THE EFFECT OF HYDROPHYTE TYPE ON NITRATE REMOVAL IN CONSTRUCTED TREATMENT WETLAND BATCH MESOCOSMS: CATTAIL(*TYPHA SPP.*) VERSUS BULRUSH (*SCIRPUS SPP.*)

By

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To the Faculty of Washington State University:

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Abstract

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Nitrate removal rates via denitrification and dissolved oxygen (DO) levels were evaluated in small batch-mode wetland mesocosms with two different plant species, cattail (*Typha spp.*) and bulrush (*Scirpus spp.*), and associated mineral-dominated sediment collected from a mature treatment wetland. Nitrate loss in both cattail and bulrush mesocosms was first-order. First order volumetric rate constants (k_v) were 0.30 d^{-1} for cattail and 0.21 d^{-1} for bulrush and rates of nitrate loss were significantly different between plant treatments ($p < 0.005$). On an areal basis, maximum rates of nitrate removal were around 500 mg N/(m^2d) early in the experiment when nitrate levels were high (> 15 mg N/L). Areal removal rates were on average 25% higher in cattail versus bulrush mesocosms. DO in mesocosm water was significantly higher in bulrush versus cattail ($p < 0.001$). DO in bulrush generally ranged between 0.5 and 2 mg/L, while DO in cattail mesocosms was consistently below 0.3 mg/L. Based on cumulative frequency analysis, DO exceeded 1 mg/L around 50% of the time in bulrush, but only 2% of the time in cattail. DO in bulrush exhibited a statistically significant diel cycle with DO peaks in the late

afternoon and DO minimums in the early morning hours. Difference in nitrate removal rates between wetland plant treatments may have been due to differing plant carbon quality. Cattail litter, which has been shown in other studies to exhibit superior biodegradability, may have enhanced biological denitrification by fueling heterotrophic microbial activity, which in turn may have depressed DO levels, a prerequisite for denitrification. The results of this study show that cattail is more effective than bulrush for treating nitrate-dominant wastewaters.

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Dedication

This work is dedicated to my father, Yami Robi Kacha, who greatly wished and desired to send me to school up until the day he passed away, when I was six, and that was all that I could not forget about him. Be it on the first day I enrolled myself to a school far away from my home or when I had to look for other schools when expelled from the first two for not speaking Amharic, the Ethiopian national language, my father's visions and love were my resources to stay the course. I have been homeless but never been school-less. He inherited me the desire to learn, the kinship that gives the right to inherit the knowledge and wisdom, which many well-to-do pioneers left behind, and are far better than any treasured wealth one can imagine to inherit. Although the culmination of this journey and that of my father's dream seemed a step away, when I first moved to the US for postgraduate study in 2004, it never became much more evident than now that it is yet far from being over. I feel that there is still more to learn.

Chapter 1

Introduction

1.1 Background

The manufacture and use of nitrogen (N) fertilizer, mainly in developed countries, has increased from less than 5 Tg N/Year in 1950 (Smil, 1991) to over 100 Tg N/year in 2009 (Prud'homme and Heffer, 2010). In the U. S. alone, nitrogenous fertilizers accounts for about 56 percent of total fertilizer use, up from 37 percent in 1960 (USEPA, 2008), while worldwide N fertilizer use is expected to double or triple over the next 40 years (Tilman et al., 2001). Widespread use of N fertilizer has polluted water resources throughout the developed world with nitrate (NO_3^-). In Europe, for example, increases in nitrate levels in rivers over the past 50 years correlated with increased use of N fertilizer (Howarth et al., 1996). In the US, an estimated 7% of US drinking waterwells have been shut down because of agricultural-related nitrate contamination, and 44,000 infants are at risk for nitrate toxicity from contaminated drinking water (Horne, 2001). Enhanced algal productivity stimulated by nitrate pollution from agricultural activities is a primary cause of the growing number of hypoxic zones in coastal waters around the world (Daigle, 2003), including the 'dead zone' in the Gulf of Mexico (Weir, 2005).

Concerns related to N pollution have stimulated interests in the use of constructed treatment wetlands (CTWs) to treat nitrate pollution from non-point sources (Braskerud, 2002; Kovacic et al., 2006; Mitsch et al., 2005; Reilly et al., 2000) as well as domestic, industrial and livestock wastewaters, and land-fill leachate (Moshiri, 1993). Application of CTWs to water quality management attracted a greater interest due to their inherent characteristics such as low cost and simple operation, and has been implemented in many places including in developing countries (Haberl, 1999), thus, resulting in a globally increased portion of land covered by wetlands (IWA, 2000).

1.2 Constructed Treatment Wetlands

Constructed treatment wetlands are engineered natural treatment systems primarily composed of aquatic plants (hydrophytes), hydric soil and water to keep the soils waterlogged for extended period and are commonly designed as a flow-through system having a hydrologic feature of either a free water surface system (FWS) or subsurface flow system (SFS)(Greenway, 2004). While they are commonly designed for water quality improvement, they also advance enhanced aestheics, landscape, and storm mitigation. However, their treatment efficiency appears to be critically dependent on weather conditions. Variation in the amount of rainfall and evapotranspiration leads to variable detention time and inconsistent treatment rates. At times when influent flow volume exceeds storage volume of the wetland, portion of the inflow exits the wetland untreated, thus leading to difficulties to achieve treatment goals consistently. Flood induced loss of carbon from wetland has also been reported to cause substantial decrease in nitrate removal (Reilly et al., 2000).

1.3 Nitrate Removal in Constructed Treatment Wetlands

Nitrate removal in CTWs is assumed to be accompolished via dissimilatory denitrification by heterotrophic bacteria utilizing nitrate as a terminal electron acceptor to produce energy in anaerobic environment. The complete denitrification process involves sequential reductive steps starting with reduction of NO_3^- to nitrite (NO_2^-). Nitrite can then be further reduced to ammonium or nitric oxide (NO), which in turn gets reduced to nitrous oxide (N_2O), and finally to dinitrogen (N_2) gas according to Eqn. (1.1) (Madigan and Martinkor, 2006). While nitrate can also undergo assimilatory reduction by plants, fungi and bacteria (Guerrero et al., 1981), dissimilatry denitrification is considered to be a major pathway for nitrate removal in CTWs (Bachand and Horne, 2000b,c; Lund et al., 2000). The inability of denitrifiers to synthesize denitrifying enzymes in the presence of oxygen (Madigan and Martinkor, 2006) makes the predominantly reduced environment of CTWs an ideal ecosystems for nitrate reduction (Kadlec and Knight, 1996). Denitrification in CTWs occurs at the reduced layer of episediment (Fleming-Singer and Horne, 2002). Nitrate concentration (Poe et al., 2003), quality of organic matter at the sediment/water interface (Russell et al., 1994), activity of the periphytons (Sirivedhin and Gray, 2006), type of hydrophytes (Bachand and Horne, 2000c; Bastviken et al., 2005), quantity and quality of plant biomass (Wen et al., 2010) and temperature (Bachand and Horne, 2000c; Wood et al., 1999) were reported as important factors in cotrolling denitrification in CTWs.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{1.1}$$

1.4 Thesis Objectives

Emergent wetland plants are important component of CTWs, and these plants affect N processing in many ways including: (1) partially controlling flow patterns through the wetland, (2) blocking the wetland water surface from wind and sunlight, thereby lowering phytoplankton growth, rates of reaeration, and the potential for warming of water, (3) providing submerged surfaces for microbial attachment, and (4) supplying the degradable organic carbon (C) that drive heterotrophic bacterial activity (Greenway, 2007; Kadlec, 2008; Thullen et al., 2005). The importance of hydrophytes to nitrate removal in CTWs has been repeatedly shown through experiments comparing removal rates of planted versus unplanted CTWs (Kyambadde et al., 2005; Lin et al., 2007; Tanner, 2001). Reduction in biomass due to increased harvesting of wetland plants have also been reported to decrease nitrate removal from wetland (Martin et al., 2003). Because wetland plants vary by the quality and quantity of C they supply to wetlands, as well as by the size of surface area they provide for microbial attachment, different plant species can differently affect removal rates of pollutants (Corstanje et al., 2006; Horne and Fleming-Singer, 2005). The few studies that have evaluated nitrate removal as a function of wetland plant type, commonly comparing *Typha spp.* (cattail) with *Scirpus spp.* (bulrush), have shown either little effect (McIntyre and Riha, 1991; Zhu and Sikora, 1995), or greater removal by cattail (Bachand and Horne, 2000a; Hume et al., 2002).

The objective of this research was to test the working hypothesis that nitrate removal in CTWs is greater using cattail versus bulrush. These two plant species were selected because they are commonly used in CTWs, and they are two species that are commonly compared in the literature regarding nitrate removal. Water in the small, batch-mode mesocosms with plants and associated sediments were spiked with nitrate, and nitrate was monitored over time to estimate relative rates

of nitrate removal. Experimental mesocosms were used because the approach captures some of the ecological complexity of full-scale treatment wetlands while allowing for the replication needed for statistical comparison between treatments (Kangas and Adey, 1996). In contrast to other mesocosms studies that used sand or gravel (Iamchaturapatr et al., 2007; Zhu and Sikora, 1995), sediments collected from cattail and bulrush stands from a regional CTW were used. An additional unique aspect of this study was the intensive monitoring of dissolved oxygen (DO) in the water column of the mesocosms. Given that cattail litter typically has more labile C and N relative to bulrush (Hume et al., 2002), higher microbial activity, higher nitrate removal, and lower DO were expected in the cattail mesocosms.

Chapter 2

Materials and Methods

2.1 Experimental Design

Triplicate mesocosms were constructed consisting of two plant treatments and associated mineral-dominated sediment: cattail (*Typha spp.*) and bulrush (*Scirpus spp.*). Plants, sediment and water from cattail and bulrush stands were collected during the summer of 2006 from a mature CTW in Moscow, Idaho, used to polish secondary effluent. In the laboratory, sediment and plants were transferred into glass aquariums measuring 50.8 cm in length, 25.4 cm in width, and 30.5 cm in height. Plant density in each mesocosm was approximately 50 plants/m^2. Once plants took root and sediments stabilized to a thickness of around 15 cm, mesocosms were gently flooded with 13 L of wetland water to a depth of 10 cm. The mesocosms, operated as batch systems, were spiked with nitrate to obtain a final concentration of 19 mg N/L. Minor evaporative losses of around 400 mL/d were compensated for by adding deionized water to the aquariums once every day. Room temperature was maintained at 18.5 °C and plants were exposed to 8 h/d of indoor plant lighting as well as natural light from nearby windows. Duplicate control aquaria with no plants and sediments were set up and operated in the same manner as experimental ones.

2.2 Sampling and Analysis

Sediments collected from the stands where wetland plants were collected were analyzed (in triplicate) for a range of parameters including pH, water content (drying at 105 °C for 24 h), loss on ignition (combustion at 550 °C), and total C and N content (dry combustion at 1350 °C; LECO CNS 2000 Analyzer, LECO Corp. MI). DO was measured in all six mesocosms with Hach standard luminescent DO (LDO) IntelliCAL probes attached to HQ40d digital meter/data loggers (Hach Company, Loveland, CO). DO probes were deployed at a water depth of 5 cm and water column DO was automatically measured and logged every 30 min over a 2-month duration. Approximately 1140 DO data points were collected in each mesocosm. Three weeks after the start of DO monitoring, mesocosms were spiked with nitrate and monitored for nitrate and ammonia for 14 days. Water samples were collected at decreasing frequency as the experiment progressed, five to four times per day initially and once per day towards the end of the incubation. For each sampling event, duplicate 2 mL water samples were collected from each mesocosm at a depth of 5 cm using a syringe. Samples were filtered using a 0.45 pore size syringe tip-filter and analyzed for nitrate and ammonia by flow injection analysis on a Lachat 8500 QuikChem auto analyzer (Lachat Instruments, Milwaukee,WI). Ammonia rarely exceeded 0.1 mg N/L, suggesting that N cycling in the wetland mesocosms was dominated by nitrate. Thus, the remainder of this paper focuses on nitrate dynamics. Various physico-chemical properties of the sediments used for the experiment are provided in Table (2.1).

Table 2.1: Physical and chemical properties of wetland sediments

Sediment	pH	Conductivity mS/cm	Loss on Ignition%	Total Nitrogen%	Total Carbon%	Texture
Cattail	7.19	0.78	5	0.15	2.1	Silt Loam
Bulrush	7.21	0.79	7	0.17	2.5	Silt Loam

2.3 Data Analysis

2.3.1 Data Modeling

Assuming a first-order rate for nitrate removal under batch conditions at constant volume and uniform nitrate concentration, the equation for concentration over time is

$$C(t) = C_o e^{-k_v t} \tag{2.1}$$

where C(t) (mg N/L) is concentration of nitrate at time t (d), Co (mg N/L) is initial nitrate concentration, and k_v (d^-1) is the volumetric rate constant for nitrate removal. Values for k_v in the two wetland plant treatments were estimated by pooling triplicate data sets of concentration with time and, based on the linearized form of Equation (2.1), calculating the slopes of the linear regression of the natural log of nitrate versus time. An area based rate constant k_a (m/d) was estimated by multiplying k_v by the constant water depth of the mesocosm (10 cm). Areal nitrate removal rates (mg/m^2d)were calculated as the difference in nitrate over 24 h divided by the area of the mesocosms ($0.129m^2$).

2.3.2 Statistical Analysis

2.3.2.1 Parametric Tests

Data sets of DO (n $\sim$ 1140 per mesocosm over 8 weeks) and nitrate (n = 35 per mesocosm over 2 weeks) over time for the two plant treatments were analyzed using SAS software (SAS Institute Inc., Cary, NC). A general linear model was used to estimate variance, slopes and intercepts of curves fitting the data sets. Statistical significance of differences among parameters and treatments were determined using a two-tailed *F*-test. Inferences about statistical differences between nitrate removal rate constants between cattail and bulrush treatments

were obtained from an F-test for the null hypothesis that the reaction term (β_3) in the complete general linear model (Eqn. 2.2) was zero if the two regression lines were parallel (Ott and Longnecker, 2001).

$$Y_c = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \epsilon \tag{2.2}$$

The F-value was calculated using estimates obtained from (Eqn. 2.2) and the reduced regression model (predictors with coefficients not hypothesized to be zero) (Eqn. 2.3):

$$Y_r = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \epsilon \tag{2.3}$$

using the following formula:

$$F = \frac{\frac{SS(regressin,Y_c) - SS(regression,Y_r)}{k-g}}{\frac{SS(residual,Y_c)}{n-(k+1)}} \tag{2.4}$$

where SS is sum of squares, k is number of all predictors, g is number of predictors hypothesized not to be zero and n is number of observations.

Differences in data sets were considered statistically significant if p values were less than 0.05.

2.3.2.2 Non-parametric Tests

Data sets of DO versus time for the cattail and bulrush treatments were also tested for periodicity using Matlab software (The Mathworks Inc., Natick, MA). Data sets were transformed via Fast Fourier Transform. A periodogram, constructed using the transformed data, was then used to detect any diel cycle in DO. Also, a simple moving average was calculated for the time series DO data to filter out short-term fluctuations and to detect long-term trends.

Chapter 3

Results and Discussion

3.1 Results and Discussion

3.1.1 Nitrate Removal

Nitrate loss in both cattail and bulrush mesocosms was first order in nature (Fig. 3.1) and statistical analysis confirmed that nitrate loss rates in cattail were significantly higher ($p < 0.005$) than in bulrush. Nitrate first-order volumetric rate constants (k_v) were 0.30 and 0.21 d^{-1} for cattail and bulrush, respectively. On an area basis, nitrate removal rate constants (k_a) were 10.8 m/year for cattail and 7.7 m/year for bulrush. The k_v values found in these study are in the low to middle range of those reported in the literature (Kadlec and Knight, 1996). Our k_a values were also on the low end of those reported for surface flow CTWs, which range from around 10 to 60 m/year (Fleming-Singer and Horne, 2007; Kadlec, 2008; Kadlec and Knight, 1996).

Nitrate loss was also evaluated on an areal removal basis (Fig. 3.2). Maximum rates of nitrate removal of 400-500 mg N/(m^2d) were observed during the initial stage of the experiment when nitrate was above 15 mg N/L. Nitrate removal rates decreased over time as nitrate concentration dropped. Rates were around

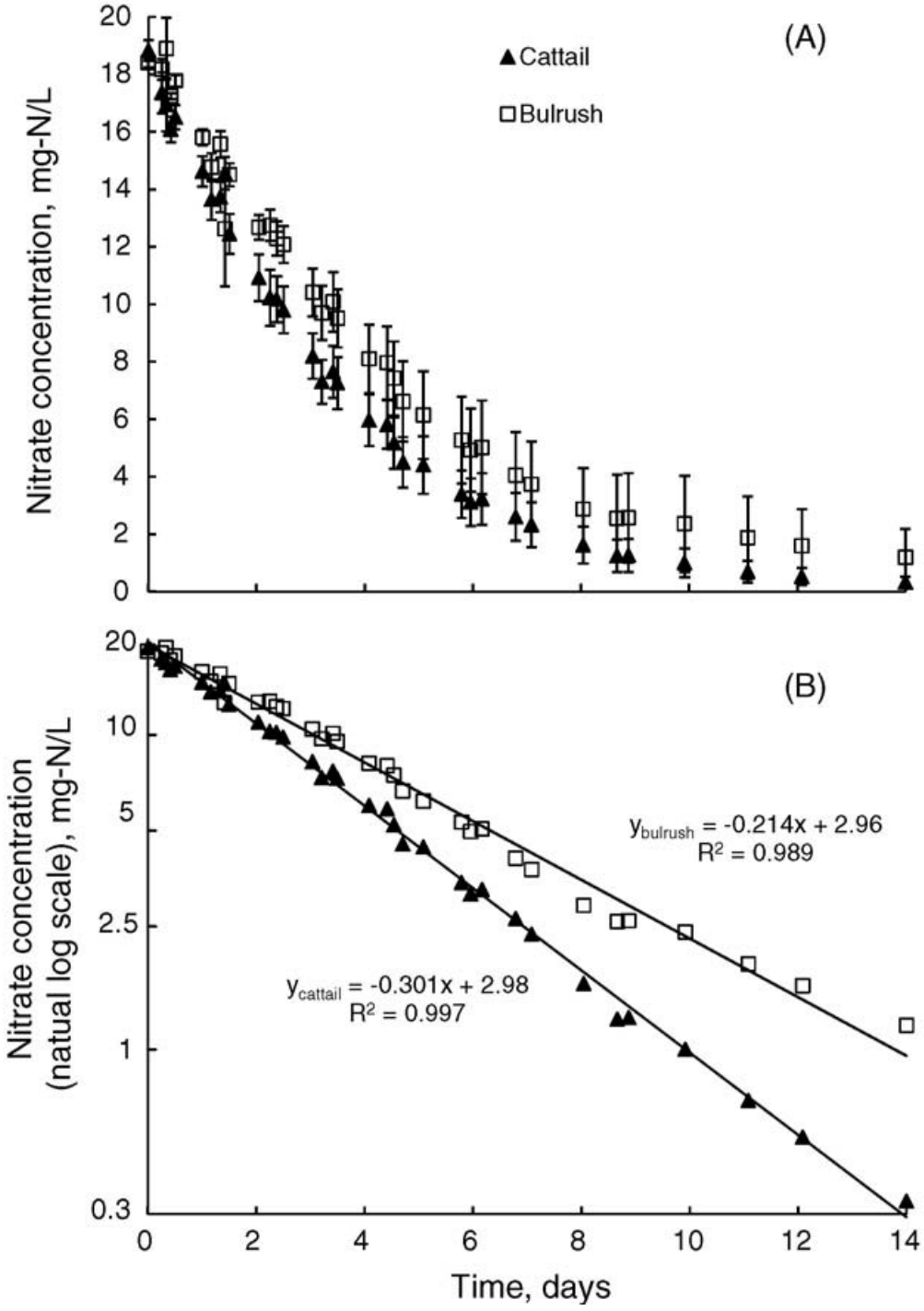

FIGURE 3.1: Nitrate loss in cattail and bulrush mesocosms mesocosms. (A) Nitrate versus time and (B) natural log-transformed nitrate versus time. Values are average of duplicate samples from triplicate treatments (n = 6). Error bars in (A) are plus/minus one standard deviation. Lines in (B) are linear regression of data sets.

300 mg N/(m^{2}d) at 8 mg N/L and 175 mg N/(m^{2}d) at 5mg N/L. Areal removal rates were on average 25% higher in cattail versus bulrush mesocosms.

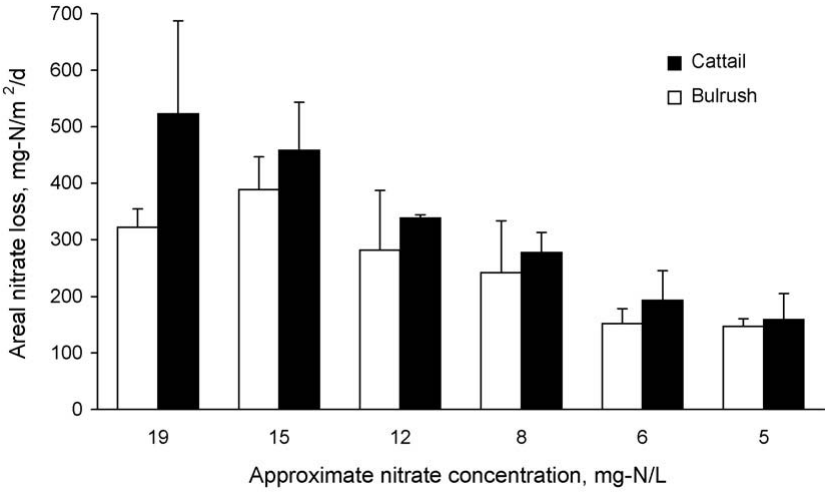

FIGURE 3.2: Area-based nitrate loss versus nitrate concentration in cattail and bulrush mesocosms. Values are average plus one standard deviation (n = 3).

Differences in the quality of organic matter that the two plant species supply to wetland sediments may explain the observed difference in nitrate removal. Many plants, including wetland species, differ in the quality of C and the relative amount of N they supply to the sediment (Corstanje et al., 2006; Hobbie, 1996; Sirivedhin and Gray, 2006; Taylor et al., 1989). Hume et al. (2002) reported that cattail litter had lower lignin content and lower C:N than bulrush, implying that cattail degrade more easily and support greater microbiological activity than bulrush. An examination of sediment characteristics from the sampling sites for the two wetland plant types somewhat support this argument. While many characteristics of the mineral sediments were fairly similar (e.g., pH ∼7.2; bulk density ∼1.4 g/cm^3), loss on ignition (5% versus 7%), C content (2.1% versus 2.5%), and C:N (14.0 versus 14.3) were lower in cattail sediments.

Our overall rates were comparable to a number of other studies of nitrate-dominated CTWs in which nitrate removal rates generally ranged from 100 to 1000 mg N/(m^{2}d) (Fleming-Singer and Horne, 2007; Gale et al., 1993; Kadlec, 2008; Phipps and Crumpton, 1994). With regard to the effect of wetland plant species,

our findings parallel those of Bachand and Horne (2000a) and Hume et al. (2002) that showed higher nitrate loss in cattail compared to bulrush. Bachand and Horne (2000a) observed a more dramatic differential in nitrate removal between cattail and bulrush with nitrate removal rates averaging 565 mg N/(m^2d) for cattail and 261 mg N/(m^2d) for bulrush at nitrate levels of around 9 mg N/L. Iamchaturapatr et al. (2007) also observed higher areal removal rates of nitrate by T. *latifolia* versus S. *radicans* and S. *triqueter* in experimental phyto-batch reactors containing wetland plants in sand. In contrast, Zhu and Sikora (1995) found no difference in nitrate removal in batch wetland mesocosms containing T. *latifolia* and S. *atrovirens georgianus* in gravel.

3.1.2 Dissolved Oxygen

DO in bulrush mesocosms generally ranged between 0.5 and 2 mg/L while DO in cattail mesocosms was consistently below 0.3 mg/L, and statistical analysis confirmed that DO was significantly higher ($p < 0.001$) in bulrush mesocosms. Evaluation of the cumulative frequency distribution of pooled DO data set for each plant treatment highlighted the dramatic differences in DO between the two treatments (Fig. 3.3). DO exceeded 1 mg/L around 50% of the time in bulrush, but only 2% of the time in cattail. DO was less than 0.1 mg/L over 40% of the time in cattail and only 1% of the time in bulrush.

These observations unequivocally showed that DO was higher in bulrush versus cattail, and that the water and the sediment/water interface were mildly aerobic in bulrush mesocosms and anaerobic in cattail mesocosms. Periodicity analysis also confirmed that DO exhibited a detectable diel cycle in bulrush mesocosms with DO peaks in the late afternoon and DO minimums in the early morning hours (Fig. 3.4).

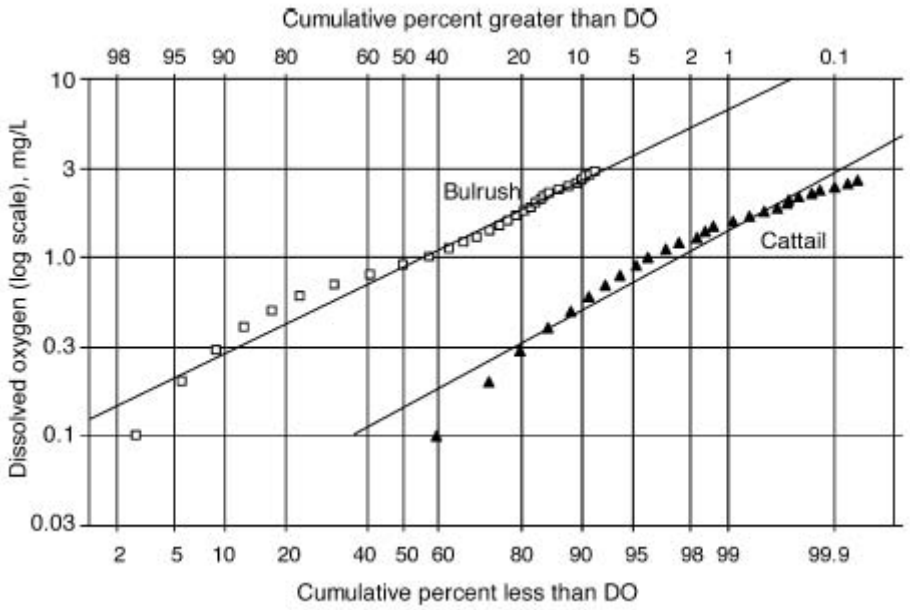

FIGURE 3.3: Cumulative frequency distribution of DO in cattail and bulrush mesocosms; n ∼3400 for each treatment. Lines are linear regression of cumulative frequency data sets.

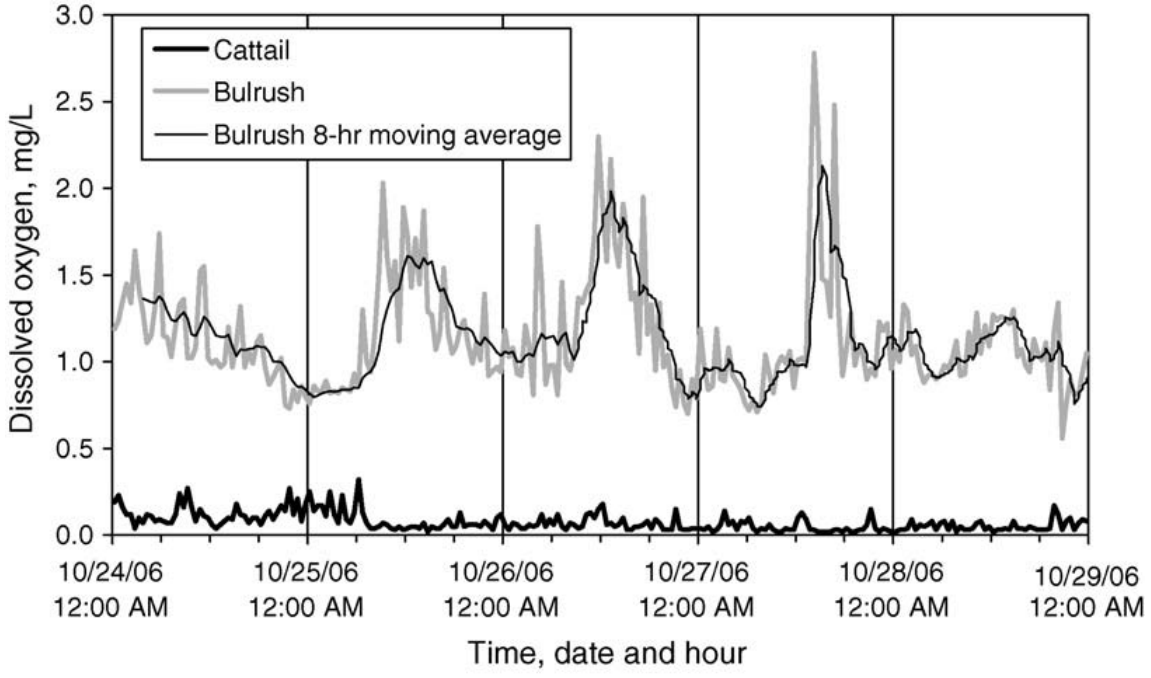

FIGURE 3.4: Typical data set of dissolved oxygen in cattail and bulrush mesocosms over a 5-day period.

A close examination of the periodogram (Fig. 3.5) reveals that the DO data from the blurush mesocosms had one of the highest peaks at a period of 1.0 day per/cycle, while dominant frequencies indicating presence of periodic pattern were absent from DO data obtained from cattail mesocosms. The DO cycle observed in the bulrush mesocosms was likely the result of photosynthesis by periphyton, enhanced by the shallow water depth and glass siding of the mesocosms, as well as the addition of nitrate. There are two potential explanations for the fact that a diel cycle of DO was observed in the bulrush mesocosms and not the cattail mesocosms. First, there may have been no periphyton in the cattail mesocosms. Second, higher biological oxygen demand in the cattail mesocosm may have acted as a rapid sink for photosynthetically produced oxygen. Two key observations support the latter explanation. Periphyton was observed on plant stems and aquarium walls in both cattail and bulrush mesocosm, thus DO was likely produced during day-light hours in all mesocosms. In addition, DO levels were higher in the bulrush versus cattail mesocosms during the dark when the periphyton was not photosynthetically active, suggesting that DO uptake was higher in cattail versus bulrush mesocosms.

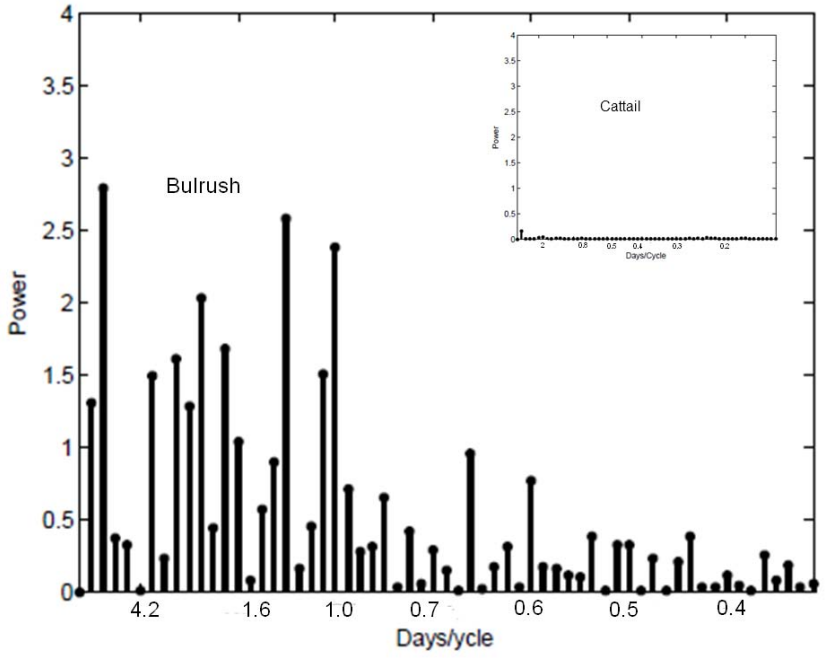

FIGURE 3.5: Periodogram of the DO data indicating presence of a diel cycle in Bulrush mesocosms; n=1024.

3.2 Conclusion

The results of this study showed that cattail exhibited significantly higher rates of nitrate removal and lower DO levels in water compared to bulrush in wetland mesocosms. From a management perspective, our results confirm that cattail should be used when treating nitrate, a pollutant that requires the activity of anaerobic microorganisms to be transformed to harmless dinitrogen gas. Bulrush, which exhibited higher DO levels in wetland water, may be more suitable to treat ammonia-dominated wastewaters, because higher DO levels should stimulate biological nitrification, a transformation that is generally recognized to be oxygen-limited (Keeney, 1973; Reddy and Patrick, 1983). Bulrush may have the added benefit of higher rates of rhizosphere oxygenation compared to cattail, which could further enhance nitrification (Reddy et al., 1990; Szogi et al., 2004; Winthrop et al., 2002). The use of bulrush could be used in conjunction with, or even preclude the need for, vegetation management strategies (e.g., open water, hummocks) to enhance nitrification, and subsequent denitrification, in CTWs treating ammonia-rich wastewaters (Thullen et al., 2005, 2002). For ammonia-dominated wastewaters, the two plant types in series, bulrush followed by cattail, could optimize N removal in CTWs by first enhancing nitrification of ammonia to nitrate, then promoting denitrification of nitrate to dinitrogen gas.

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