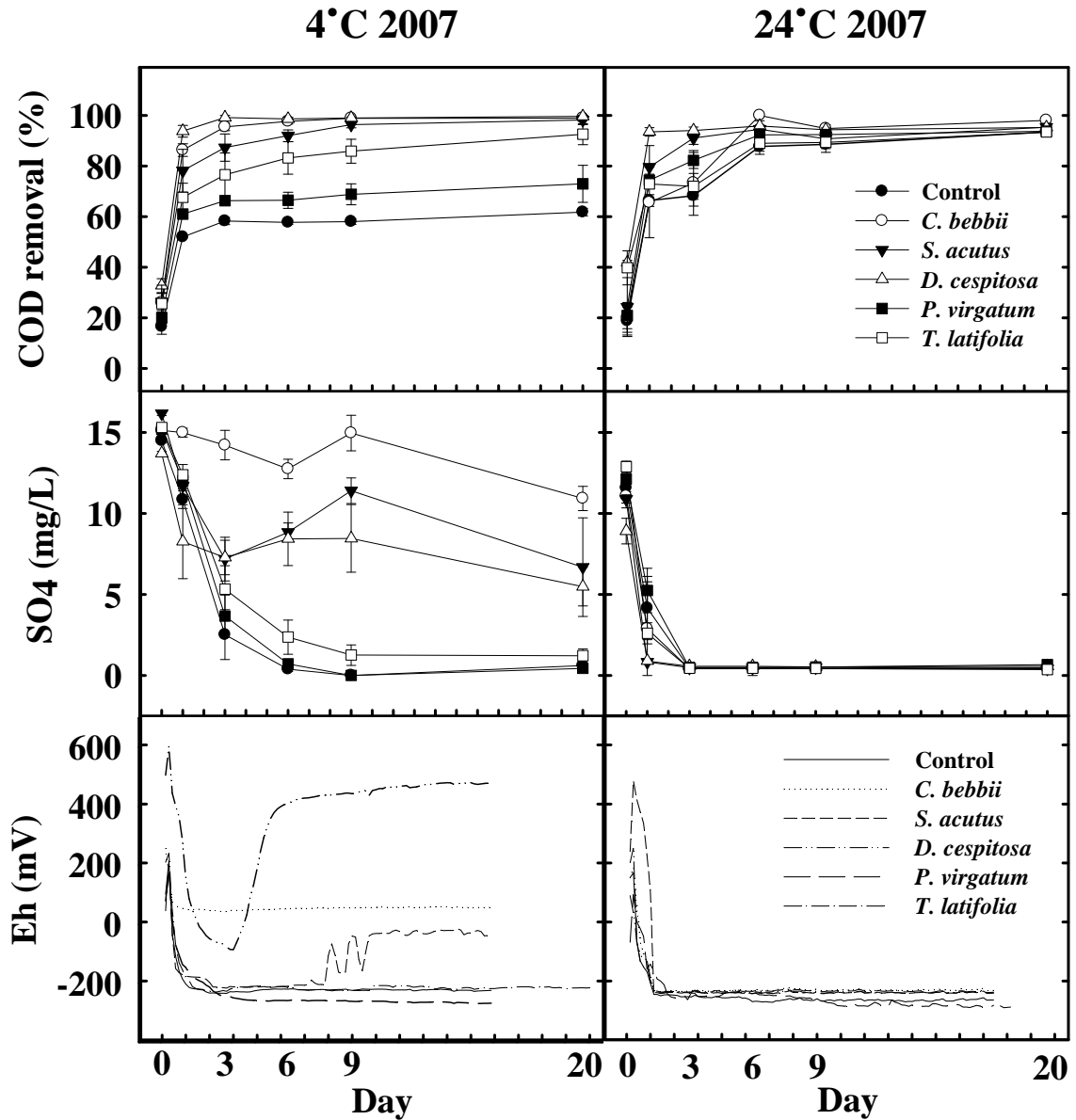
Figure 1: COD removal (%), SO_4 concentration (mg/L), and Eh (mV) over 20-day batch incubations at 4˚C in 2007 and 24˚C in 2007. Symbols in COD removal and SO_4 graphs represent the mean of three replicates. Error bars represent ± one standard error. Day zero represents data collected immediately after filling the columns with wastewater. See Tables 3-6 for means, standard errors, and statistical comparisons for COD and SO_4. Lines in Eh graphs represent the mean of two replicates with measurements taken at 4-hour intervals.

and temperature when using data from all temperature incubations (Table 3).

Temperature had a positive effect on COD removal only in the unplanted controls and

columns with *I. missouriensis*, which had little biomass. Removal increased at lower

temperatures in columns planted with *C. nebrascensis* and *C. utriculata*.

Table 2: COD removal (%) on day 6 of 4˚C batches in 2007 and 2008 and the 24˚C batch
in 2007. COD values are means of three replicates ± one standard error. Values with an
asterisk (*) are significantly different from other values in the same row (p<0.05,
Repeated Measures ANOVA).

	4˚C 2007		24˚C 2007		4˚C 2008	
Control	58 ± 1		88 ± 2	*	66 ± 2	
C. aquatilis	90 ± 3		98 ± 0		97 ± 1	
C. bebbii	98 ± 1		100 ± 0		98 ± 1	
C. microptera	83 ± 4		82 ± 3		96 ± 3	*
C. nebrascensis	96 ± 2		93 ± 3		97 ± 1	
C. praegracilis			97 ± 3		97 ± 1	
C. utriculata	96 ± 2		83 ± 1	*	99 ± 1	
S. acutus	92 ± 2		94 ± 2		99 ± 0	
J. arcticus	89 ± 4		96 ± 4		96 ± 2	
J. torreyi	94 ± 2		92 ± 6		91 ± 2	
C. canadensis	73 ± 11		94 ± 4		85 ± 2	
D. cespitosa	99 ± 1		96 ± 2		98 ± 1	
H. jubatum	71 ± 3		86 ± 4		79 ± 1	
L. cinereus	69 ± 2		79 ± 2	*	72 ± 1	
P. virgatum	66 ± 3		92 ± 2	*	71 ± 3	
P. arundinacea	87 ± 1		79 ± 4	*	92 ± 4	
P. australis	67 ± 5		67 ± 6		89 ± 1	
I. missouriensis	67 ± 5		92 ± 1	*	67 ± 3	
P. vulgaris	87 ± 1	*	77 ± 8		74 ± 2	
T. latifolia	83 ± 6		89 ± 4		92 ± 2	

COD removal was consistently high across all seasons with five of the six sedges

(*C. aquatilis, C. bebbii, C. nebrascensis, C. praegracilis*, and *C. utriculata*, 83-100%

removal), both rushes (*J. arcticus* and *J. torreyi*, 89-97%), *S. acutus*, (87-100%), and *D.

cespitosa* (95-100%) (Figures 2-5, Table 4). Removal was more variable for other

species (53-94%) with lower average removal across seasons (70-88%). Plant species

rankings for COD removal were similar across batches. Species with the highest removal

at cold temperatures also had the highest removal at warm temperatures (Figure 6).

Table 3: Correlations between temperature and COD removal (%) and temperature and
SO_4 concentrations (mg/L). COD removal and SO_4 concentration values are from the
sixth day of each wastewater batch. Analyses include data for batches from 16˚C in 2006
through 4˚C in 2008. The coefficient of correlation is significantly different than 0 if
followed by an asterisk (*).

	COD removal and Temperature		SO_4 and Temperature	
	r		r	
Control	+ 0.77	*	+ 0.35	
C. aquatilis	+ 0.37		- 0.73	*
C. bebbii	- 0.20		- 0.84	*
C. microptera	- 0.55		- 0.63	*
C. nebrascensis	- 0.67	*	- 0.79	*
C. praegracilis	- 0.26		- 0.77	*
C. utriculata	- 0.93	*	- 0.85	*
S. acutus	- 0.20		- 0.77	*
J. arcticus	+ 0.32		- 0.77	*
J. torreyi	- 0.37		- 0.79	*
C. canadensis	+ 0.49		- 0.14	
D. cespitosa	- 0.40		- 0.78	*
H. jubatum	+ 0.51		- 0.33	
L. cinereus	+ 0.40		- 0.32	
P. virgatum	+ 0.60		+ 0.40	
P. arundinacea	- 0.40		- 0.69	*
P. australis	- 0.24		- 0.48	
I. missouriensis	+ 0.67	*	+ 0.05	
P. vulgaris	- 0.45		- 0.25	
T. latifolia	- 0.10		- 0.30	

Seasonal differences in plant effects are also apparent in statistical comparison

among plants and controls during individual batches (Table 4). There were few

significant differences between plant species and the unplanted control at 24 and 16˚C in

2006 and 24˚C in 2007; the species that differed from controls were not consistent for

these batches. In contrast, 13-18 species differed from controls during the other batches;

in every case, removal was greater in planted columns than controls.
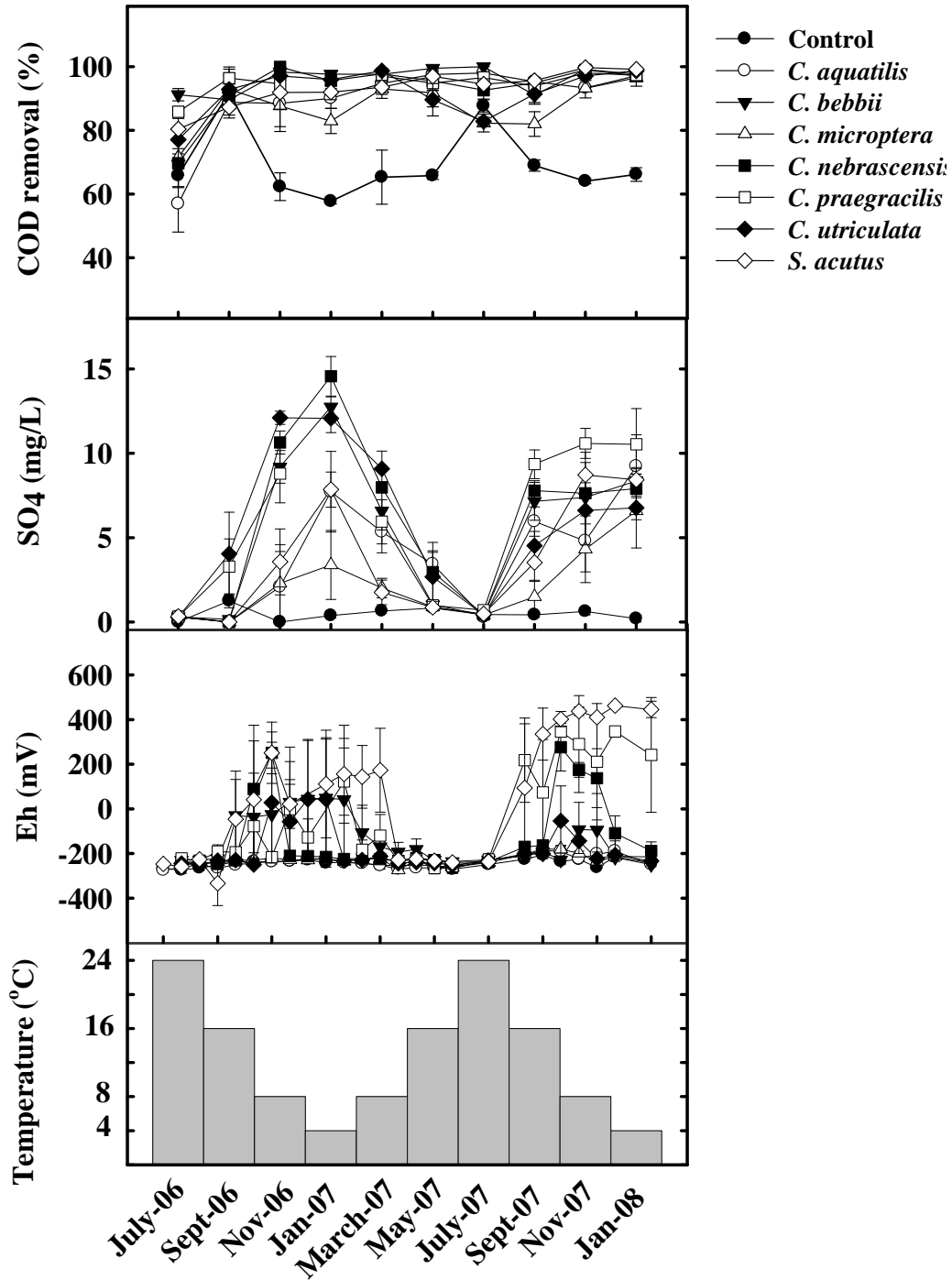
Figure 2: Seasonal variation in day-6 COD removal (%), SO_4 concentration (mg/L), and Eh (mV) for *Cyperaceae*. SO_4 and COD removal symbols represent the mean of three replicates. Eh symbols represent the mean of two replicates. Error bars represent ± one standard error. See Tables 2, 4, 5, and 6 for means, standard errors, and statistical comparisons.
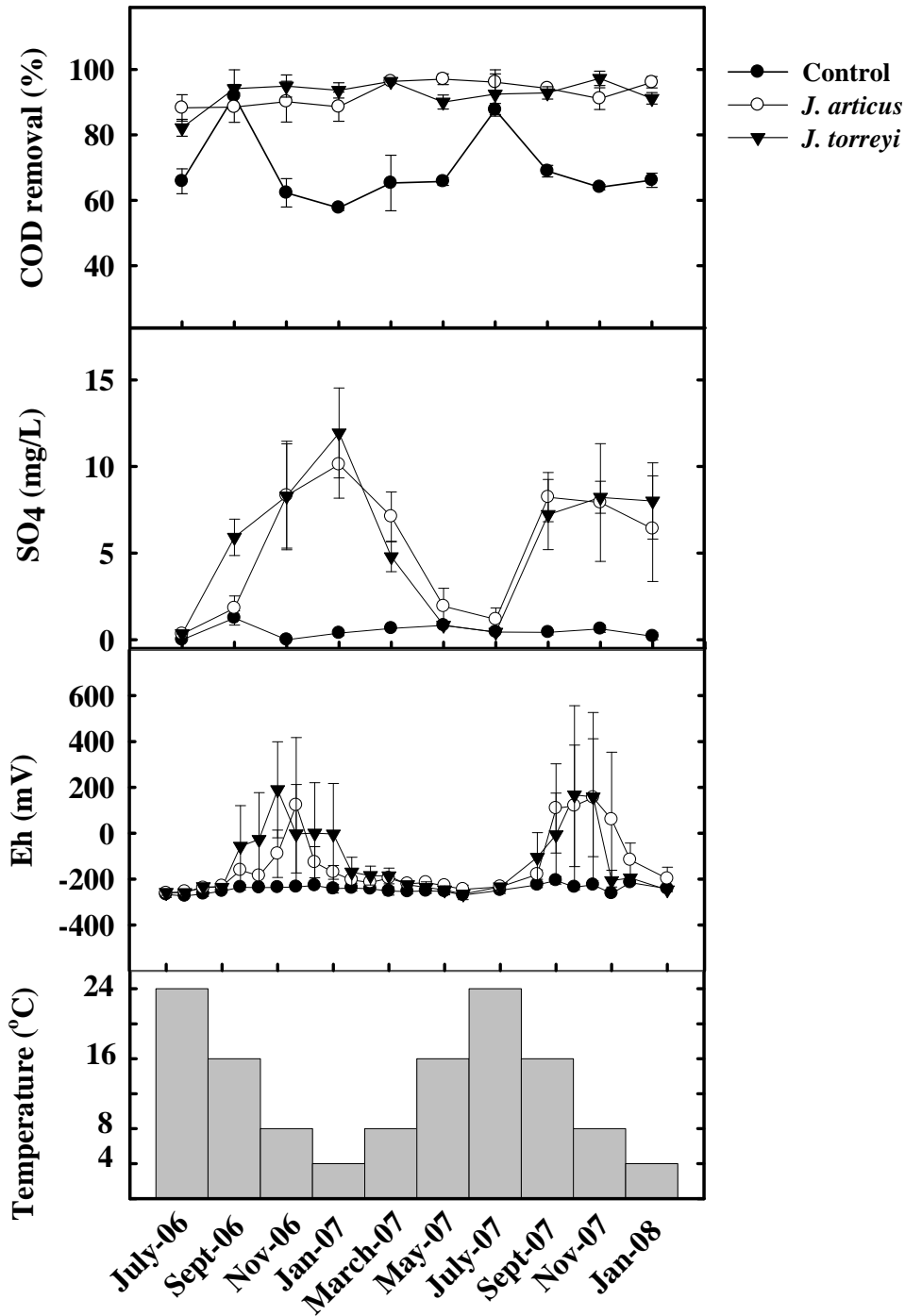
Figure 3: Seasonal variation in day-6 COD removal (%), SO_4 concentration (mg/L), and Eh (mV) for *Juncaceae*. SO_4 and COD removal symbols represent the mean of three replicates. Eh symbols represent the mean of two replicates. Error bars represent ± one standard error. See Tables 2, 4, 5, and 6 for means, standard errors, and statistical comparisons.
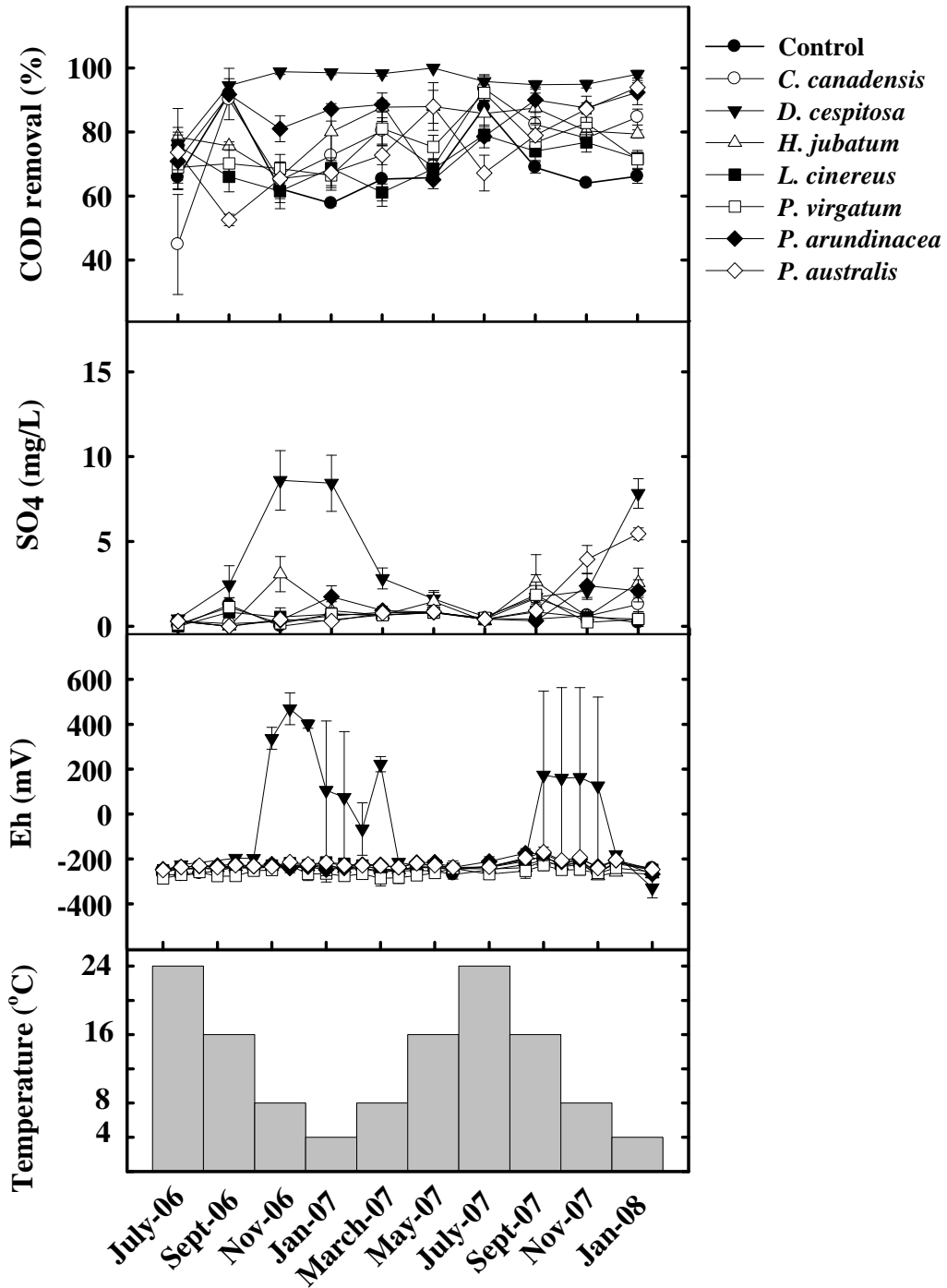
Figure 4: Seasonal variation in day-6 COD removal (%), SO_4 concentration (mg/L), and Eh (mV) for *Poaceae*. SO_4 and COD removal symbols represent the mean of three replicates. Eh symbols represent the mean of two replicates. Error bars represent ± one standard error. See Tables 2, 4, 5, and 6 for means, standard errors, and statistical comparisons.
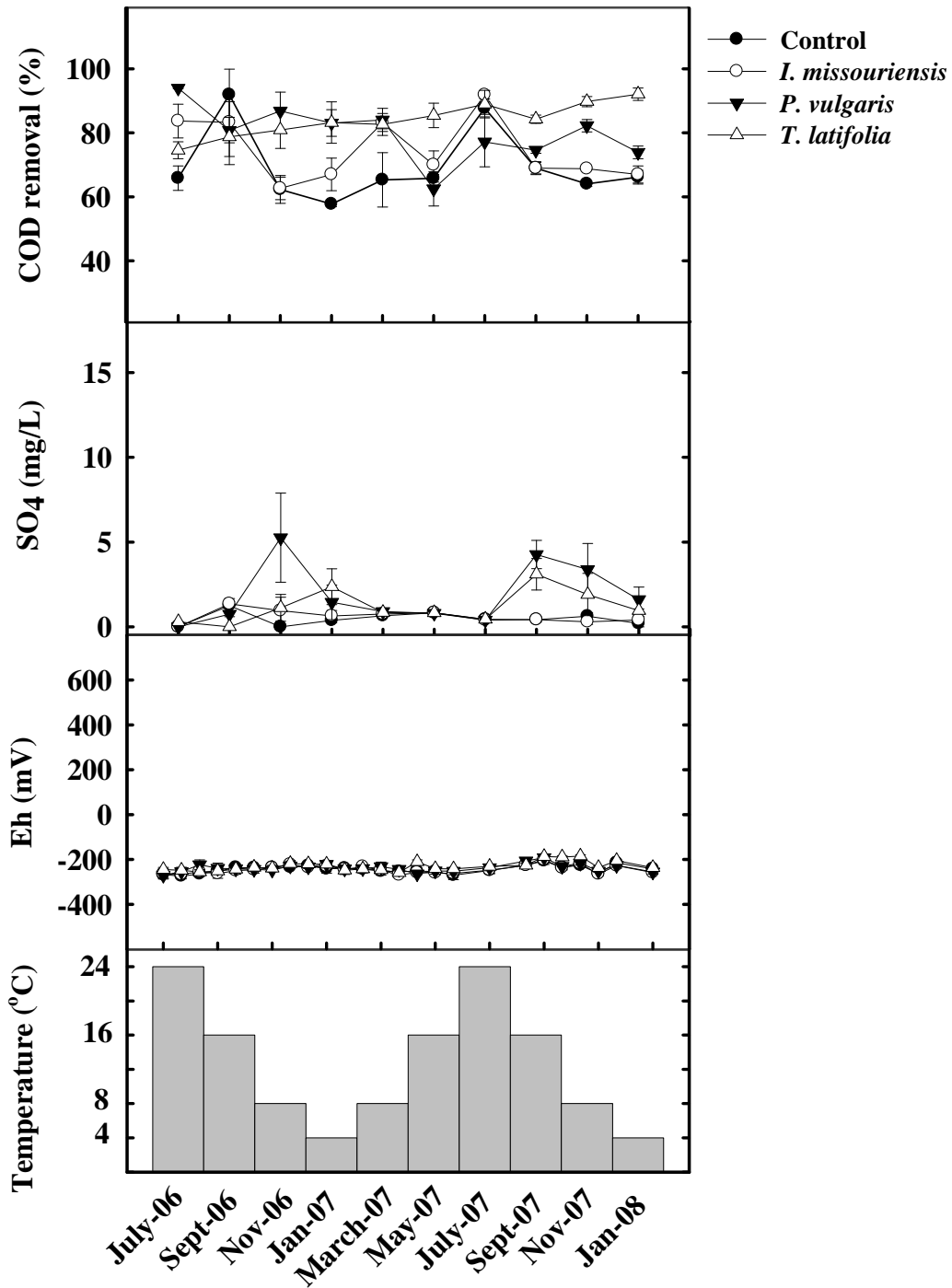
Figure 5: Seasonal variation in day-6 COD removal (%), SO_4 concentration (mg/L), and Eh (mV) for *Iridaceae*, *Lamiaceae*, and *Typhaceae*. SO_4 and COD removal symbols represent the mean of three replicates. Eh symbols represent the mean of two replicates. Error bars represent ± one standard error. See Tables 2, 4, 5, and 6 for means, standard errors, and statistical comparisons.

Table 4: COD removal (%) on day 6 of batches at all temperatures. COD values (% removal) are means of three replicates ± one standard error. Within each column, values in bold font are significantly different than the control (p<0.05, planned contrasts). Values in a column with the same letter are not significantly different from each other (p≥0.05, Tukey HSD).

	24˚C 2006		16˚C 2006		8˚C 2006		4˚C 2007		8˚C 2007		16˚C 2007	
Control	66 ± 4	abc	92 ± 1	cd	62 ± 4	ab	58 ± 1	a	65 ± 9	ab	66 ± 1	a
C. aquatilis	57 ± 9	ab	89 ± 1	cd	**88 ± 7**	cdef	**90 ± 3**	cde	**95 ± 2**	def	**98 ± 0**	e
C. bebbii	**91 ± 2**	c	90 ± 2	cd	98 ± 1	f	98 ± 1	e	98 ± 0	def	99 ± 0	e
C. microptera	72 ± 1	abc	**80 ± 6**	bcd	**88 ± 8**	bcdef	**83 ± 4**	bcde	93 ± 3	def	92 ± 4	de
C. nebrascensis	70 ± 7	abc	91 ± 2	cd	**100 ± 0**	f	**96 ± 2**	e	98 ± 1	ef	96 ± 3	e
C. praegracilis	**86 ± 1**	bc	96 ± 1	d	**95 ± 2**	f			97 ± 1	def	95 ± 2	e
C. utriculata	77 ± 3	bc	93 ± 2	d	**97 ± 1**	f	**96 ± 2**	e	99 ± 1	f	90 ± 5	de
S. acutus	80 ± 3	bc	87 ± 3	bcd	**92 ± 5**	ef	**92 ± 2**	de	94 ± 2	def	97 ± 1	e
J. arcticus	**88 ± 4**	c	89 ± 0	cd	**90 ± 6**	def	**89 ± 4**	bcde	96 ± 0	def	97 ± 2	e
J. torreyi	82 ± 3	bc	94 ± 1	d	**95 ± 3**	f	**94 ± 2**	de	96 ± 0	def	90 ± 2	de
C. canadensis	**45 ± 16**	a	90 ± 1	cd	65 ± 6	abcd	**73 ± 11**	abcd	80 ± 4	bcd	69 ± 2	abc
D. cespitosa	75 ± 13	abc	95 ± 2	d	**99 ± 1**	f	**99 ± 1**	e	98 ± 1	ef	**100 ± 0**	e
H. jubatum	78 ± 3	bc	**76 ± 1**	bcd	66 ± 5	abcd	**71 ± 3**	abcd	88 ± 4	cdef	88 ± 7	cde
L. cinereus	76 ± 4	abc	**66 ± 5**	ab	62 ± 5	a	69 ± 2	abc	61 ± 3	a	69 ± 3	ab
P. virgatum	69 ± 4	abc	**70 ± 6**	abc	69 ± 4	abcde	66 ± 3	ab	**81 ± 5**	bcde	75 ± 4	abcd
P. arundinacea	71 ± 7	abc	92 ± 1	cd	**81 ± 4**	abcdef	**87 ± 1**	bcde	89 ± 1	cdef	65 ± 3	a
P. australis	74 ± 5	abc	**53 ± 2**	a	65 ± 5	abcd	67 ± 5	ab	73 ± 3	abc	**88 ± 5**	cde
I. missouriensis	**84 ± 5**	bc	83 ± 6	bcd	63 ± 4	abc	67 ± 5	ab	**83 ± 2**	cdef	70 ± 4	abc
P. vulgaris	**94 ± 0**	c	81 ± 11	bcd	**87 ± 6**	abcdef	**87 ± 1**	bcde	84 ± 4	cdef	62 ± 5	a
T. latifolia	75 ± 3	abc	**79 ± 6**	bcd	**81 ± 6**	abcdef	**83 ± 6**	bcde	83 ± 3	cdef	85 ± 4	bcde
All species	76 ± 3		84 ± 3		83 ± 3		84 ± 3		89 ± 2		86 ± 3	

Table 4 continued: COD removal (%) on day 6 of batches at all temperatures. COD values (% removal) are means of three replicates ± one standard error. Within each column, values in bold font are significantly different than the control (p<0.05, planned contrasts). Values in a column with the same letter are not significantly different from each other (p≥0.05, Tukey HSD). Averages in the right-hand column are for batches 16˚C 2006 through 4˚C 2008; the first batch reflected start-up effects and is excluded. Averages were not compared statistically.

	24˚C 2007		16˚C 2007		8˚C 2007		4˚C 2008		Average
Control	88 ± 2	abc	69 ± 2	a	64 ± 1	a	66 ± 2	a	70 ± 3
C. aquatilis	98 ± 0	bc	**95 ± 0**	e	**93 ± 1**	fghi	**97 ± 1**	e	94 ± 2
C. bebbii	**100 ± 0**	c	**91 ± 2**	de	**99 ± 0**	hi	**98 ± 1**	e	97 ± 1
C. microptera	82 ± 3	abc	**82 ± 4**	abcde	**93 ± 3**	fghi	**96 ± 3**	e	88 ± 2
C. nebrascensis	93 ± 3	bc	**95 ± 0**	e	**99 ± 0**	hi	**97 ± 1**	e	96 ± 1
C. praegracilis	97 ± 3	bc	**94 ± 1**	e	**98 ± 2**	hi	**97 ± 1**	e	96 ± 1
C. utriculata	83 ± 1	abc	**91 ± 3**	de	**97 ± 1**	hi	**99 ± 1**	e	94 ± 2
S. acutus	94 ± 2	bc	**96 ± 1**	e	**100 ± 0**	i	**99 ± 0**	e	95 ± 2
J. acrticus	96 ± 4	bc	**94 ± 0**	e	**91 ± 3**	efghi	**96 ± 2**	e	93 ± 2
J. torreyi	92 ± 6	bc	**93 ± 2**	e	**97 ± 2**	hi	**91 ± 2**	de	94 ± 1
C. canadensis	94 ± 4	bc	**82 ± 2**	abcde	**78 ± 2**	bcd	**85 ± 2**	cd	80 ± 4
D. cespitosa	96 ± 2	bc	**95 ± 1**	e	**95 ± 1**	ghi	**98 ± 1**	e	97 ± 2
H. jubatum	86 ± 4	abc	**87 ± 5**	bcde	**80 ± 4**	bcde	**79 ± 1**	bc	80 ± 2
L. cinereus	79 ± 2	abc	74 ± 1	ab	**77 ± 3**	bc	**72 ± 1**	ab	70 ± 2
P. virgatum	92 ± 2	bc	**77 ± 6**	abc	**83 ± 3**	cdefg	71 ± 3	ab	76 ± 3
P. arundinacea	79 ± 4	ab	**90 ± 4**	cde	**88 ± 2**	cdefgh	**92 ± 4**	de	85 ± 3
P. australis	**67 ± 6**	a	**79 ± 2**	abcd	**87 ± 4**	cdefgh	**89 ± 1**	de	74 ± 3
I. missouriensis	92 ± 1	bc	69 ± 2	a	69 ± 0	ab	67 ± 3	a	74 ± 3
P. vulgaris	77 ± 8	ab	75 ± 1	ab	**82 ± 2**	cdef	**74 ± 2**	ab	79 ± 3
T. latifolia	89 ± 4	bc	**84 ± 1**	bcde	**90 ± 2**	defghi	**92 ± 2**	de	85 ± 2
All species	89 ± 2		86 ± 2		89 ± 2		89 ± 2		87 ± 2

Figure 6: COD removal (%) and SO$_4$ concentration (mg/L) for each species and unplanted control at 4˚C and 24˚C. 4˚C bars are averages of means of three replicates from two 4˚C incubations in 2007 and 2008. 24˚C bars are means of three replicates during the 24˚C 2007 incubation. Error bars represent one standard error.
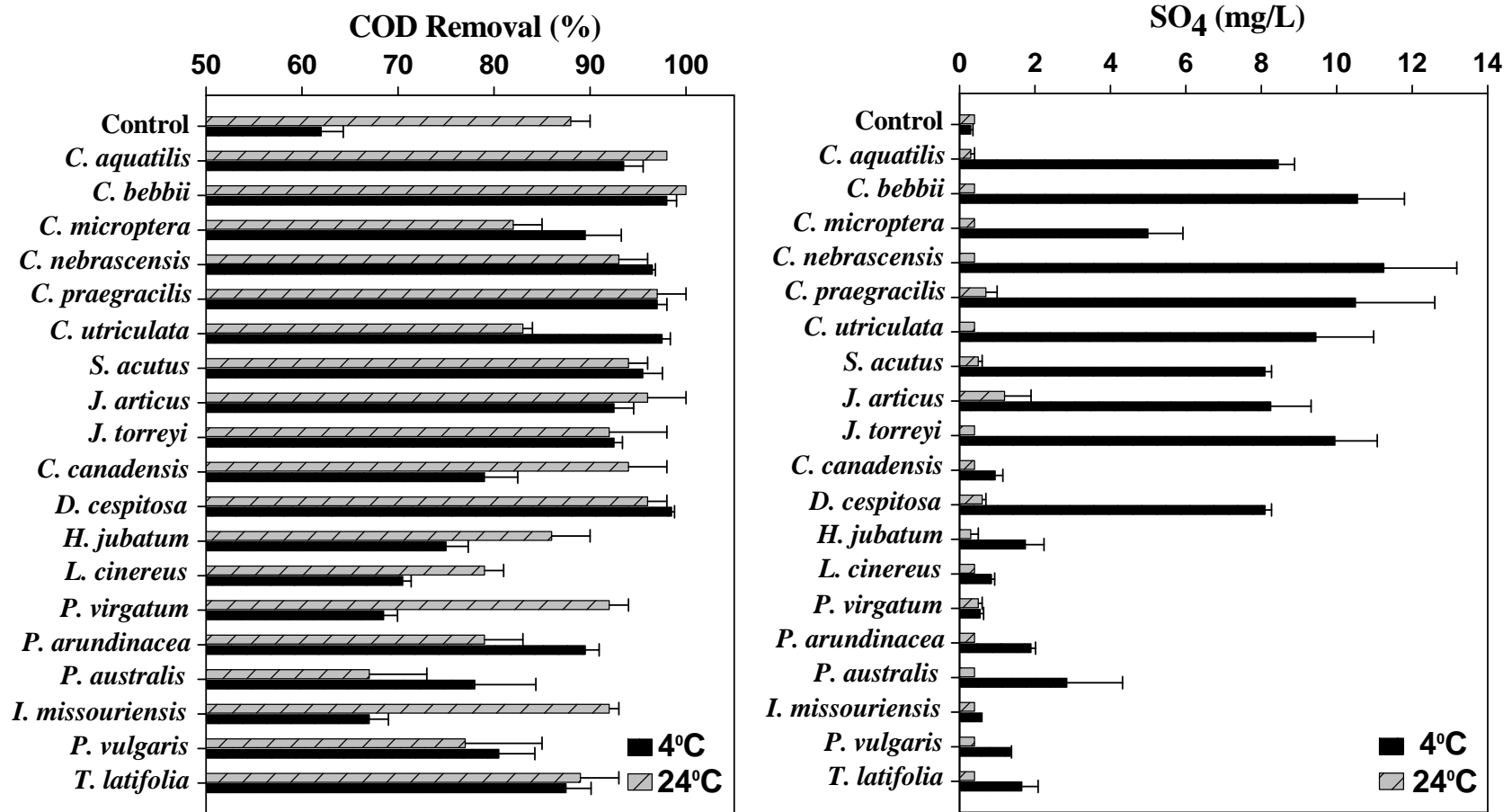
Sulfate Concentration

Like COD removal, sulfate concentrations during 20-day wastewater batches

varied among plant species and temperatures (Figure 1). Some plant species' effects on

SO_4 concentration varied strongly by temperature, while others' did not (Table 3,

Table 5). Many more species showed temperature dependence for SO_4 than for COD

removal. For all species with seasonal differences, SO_4 concentrations were higher at

cooler temperatures (Figure 6).

At 24˚C, a rapid decrease in SO_4 concentration occurred in all columns within the

first day of the batch (Figure 1). By day 3 at 24˚C, SO_4 concentrations in all planted and

control columns were low, ranging from 0 to 1.2 mg/L. SO_4 remained low with little

change in concentrations throughout the 20-day batch. Concentrations did not differ

significantly among treatments at 24˚C in 2007 except for a small difference between *J.

arcticus* and the control (Table 6). Concentrations were significantly different at 24˚C in

2006; however, this was the result of low variability of near-zero values (0.0 – 0.4 mg/L).

During the 4˚C incubation, initial declines in SO_4 concentrations were not as rapid as

at 24˚C, and concentrations remained above 5 mg/L for several species (Figure 1). On

day 6, SO_4 concentrations ranged from 0.3 to 14.6 mg/L in the 4˚C 2007 batch and from

0.2 to 10.5 mg/L in the 4˚C 2008 batch (Table 5, Table 6). Time series for the unplanted

control, *H. jubatum, C. canadensis, P. virgatum, P. arundinacea, L. cinereus, T. latifolia,

I. missouriensis*, and *P. vulgaris* followed a pattern similar to that seen at 24˚C with SO_4

concentrations declining to near 0 mg/L by day 6 and little change throughout the

remainder of the batch. Day-6 SO_4 concentrations in columns with these plants were not

significantly different from the unplanted control. In contrast, 4˚C 2007 day-6 SO_4

concentrations ranged from 3.4 – 12.7 mg/L in columns planted with sedges, rushes, and

D. cespitosa; all of these differed significantly from the controls except *C. microptera.*

Table 5: SO_4 concentrations (mg/L) on day 6 of 4˚C batches in 2007 and 2008 and the 24˚C batch in 2007. SO_4 concentrations are means of three replicates ± one standard error. Values with an asterisk (*) are significantly different from other values in the same row (p<0.05, Repeated Measures ANOVA). Influent wastewater SO_4 concentrations averaged 14 ± 0.5 mg/L.

	4 ˚C 2007		24 ˚C 2007		4 ˚C 2008	
Control	0.4 ± 0.1		0.4 ± 0.0		0.2 ± 0.2	
C. aquatilis	7.7 ± 2.4		0.3 ± 0.1	*	9.2 ± 1.9	
C. bebbii	12.7 ± 0.6		0.4 ± 0.0	*	8.4 ± 0.7	
C. microptera	3.4 ± 2.0		0.4 ± 0.0		6.6 ± 2.2	
C. nebrascensis	14.6 ± 1.2		0.4 ± 0.0	*	7.9 ± 1.2	
C. praegracilis			0.7 ± 0.3	*	10.5 ± 2.1	
C. utriculata	12.1 ± 0.8	*	0.4 ± 0.0	*	6.8 ± 0.7	*
S. acutus	7.8 ± 1.0		0.5 ± 0.1	*	8.4 ± 0.3	
J. acrticus	10.1 ± 1.9	*	1.2 ± 0.7		6.4 ± 3.0	
J. torreyi	11.9 ± 2.6		0.4 ± 0.0	*	8.0 ± 2.2	
C. canadensis	0.6 ± 0.2		0.4 ± 0.0		1.3 ± 0.5	
D. cespitosa	8.4 ± 1.7		0.6 ± 0.1	*	7.8 ± 0.9	
H. jubatum	0.9 ± 0.5		0.3 ± 0.2		2.6 ± 0.9	
L. cinereus	0.7 ± 0.3		0.4 ± 0.0		1.0 ± 0.5	
P. virgatum	0.7 ± 0.2		0.5 ± 0.1		0.4 ± 0.2	
P. arundinacea	1.7 ± 0.7		0.4 ± 0.0		2.1 ± 0.7	
P. australis	0.3 ± 0.0	*	0.4 ± 0.0	*	5.4 ± 0.4	*
I. missouriensis	0.6 ± 0.3		0.4 ± 0.0		0.6 ± 0.3	
P. vulgaris	1.4 ± 1.0		0.4 ± 0.0		1.3 ± 0.9	
T. latifolia	2.4 ± 1.1		0.4 ± 0.0		0.9 ± 0.2	

Day-6 SO_4 concentrations in the columns containing sedges, rushes, *D. cespitosa,*

and *P. arundinacea* varied by seasons, while other treatments showed no to little seasonal

change (Figures 2-5). Negative correlations between temperature and SO_4 were found

for sedges, rushes, *D. cespitosa,* and *P. arundinace* (Table 3). Correlations were not

significant for other species.

Table 6: SO_4 concentrations (mg/L) on day 6 of batches at all temperatures. SO_4 values (mg/L) are means of three replicates ± one standard error. Within each column, values in bold are significantly different than the control ($p<0.05$, planned contrasts). Values in a column with the same letter are not significantly different from each other ($p\geq0.05$, Tukey HSD).

	24°C 2006	16°C 2006	8°C 2006	4°C 2007	8°C 2007	16°C 2007
Control	0.0 ± 0.0 a	1.3 ± 0.4 a	0.0 ± 0.0 a	0.4 ± 0.1 a	0.7 ± 0.0 a	0.8 ± 0.0 a
C. aquatilis	**0.3 ± 0.0** bc	0.0 ± 0.0 a	2.1 ± 2.1 abcde	**7.7 ± 2.4** bcdef	**5.3 ± 1.2** bcde	**3.4 ± 0.8** a
C. bebbii	**0.3 ± 0.0** c	0.1 ± 0.1 a	**9.2 ± 1.0** def	**12.7 ± 0.6** f	**6.6 ± 1.1** cde	1.0 ± 0.1 a
C. microptera	**0.3 ± 0.0** c	0.0 ± 0.0 a	2.3 ± 2.3 abcde	3.4 ± 2.0 abcde	2.0 ± 0.6 ab	0.9 ± 0.1 a
C. nebrascensis	**0.3 ± 0.0** c	0.0 ± 0.0 a	**10.6 ± 0.7** ef	**14.6 ± 1.2** f	**8.0 ± 0.3** de	**3.0 ± 1.8** a
C. praegracilis	**0.3 ± 0.0** c	3.3 ± 1.6 ab	**8.8 ± 1.7** cdef		**5.9 ± 1.3** cde	1.0 ± 0.1 a
C. utriculata	**0.3 ± 0.0** bc	**4.0 ± 2.5** ab	**12.1 ± 0.4** f	**12.1 ± 0.8** f	**9.1 ± 1.1** e	**2.7 ± 1.5** a
S. acutus	**0.3 ± 0.0** c	0.0 ± 0.0 a	3.6 ± 2.0 abcdef	**7.8 ± 1.0** cdef	1.8 ± 0.7 ab	0.9 ± 0.0 a
J. acrticus	**0.3 ± 0.0** c	1.8 ± 0.7 ab	**8.3 ± 3.1** abcdef	**10.1 ± 1.9** ef	**7.1 ± 1.4** de	1.9 ± 1.0 a
J. torreyi	**0.3 ± 0.0** c	**5.9 ± 1.0** b	**8.3 ± 3.0** abcdef	**11.9 ± 2.6** f	**4.8 ± 0.9** bcd	0.8 ± 0.0 a
C. canadensis	**0.3 ± 0.0** bc	0.2 ± 0.2 a	0.3 ± 0.1 abc	0.6 ± 0.2 a	0.8 ± 0.2 a	0.8 ± 0.0 a
D. cespitosa	**0.4 ± 0.1** c	2.4 ± 1.1 ab	**8.6 ± 1.8** bcdef	**8.4 ± 1.7** def	**2.8 ± 0.6** abc	1.6 ± 0.4 a
H. jubatum	**0.3 ± 0.0** c	0.0 ± 0.0 a	3.1 ± 1.0 abcde	0.9 ± 0.5 ab	0.7 ± 0.0 a	1.4 ± 0.6 a
L. cinereus	0.0 ± 0.0 a	0.8 ± 0.0 a	0.5 ± 0.5 abc	0.7 ± 0.3 a	0.7 ± 0.0 a	0.8 ± 0.0 a
P. virgatum	0.1 ± 0.1 ab	1.1 ± 0.6 a	0.2 ± 0.2 ab	0.7 ± 0.2 a	0.6 ± 0.0 a	0.8 ± 0.0 a
P. arundinacea	**0.3 ± 0.0** bc	0.0 ± 0.0 a	0.4 ± 0.0 abc	1.7 ± 0.7 abcd	0.9 ± 0.2 a	0.8 ± 0.0 a
P. australis	**0.3 ± 0.0** bc	0.0 ± 0.0 a	0.4 ± 0.1 abc	0.3 ± 0.0 a	0.8 ± 0.1 a	0.8 ± 0.0 a
I. missouriensis	0.0 ± 0.0 a	1.4 ± 0.1 a	1.0 ± 0.8 abcd	0.6 ± 0.3 a	0.8 ± 0.1 a	0.8 ± 0.0 a
P. vulgaris	0.0 ± 0.0 a	0.7 ± 0.1 a	**5.3 ± 2.6** abcdef	1.4 ± 1.0 abc	0.9 ± 0.1 a	0.8 ± 0.0 a
T. latifolia	**0.3 ± 0.0** c	0.0 ± 0.0 a	1.1 ± 0.8 abcd	2.4 ± 1.1 abcd	0.8 ± 0.2 a	0.8 ± 0.0 a
All species	0.2 ± 0.0	1.1 ± 0.4	4.5 ± 0.9	5.4 ± 1.2	3.2 ± 0.7	1.3 ± 0.2

Table 6 continued: SO_4 concentrations (mg/L) on day 6 of batches at all temperatures. SO_4 values (mg/L) are means of three replicates ± one standard error. Within each column, values in bold are significantly different than the control ($p<0.05$, planned contrasts). Values in a column with the same letter are not significantly different from each other ($p\geq0.05$, Tukey HSD). Averages from the right-hand column are for batches 16°C 2006 through 4°C 2008: the first batch reflected start-up effects and is excluded. Averages were not compared statistically.

	24°C 2007		16°C 2007		8°C 2007		4°C 2008		Average
Control	0.4 ± 0.0	a	0.4 ± 0.1	a	0.6 ± 0.2	ab	0.2 ± 0.2	a	0.5 ± 0.1
C. aquatilis	0.3 ± 0.1	a	**6.0 ± 0.9**	bcde	4.8 ± 1.8	abcd	**9.2 ± 1.9**	bc	4.3 ± 1.0
C. bebbii	0.4 ± 0.0	a	**7.2 ± 1.1**	cde	**7.4 ± 0.9**	abcd	**8.4 ± 0.7**	abc	5.9 ± 1.4
C. microptera	0.4 ± 0.0	a	1.5 ± 1.0	ab	4.3 ± 2.0	abcd	**6.6 ± 2.2**	abc	2.4 ± 0.7
C. nebrascensis	0.4 ± 0.0	a	**7.8 ± 0.6**	cde	**7.6 ± 1.8**	abcd	**7.9 ± 1.2**	abc	6.7 ± 1.6
C. praegracilis	0.7 ± 0.3	a	**9.4 ± 0.9**	e	**10.6 ± 0.9**	d	**10.5 ± 2.1**	c	6.3 ± 1.5
C. utriculata	0.4 ± 0.0	a	**4.5 ± 0.9**	abcde	**6.6 ± 2.0**	abcd	**6.8 ± 0.7**	abc	6.5 ± 1.4
S. acutus	0.5 ± 0.1	a	**3.5 ± 1.1**	abcd	**8.7 ± 1.3**	cd	**8.4 ± 0.3**	abc	3.9 ± 1.1
J. acrticus	**1.2 ± 0.7**	a	**8.2 ± 1.4**	de	**7.9 ± 3.4**	abcd	**6.4 ± 3.0**	abc	5.9 ± 1.1
J. torreyi	0.4 ± 0.0	a	**7.2 ± 2.0**	cde	**8.2 ± 0.9**	bcd	**8.0 ± 2.2**	abc	6.2 ± 1.3
C. canadensis	0.4 ± 0.0	a	1.6 ± 1.4	ab	0.6 ± 0.2	ab	1.3 ± 0.5	ab	0.7 ± 0.1
D. cespitosa	0.6 ± 0.1	a	1.7 ± 0.2	ab	2.1 ± 0.5	abc	**7.8 ± 0.9**	abc	4.0 ± 1.0
H. jubatum	0.3 ± 0.2	a	2.6 ± 1.6	abc	0.6 ± 0.1	ab	2.6 ± 0.9	abc	1.4 ± 0.4
L. cinereus	0.4 ± 0.0	a	0.8 ± 0.4	ab	0.5 ± 0.3	ab	1.0 ± 0.5	ab	0.7 ± 0.1
P. virgatum	0.5 ± 0.1	a	1.8 ± 0.8	ab	0.2 ± 0.2	a	0.4 ± 0.2	a	0.6 ± 0.2
P. arundinacea	0.4 ± 0.0	a	0.3 ± 0.1	a	2.4 ± 0.7	abc	2.1 ± 0.7	abc	1.0 ± 0.3
P. australis	0.4 ± 0.0	a	0.9 ± 0.5	ab	3.9 ± 0.8	abcd	**5.4 ± 0.4**	abc	1.4 ± 0.6
I. missouriensis	0.4 ± 0.0	a	0.4 ± 0.1	a	0.3 ± 0.1	ab	0.6 ± 0.3	abc	0.7 ± 0.1
P. vulgaris	0.4 ± 0.0	a	**4.3 ± 0.8**	abcde	3.4 ± 1.5	abcd	1.3 ± 0.9	ab	2.0 ± 0.6
T. latifolia	0.4 ± 0.0	a	3.1 ± 0.9	abcd	1.9 ± 1.2	abc	0.9 ± 0.2	abc	1.3 ± 0.3
All species	0.5 ± 0.0		3.8 ± 0.7		4.3 ± 0.8		5.0 ± 0.8		3.2 ± 0.6

Relationships Between COD Removal and Sulfate Concentration

The species which showed the greatest and most consistent COD removal

throughout the year had the highest SO_4 concentrations at colder temperatures (Figures 2-

5, Figure 6). Columns with less than 80% average COD removal had SO_4 concentrations

consistently near 0 mg/L. Relationships between COD removal and SO_4 concentration

were strongest at colder temperatures (Figure 7). The strongest relationship, with $R^2 =$

0.85, occurred at 4˚C in both 2007 and 2008. The weakest relationship with $R^2 = 0.10$,

occurred during the 24˚C 2007 period. The weak relationship was a result of the low

variability of SO_4 concentration among species at warm temperatures. The magnitude of

SO_4 variation and the strength of the relationship between COD removal and SO_4
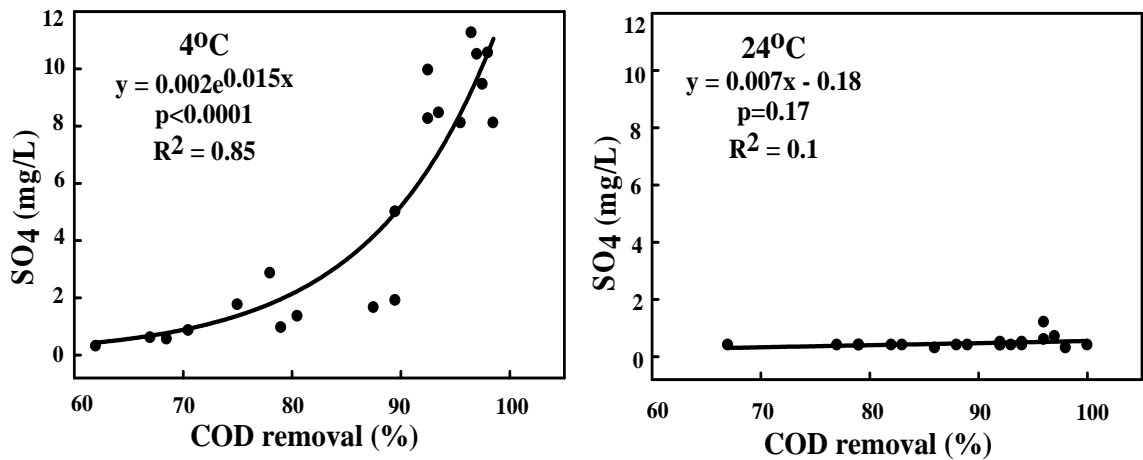
concentration were intermediate at 8˚C and 16˚C.

Figure 7: Relationships between COD removal (%) and SO_4 concentrations (mg/L) at
4˚C and 24˚C. Each point represents day-6 measurements for one species or unplanted
controls. Values at 4˚C are averages of 4˚C 2007 and 2008 batches. Values at 24˚C are
for the 24˚C 2007 batch.

Redox Potential

Redox potential was also influenced by plant species and seasons. At 24˚C in 2006 and 2007, the redox potential in all planted and control columns dropped sharply within the first day and remained in the range of -225 to -275 mV throughout the 20-day wastewater batches (Figure 1). In contrast, greater variation occurred during the 4˚C batches. Redox potential ranged from -236 to 401 mV on day 6 of the 4˚C batch in 2007 and from -260 mV to 445 mV on day 6 of the 4˚C batch in 2008. At 4˚C in 2007 and 2008 the control columns, and columns with *C. microptera, C. canadensis, H. jubatum, L. cinereus, P. arundinacea, P. australis, P. virgatum, P.vulgaris*, and *T. latifolia* followed a pattern similar to that seen at 24˚C. Columns planted with other species behaved differently at 4˚C and 24˚C. At 4˚C columns with *C. aquatilis, C. bebbii, C. praegracilis, C. utriculata, S. acutus, J. arcticus, J. torreyi*, and *D. cespitosa* either declined less initially or increased throughout the batch from low initial levels (Figure 1 shows patterns for representative species).

Redox potential varied little among species at warm temperatures but varied substantially during cold temperature batches (Figures 2-5). Day-6 redox potential remained below -200 mV and was virtually unchanged across seasons in controls and with the majority of plant species: *C. aquatilis, C. microptera, C. canadensis, H. jubatum, L. cinereus, P. virgatum, P. arundinacea, P. australis, I. missouriensis, P. vulgarsis, and T. latifolia.* Species that showed greater variation and rose above -200 mV by the sixth day of cold temperature batches were *C. bebbii, C. nebrrascensis, C. praegracilis, C. utriculata, S. acutus, J. arcticus, J. torreyi,* and *D. cespitosa*; Eh rose

above -200 mV by the ninth day in columns planted with *C. aquatilis*. Columns planted with these species also had average COD removal greater than 90% and average SO_4 concentrations greater than 3 mg/L.

Discussion

Patterns of COD removal, sulfate concentration, and redox potential during 20-day batch incubations demonstrated that wastewater treatment was affected by both seasons and plants. Most plants affected seasonal patterns to some degree, but plant effects were species specific. In unplanted controls, seasonal temperature variation had little to no influence on SO_4 concentrations and redox potential, and COD removal declined at lower temperatures.

Columns containing some plant species had water chemistry patterns similar to unplanted controls, while other plant species behaved very differently from the controls. Many species had consistently high COD removal throughout the year; columns with these species had the highest average COD removal and had elevated SO_4 concentrations and, sometimes, redox potentials at lower temperatures. Other species had intermediate or inconsistent seasonal COD removal, SO_4 concentrations, and redox potential.

Lower COD removal in the unplanted controls during cold temperature incubations was presumably caused by a decrease in biological activity at low temperatures as conventionally assumed for wastewater treatment (USEPA 1993, Kadlec and Knight 1996). Planted columns that followed patterns similar to the control were likely also influenced by the effect of cold temperatures on biological activity. The

constant high COD removal values seen year-round with other species suggest that those plants can somehow offset the negative effects of cold temperatures.

One possibility is that increased oxygen availability stimulates microbial processes that would otherwise be negatively affected by cold temperatures. It is well documented that many wetland plants release oxygen from their roots (Armstrong 1964, Armstrong 1971b, Brix and Schierup 1990, Gries et al. 1990, Kludze et al. 1994, Kludze and DeLaune 1994, Sorrell and Armstrong 1994, Laskov et al. 2006, Matsui and Tsuchiya 2006, Tanaka et al. 2007). The ability of plants to transport oxygen from shoots to roots efficiently and release oxygen from roots into the rhizosphere has the potential to influence wastewater treatment processes. Because domestic wastewater has abundant organic carbon, an electron donor, treatment is often limited by oxygen, the most energetically favorable electron acceptor (Reed and Brown 1992, Kadlec and Reddy 2001). Leakage of oxygen from roots can create oxidized zones (Armstrong 1971b) within the predominantly reduced environment of the bulk wastewater solution, supporting more efficient aerobic treatment of COD and nitrogen (Brix 1987).

Seasonal variation in root oxygen loss could account for the observation that differences in COD removal between planted and unplanted columns were found for most species at lower temperatures but were infrequent at warmer temperatures. The amount of root oxygen loss may vary across seasons due to variation in a plant's oxygen assimilation and respiration. Armstrong (1971b) and Howes and Teal (1994) found increases in root oxygen loss from *Oryza sativa* (rice) and *Spartina alterniflora* (smooth cordgrass), respectively, when temperature was lowered. Gries et al. (1990) and Moog

and Bruggemann (1998) measured both root respiration and oxygen release from reeds and sedges. They found that with decreasing temperatures, root respiration rates decreased while radial oxygen loss increased. The interaction between temperature and root oxygen loss has been studied for only a small number of species. The SO_4 concentration and Eh results of this study suggest indirectly that elevated oxygen release at lower temperatures may be fairly common among wetland plants.

SO_4 and Eh measurements in this experiment support the hypothesis that root oxygen loss is a mechanism for plant species and seasonal effects on wastewater. At 4˚C a strong relationship between COD removal and SO_4 concentration was found; there was also a weaker relationship between COD removal and Eh. Species with low COD removal also had low SO_4 concentration and redox potential; this may have resulted from having low or no oxygen release from roots and, consequently, insufficient oxygen to support sulfide oxidation, and raise Eh. Species with high COD removal also had elevated SO_4 concentration and redox potential, which indicates elevated oxygen levels that would support sulfide oxidation and enhance overall root-zone oxidation status. In contrast, COD removal was not related to SO_4 concentrations or Eh at 24˚C because SO_4 and Eh were uniformly low in spite of variation in COD removal rates among species.

Past constructed wetland studies have also found differences in water chemistry and microbial processes that suggest greater oxygen availability in winter than summer. Kadlec and Reddy (2001) found a slight increase in BOD removal at lower temperatures, consistent with the slight increase in COD removal at colder temperatures measured in columns planted with *C. nebrascensis* and *C. utriculata* in this study. Griffin et al.

(1999) measured a lower Eh, increased ammonification and total N, and sulfate reduction leading to sulfide formation in the summer; in the winter they found elevated Eh, increased N removal, and no sulfate reduction.

Although differences were most pronounced in winter, the presence of plants, especially select species, enhanced wastewater treatment throughout the year compared to unplanted columns. Variations between plant species' COD removal during colder temperatures is likely driven by differing amounts of root oxygen loss between species. Past studies have found that plant species differ in the amount of oxygen released from roots (Bedford et al. 1991, Sorrell 1999, Chabbi et al. 2000, Visser et al. 2000b, Wiessner et al. 2002). Differences in plants' physiological and morphological traits adaptive to their environment may influence the amount of root oxygen loss.

Columns planted with *C. aquatilis*, *C. bebbii*, *C. praegracilis*, *C. utriculata*, *S. acutus*, *J. arcticus*, *J. torreyi*, and *D. cespitosa* had the greatest COD removal throughout the year, with experiment average removals over 90%. These plant species, with the exception of *S. acutus*, are not currently widely used in constructed wetlands and are not well researched. *T. latifolia*, *P. australis*, and *P. arundinaceae* are widely used and well researched, yet these species provided less improvement in treatment than many of the species studied (average COD removal across temperatures = 84%, 74%, and 83%, respectively). Because these three species are weedy in wetland ecosystems and have unexceptional performance, other species may be preferable. In addition to the species studied here, other members of the *Cyperaceae* and *Juncaceae* are likely candidates; all of the species with >90% COD removal, except *D. cespitosa*, were in these two families.

All species with high COD removal were classified as Obligate Wetland or Facultative

Wetland species by the U.S. Fish and Wildlife Service (U.S. Fish and Wildlife Service

1988), but some species with the same Wetland Indicator Status did not perform as well.

Interestingly, *C. microptera*, the *Carex* species with the lowest Wetland Indicator Status

(Facultative), had the lowest average COD removal and SO_4 concentration of the *Carex*

species throughout the experiment (86% and 2 mg/L).

Results of this study suggest that choosing appropriate plants can increase

constructed wetland efficiency throughout the year and may be especially important in

cold climates. In spite of the normal tendency for microbial activity to decline during

cold temperatures, select plant species may create conditions for constant or even

increased COD removal at low temperatures. However, species must be matched to

wastewater goals. Plant species which promote aerobic processes will increase the

efficiency of wetlands designed for organic carbon and nutrient removal from domestic

wastewater (Burgoon et al. 1995, Cronk and Fennessy 2001, Nivala et al. 2007). On the

other hand, constructed wetlands designed for sulfate reduction and metal removal

require limiting oxygen to promote low redox conditions (Machemer and Wildeman

1992, Nelson et al. 2006, Stein et al. 2007), and plants that do not facilitate oxidation

would be most effective.

Results from this study that demonstrate pronounced differences between species

in the winter justify further investigation of plants' seasonal effects on wastewater

treatment. A limited number of studies have compared plant species (Gersberg et al.

1986, Coleman et al. 2001, Allen et al. 2002, Fraser et al. 2004, Picard et al. 2005, Stein

and Hook 2005, Iamchaturapatr et al. 2006, Akratos and Tsihrintizis 2007, Yang et al. 2007) and even fewer have examined plant influences across all seasons (Allen et al. 2002, Picard et al. 2005, Stein and Hook 2005, Akratos and Tsihrintizis 2007, Yang et al. 2007). This study is unique in that it examines seasonal effects of a large number of species for potential use in constructed wetlands including many regionally native plants as well as commonly used species. Greenhouse-scale studies with controlled environments will continue to be valuable for studying underlying biological processes. However, because small-scale greenhouse studies may not predict performance in the field due to scale differences and environmental and wastewater variation, field studies are needed. This study and Allen et al. (2002) provide evidence that start-up effects occur in model wetlands: COD removal was lower in the experiments' initial batches. This may be because plants were still growing into their containers and microbial communities were developing. Both greenhouse and field studies will provide more useful information if observed over two or more years. Additionally, rather than studying only monocultures, further research should investigate wastewater treatment with mixed plantings (Callaway and King 1996, Coleman et al. 2001, Picard et al. 2005). Additional studies which make comparisons between a large number of species, both at small scales and in the field, and studies of mixed planting will collectively contribute to plant selection for improving constructed wetland function.

PLANT INFLUENCES ON WASTEWATER TREATMENT

Introduction

Constructed wetlands (CWs) are a natural alternative to conventional methods of wastewater treatment (Kadlec and Knight 1996). Wastewater is treated by physiochemical and biological interactions between wastewater, wetland media, microorganisms, and plants. Although plants are a major component of wetlands, relatively little is known about their effects on the processes responsible for wastewater treatment. A greater understanding of plants' influences would allow designers to select appropriate plant species to increase the efficiency of the desired treatment, whether it is organic carbon, nutrient, or heavy metal removal.

While comparisons of unvegetated and vegetated wetlands have found increased treatment with plants present (Coleman et al. 2001, Hunter et al. 2001, Lin et al. 2002, Picard et al. 2005, Iamchaturapatr et al. 2006, Yang et al. 2007) and species-specific effects (Gersberg et al. 1986, Allen et al. 2002, Fraser et al. 2004, Stein and Hook 2005, Akratos and Tsihrintizis 2007), the mechanisms underlying those effects are still the subject of speculation. Plants could improve treatment through direct uptake of nutrients, but plant nutrient content cannot account for the enhanced nutrient removal from wastewater (Gersberg et al. 1986, Bachand and Horne 2000, Lin et al. 2002, Akratos and Tsihrintizis 2007). Instead, influencing microbial activity is believed to be plants' primary role in treatment wetlands by creating attachment sites and releasing exudates and oxygen (Reed and Brown 1992, Brix 1997, Tanner 2001).

A plant's influence on wastewater and microbial activity occurs primarily in the rhizosphere, the area immediately surrounding the root that is influenced by root exudates (Lynch and Whipps 1990). Abundant microbial communities, increased chemical processing, and elevated dissolved oxygen have been measured in the root zones in constructed wetlands (Reddy et al. 1989, Hatano et al. 1993, Bodelier et al. 1996, Ottova et al. 1997, Weiss et al. 2003, Bezbaruah and Zhang 2004, Munch et al. 2005, Vacca et al. 2005, Gagnon et al. 2006). These measurements provide evidence that roots increase microbial processes due, in part, to oxygen release (Armstrong 1964, Wiessner et al. 2002, Bezbaruah and Zhang 2004). However, past studies measuring oxygen release have conflicting results with roots either taking up or releasing oxygen (Armstrong 1971b, Brix and Schierup 1990, Gries et al. 1990, Bedford et al. 1991, Kludze et al. 1994, Kludze and DeLaune 1994, Sorrell and Armstrong 1994, Sorrell 1999, Chabbi et al. 2000, Visser et al. 2000b, Laskov et al. 2006, Matsui and Tsuchiya 2006, Tanaka et al. 2007). There is disagreement regarding the potential influence of rhizodeposition – the transfer of substances from roots to the surrounding medium – on the bulk soil or water (Bedford et al. 1991, 1994; Sorrell and Armstrong 1994).

The debate over root influences in soil and water results partly from differences in study methods and scale. Most studies of root effects involve short-term and small-scale measurements of isolated roots, as opposed to studies that examine the net effect of the whole plant in a wetland system taking into account the temporal and spatial variability of roots acting as a source and a sink of oxygen (Bedford et al. 1991, 1994). While isolated measurements in the rhizosphere provide evidence of the mechanisms underlying

plant influences on the bulk solution, the extent of these influences on wastewater treatment also needs to be determined at appropriate scales.

Comparisons among COD removal, sulfate concentration, and redox potential (Eh) suggest that plants do affect oxygen-dependent processes in constructed wetland microcosms with mature plants (Chapter 2, Allen et al. 2002). COD removal from wastewater declined at cold temperatures in microcosms planted with some plant species but remained high at all temperatures with other species. SO_4 and Eh indicated that conditions were highly reduced at all times in microcosms which had a decline in COD removal at cold temperatures. In contrast, SO_4 and Eh indicated that a degree of oxidation occurred in microcosms with consistent high COD removal. Allen et al. (2002), Hook et al. (2003), and Stein and Hook (2005) hypothesized that the latter plant species release greater amounts of oxygen in the winter when root respiration slows and that this offsets the negative effects of temperature on COD breakdown by microbes.

If root oxygen loss does affect wastewater treatment in this way, plant species could potentially be selected to increase the efficiency of COD removal and other oxidative treatment processes based on the quantity of oxygen released from their roots. Since root oxygen loss is problematic to measure and discrepancies occur with different experimental methods (Sorrell and Armstrong 1994), other plant traits might prove to be more useful for plant selection; possibilities include flooding tolerance, Wetland Indicator Status (WIS), and root morphology, each of which is related to plants' abilities to avoid flooding stress caused by anoxia.

Plants differ in their physiological and morphological traits and ecological distribution as a result of adaptation, or lack of adaptation, to wetland environments (Laan et al. 1989, Sorrell et al. 2000, Visser et al. 2000a). Plants in wetland ecosystems must survive in water-saturated substrates which provide inadequate oxygen for root respiration and expose plants to reduced substances that can be toxic (Ponnamperuma 1972). Wetland plants have various traits and mechanisms for avoiding or tolerating internal anoxia when flooded (McManmon and Crawford 1971, Drew 1997, Crawford 2003). In some species, oxygen is transported from shoots to roots, where it is used in respiration; some of the oxygen may be released to the surrounding soil or water, resulting in the oxidation of reduced, potentially harmful substances (Armstrong 1971b). Thus, individual species adaptations to anoxic conditions may have important implications for wastewater treatment processes affected by oxygen availability and may also help explain species-specific effects on biogeochemical processes in wastewater (Chapter 2).

My study investigated root oxygen loss (ROL) as a mechanism contributing to plants' influence on wastewater treatment in constructed wetlands and the potential of ROL and related plant traits to guide species selection for use in CWs. Seasonal and plant effects on water chemistry in wetland microcosms ("columns") containing monocultures of 19 different plant species were investigated in Chapter 2. In the study presented here, root oxygen loss was measured while plants were dormant and while plants were growing and compared to COD removal rates reported in Chapter 2. Relationships with traits that may be related to ROL – aerenchyma, root diameter

distribution, flooding tolerance, Wetland Indicator Status, and family or genus – were also investigated.

Materials and Methods

Species Selection

Nineteen plant species were selected so that species associated with different hydrologic regimes and flooding tolerances could be compared. The Wetland Indicator Status, a ranking that indicates the probability that a plant grows in a wetland, was used to choose plants with varying degrees of adaptation to the wetland environment (U.S. Fish and Wildlife Service 1988). Additionally, plant species were selected based on their abundance in North America, particularly in the region surrounding Montana, their use by professionals in the field of wetland restoration, and their use in past constructed wetland research. The 19 species included sedges, rushes, grasses, and forbs (Table 7). The plant species were either purchased from nurseries or transplanted from the field. Plants were grown and experiments were conducted in the Plant Growth Center at Montana State University in Bozeman, MT.

Root Oxygen Loss

Experimental Design: Root oxygen loss was measured on a subset of the 19 species during the winter and summer. Three replicates of an unplanted control and 14 plant species were measured in January 2007, when the greenhouse temperature was 4˚C and plants were dormant (Table 7). Four replicates of the control and 10 plant species

were measured in July 2007, when the plants were actively growing and the greenhouse

temperature was 24˚C.

Table 7: Plant species studied and their Wetland Indicator Status. Obligate Wetland species occur in wetlands >99% of the time, Facultative Wetland species 67-99% of the time, and Facultative species 34-66% of the time. Root oxygen loss was measured at 4˚C and/or 24˚C for species indicated with an X in the respective columns.

Family	Species	Common name	4˚C ROL	24˚C ROL
	Obligate Wetland Species			
Cyperaceae	*Carex aquatilis* Wahlenb.	water sedge	X	
	Carex bebbii Olney ex Fernald	Bebb's sedge	X	X
	Carex nebrascensis Dewey	Nebraska sedge	X	
	Carex utriculata Boott	Northwest Territory sedge	X	X
	Schoenoplectus acutus (Muhl. ex Bigelow) A. Love & D. Love	hardstem bulrush	X	X
Typhaceae	*Typha latifolia* L.	broadleaf cattail	X	X
	Facultative Wetland Species			
Cyperaceae	*Carex praegracilis* W. Boott	clustered field sedge	X	X
Juncaceae	*Juncus arcticus* Willd.	arctic rush	X	
	Juncus torreyi Coville	Torrey's rush	X	X
Poaceae	*Calamagrostis canadensis* (Michx.) P. Beauv.	bluejoint	X	X
	Deschampsia cespitosa (L.) P. Beauv.	tufted hairgrass	X	X
	Phalaris arundinacea L.	reed canarygrass		
	Phragmites australis (Cav.) Trin. ex Steud.	common reed		
Iridaceae	*Iris missouriensis* Nutt.	Rocky Mountain iris		
	Facultative Species			
Cyperaceae	*Carex microptera* Mack.	smallwing sedge	X	X
Poaceae	*Hordeum jubatum* L.	foxtail barley	X	X
	Leymus cinereus (Scribn. & Merr.) A. Love	basin wildrye		
	Panicum virgatum L.	switchgrass	X	
Lamiaceae	*Prunella vulgaris* L.	common selfheal		

Before root oxygen loss was measured, plants were grown in saturated conditions. Plants used for winter measurements were planted in May 2006 and grew until January 2007. Plants used for summer measurements were planted in April 2007 and grew until July 2007. Nursery or field soil was removed from the roots, plants were trimmed for uniform root and shoot biomass, and planted in sand (20/30 sand, Bozeman Brick Block and Tile, Bozeman, MT) in Deepots ® (5.1 cm diameter x 22.9 cm, Stuewe & Sons, Inc., Corvallis, OR). Deepots ® were placed in Rubbermaid ® bins (51 cm long x 37 cm wide x 31 cm high) and bins were filled with ¼ strength Hoagland's nutrient solution so that sand was saturated. Tap water was added to the base of the bins through a continuous water delivery system to replace water losses from evapotranspiration. Every two weeks, bins were drained and plants were randomized between and within bins to provide uniform average conditions for plant growth. Bins were refilled with ¼ strength Hoagland's nutrient solution. Photoperiod followed seasonal light availability; no supplemental lighting was used. The greenhouse temperature varied between 4, 8, 16, and 24˚C in 60-day intervals to mimic seasonal cycles.

Root Oxygen Loss Measurements: Root oxygen loss was measured colorimetrically by the titanium citrate method described by Kludze et al. (1994). Titanium-citrate solution was prepared according to methods of Zehnder and Wuhrmann (1976), using water which was deoxygenated by purging with nitrogen gas. Oxygen contamination was avoided by creating solutions in an anaerobic glovebox (Protector Controlled Atmosphere Glove Box, Labconco Corporation, Kansas City, MO). Ti^{3+} citrate solution was created by mixing 300 mL of 0.2 M sodium citrate solution, 30 mL of

1.16 M titanium chloride, and saturated sodium carbonate to adjust the pH to 5.6. This solution was then diluted by a factor of ten with deoxygenated water.

In the greenhouse, sand was removed from plant roots, and an individual plant and its sampling tube were suspended in a glass jar (Ball canning jars, 12 oz.). A known amount of Ti^{3+} citrate solution was poured into the glass jar to immerse the roots. Paraffin oil was applied at the root-shoot junction to hinder atmospheric oxygen contamination of the root zone. Control treatments had the same apparatus with no plant.

After 24 hours, a 5 ml solution sample was extracted and stored in a Vacutainer (BD Vacutainers, Franklin Lakes, NJ). Absorbance of the solution was measured at 527 nm in a spectrophotometer (Spectronic Genesys 5, Spectronic Instruments, Rochester, NY). Oxygen concentrations were extrapolated from a standard curve (Oxygen = -0.0011(Absorbance) + 0.3738) and the whole-plant oxygen loss was calculated using the following equation:

$$ROL = v \, (p - c)$$

where ROL = root oxygen loss, $\mu mol \; O_2 \; plant^{-1} \; day^{-1}$; v = standardized volume of Ti^{3+} citrate solution (volume of Ti^{3+} citrate solution for individual measurments / largest volume of solution used in the experiment); p = oxygen concentration in the root solution after 24 hours with plants, $\mu mol \; O_2 \; plant^{-1}$; c = oxygen concentration in the root solution after 24 hours in the control without plants, $\mu mol \; O_2 \; plant^{-1}$. Roots and shoots were dried to constant weight so that root oxygen loss measurements could be calculated per gram of dry root.

<u>Root Anatomy and Morphology</u>

<u>Experimental Design:</u> Plant material was purchased or collected from the field in April 2007. Plants were grown as described above until October 2007, at which time they were harvested for root measurements.

<u>Aerenchyma Measurement:</u> The proportion of aerenchyma tissue was measured in three roots from two replicates of *C. canadensis*, *C. utriculata*, *D. cespitosa*, *S. acutus,* and *T. latifolia* following the methods of Visser and Bogemann (2003). Cross sections were taken from 2-3 cm segments from the stem-root junction, stained with Cotton Blue, and photographed with a Nikon Coolpix 990 and Eclipse E600 photomicroscope (Nikon Inc., Japan). The diameters of the inner and outer root tissues and aerenchyma tissue were measured using Adobe Photoshop CS3 (Adobe Systems Inc., San Jose, CA). The proportion of aerenchyma tissue was calculated by dividing the area of aerenchyma tissue by the area of the root cross section.

<u>Root Measurement:</u> Root morphology was measured for two replicates of five plant species, *C. canadensis, C. utriculata, D. cespitosa, H. jubatum,* and *S. acutus.* Dried roots from plants previously used to measure ROL were rehydrated and roots were scanned using WinRhizo Pro Version 2002c and XLRhizo Version 2003a software (Regent Instruments, Inc., Montréal, QE, Canada). Roots were divided into four diameter classes ranging from $0.0 \leq 0.60$ mm up to >2.25 mm. The proportion of root length, surface area, and volume in each diameter class was calculated.

<u>Flooding Tolerance</u>

To test the effects of flooding on plant growth, the 19 plant species were

subjected to two different hydrologic regimes. Four replicates of each species were

drained freely and four replicates were flooded to 5 cm above the surface of the soil.

Redox potential three centimeters below the soil surface averaged -30 mV in 12 flooded

pots and 245 mV in 12 drained pots.

Plant material was purchased or collected from the field, cut for uniform root and

shoot biomass, and planted in May 2006 in a 1:1:2 topsoil:peat:sand mixture in Treepots

® (11.4 cm x 11.4 cm x 35.6 cm, Stuewe & Sons, Inc., Corvallis, OR). Throughout the

experiment, the greenhouse temperature was maintained at 25˚C for 16 hours during the

day and 16˚C for 8 hours during the night. No nutrients or supplemental light were

provided. Plants were randomly assigned positions in the greenhouse and rotated to a

new position every two weeks to minimize differences in growing conditions. After five

months of growth, plants were harvested. Root and shoot tissues were separated,

cleaned, dried to constant mass, and weighed.

<u>Statistical Analysis</u>

Differences among plant species' root oxygen loss, proportion of aerenchyma

tissue, and proportion of roots in each diameter class were determined by ANOVA (SPSS

Version 15.0, SPSS Inc., Chicago, IL). Planned contrasts were used to test whether

oxygen concentrations in planted treatments were different than in the unplanted controls.

Post hoc Tukey HSD comparisons were used to test for differences between plant species

in all experiments. Differences in shoot biomass, root biomass, and total biomass

between drained and flooded treatments were analyzed for each plant species

independently with a t-test using SPSS (SPSS, Inc., Chicago, IL Version 15.0). The

significance level was set at $\alpha = 0.05$.

Pearson's correlation (SPSS Version 15.0, SPSS Inc., Chicago, IL) was used to

test for associations between COD removal and root oxygen loss. COD removal data

from the 4˚C 2007 batch (Chapter 2) was correlated to the winter root oxygen loss

measurements while COD removal from the 24˚C 2007 batch was correlated to the

summer root oxygen loss measurements. To determine whether the correlation was

significant, the null hypothesis $\rho = 0$ was tested at a significance level of $\alpha = 0.05$.

Least squares linear regression (SPSS, Inc., Chicago, IL Version 15.0) was used

to analyze relationships between COD removal (Chapter 2) and Wetland Indicator Status

and flooding tolerance for all species. COD removal data from the 4˚C 2007 incubation

(Chapter 2) was used. Wetland Indicator Status and flooding tolerance were treated as

categorical variables: facultative species were assigned a value of one, facultative

wetland species assigned a value of two, and obligate wetland species assigned a value of

three. Plants with greater biomass when flooded were assigned a value of one, and plants

with no biomass increase when flooded were assigned a value of two.   Relationships

were considered significant with $\alpha = 0.05$. The coefficient of determination (R^2) was

obtained to determine the strength of the relationship.

Results

Root Oxygen Loss

During the winter, root oxygen release per plant and per unit mass ranged from

0.0 μmol O_2 plant^{-1} day^{-1} for the control to a maximum of 85.7 μmol O_2 plant^{-1} day^{-1} for

D. cespitosa and from 0.0 to 26.2 μmol O_2 g^{-1} dry root day^{-1} for the control and *H.*

jubatum, respectively (Table 8). Ten of 14 plant species released more oxygen than the

control based on whole plant measurements. Nine species released more oxygen than the

control when ROL was expressed on root mass basis. The plants identified as releasing

more oxygen differed between the two methods for calculating ROL because of the large

range in root biomass. Under the same growing conditions, root mass for individual *T.*

latifolia planted averaged 15.0 grams and *H. jubatum* roots averaged 0.77 grams.

During the summer, ROL ranged from -0.7 to 45.3 μmol O_2 plant^{-1} day^{-1} for the

control and *S. acutus* and from -0.7 to 25.6 μmol O_2 g^{-1} dry root day^{-1} for the contral and

S. acutus, respectively. Four of 10 species released more oxygen than the control based

on whole plant measurements, and two of 10 released more based on dry root

measurements. During the summer, there was less variation among plant species and

fewer species released more oxygen than the control. Summer and winter measurements

fell within the range of oxygen release measured in past studies: -270 μmol O_2 g^{-1} dry

mass h^{-1} (oxygen uptake) to 126 μmol O_2 g^{-1} dry mass h^{-1} (oxygen loss) (Bedford et al.

1991, Kludze et al. 1994, Sorrell 1999, Laskov et al. 2006).

So that these measurements could be compared to other studies, the amounts of

oxygen released by *D. cespitosa* and *S. acutus* were expressed based on root surface area

Table 8: Root oxygen loss at 4°C and 24°C. 4°C ROL values (reported as μmol O_2 plant^{-1} day^{-1} and μmol O_2 g^{-1} dry root day^{-1}) are means of three replicates ± one standard error. 24°C ROL values (reported as μmol O_2 plant^{-1} day^{-1} and μmol O_2 g^{-1} dry root day^{-1}) are means of four replicates ± one standard error. Values in a column with an asterisk (*) are significantly different than the control (p<0.05, planned contrast).

	4°C				24°C			
	Soln. used (mL)	Dry root (g)	Root oxygen loss μmol O_2 plant^{-1} day^{-1}	Root oxygen loss μmol O_2 g^{-1} dry root day^{-1}	Soln. used (mL)	Dry root (g)	Root oxygen loss μmol O_2 plant^{-1} day^{-1}	Root oxygen loss μmol O_2 g^{-1} dry root day^{-1}
D. cespitosa	217	5.7	85.7 ± 5.5 *	20.0 ± 5.7 *	250	1.7	13.8 ± 11.3	5.9 ± 7.4
S. acutus	205	4.8	73.4 ± 8.4 *	17.6 ± 4.8 *	255	4.3	45.3 ± 12.4 *	25.6 ± 13.5 *
C. microptera	217	4.1	67.9 ± 7.0 *	16.4 ± 1.3 *	250	1.5	4.0 ± 2.2	2.5 ± 1.2
J. torreyi	217	3.8	60.3 ± 3.1 *	16.2 ± 2.1 *	250	2.3	26.8 ± 9.7 *	12.0 ± 3.2
C. nebrascensis	223	3.0	55.3 ± 17.7 *	18.4 ± 5.5 *				
C. bebbii	217	2.4	47.7 ± 15.3 *	23.2 ± 9.5 *	250	1.2	4.2 ± 4.3	2.8 ± 3.4
C. aquatilis	212	5.6	40.0 ± 23.3 *	6.7 ± 2.4				
T. latifolia	217	15.0	35.2 ± 4.5 *	3.0 ± 1.3	340	5.6	30.7 ± 19.9 *	5.0 ± 2.9
C. canadensis	233	1.5	26.6 ± 5.1 *	16.9 ± 2.1 *	250	1.3	-0.2 ± 2.7	-0.4 ± 2.0
H. jubatum	208	0.8	25.5 ± 16.1 *	26.2 ± 13.1 *	250	1.3	0.1 ± 7.4	0.1 ± 5.8
C. utriculata	217	1.9	20.0 ± 14.2	8.2 ± 4.5	250	1.6	20.8 ± 5.1 *	13.5 ± 3.1 *
P. virgatum	217	1.6	17.7 ± 11.6	9.4 ± 5.9				
J. arcticus	213	1.9	17.6 ± 0.5	9.9 ± 2.2				
C. praegracilis	207	1.1	13.1 ± 2.2	13.1 ± 2.9 *	250	1.0	10.5 ± 5.1	7.9 ± 4.2
Control	154		0.0 ± 1.5	0.0 ± 1.5	250		-0.7 ± 1.6	-0.7 ± 1.6

as determined with WinRhizo Pro Version 2002c and XLRhizo Version 2003a software (Regent Instruments, Inc., Montréal, QE, Canada) and planted area based on the area of the mason jar used in the root oxygen loss measurements. *D. cespitosa* released 2.1 x 10^{-6} g O_2 cm^{-2} root surface area day^{-1} and 0.034 g O_2 m^{-2} planted area day^{-1}. *S. acutus* released 1.6 x 10^{-6} g O_2 cm^{-2} root surface area day^{-1} and 0.018 g O_2 m^{-2} planted area day^{-1}. These values also fall in the range reported in the literature (Armstrong 1971b, Brix and Schierup 1990, Gries et al. 1990, Visser et al. 2000b, Matsui and Tsuchiya 2006, Tanaka et al. 2007).

Root Anatomy and Morphology

The percentage of aerenchyma tissue measured below the root-shoot junction varied between species (Table 9, Figure 8). The proportion of aerenchyma tissue ranged from 12% for *C. canadensis* to 59% for *S. acutus*.

Table 9: Percent aerenchyma below the root-shoot junction. Values are means of six replicates ± one standard error. Values in a column with the same letter are not significantly different ($p \geq 0.05$, Tukey HSD).

	Aerenchyma Tissue (%)	
Calamagrostis canadensis	11.8 ± 7.9	a
Deschampsia cespitosa	38.3 ± 3.7	b
Typha latifolia	55.2 ± 4.2	bc
Carex utriculata	56.9 ± 3.7	bc
Schoenoplectus acutus	58.7 ± 3.4	c

For all species, the majority of roots were less than 0.6 mm in diameter. As seen in Table 10, plants differed in the relative proportion of roots less than 0.6 mm and within the range from 0.6 mm to 1.5 mm. *C. canadensis* and *H. jubatum* had a greater

proportion of roots with the smallest diameter than *D. cespitosa* and *S. acutus,* which had

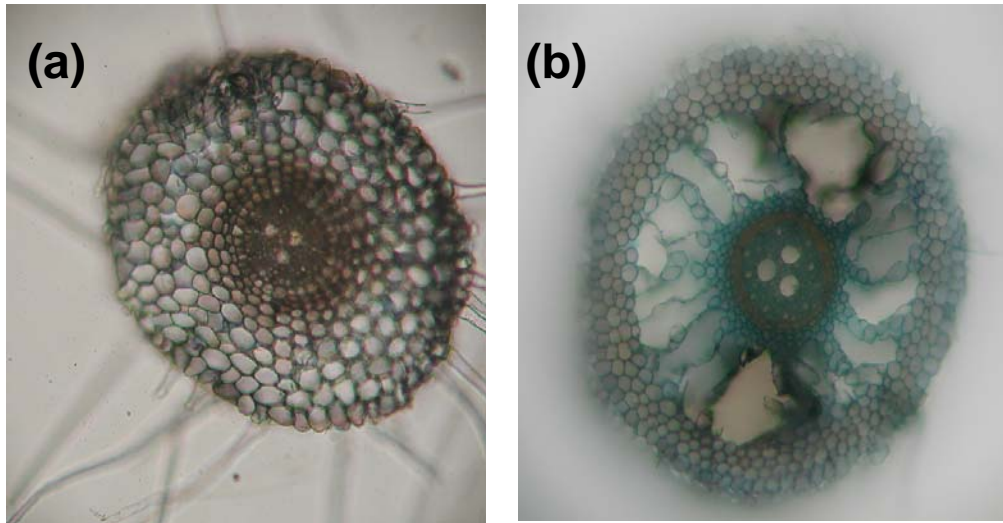a greater proportion of 0.6 to 1.5 mm diameter roots.

Figure 8: Root cross sections of (a) *C. canadensis* and (b) *D. cespitosa.*
Photo (a) shows no aerenchyma tissue; aerenchyma structure is present in (b).

Table 10: Length per root diameter class (%). Values are the mean ± one standard error
of two replicates. Values in a column with the same letter are not significantly different
($p \geq 0.05$, Tukey HSD).

	Length per root diameter class (%)			
	$0.0 \leq 0.60$ mm	$0.60 \leq 1.5$ mm	$1.5 \leq 2.25$ mm	> 2.25 mm
C. canadensis	96.2 ± 1.1 a	3.4 ± 0.9 c	0.3 ± 0.1 a	0.2 ± 0.1 a
H. jubatum	94.7 ± 0.7 a	4.9 ± 0.7 bc	0.3 ± 0.0 a	0.1 ± 0.0 a
C. utriculata	88.3 ± 2.8 ab	11.0 ± 2.5 ab	0.4 ± 0.2 a	0.3 ± 0.1 a
D. cespitosa	86.7 ± 0.5 b	12.6 ± 0.5 a	0.5 ± 0.0 a	0.2 ± 0.0 a
S. acutus	86.6 ± 0.5 b	12.5 ± 0.3 a	0.6 ± 0.1 a	0.4 ± 0.0 a

Flooding Tolerance

The plant species varied widely in their tolerance and growth response to

hydrologic regimes. Three replicates of *L. cinereus* and *I. missouriensis*, and one

replicate of *P. vulgaris* died in the flooded treatment. Two replicates of *T. latifolia* died

in the drained treatment. No significant difference in biomass between flooded and

drained treatments occurred with *C. bebbii, J. arcticus, H. jubatum, D. cespitosa, C.*

canadensis, P. virgatum, and *P. vulgaris*. The total biomass of *C. aquatilis, C.*

microptera, C. nebrascensis, C. praegracilis, C. utriculata, S. acutus, J. torreyi, P.

arundinacea, P. australis, and *T. latifolia* was greater in the flooded treatment; *L.*

cinereus and *I. missouriensis*'s total biomass was greater in the drained treatment (Figure

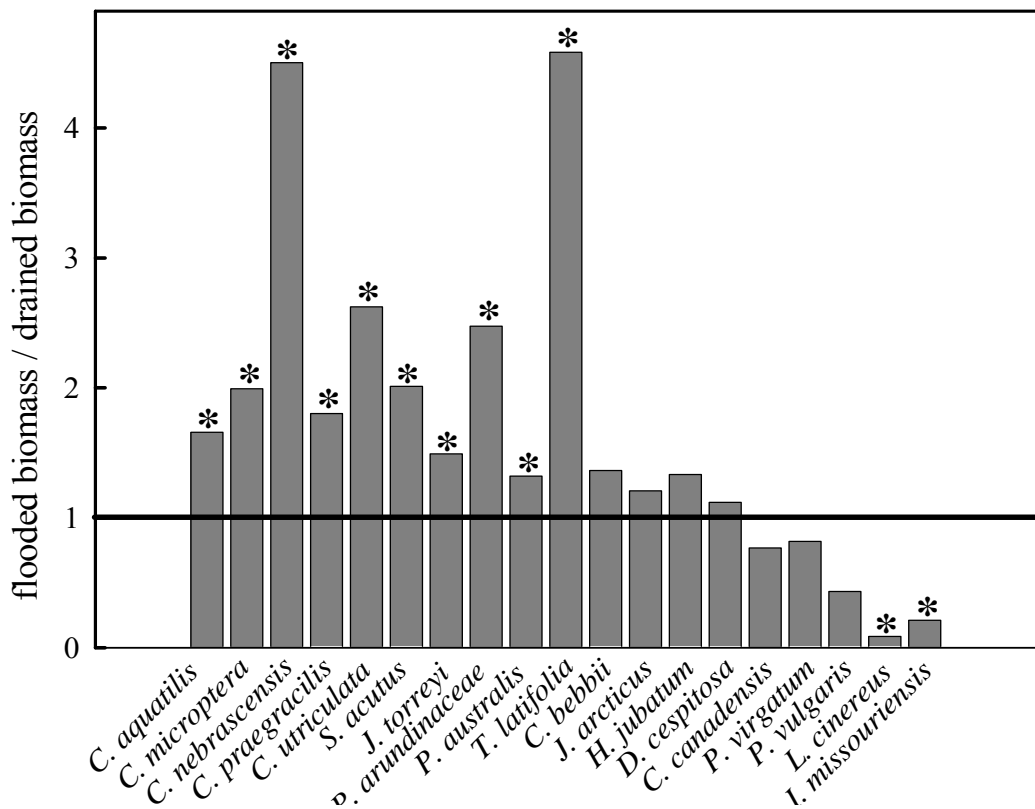9). Some plant species allocated biomass differently in flooded than drained conditions.

Figure 9: Biomass of plant species when flooded divided by biomass when drained.
Plant biomass values with an asterisk (*) differed significantly between flooded and
drained treatments (p<0.05).

*C. praegracilis, J. torreyi,*and *P. australis*'s total biomass was greater in flooded

conditions because their shoot biomass was significantly greater; however, their root

biomass was not greater with flooding (data not shown). Additionally, all plant species

except *H. jubatum, P. virgatum, L. cinereus, I. missouriensis*, and *P. vulgaris* developed

adventitious roots in the upper soil profile under flooded conditions. The formation of

adventitious roots is an adaptation to flooded conditions that increases the diffusion of

gases between shoots and roots (Visser et al. 1996). A possible factor contributing to *I.*

missouriensis's greater biomass in the drained treatment was that the plants purchased

were approximately 5 cm tall and were therefore submerged in the flooded treatment.

Relationships Between Plant Traits and Plant Effects on Wastewater Treatment

ROL and COD Removal: COD removal on day 6 of the 4˚C 2007 and 24˚C 2007

wastewater batches were compared to root oxygen loss per plant taken during the same

temperature regime. COD removal and ROL were positively correlated at 4˚C ($p = 0.001$

and $r = 0.67$) but not at 24˚C ($p = 0.74$ and $r = 0.10$) (Figure 10).

The amount of oxygen required to facilitate plants' influence on COD removal

was estimated and compared to measured root oxygen loss. During the 2007 batch at

4˚C, the difference in COD removal between the unplanted control and *D. cespitosa* was

201 mg/L after six days, equivalent to 1.05 mmols O_2 L^{-1} $days^{-1}$. Root oxygen loss for *D.*

cespitosa during the winter was 86 μmol O_2 $plant^{-1}$ day^{-1}, which would account for only

8% of the required oxygen needed to explain the difference in COD removal between

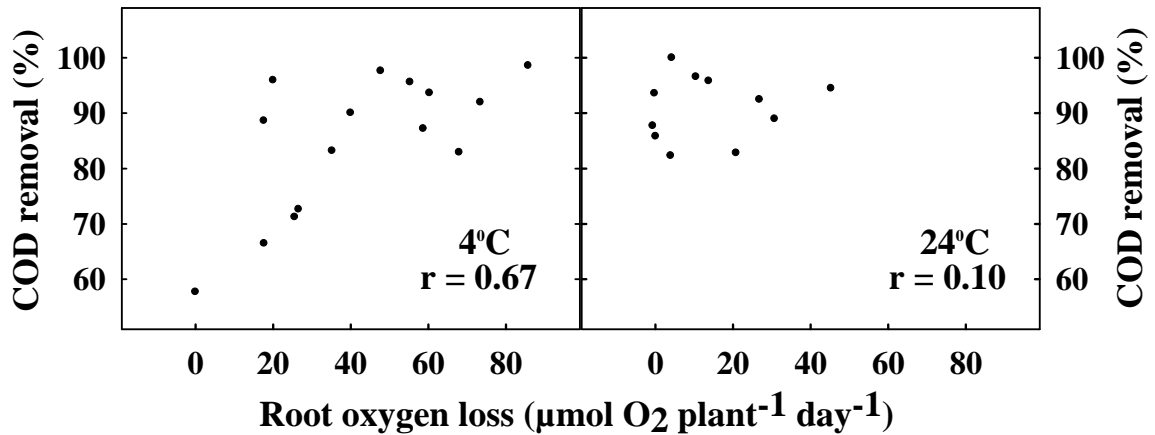planted and control columns. Similarly, differences in COD removal between

Figure 10: Correlation between COD removal and root oxygen loss at 4°C and 24°C. Each point in the 4°C plot represents one species or unplanted control's day-6 COD removal (%) from the 4°C 2007 batch incubation and ROL measured in winter. Each point in the 24°C plot represents one species or unplanted control's day-6 COD removal (%) from the 24°C 2007 batch and ROL measured in summer.

the unplanted control and *C. utriculata* were equivalent to 0.98 mmols O_2 L^{-1} days^{-1}. *C. utriculata* released 20 μmol O_2 L^{-1} days^{-1}, which was only 2% of the oxygen required to facilitate COD removal.

Wetland Indicator Status, Flooding Tolerance, and COD Removal: A relationship was found between WIS and COD removal (p = 0.001 and R^2 = 0.35). All Facultative species had relatively low COD removal with maximum removal of 83%. Facultative wetland species had a large range from 67% to 99% COD removal, and all obligate species had relatively high COD removal from 83% to 97%.

No relationship was found between species' flooding tolerance and COD removal (p = 0.19 and R^2 = 0.10). The average COD removal for plants which had greater biomass when flooded was 87%. In comparison, the average COD removal for plants with no significant biomass difference between flooded and drained treatments was 81%.

Discussion

To survive in water-saturated soil conditions, wetland plants have adaptations to meet their oxygen needs for root metabolism; some of that oxygen is released to the surrounding soil or water (Beals 1917, Armstrong 1971a, Crawford 2003). Many authors (Gersberg et al. 1986, Griffin et al. 1999, Kadlec and Reddy 2001, Allen et al. 2002, Fraser et al. 2004) attribute the observed differences in wastewater treatment between planted and unplanted wetlands to root oxygen loss. This study and a parallel study (Chapter 2) compared root oxygen loss and constructed wetland water chemistry across numerous plant species and between winter and summer, and also examined relationships with other traits that may be related to root oxygen loss. My results provided some support for the hypothesis that constructed wetland processes and chemistry are related to root oxygen loss and that root oxygen loss contributes to greater effects of plants in winter.

Comparisons between COD removal in model constructed wetlands, root oxygen loss measurements, and plant attributes allow for correlations but not causal relationships. The strength of the relationships between these measurements may be affected by the complexity of these factors, the accuracy of measurements or rankings, or the differences in experimental conditions and scale. The variables studied were measured at different scales, under different conditions, and/or at different times. COD was measured in model wetlands with mature plants, biofilms, and gravel substrate; root oxygen loss was measured from small, young plants in oxygen-scavenging solution in a small jar; flooding

tolerance was measured with young plants in potting soil; aerenchyma was measured from young isolated roots; and root dimensions were measured from the plants in the root oxygen loss experiment. The Wetland Indicator Status is a ranking from professionals' judgment of ecological distribution in the field. This study approach was chosen to look for general patterns across many species and to identify practical, easily measured indices to guide plant selection.

Relationship Between Wastewater COD Removal and ROL

The amount of root oxygen loss measured from the subset of species varied widely, especially in winter. Ten of 14 plant's root oxygen loss were significantly different than the unplanted control in the winter versus 4 of 10 in the summer. Species' rankings for amount of root oxygen loss also differed between seasons. Root oxygen loss would be expected to vary among species based on differences in anatomy, morphology, and physiology, and between seasons due to seasonal growth and dormancy cycles (Luxmore et al. 1970, Howes and Teal 1994, Sorrell et al. 2000).

COD removal and ROL were correlated in the winter. Species with higher ROL generally had higher COD removal. This correlation was not found in the summer. These results parallel those found for COD, SO_4, and redox potential in model constructed wetlands reported in Chapter 2. In winter, differences in COD removal were related to two indicators of root-zone oxygen availability: SO_4, which requires oxygen for sulfide oxidation, and Eh, a measurement of the oxidation-reduction potential. In Chapter 2, nine of 19 species tested had consistently high COD removal year-round (average >90%) and showed cold season increases in SO_4; all but one of these species

also had periods of elevated Eh at low temperatures. Six of these species had statistically significant ROL compared to controls in the winter: *D. cespitosa*, *S. acutus*, *J. torreyi*, *C. nebrascensis*, *C. bebbii*, and *C. aquatilis*. They also had six of the seven highest ROL rates. However, three of those nine species with high COD removal, *J. arcticus*, *C. praegracilis*, and *C. utriculata*, did not have significant ROL. The other 10 species tested in Chapter 2 had lower and more variable COD removal (70-88%) and a consistently anaerobic root-zone year-round. Winter ROL was measured in five of these species; four (*C. microptera*, *T. latifolia*, *C. canadensis*, *H. jubatum*) had significant ROL compared to the unplanted control, but only one (*C. microptera*) had above-average ROL. *P. virgatum* did not have significant ROL. Together, the relationships between COD removal and ROL, SO_4, and Eh suggest that increased root-zone oxygen supply does enhance COD removal in the winter.

Seasonal differences in the amount of ROL and relationships between ROL and processes that consume oxygen in the root-zone may be explained by plants' seasonal growth and dormancy cycles. When temperatures are low and plants are dormant, roots may act less as oxygen sinks due to a reduction in their respiration, and this can results in increased oxygen loss (Armstrong 1971b, Greis et al. 1990, Howes and Teal 1994, Moog 1998, Moog and Bruggemann 1998). This could explain the increase in BOD and COD removal with decreased temperatures that Kadlec and Reddy (2001) observed as well as the constant COD removal measured with some plant species in Chapter 2 and previously reported for *C. utriculata* and *S. acutus* by Allen at al. (2002). According to this interpretation, certain species can increase oxygen availability in the root-zone at lower

temperatures, and this can in turn offset the negative effect of cold temperatures on aerobic microbial processes (Allen et al. 2002, Hook et al. 2003, Stein and Hook 2005). On the other hand, if plants release less oxygen during the summer due to higher respiration, they would not influence the root-zone oxidation as much.

Although there was evidence that differences in ROL among species and seasons might be related to differences in COD removal, it was inconclusive. The correlation between ROL and COD removal was statistically significant in winter, but not particularly strong ($r = 0.67$). Additionally, measured amounts of oxygen released from plant roots could only account for a small portion of the additional COD removed in columns planted with species that most enhanced removal.

There are several possible reasons for the discrepancy between ROL measured in vitro and COD removal in the columns. The columns used to measure COD removal had more root mass per volume of solution than the jars used to measure root oxygen loss: 5 times greater for *D. cespitosa* and 12 times greater for *C. utriculata*. These differences in root mass and the volume of the solution help explain some of the discrepancies between measurements. When measuring COD removal and root oxygen loss, more root mass would affect the results obtained, since measurements were taken from the bulk solution. Root oxygen release in vitro was diluted over a relatively larger volume of solution. The rhizosphere generally extends 1 to 30 mm from the root (Munch et al. 2005, Edwards et al. 2006). Therefore, a greater proportion of the wastewater in the water quality experiment was influenced directly by the roots. It is likely that the comparison between root masses underestimates the differences between the rhizosphere influences on COD

removal and root oxygen loss. Greater root mass and the rhizosphere effect would increase root oxygen loss values and could explain the COD removal difference between planted and control columns.

It is not surprising that relationships between ROL and wastewater data are ambiguous. The use of ROL measurements to explain processes at larger scales has been questioned because of differences in media, plant size and age, and presence or absence of organic matter and biofilms (Bedford et al. 1991 and 1994, Sorrell and Armstrong 1994). Relating wetland processes to ROL also depends on how ROL is characterized (per plant, per unit of root mass, or per surface area). Although ROL measurements in this study were consistent with other reported values (Armstrong 1971b, Brix and Schierup 1990, Gries et al. 1990, Bedford et al. 1991, Kludze et al. 1994, Sorrell 1999, Visser et al. 2000b, Matsui and Tsuchiya 2006, Laskov et al. 2006, Tanaka et al. 2007), those values would be subject to the same questions about measurement methods and scale. Discrepancies could also reflect the complexity of interactions between plants and root-zone processes. For example, rhizodeposition and enhanced surface area for growth of attached microbes may contribute to plant influences.

Relationships Between Wastewater COD Removal and Plant Attributes

Because *in vitro* root oxygen loss is difficult to measure, I investigated other variables that could be related to ROL. These included: anatomical and morphological traits that can affect oxygen transport directly; flooding tolerance and ecological association with wetlands, which typically involve adaptations to maintain adequate

internal oxygen levels; and membership in plant families with a disproportionate number of wetland species.

Aerenchyma tissue, which can help transport oxygen to roots and increase the release of oxygen, and anatomical barriers to oxygen loss, such as suberized cells, can differ among wetland species (Armstrong 1979, Jackson and Drew 1984, Justin and Armstrong 1987, Armstrong and Armstrong 1988, Moog 1998, Jackson and Armstrong 1999, Chabbi et al. 2000, Visser et al. 2000b). Of the five species examined, *C. canadensis*, the species with the lowest proportion of aerenchyma below the root-shoot junction (12%) had the lowest COD removal rates and SO_4 concentrations in wastewater incubations. The other four species had 38% to 59% aerenchyma; their COD removal rates were intermediate to high and unrelated to the amount of aerenchyma.

Of five species characterized, those with proportionally more 0.6 - 1.5 mm diameter roots (*D. cespitosa*, *C. utriculata*, and *S. acutus*) had >90% average COD removal, while those with more 0.0 - 0.6 mm diameter roots (*C. canadensis* and *H. jubatum*) averaged approximately 80% COD removal. Thicker roots had more aerenchyma tissue, which would facilitate efficient gas transport. Visser et al. (2000) found that the diameter of flooded roots increased as a result of increased aerenchyma.

Wetland Indicator Status, a rating indicating the occurrence of a plant species in a wetland, is easily obtained from U.S. Fish and Wildlife Service lists. Because plants' distributions and flood tolerances are influenced by differences in root anatomy, morphology, and gas flow (Justin and Armstrong 1987, Laan et al. 1989, Sorrell et al. 2000), WIS may give some indication of a species' capacity to transport oxygen to roots

and, potentially, into the surrounding medium. Comparing plants' WIS and COD removal revealed that Facultative species had relatively low but inconsistent COD removal (70%-88%), Facultative Wetland species varied widely (74%-97%), and Obligate Wetland species consistently had high COD removal (≥85%). Five out of six Obligate Wetland species, three of eight Facultative Wetland species, and none of the five Facultative species had ≥90% average COD removal.

Greenhouse ratings of flood tolerance showed a limited relationship to COD removal. The two species that had significantly less biomass when flooded had the lowest average COD removal. Species that had no significant biomass differences between flooded or drained conditions and species with greater biomass in the flooded condition had equally wide ranges of COD removal, approximately 75% to nearly 100%. Four of seven species which had no significant biomass differences between flooded or drained conditions had greater COD removal than the control, three had an average COD removal >90%. Nine of ten species with greater biomass in the flooded condition had greater COD removal than the control; six of them had an average COD removal >90%.

Additional observations made during these studies revealed differences between plant species. Three of the selected 19 plant species, *L. cinereus*, *P. vulgaris*, and *P. viragatum* would not be good candidates for constructed wetlands, since some replicates of these species did not survive in the flooding-tolerance and wastewater treatment experiments; these three species were among the species with the lowest average COD removal. Photosynthesis was not quantified in this study during the winter; however, photosynthesis has been measured during low temperature winters in coniferous trees

(Freeland 1944, Parker 1953). Since photosynthetic oxygen is transported to the

belowground roots, plant species that conduct photosynthesis during the winter possibly

assimilate more oxygen and could influence root oxygen loss. When the greenhouse

temperature was set at 4°C, *C. aquatilis*, *C. bebbii*, *C. nebrascensis*, *C. microptera*, *C.*

praegracilis, *C. utriculata*, *S. acutus*, *J. arcticus*, *J. torreyi*, *D. cespitosa*, and *P. vulgaris*

had green leaves, the characteristic pigment of chlorophyll in photosynthetic organisms;

other species were brown. Species with green leaves, with the exception of *P. vulgaris*,

had the greatest average COD removal. While harvesting the flooding-tolerance

experiment, I made observations as to whether plants grown in flooded conditions grew

adventitious roots. Some species have the ability to grow new adventitious roots near the

soil surface for increased oxygen assimilation (Laan et al. 1989, Visser et al. 2000b,

Kyambadde et al. 2004). All species grew adventitious roots except for *H. jubatum*, *P.*

virgatum, *L. cinereus*, *I. missouriensis*, and *P. vulgaris*; these species without

adventitious roots were among the species with lowest average COD removal. Rooting

depth was observed while destructively harvesting three of 19 plant species at the end of

the water quality experiment. *L. cinereus*, which had the lowest average COD removal

among plant species, had root growth only in the top 10 cm of the microcosm. In

contrast, *C. utriculata* and *D. cespitosa*, which are two species with average COD

removal >90% grew roots to the bottom of the microcosm.

Plant family was related to COD removal as strongly as any other attribute. Most

species removed significantly more COD than unplanted controls at least some of the

time, but plants with the greatest influence on COD, SO_4, and Eh were mostly members

of the *Cyperaceae* and *Juncaceae*. Plants with >90% average COD removal and

seasonally elevated SO_4 and Eh included hardstem bulrush and five of six sedges

(*Cyperaceae*), both rushes (*Juncaceae*), and one of seven grasses, *D. cespitosa*

(*Poaceae*). Plants with lower influence on COD removal and rootzone oxidation

included six grasses and one sedge, *C. microptera*; *C. microptera* was the only sedge

rated as Facultative. With one species each, no generalizations can be made for the

Iridaceae, *Lamiaceae*, and *Typhaceae*.

Selecting Appropriate Species for Constructed Wetlands

Species affect seasonal performance, with respect to COD removal, in cold

climates (Chapter 2) and this may be related to effects on root-zone oxygen. Chapter 2

results support the proposition that species selection is probably more important in cold

regions than warm. This study (Chapter 3) found large variations in ROL and plant

attributes related to ROL; these were related in differing degrees to differences in COD

removal, SO_4, and Eh reported in Chapter 2. Although not definitive, these results

generally support the hypothesis that root oxygen loss is a mechanism that contributes to

plant species effects on water chemistry. They also suggest that plants' adaptive traits for

living in a wetland environment influence their effects on wastewater treatment.

Although intuitive, this is not inevitable; for example, plant effects could reflect their

organic carbon production, support of biofilms, or other factors unrelated to oxygen.

Results from this study suggest that plant selection can help optimize wastewater

treatment. The appropriate species for a given treatment wetland depends on the limiting

factors and the chemical transformations needed for the relevant treatment processes.

Plant species that promote aerobic processes will increase the efficiency of wetlands designed to remove organic compounds and nutrients from domestic wastewater (Burgoon et al. 1995, Cottingham et al. 1999, Nivala et al. 2007). In cold climates, root oxygen loss can apparently offset the expected effect of low temperatures on microbial activity and maintain constant COD removal over the seasons. On the other hand, constructed wetlands designed for sulfate reduction and metal removal require the exclusion of oxygen to promote low redox conditions (Machemer and Wildeman 1992, Nelson et al. 2006, Stein et al. 2007). Plants that release significant amounts of oxygen should be avoided in this case.

Results provide insights into the mechanisms responsible for plants' effects on biogeochemical processes. Previous studies have shown that differences in adaptive traits for living in the wetland environment can affect transport of oxygen to the root-zone (Justin and Armstrong 1987, Laan et al. 1989, Sorrell et al. 2000). Results reported here and in Chapter 2 indicate that such differences can be important to plants' effects on biogeochemical processes that use oxygen. Few studies have examined variation of these effects over enough species to draw generalizations. Basic understanding of how plants enhance aerobic processes in wetlands could be advanced using a combination of field studies and rhizosphere-scale sampling in wetland microcosms. Most of the species with the highest COD removal rates in Chapter 2 were natives that are not widely used or well tested. This study provides support for using native species and guidance for selecting them. The influence of a diverse selection of species could further enhance constructed

wetland performance through differences in phenology, rooting patterns, and their influence on root-zone processes.

The measurements of plant attributes used here to evaluate relationships with COD removal differ in their ease of obtainability. Root oxygen loss measurements are technically difficult and values in the literature are not comparable between plant species due to different experimental designs (Sorrell and Armstrong 1994). Root characteristics were also technically difficult to measure and values in literature could also be variable depending on experimental conditions. Flooding tolerance measurements were time consuming and not predictive. From a constructed wetland designer's perspective, Wetland Indicator Status and taxonomy would be the most easily obtained plant attributes for selecting species. All Obligate Wetland species and members of the *Cyperaceae* and *Juncaceae* families had greater COD removal than the control and comprised a large proportion of species with COD removal ≥90%. These are likely plant groups that would be appropriate for constructed wetlands but exceptions will certainly emerge. These groups also include plants with intermediate COD removal: *T. latifolia*, an Obligate Wetland species, had average COD removal of 85%, and *C. microptera*, a member of the *Cyperaceae* but a Facultative species, had an average COD removal of 88%. *D. cespitosa*, a Facultative Wetland species and a member of *Poaceae*, had the highest average COD removal (97%) but is not in the mentioned plant groups. In spite of these inconsistencies, family and WIS can still serve as "coarse filters" to identify candidates for further evaluation.

CONCLUSION

Seasonal Plant Effects on Wastewater Treatment

Because plants influence the biology and chemistry around their roots in a zone referred to as the *rhizosphere*, it has long been thought that they could influence wastewater treatment in constructed wetlands. However, plants' effects on wastewater treatment have been debated due to contradictory results comparing treatment efficacy between planted and unplanted wetlands.

My research showed that plants had seasonal effects on COD removal, SO_4 concentrations, and Eh, and that those effects were species-specific. COD removal in the control declined in the winter, probably due to the decline of microbial activity at colder temperatures. Cold temperature did not affect COD removal negatively in most planted columns, except for those planted with *I. missouriensis*, *L. cinereus*, and *P. virgatum*. COD removal remained constant with many species or, in the case of columns planted with *C. nebrascensis* and *C. utriculata,* increased at low temperatures. These results provide experimental support for the feasibility of constructed wetlands in cold climates, adding to the evidence from case studies presented in Mander and Jenssen (2003) and elsewhere.

The strong differences found among species suggest that specific plant species could be used to improve wastewater treatment. Columns with *C. aquatilis*, *C. bebbii*, *C. nebrascensis*, *C. praegracilis*, *C. utriculata*, *S. acutus*, *J. arcticus*, *J. torreyi*, and *D. cespitosa* had high COD removal across all seasons, with an average over the entire

experiment greater than 90%. In contrast, columns planted with some species had lower

COD removal at 4˚C than at 24˚C, and in other columns, COD removal was mediocre

throughout the seasons with no apparent temperature influence. The year-round high

COD removal by select species suggests that these plants can offset the effects of cold

temperature that were observed in the control columns and that these plants have traits

that result in greater influence on wastewater treatment than other plants.

Positive correlations between plants' COD removal and both SO_4 concentration

and Eh during colder temperatures indicated, indirectly, that species with high COD

removal have elevated root zone oxygen availability in the winter. Sustained aerobic

respiration by microbes may offset the negative temperature effects on microbial

processes and keep COD removal constantly high in columns planted with species that

enhance oxygen supply. Root oxygen loss could be the reason for both elevated oxygen

levels in select columns and the higher COD removal.   In contrast, in unplanted controls

or columns planted with other species for which COD removal declined, SO_4

concentrations and redox potentials remained low during cold temperatures.

Results from Chapter 3 showed that at 4˚C root oxygen loss per plant was

positively correlated with COD removal; species with greater root oxygen loss had

greater COD removal. No correlation existed at 24˚C. However, comparisons between

root oxygen loss values and the oxygen needed to account for elevated COD removal in

planted columns showed that root oxygen loss only accounted for a small fraction of the

oxygen required. Discrepancies may result from differences in root mass densities

between root oxygen loss and wastewater treatment experiments or from flaws in

measuring root oxygen loss. The reliability and meaning of root oxygen loss
measurements is debated (Bedford et al. 1991, Sorrel and Armstrong 1994) and
experimental methods can yield inaccurate oxygen measurements. Regardless, results
suggest that differences in root oxygen loss may be a factor in COD removal differences
between seasons and plant species. Since root oxygen loss measurements do not fully
explain patterns of COD removal, other mechanisms for plant and seasonal influences on
wastewater treatment should be investigated.

Attributes related to root oxygen loss were consistent with patterns of COD
removal. Root anatomy and morphology can influence root oxygen loss and, therefore,
could influence COD removal. Greater amounts of aerenchyma tissue and larger roots
are factors which would increase root oxygen loss (Justin and Armstrong 1987, Visser et
al. 2000b). Of the five species studied, *C. canadensis* had the least amount of
aerenchyma tissue and the greatest portion of small diameter roots, and the lowest COD
removal. The others had more aerenchyma tissue, larger diameter roots, and higher COD
removal.

Plant Selection for Constructed Wetlands

The presence of plants can improve wastewater treatment, and may be especially
important in cold climates. The average COD removal in unplanted model wetlands
across all seasons was 67% and COD removal declined during colder temperatures. In
contrast, the average COD removal in planted model wetlands was 87%. Unlike the
unplanted model wetlands, cold temperature generally did not have a negative effect on

COD removal in planted wetlands. COD removal remained relatively constant across seasons in planted wetlands indicating that plants can promote more predictable treatment.

Based on my results, species selection may also be used to increase the efficacy of treatment. *C. aquatilis*, *C. bebbii*, *C. praegracilis*, *C. utriculata*, *S. acutus*, *J. articus*, *J. torreyi*, and *D. cespitosa* had high COD removal throughout the year, with average COD removals over 90%. These plant species, with the exception of *S. acutus*, are not frequently used in constructed wetlands, but they had greater removal than three widely used plants species, *T. latifolia*, *P. australis*, and *P. arundinaceae,* which had average COD removal of 84%, 74%, and 83% COD removal, respectively. In addition *T. latifolia*, *P. australis*, and *P. arundinaceae* are weedy in many North American wetland ecosystems; other species may provide better wastewater treatment while enhancing ancillary benefits including better habitat and a more aesthetic greenspace with a diversity of species.

Comparing 19 plant species and unplanted model wetlands demonstrated that certain groups of plants had similar patterns of COD removal (Table 11). Taxonomic group and Wetland Indicator Status, which are easily obtained, can be useful for selecting plant species that promote COD removal. All *Cyperaceae* and *Juncaceae* species used in this study had greater COD removal than the unplanted wetland, generally better than 90%. Other members of these families that occur primarily in wetlands would also likely be good candidates for treatment wetlands. All the species classified as Obligate Wetland

species (U.S. Fish and Wildlife Service 1988) had greater COD removal than the unplanted control, again usually over 90%.

Future research could provide additional insight for selecting appropriate plant species for the goals of the treatment wetland. Tests in large-scale wetlands in the field would provide more realistic performance evaluations. Additionally, wastewater treatment with mixed species plantings should be investigated (Callaway and King 1996, Coleman 2001, Picard et al. 2005). Collectively, such studies will contribute to rational plant selection for improving constructed wetland function.

Table 11: Summary of plant species studied and their attributes and influences on wastewater. Obligate Wetland species occur in wetlands >99% of the time, Facultative Wetland species 67-99% of the time, and Facultative species 34-66% of the time. COD removal averages (%) are for batches 16˚C 2006 through 4˚C 2008. SO_4 concentrations (mg/L) and Eh (mV) increased in the winter and biomass (g) increased in flooded conditions for species indicated with an X in the respective columns. Winter ROL values ($\mu molO_2 plant^{-1}day^{-1}$) are averages of three replicates. ROL and aerenchyma (%) were measured for only a subset of species.

Species	Family	Wetland Indicator Status	COD removal average ± SE (%)	Elevated SO_4 in winter	Elevated Eh in winter	Increased biomass in flooded	Winter ROL $\mu molO_2$ plant^{-1}day^{-1}	Aerenchyma (%)
Control			70 ± 3				0.0	
C. aquatilis	Cyperaceae	Obligate Wetland	94 ± 2	X		X	40.0	
C. bebbii	Cyperaceae	Obligate Wetland	97 ± 1	X	X	X	47.7	
C. microptera	Cyperaceae	Facultative	88 ± 2	X		X	67.9	
C. nebrascensis	Cyperaceae	Obligate Wetland	96 ± 1	X	X	X	55.3	
C. praegracilis	Cyperaceae	Facultative Wetland	96 ± 1	X	X	X	13.1	
C. utriculata	Cyperaceae	Obligate Wetland	94 ± 2	X		X	20.0	56.9
S. acutus	Cyperaceae	Obligate Wetland	95 ± 2	X	X	X	73.4	58.7
J. arcticus	Juncaceae	Facultative Wetland	93 ± 2	X	X		17.6	
J. torreyi	Juncaceae	Facultative Wetland	94 ± 1	X	X	X	60.3	
C. canadensis	Poaceae	Facultative Wetland	80 ± 4				26.6	11.8
D. cespitosa	Poaceae	Facultative Wetland	97 ± 2	X	X		85.7	38.3
H. jubatum	Poaceae	Facultative	80 ± 2				25.5	
L. cinereus	Poaceae	Facultative	70 ± 2					
P. virgatum	Poaceae	Facultative	76 ± 3				17.7	
P. arundinacea	Poaceae	Facultative Wetland	85 ± 3	X		X		
P. australis	Poaceae	Facultative Wetland	74 ± 3			X		
I. missouriensis	Iridaceae	Facultative Wetland	74 ± 3					
P. vulgaris	Lamiaceae	Facultative	79 ± 3					
T. latifolia.	Typhaceae	Obligate Wetland	85 ± 2			X	35.2	55.2

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