NATIVE PLANT MATERIAL SELECTION FOR WATER TREATMENT WETLANDS[1]

C. R. Taylor[2], P. B. Hook, C. A. Zabinski, and O.R. Stein

Abstract: Treatment wetlands (TWs) are widely used for treating domestic, agricultural, and industrial wastewater, stormwater runoff, and acid mine drainage; natural wetlands are also exposed to these pollutant sources. Currently, few plant species are used in the majority of TWs, and these are often non-native and/or weedy. We are working to identify native species for year-round use in cold-region TWs, particularly the Rocky Mountain region, and explore the basis of differences in performance. In studies presented here, we evaluated chemical oxygen demand (COD) removal from simulated wastewater in microcosms planted with monocultures of 19 species. Experiments were conducted over one year at seasonal temperatures of 4-24°C. With some species and in unplanted controls, COD removal declined at cold temperatures during dormancy, as expected with normal temperature dependence of microbial processes. However, COD removal was constant across seasons with the majority of species. Average COD removal exceeded 90% for *Carex aquatilis*, *C. bebbii*, *C. praegracilis*, *C. utriculata*, *Schoenoplectus acutus*, *Juncus arcticus*, *J. torreyi*, and *Deschampsia cespitosa*; of these, only *S. acutus* is widely used. In contrast, the widely used (and frequently invasive) species *T. latifolia*, *P. australis*, and *P. arundinacea* were somewhat less effective, with average COD removals of 84%, 74%, and 83%, respectively. Redox, sulfate, and root oxygen loss measurements suggest that plant-mediated oxygen transfer may explain the ability of some species to offset the effect of temperature on microbial processes and maintain high COD removal in all seasons. Results indicate that many non-weedy, regionally native species may be candidates for use in TWs or for rehabilitation of natural wetlands exposed to certain pollutants. In addition to the species we studied, other Obligate Wetland and Facultative Wetland species of the Cyperaceae and Juncaceae merit investigation.

Additional Key Words: wastewater treatment, sedges, rushes, wetland indicator status, root oxygen loss, chemical oxygen demand, COD, biological oxygen demand, BOD.

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Introduction

In treatment wetlands, a variety of physiochemical and biological interactions between wastewater, wetland media, microorganisms, and plants are responsible for removal of pollutants from domestic, agricultural, and industrial wastewater, stormwater runoff, and acid mine drainage. Although plants are a major component of wetlands, relatively little is known about the effects of plant material selection on the processes responsible for wastewater treatment. A limited number of previous studies have compared different plant species' performance in treatment wetlands (e.g. Gersberg et al., 1986; Coleman, 2001; Fraser et al., 2004). Few have examined plant influences across all seasons (Allen et al., 2002; Picard et al., 2005; Akratos and Tsihrintizis, 2007; Yang et al., 2007) or more than four species at a time (Tanner, 1996; Lin et al., 2002; Iamchaturapatr et al., 2007; and Yang et al., 2007). 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.

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 (Brix, 1997; Tanner, 2001). While variation in temperature affects microbial activity directly, seasonal cycles of plant growth and physiology may also influence root oxygen loss (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 treatment wetland performance may also vary. In previous research, we evaluated effects of three plant species on seasonal patterns of chemical oxygen demand (COD). Chemical oxygen demand measures the mass of oxygen consumed per liter of solution when heated with strong oxidizing reagents; it mainly reflects the amount of dissolved and suspended organic carbon and is one of the primary pollutants of concern in domestic and agricultural wastewater. We reported that plants influenced seasonal patterns of COD removal and hypothesized that this resulted from differences in ROL, particularly at low temperatures (Allen et al., 2002; Hook et al., 2003).

Currently, relatively few plant species are used in the majority of treatment wetlands, and these are often non-native and/or weedy. The purpose of this study was to compare the effects of a large number of species on wastewater treatment to identify native species for year-round use in cold-region treatment wetlands, particularly the Rocky Mountain region, and to explore the basis of differences in performance. The questions addressed included: Do plants affect seasonal patterns of COD removal? Are such plant effects common for many species, particularly regionally native, non-invasive species? Are they more prevalent in certain groups of species? Is variation in COD removal related to indicators of root-zone oxidation status? Do relationships exist between plant influences on wastewater treatment and plant traits related to flooding tolerance? Results are most applicable to wastewaters rich in organic carbon, such as those from domestic, municipal, or livestock facility sources, but they may also be relevant to treatment of any pollutants by oxidation or reduction processes.

Methods

Species Selection

A diverse selection of sedges, rushes, grasses, and forbs was chosen for comparison. Plants ranged from Facultative to Obligate wetland species (U.S. Fish and Wildlife Service, 1988), represented several families, and included plants in either common or limited use in treatment wetlands. The species used were: *Carex aquatilis*, *Carex bebbii*, *Carex microptera, Carex nebrascensis, Carex praegracilis*, *Carex utriculata, and Schoenoplectus acutus* (*Cyperaceae*); *Juncus arcticus* and *Juncus torreyi* (*Juncaceae); Calamagrostis canadensis, Deschampsia cespitosa, Hordeum jubatum, Leymus cinereus, Panicum virgatum, Phalaris arundinacea, and Phragmites australis (Poaceae); Typha latifolia (Typhaceae); Iris missouriensis* (*Iridaceae); Prunella vulgaris* (*Lamiaceae).*

Wastewater Treatment and Chemistry

Plant effects on COD removal were tested using wetland microcosms operated in a controlled environment over 20 months. The experiment was conducted in a greenhouse at the Plant Growth Center at Montana State University in Bozeman, MT (46˚N, 111˚W). Three replicates of unplanted controls and monocultures of each species were planted in model subsurface wetlands consisting of 15 cm diameter by 30 cm tall polyvinyl chloride (PVC) columns filled with 1-5 mm gravel (Fig. 1). From June 20, 2006, through February 12, 2008, greenhouse temperature was changed every 60 days to mimic natural seasonal cycles; the temperature sequence was 24, 16, 8, 4, 8, 16, 24, 16, 8, and 4˚C. Supplemental lighting was not used. Patterns of natural light and controlled temperature induced normal seasonal cycles of plant dormancy and growth. To simulate batch operation in subsurface flow treatment wetlands, columns were drained and filled every 20 days with synthetic wastewater simulating secondary domestic effluent. 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 $FeCl_3$; influent wastewater had 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. Pollutant concentrations and loading rates for onsite septic systems, municipal wastewater plants, and livestock facilities, as well as influent concentrations and loading rates for treatment wetlands all vary widely (Kadlec and Wallace 2009; USEPA 2002; USEPA 2000); the wastewater composition and COD loading rate (33 kg/ha*day) used here are well within typical observed and recommended ranges. A standpipe supplied fresh water to replace evaporative losses and maintained the water level just below the gravel surface.

Chemical oxygen demand, sulfate (SO_4), and redox potential (Eh) were measured to evaluate the effects of plants and seasons on wastewater treatment. Because this study concerned domestic wastewater, COD was regarded as a pollutant. Sulfate concentrations and redox potential were measured as indirect indicators of rootzone oxidation to evaluate whether differences in COD removal were related to oxygen availability. Chemical oxygen demand and SO_4 were measured in the wastewater mixing tank and day 6 of all batches. During 4˚C and 24˚C batches, COD and SO_4 were also measured on days 1, 3, 9, and 20. We report only day 6 data here. Based on previous work using the same methods, and 4˚C and 24˚C data from this study, we determined that results for day 6 are similar to those for days 3-20 and result in the same inferences about effects of plants and seasons (Allen 1999; Taylor 2009). This is because differences among plants and batches, when they occur, generally emerge rapidly and persist throughout a batch; most COD time series show a rapid decline over days 0-3 followed by gradual change thereafter. Six days is a reasonable duration for TW batch operation. Effects of plants and seasons on day-6 COD removal and SO_4 were tested statistically using a significance level of $p \leq 0.05$. Data for the first batch were excluded because they showed clear start-up effects. Plant species and controls were compared within each batch using analysis of variance (ANOVA) and planned contrasts. Seasonal temperature effects on COD and SO_4 were analyzed for each species in two ways: (1) 4 and 24˚C batches were compared using repeated measures ANOVA; (2) relationships with temperature were quantified with Pearson's correlation coefficient. Redox potential was measured every 4 hours using platinum electrodes; only two replicates were sampled due to the limited capacity of the multiplexer used for automation.

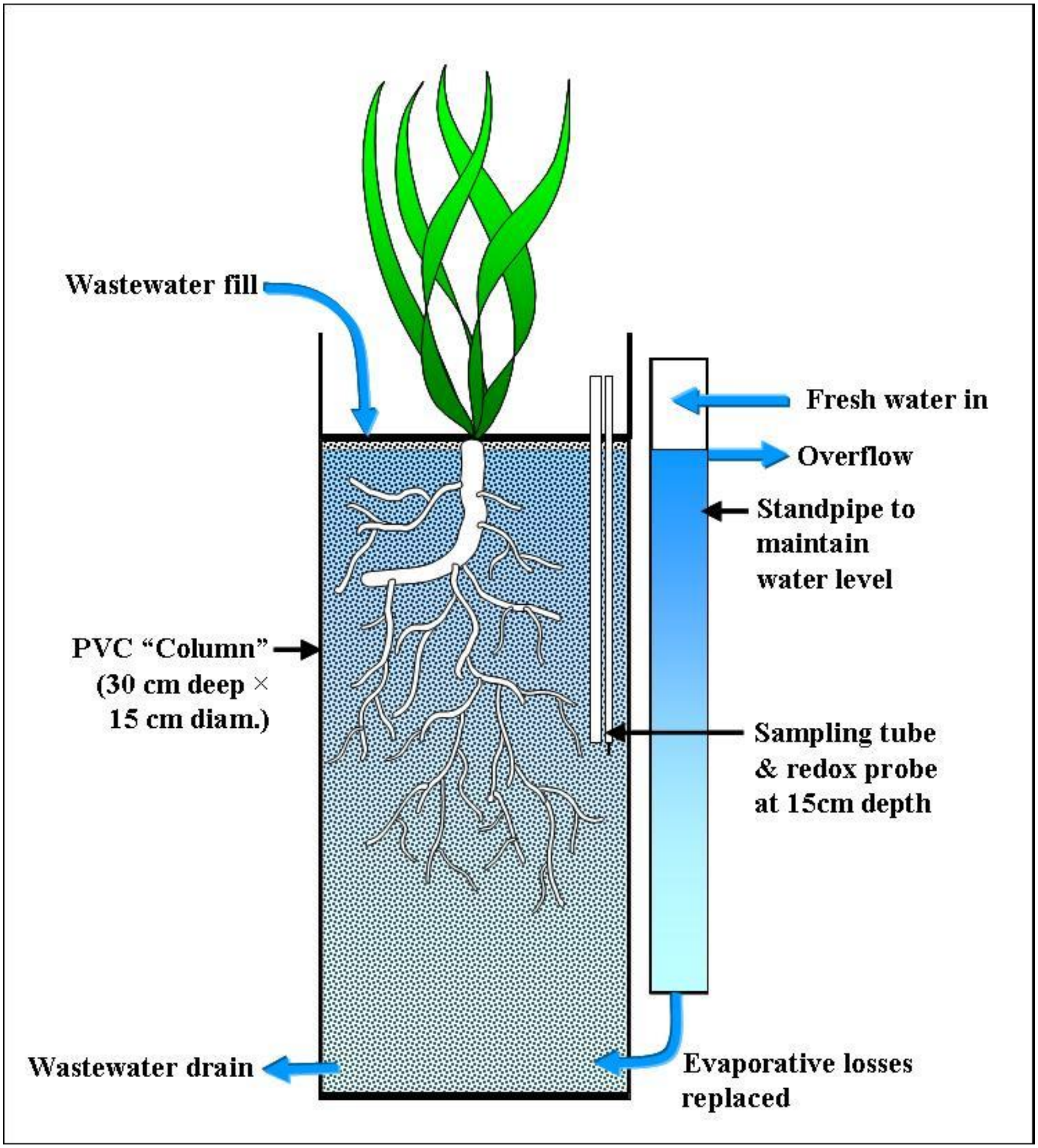


Figure 1. Model treatment wetlands ("columns") were constructed from PVC pipe, filled with 1-5 mm gravel, and planted with one of 19 plant species or left unplanted. At the end of each 20-day batch, columns were drained by gravity and refilled with synthetic wastewater. During each batch, fresh water replaced evaporative losses and maintained a constant water level just below the gravel surface. A vinyl tube open at 15 cm depth was used to sample wastewater. In two replicates of each species, a platinum electrode was used to measure redox potential at 15 cm.

Root Oxygen Loss

Root oxygen loss was measured during winter and summer. A subset of the 19 species was evaluated due to time limitations and the demanding nature of the measurements; species were selected to represent differing degrees of influence on COD removal and SO_4 concentration shown in preliminary results. Three replicates of 14 species were measured in winter; four replicates of ten species were measured in summer to improve power of between-species statistical comparisons. Plants used for root oxygen loss measurements were grown in saturated conditions. Root oxygen loss was measured colorimetrically by the titanium (Ti^{3+}) citrate method described by Kludze et al. (1994). A known amount of Ti^{3+} citrate solution was poured into a glass jar (Ball canning jars, 0.35 L [12 oz.]) to immerse the roots of a single plant. Paraffin oil was applied at the root-shoot junction to hinder atmospheric oxygen diffusion into the root zone. Control treatments used the same apparatus with no plant.

After 24 hours, a 5 ml solution sample was extracted from each jar and stored in a Vacutainer (BD Vacutainers, Franklin Lakes, NJ). Absorbance of the solution was measured at 527 nm wavelength in a spectrophotometer (Spectronic Genesys 5, Spectronic Instruments, Rochester, NY). Oxygen concentrations were estimated from a standard curve calibrated to a series of samples with known Ti^{3+} concentrations (Oxygen = -0.0011(Absorbance) + 0.3738) and whole-plant oxygen loss was calculated using the equation:

$$\mathrm{ROL} = v\,(p - c) \tag{1}$$

where ROL = root oxygen loss, μmol O_2 plant^{-1} day^{-1}; v = standardized volume of Ti^{3+} citrate solution (volume of Ti^{3+} citrate solution for individual measurements / largest volume of solution used in the experiment); p = oxygen concentration in the root solution after 24 hours with plants, μmol O_2 plant^{-1} day^{-1}; c = oxygen concentration in the root solution after 24 hours in the control without plants, μmol O_2 plant^{-1} day^{-1}.

Results

COD Removal

The majority of the 19 species had relatively constant COD removal across seasons; values were either uniform or variation was erratic rather than seasonal (Fig. 2). Removal of COD declined at cold temperatures with only a few of the species and the unplanted control. Repeated measures ANOVA comparisons found that controls and columns with *I. missouriensis*, *L. cinereus* and *P. virgatum* had significantly lower COD removal at 4˚C than 24˚C, while *C.*

utriculata and *P. arundinacea* had significantly higher removal at 4˚C. Only the controls and three species showed significant correlations between COD removal and temperature. Removal increased with temperature only in unplanted controls and columns with *I. missouriensis* (r=0.77 and 0.67, respectively). With *C. nebrascensis* and *C. utriculata,* removal decreased as temperature increased (r=-0.67 and -0.93, respectively).

Removal of COD differed between plant species, especially at cold temperatures. Overall, plant species rankings for COD removal were similar across seasons: species with the highest COD removal at cold temperatures generally also had the highest removal at warm temperatures (Fig. 3). Removal of COD was consistently high across all seasons with nine species: five of the six sedges (*C. aquatilis, C. bebbii, C. nebrascensis, C. praegracilis,* and *C. utriculata* with 83-100% removal from different species and batches), both rushes (*J. arcticus* and *J. torreyi*, 89-97% removal), *S. acutus,* (87-100% removal), and *D. cespitosa* (95-100% removal) (Fig. 2). Study-long average COD removal was ≥93% for each of these species. Removal was more variable for the other ten species, ranging from 53% to 94% for different species and batches; study-long average COD removal was 70% to 88% for these species.

Oxidation indicators

At 24˚C, SO_4 concentrations were uniformly low for all species (generally ≤1.2 mg/L) on days 3-20. At 4˚C, SO_4 differed strongly among species. Day 6 concentrations ranged from 0.2 to 14.6 mg/L at 4˚C. For the majority of species, SO_4 concentration increased as temperature decreased (Fig. 2). Significant negative correlations (r≤-0.63) between temperature and SO_4 were found for all sedges and rushes, *S. acutus*, *D. cespitosa,* and *P. arundinacea*; mean SO_4 concentration at 4˚C exceeded 5 mg/L for all of these species except *P. arundinacea* (1.9 mg/L). Temperature and SO_4 were not correlated for the other grasses, *I. missouriensis*, *P. vulgaris*, and *T. latifolia*; although *P. vulgaris* and *T. latifolia* showed a tendency for slight rises in SO_4 at low temperatures, concentrations were never significantly greater than for controls.

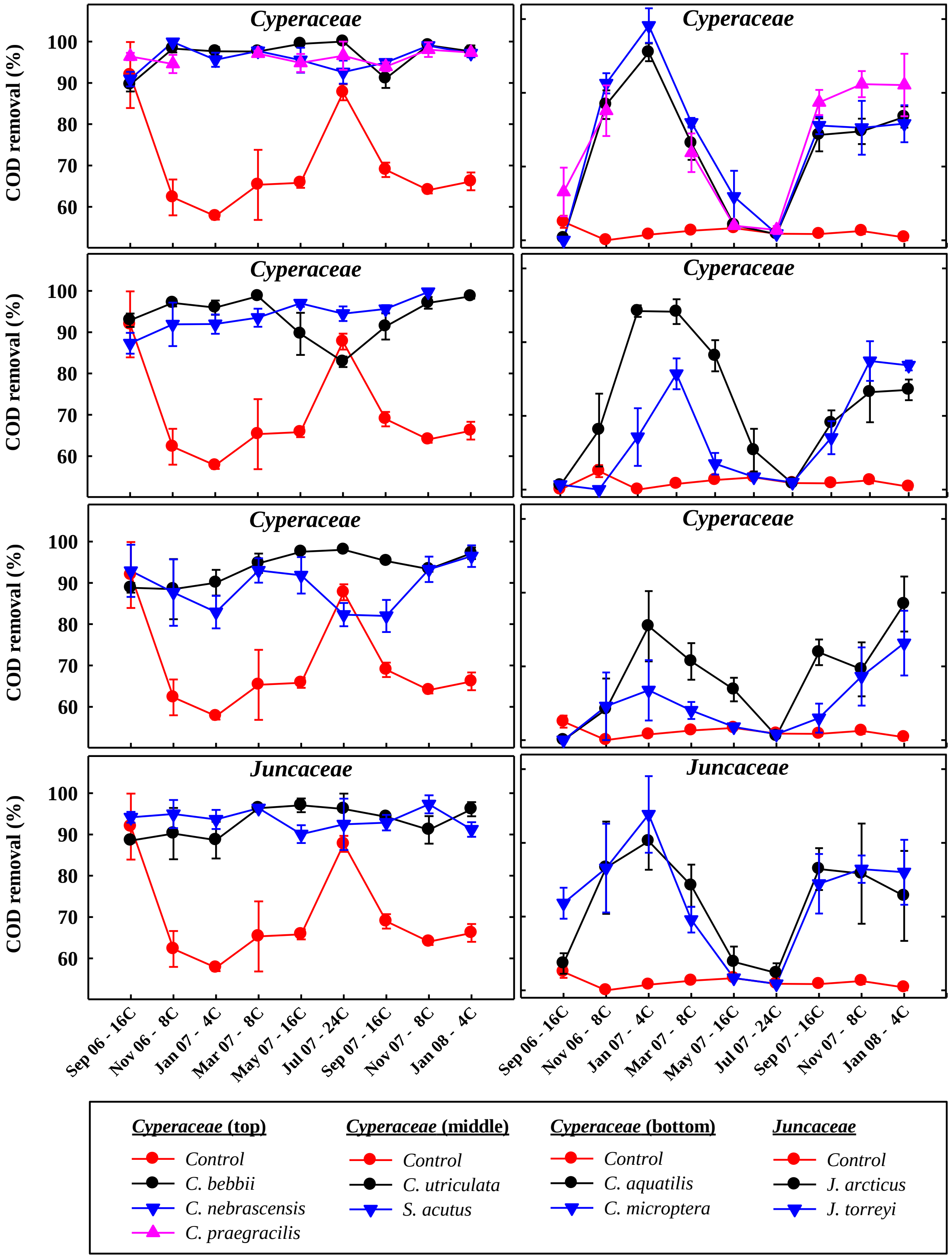
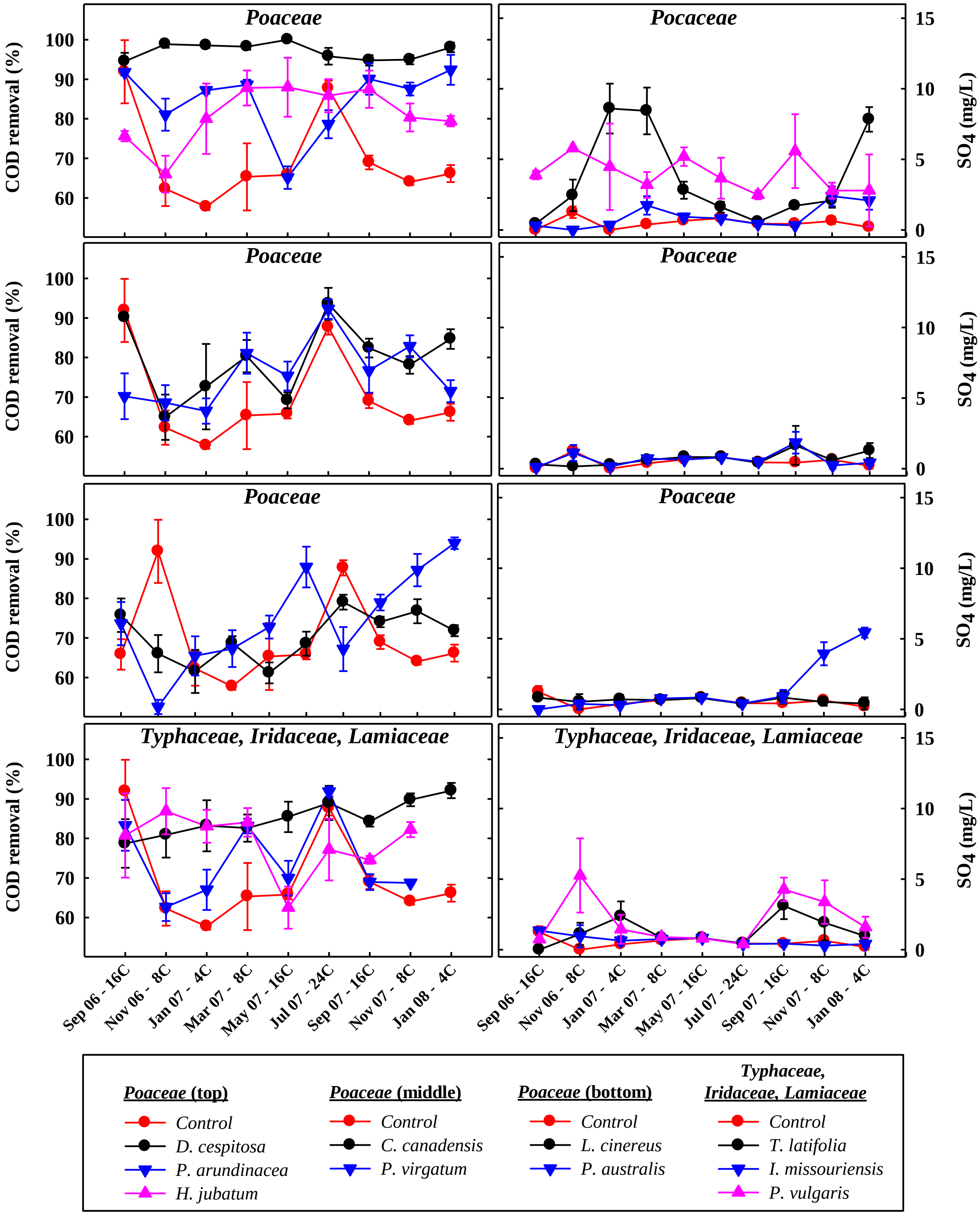


Figure 2. Seasonal variation in day-6 COD removal (left) and SO_4 concentration (right) for 19 species in 6 families and unplanted controls. Symbols represent the mean of 3 replicates; error bars represent ± one standard error.



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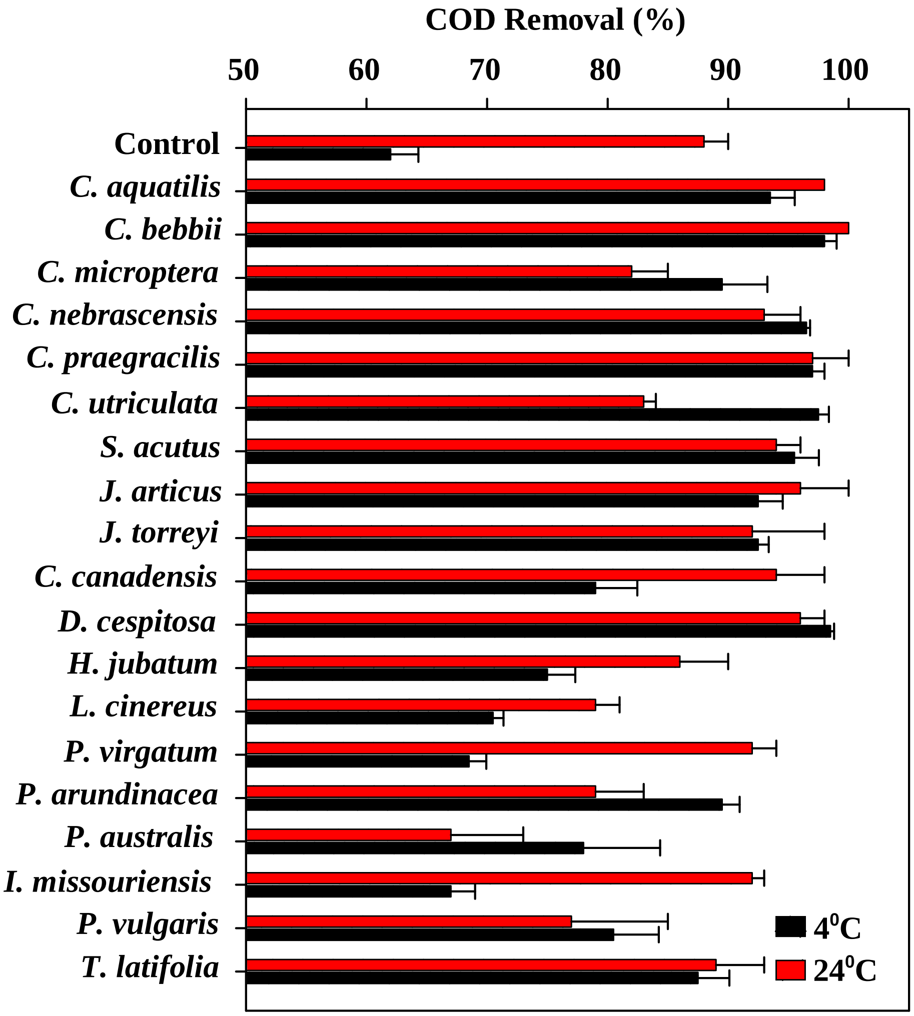

Figure 3. COD removal (%) for each species and control at 4˚C and 24˚C. 4˚C bars are means of 3 replicates from two 4˚C incubations in 2007 and 2008. 24˚C bars are means of three replicates during the 24˚C 2007 incubation. Error bars represent one standard error.

Redox potential results loosely paralleled those for SO_4 but were more variable. Day-6 Eh remained below -200 mV and was virtually unchanged across seasons in controls and with the majority of plant species. Species that showed greater variation and rose above -200 mV at lower temperatures were *C. bebbii, C. nebrascensis, C. praegracilis, C. utriculata, S. acutus, J. arcticus, J. torreyi,* and *D. cespitosa*. Among these species, readings between -100 mV and +200 mV were common, and several readings were >400 mV.

Root oxygen loss

During the winter, root oxygen release 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* (Table 1). Ten of 14 plant species released more oxygen than the control. During the summer, ROL ranged from -0.7 for the control to 45.3 µmol O_2 plant^{-1} day^{-1} for *S. acutus*. Only 4 of 10 species released more oxygen than the control in the summer.

Table 1: Root oxygen loss at 4˚C and 24˚C. At 4˚C ROL values (reported as µmol O_2 plant^{-1} day^{-1}) are means of three replicates ± one standard error. At 24˚C ROL values are means of four replicates ± one standard error.

Species	**4˚C**		**24˚C**	
	----- Root oxygen loss, µmol O_2 plant^{-1} day^{-1} -----			
D. cespitosa	85.7[1] ± 5.5	*	13.8 ± 11.3	
S. acutus	73.4 ± 8.4	*	45.3 ± 12.4	*
C. microptera	67.9 ± 7.0	*	4.0 ± 2.2	
J. torreyi	60.3 ± 3.1	*	26.8 ± 9.7	*
C. nebrascensis	55.3 ± 17.7	*		
C. bebbii	47.7 ± 15.3	*	4.2 ± 4.3	
C. aquatilis	40.0 ± 23.3	*		
T. latifolia	35.2 ± 4.5	*	30.7 ± 19.9	*
C. canadensis	26.6 ± 5.1	*	-0.2 ± 2.7	
H. jubatum	25.5 ± 16.1	*	0.1 ± 7.4	
C. utriculata	20.0 ± 14.2		20.8 ± 5.1	*
P. virgatum	17.7 ± 11.6			
J. arcticus	17.6 ± 0.5			
C. praegracilis	13.1 ± 2.2		10.5 ± 5.1	
Control	0.0 ± 1.5		-0.7 ± 1.6	

* Significantly different from the control at the 0.05 level of probability based on ANOVA planned contrast.

Discussion

Results demonstrate that most plant species tested enhance year-round COD removal compared to unplanted controls and they negate the seasonal effects of cold temperature observed in controls. Somehow, the species with consistently high COD removal rates can offset the negative effects of cold temperatures. One possibility is that increased oxygen availability stimulates microbial processes that would otherwise be retarded by cold. It is well documented that many wetland plant species release oxygen from their roots in varying amounts (Armstrong, 1971; Gries et al., 1990; Sorrell, 1999), and our results confirm this for many of the species studied. Because domestic wastewater has abundant organic carbon, treatment is often limited by oxygen, the most energetically favorable electron acceptor. Enhanced oxygen supply near roots could alleviate this limitation and facilitate more efficient organic carbon removal by aerobic respiration, especially in the winter when plant respiration is reduced and greater net oxygen loss is expected (Howes and Teal, 1994). It appears that in cold climates, root oxygen loss may offset the expected effect of low temperatures on microbial activity and maintain reliable COD removal over the seasons.

Associations between plants' COD removal and SO_4 concentrations, root-zone redox potential, and root oxygen loss measurements indicate that species with high COD removal also have elevated oxygen availability in the winter. Distinctions between species associated with higher or lower SO_4 concentrations matched those described for COD removal: *Juncus* species, *S. acutus*, *Carex* species other than *C. microptera*, and *D. cespitosa* all averaged >90% COD removal and ≥4 mg/L SO_4 across batches; other grasses, *T. latifolia*, *P. vulgaris*, and *I. missouriensis* averaged ≤85% COD removal and ≤2 mg/L SO_4; *C. microptera* was intermediate. Exponential regressions showed a strong relationship between experiment-long average COD and SO_4 ($R^2 = 0.82$); seasonally, the strongest relationships were at 4˚C ($R^2 = 0.85$ in both 2007 and 2008) and the weakest at 24˚C ($R^2 = 0.10$). Because oxidation of sulfide and elemental sulfur to sulfate requires oxygen and occurs rapidly in its presence (Brune et al. 2000), the relative proportion of sulfur in the form of sulfate reflects variation in rootzone oxygen availability. Redox potential data were broadly consistent with COD-SO_4 relationships. Species with average COD removal >90% also included those with Eh exceeding -200 mV at low temperatures; redox potential remained low at -200 mV for all species at warmer temperatures. Environments with Eh below -100 mV are characterized by sulfate reduction to sulfide and considered anoxic, whereas those with Eh above 300-400 mV are dominated by aerobic respiration; intermediate Eh values are associated with shifts in the relative importance of these and other microbial metabolic processes and are sometimes called "suboxic" (Fiedler et al. 2007; Faulwetter et al. 2009). Finally, greater variation among different species' root oxygen loss occurred during winter than the summer; COD removal and ROL were positively correlated at 4˚C ($p = 0.001$, $r = 0.67$) but not at 24˚C ($p = 0.74$, $r = 0.10$).

Evidence that plants enhanced root-zone oxidation more in winter than summer is consistent with several other wetland studies. Kadlec and Reddy (2001) found a slight increase in TW BOD removal at lower temperatures similar to observations for *C. nebrascensis* and *C. utriculata* in this study. Griffin et al. (1999) measured lower Eh, increased ammonification and total N, and sulfide formation in summer, versus elevated Eh, increased N removal, and no sulfate reduction in winter. Armstrong (1971) and Howes and Teal (1994) found greater ROL at lower temperatures. Gries et al. (1990) and Moog and Bruggemann (1998) found that enhanced ROL was associated with reduced root respiration at low temperatures.

In addition to improving performance of TWs, one of our goals is to identify non-invasive native plants for effective use in TWs. 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-long average removals exceeding 90%. These plants are native to the Rocky Mountain region but of these, only *S. acutus* is widely used in treatment wetlands. The widely used species - *T. latifolia*, *P. australis*, and *P. arundinacea* - were somewhat less effective, with average COD removals of 84%, 74%, and 83%, respectively. All three of these species can be invasive or weedy in natural wetland ecosystems. Because they have unexceptional performance, it may be preferable to use other native species that are less weedy, have higher ecological value, are more aesthetically attractive, and have equal or better performance with respect to pollutant removal.

We have identified several native species with strong potential for use in TWs. How might other candidate species be identified efficiently? Table 2 summarizes the results presented above as well each species' Wetland Indicator Status and results of test of flooding tolerance not reported here. Both plant family and Wetland Indicator Status appear to have some value for preliminary screening of species for COD removal. In addition to those studied here, other members of the *Cyperaceae* and *Juncaceae* are likely candidates for use in TWs. All of the

Table 2: Summary of plant species studied and their attributes and influences on wastewater. Species are sorted in ascending order of COD removal.

Species	Family	Wetland Indicator Status[1]	Average COD removal[2] (%)	Elevated SO_4 in winter[3]	Elevated Eh in winter[3]	Increased biomass when flooded[3]	Winter ROL (μmolO_2 $plant^{-1}day^{-1}$)[4]
Control			70 ± 3				0.0
L. cinereus	*Poaceae*	Facultative	70 ± 2				—
P. australis	*Poaceae*	Fac. Wetland	74 ± 3			X	—
I. missouriensis	*Iridaceae*	Fac. Wetland	74 ± 3				—
P. virgatum	*Poaceae*	Facultative	76 ± 3				17.7
P. vulgaris	*Lamiaceae*	Facultative	79 ± 3				—
C. canadensis	*Poaceae*	Fac. Wetland	80 ± 4				26.6
H. jubatum	*Poaceae*	Facultative	80 ± 2				25.5
P. arundinacea	*Poaceae*	Fac. Wetland	85 ± 3			X	—
T. latifolia.	*Typhaceae*	Obl. Wetland	85 ± 2			X	35.2
C. microptera	*Cyperaceae*	Facultative	88 ± 2	X		X	67.9
J. arcticus	*Juncaceae*	Fac. Wetland	93 ± 2	X	X		17.6
C. aquatilis	*Cyperaceae*	Obl. Wetland	94 ± 2	X		X	40
C. utriculata	*Cyperaceae*	Obl. Wetland	94 ± 2	X	X	X	20
J. torreyi	*Juncaceae*	Fac. Wetland	94 ± 1	X	X	X	60.3
S. acutus	*Cyperaceae*	Obl. Wetland	95 ± 2	X	X	X	73.4
C. nebrascensis	*Cyperaceae*	Obl. Wetland	96 ± 1	X	X	X	55.3
C. praegracilis	*Cyperaceae*	Fac. Wetland	96 ± 1	X	X	X	13.1
C. bebbii	*Cyperaceae*	Obl. Wetland	97 ± 1	X	X	X	47.7
D. cespitosa	*Poaceae*	Fac. Wetland	97 ± 2	X	X		85.7

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[1] Obligate Wetland ("Obl. Wetland") species occur in wetlands >99% of the time, Facultative Wetland ("Fac. Wetland") species 67-99% of the time, and Facultative species 34-66% of the time.

[2] COD removal averaged across seasonal batches from 16˚C 2006 through 4˚C 2008.

[3] Species for which SO_4 concentrations and Eh increased in the winter and biomass was greater in flooded than drained conditions are indicated with an "X" in the respective columns.

[4] ROL was not measured for species indicated with a horizontal bar "—".

species with >90% COD removal, except *D. cespitosa*, were in these two families. All species with high COD removal were also classified as Obligate Wetland or Facultative Wetland species (U.S. Fish and Wildlife Service, 1988), although some Obligate Wetland and Facultative Wetland species did not perform as well. Interestingly, *C. microptera*, the *Carex* species with the lowest average COD removal (86%) also had the lowest Wetland Indicator Status ranking of the sedges (Facultative).

A somewhat more intensive approach to screening might be to test for root-zone sulfate levels under standardized conditions. We found a strong relationship between COD removal and sulfate concentration at low temperatures. This relationship accounted for most of the variation among species, families, and Wetland Indicator Status ranks. In fact occurrence of elevated sulfate predicted effective COD removal better than ROL did (Table 2), probably because of the difficulty and questionable reliability of ROL measurement methods. Because the conversion of sulfur between sulfide and sulfate is so sensitive to oxygen availability (Brune et al. 2000), presence of significant amounts of sulfate is a plausible proxy for root-zone oxygen availability and much easier to measure than ROL.

Conclusion

These results indicate that many non-weedy, regionally native species may be candidates for use in treatment wetlands or for rehabilitation of natural wetlands exposed to certain pollutants. The greater difference in COD removal between planted wetland microcosms and unplanted controls at lower temperature indicates that plants, and plant species selection, are probably more important for improving COD removal in locations with low winter temperatures.

The appropriate species to optimize wastewater treatment depends on the limiting factors and the chemical transformation involved in the treatment of specific pollutants of concern. Plant species that promote aerobic processes will generally increase the efficiency of wetlands designed to remove organic compounds and nutrients from domestic and other wastewaters (Burgoon et al., 1995; Cottingham et al., 1999; Kowles-Grove and Stein, 2005; Nivala et al., 2007). On the other hand, treatment wetlands designed for sulfate reduction and metal removal, such as those used to treat mine drainage, require the exclusion of oxygen to promote low redox conditions (Machemer and Wildeman, 1992; Nelson et al., 2006). Plants that release significant amounts of oxygen and deplete organic carbon should be avoided in this case (Stein et al., 2007), and the ability to augment organic carbon supply for microbes should be the focus of plant selection instead. In this case, screening for absence of sulfate under standardized conditions may have value for identifying preferred species.

The consistency of our results with accumulating evidence that seasonal variation of COD and BOD removal is minimal or absent in operational TWs (Kadlec and Reddy, 2001; Kadlec and Wallace, 2009) lends some credence to the validity of our results. However, while greenhouse-scale studies with controlled environments are valuable for studying effects on water treatment processes, field-scale studies are needed to evaluate individual species' performance under realistic conditions. Additional research on interactions between wastewater, wetland media, microorganisms, plants, and temperature in treatment wetlands could help to improve their design and operation in cold regions. Further research should also investigate wastewater treatment with mixed species plantings rather than monocultures (Coleman et al., 2001; Picard et al., 2005). Collectively, such studies will contribute to plant selection for improving treatment wetland function.

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