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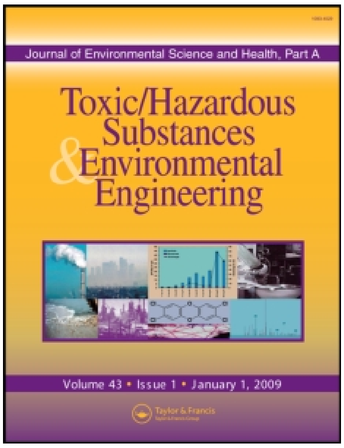

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Ammonium Removal in Constructed Wetland Microcosms as Influenced by Season and Organic Carbon Load

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We evaluated ammonium nitrogen removal and nitrogen transformations in three-year-old, batch-operated, subsurface wetland microcosms. Treatments included replicates of *Typha latifolia*, *Carex rostrata*, and unplanted controls when influent carbon was excluded, and *C. rostrata* with an influent containing organic carbon. A series of 10-day batch incubations were conducted over a simulated yearlong cycle of seasons. The presence of plants significantly enhanced ammonium removal during both summer (24°C, active plant growth) and winter (4°C, plant dormancy) conditions, but significant differences between plant species were evident only in summer when *C. rostrata* outperformed *T. latifolia*. The effect of organic carbon load was distinctly seasonal, enhancing *C. rostrata* ammonium removal in winter but having an inhibitory effect in summer. Season did not influence ammonium removal in *T. latifolia* or unplanted columns. Net production of organic carbon was evident year-round in units without an influent organic carbon source, but was enhanced in summer, especially for *C. rostrata*, which produced significantly more than *T. latifolia* and unplanted controls. No differences in production were evident between species in winter. COD values for *C. rostrata* microcosms with and without influent organic carbon converged within 24 hours in winter and 7 days in summer. Gravel sorption, microbial immobilization and sequential nitrification/denitrification appear to be the major nitrogen removal mechanisms. All evidence suggests differences between season and species are due to differences in seasonal variation of root-zone oxidation.

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Key Words: Treatment wetland; *Typha*; *Carex*; Nitrogen; Ammonia; Nitrate; Sorption; Winter.

INTRODUCTION

Constructed wetlands (CWs) are recognized as a viable, aesthetically pleasing, low-cost alternative for secondary and tertiary domestic wastewater treatment. However, misconceptions regarding potential performance and lack of understanding of specific removal mechanisms, especially for nitrogen, have frequently resulted in failed designs.[1] Identified high-priority research topics for subsurface flow (SSF) systems include: (1) better understanding of nitrogen removal and nitrogen transformations, especially as affected by temperature and season; (2) development of rational design models for nitrogen removal, and; (3) investigation of plant species other than reeds, rushes and cattails.[2]

Because wetlands can simultaneously contain aerobic and anaerobic zones and be either net producers or consumers of organic carbon depending on organic carbon load,[3] the sequential nitrification/denitrification process is often identified as a primary ammonium removal mechanism in constructed wetlands.[4–8] However, other research questions the relative strength and reliability of this sequential mechanism, especially in long-term, field-scale systems.[9–11]

In SSF wetland systems, nitrification has generally been identified as the limiting step to sequential nitrification/denitrification, as aerobic heterotrophs outcompete nitrifiers for a limited oxygen supply.[12] However, the oxygen status of the wetland root zone may be increased by species selection,[4,13] cold season conditions,[14,15] and lower organic carbon load.[16] Using the same experimental units as used in our study,[17,18] demonstrated superior year-round organic carbon removal and increased root-zone oxygenation in *Carex rostrata** over similarly performing *Typha latifolia* and unplanted controls. The *C. rostrata* (but not *T. latifolia* or control) showed increased winter performance, indicating that overall and seasonal differences in root zone oxygenation may be species specific.

Based on results outlined above, we designed an experiment to compare ammonium nitrogen removal from microcosms planted with two wetland species (*C. rostrata* and *T. latifolia*) and unplanted controls during winter and summer conditions, and for the *C. rostrata* treatment, with and without an influent organic carbon load. In addition to providing seasonal performance data for these species and carbon loads, we elucidate potential removal mechanisms and the relationship between nitrogen and carbon removal for *C. rostrata* planted wetlands.

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*Recent taxonomic revisions reclassify most North American *Carex rostrata* as *C. utriculata*. We retain the earlier name for consistency with our earlier publications.

MATERIALS AND METHODS

This study, conducted between July 1999 and August 2000, utilized 16 of the 32 batch-operated subsurface CW microcosms ("columns") that have been operating in a controlled temperature greenhouse at Montana State University since April 1997. Eight columns were planted with *Carex rostrata* Stokes (beaked sedge), four with *Typha latifolia* L. (broadleaf cattail), while four were left unplanted as controls. The columns were constructed from 60 cm tall × 20 cm diameter polyvinyl chloride (PVC) pipe and filled to a 50 cm depth with washed, noncalcareous alluvial gravel (0.3–1.3 cm), resulting a pore volume of 4.3L. Solution sampling tubes (0.3 cm diameter vinyl tubing) were installed from above with openings at depths of 5, 15, and 30 cm. Details of column design, construction and planting; typical greenhouse environmental variability; as well as operation of the system prior to June 1999 have been described previously.[17,18] Except as noted below, operation was identical during this study.

Seasonal cycles of plant dormancy and growth were induced by varying the greenhouse set temperature in conjunction with natural variation in day length. During the summer months (June, July, and August) the ambient air temperature was set to 24°C. During the fall (September, October, and November) and spring (March, April, and May), the set temperature was 14°C. During winter months (December, January, and February), the set temperature was 4°C. There were approximately week long 5°C steps between all simulated seasons. Though actual temperatures showed diurnal and spatial variability, set temperatures represent average daily greenhouse temperatures well.

Two different synthetic wastewater formulations were used to simulate two stages of domestic wastewater pretreatment (postsecondary and weak-strength postprimary). To simulate postsecondary treatment, all columns received target influent concentrations of 40 mg/L NH_4-N as the sole N source, 8 mg/L PO_4-P and appropriate micronutrients,[19] by mixing nutrient and metal salts with tap water. Four (or five, as described below) of the *Carex* columns additionally received 200 mg/L of sucrose ($C_{12}H_{22}O_{11}$) as an organic carbon source (COD $\approx$ 225 mg/L) to simulate postprimary-treatment wastewater. *Carex* treatments with and without additional carbon are denoted by (C) and (NC).

The initial experimental design included tracking of chemical constituents from four replicates of the four treatments during two 10-day incubations within each seasonal temperature setting. However, this design was revised during the second 24°C period due to a planned (destructive sampling) and unplanned (plant mortality) reduction in the number of *Carex* columns. All five surviving *Carex* columns were used together as replicates in four sequential incubations that used either no-carbon or carbon-amended wastewater, thereby maintaining at least two incubations of each treatment. Potential confounding of within-season variation with treatment effects was addressed by running one no-carbon incubation, followed by two carbon incubations, and finally the last no-carbon incubation. Data from the unplanted control and *Typha* columns were collected from all four incubations at 24°C, and this article utilizes all data collected during the two winter-season (4°C) and four summer (24°C) incubations.[19] Columns were gravity drained 3 days prior to each incubation and then again at the start of each incubation. Upon each emptying, synthetic wastewater that had been mixed 1 day prior to allow for equilibration with the greenhouse temperature was added from the top. As no vertical gradients were evident in previous experiments with the same columns,[17] aqueous samples were drawn from the 15 cm depth only. Sampling from all columns occurred at days 0 (immediately after filling), 0.3, 0.6, 1, 3, 7, and 10 of each incubation. Duplicate samples were also drawn from influent water immediately before filling columns. Parameters measured from every sampling event included: chemical oxygen demand (COD), ammonium, nitrate, nitrite, phosphate, and sulfate. Total organic carbon (TOC), total nitrogen (TN), and pH were measured for all sampling events except 0.3 and 0.6 days for the first incubation of each temperature cycle, but only from Day 0 and Day 10 for other incubations. Samples were immediately analyzed for COD (0–1500 mg/L) and TN using colorimetric procedures (Hach Corp., Loveland, CO). TOC samples were acidified with 20% H_3PO_4 and later measured using a Dohrmann DC-80 carbon analyzer (Xertex Corp., Santa Clara, CA). All other procedures were performed later on samples that were filtered through a 0.22 μm cellulose acetate filter and stored in sterile test tubes at 2°C. Ammonium was determined using the modified Berthelot-1859 colorimetric method; nitrate, nitrite, sulfate, and phosphate were measured using high performance liquid chromatography (Dionex model DX-500, Dionex Corp., Sunnyvale, CA.).

Constituents that were not present in the influent, such as nitrate and sulfate, were analyzed statistically on a mass concentration basis. The removal ratio (C/C_0, with C_0 the average measured prefill influent concentration) was used to normalize the influent variability of ammonium and in the *Carex* (*C*) columns, COD. *Carex* (*C*) and *Carex* (*NC*) columns were considered distinct statistical treatments, along with *Typha* and control columns. Treatment effects on ammonium and COD removal measured from Day 1 through Day 10 were determined simultaneously with a potential linear time effect using Minitab's (Minitab, Inc., State College, PA) analysis of covariance. Treatment effects were separated using Tukey's multiple comparison procedure for all combinations, at a confidence level of 95%. This technique's level of significance is based on a family of inferences simultaneously evaluated, and may be more conservative than individual comparisons.[20] Treatment variation prior to Day 1 was analyzed within a specific sample time using analysis of variance. Because measured nitrate was both highly variable and nonlinear with time, analysis of covariance was performed without a time effect using $\log_{10}$ transformed data. The detection limit (0.05 mg/L) was used in lieu of zero values. The geometric mean, or the antilog of the mean of the log-transformed data, is used in the presentation of the results. The standard error was approximated using the delta method.[21]

Two sorption experiments also were performed using methods adapted from Breen[9] and Sikora et al.,[8] on gravel that had been taken from a destructively sampled *Carex* column and prepared by washing with deionized water then autoclaved for 20 minutes. In the first, used to establish the gravel-water equilibration time, 100 g of prepared gravel was mixed in 500 mL acid-washed plastic bottles with 100 mL of either 40 mg/L or 5 mg/L NH_4-N. Four replicate samples were used at each concentration. Sealed bottles were continuously shaken in the dark for 3 days. Aqueous samples were drawn at selected times, filtered through a 0.22 μm cellulose acetate filter, and analyzed colorimetrically for NH_4-N. In the second experiment, used to estimate the maximum sorptive capacity of the gravel, 100 g of prepared gravel was placed in acid-washed bottles and incubated in the dark for 2 days (the equilibration time) with 100 mL of ten different NH_4-N concentrations ranging from 0 to 6000 mg/L. The gravel was removed, washed with deionized water, and shaken for an additional day in fresh bottles with 100 mL of 1N KCl to extract the sorbed ammonium. The supernatant was then filtered and analyzed as described above for NH_4-N.

RESULTS

Time series of the measured concentrations of total N, NH_4-N, NO_3-N, and COD, averaged within season and treatment are shown in Figure 1. As pH never exceeded 7.3, virtually all ammoniacal nitrogen was in the ammonium ion form. Nitrite concentrations were consistently below the detection limit (0.05 mg/L), therefore, the estimated concentration of organic nitrogen (ON, dissolved plus particulate), which was not present in the influent, was determined by the difference between measured TN and ammonium plus nitrate concentrations. The calculated ON concentration varied between 2 and 5 mg/L and displayed no apparent trend with time, treatment or season. Therefore, TN differences between season and treatment are well represented by ammonium differences described in more detail below, as can be inferred from Figure 1.

Ammonium

Removal of NH_4-N from the aqueous phase was quite rapid as treatment mean removal varied between 53% and 92% within the first eight hours. Relative performance of treatments was established within 24 hours, and little change in concentration or relative ranking of experimental treatments occurred thereafter. The overall treatment and season effects reported are based on data collected from 24 hours onward. Statistically significant ($p < 0.05$) seasonal effects were displayed only in the *Carex* (*NC*) treatment, with higher removal of NH_4-N during summer at 24°C (removal ratio, $C/C_o = 0.05 \pm 0.02$) than during the winter at 4°C ($C/C_o = 0.25 \pm 0.03$). In contrast, the

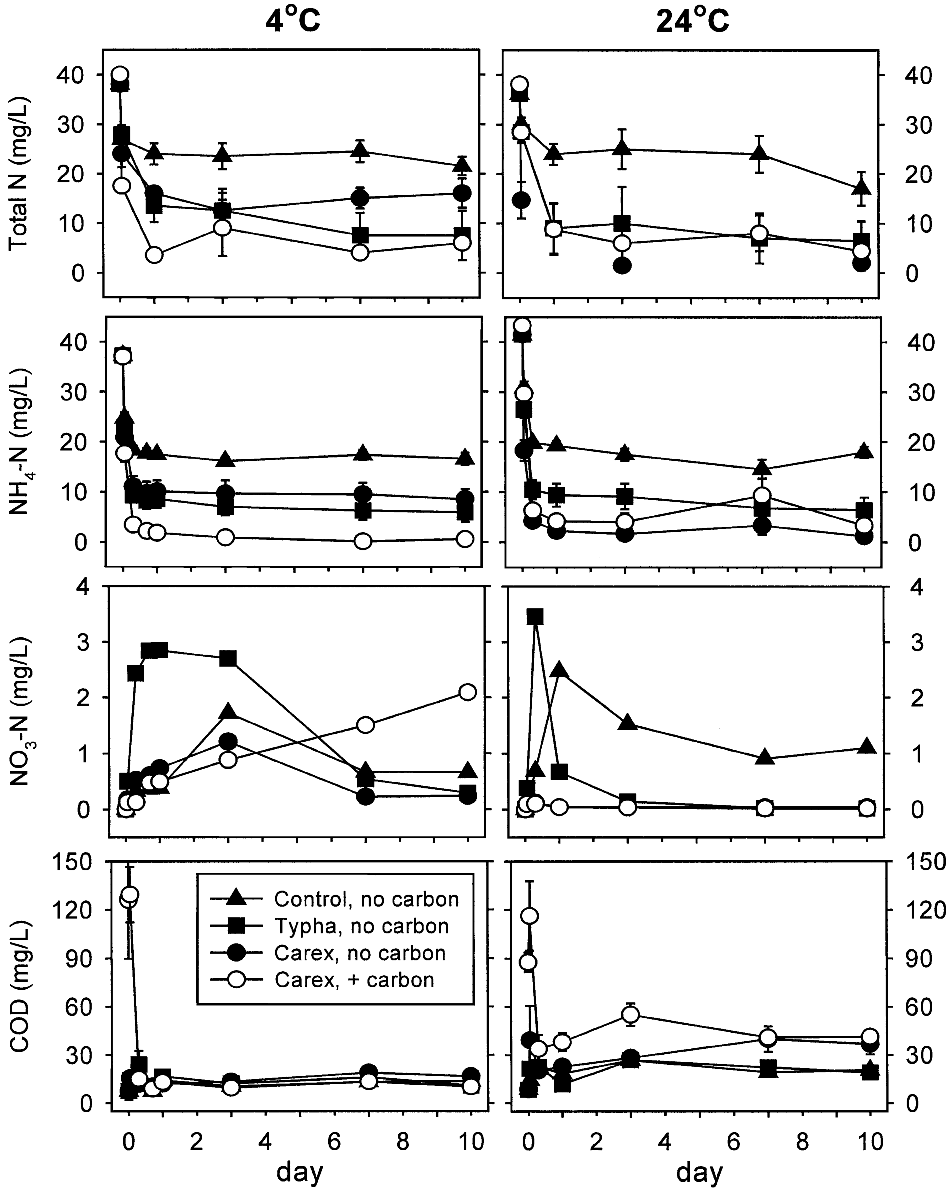

Figure 1: Total nitrogen, ammonium nitrogen, nitrate nitrogen, and chemical oxygen demand concentrations during winter and summer incubations. Symbols represent the arithmetic mean (geometric mean for nitrate nitrogen) of treatment replicates over all incubations at the given temperature ± one standard error. The two symbols at day zero represent values from influent wastewater and samples taken from columns immediately after filling.

carbon-amended *Carex* (*C*) columns tended ($p = 0.06$) to remove more NH_4-N in winter ($C/C_o = 0.02 \pm 0.03$) than in summer ($C/C_o = 0.12 \pm 0.02$). Removal in *Typha* and unplanted controls columns did not differ seasonally, with mean removal ratios of 0.19 ± 0.03 and 0.42 ± 0.02, respectively.

Ammonium removal was increased significantly by the presence of plants during both seasons, but seasonal interactions with carbon load were apparent. In winter *Carex* (*C*) columns had significantly better removal than either *Carex* (*NC*) or *Typha* columns, both of which were significantly better than unplanted controls. In summer, performance of *Carex* (*C*) decreased and *Carex* (*NC*) increased in such a way that *Carex* (*NC*) columns were significantly better at removing NH_4-N than *Typha* columns, which were significantly better than control columns. Performance of carbon-amended *Carex* (*C*) columns was intermediate and could not be statistically separated from either *Carex* (*NC*) or *Typha* columns without organic carbon.

Nitrate

Sample nitrate concentrations varied from the instrument detection limit (0.05 mg/L) to as high as 27 mg/L. High variability occurred between treatment replicates within an incubation, and individual columns typically varied between incubations of the same season. Time effects were also apparent (Fig. 1), thus post 24-hour geometric means were used to assess treatment and seasonal effects. The control treatment was significantly higher (1.38 mg/L) than the statistically-similar planted treatments (0.05–0.10 mg/L) in summer, but no significant treatment effect was observed in winter, though *Carex* (*C*) averaged 2.09 mg/L while other treatments ranged from 0.23 to 0.66 mg/L. Seasonal effects within treatments were not significant, but when averaged over all treatments, significantly higher means occurred at 4°C than 24°C.

Chemical Oxygen Demand

Only the *Carex (C)* columns, in which 200 mg/L sucrose was added, had appreciable initial concentrations of COD (mean = 64–189 mg/L; Fig. 1). This COD underwent rapid removal, which varied by season. Overall (after Day 1) removal was significantly better in winter ($C/C_0 = 0.09 \pm 0.03$) than in summer ($C/C_0 = 0.44 \pm 0.02$), consistent with earlier results.[17] All treatments lacking COD in the influent displayed initially increasing COD concentrations, indicating an internal net release of organic carbon, averaging 15 ± 3 mg/L after 24 hours in winter. In summer, *Typha* and control COD values had comparable values of 21 ± 1 mg/L after 24 hours and the *Carex* (*NC*) treatment displayed increasing COD concentration up to 40 mg/L on Day 7, nearly identical to the *Carex* (*C*) treatment. Thus, in summer both *Carex* (*C*) and *Carex* (*NC*) treatments had about twice the COD of the seasonally invariant *Typha* and control treatments, but no differences in COD were evident in winter.

Gravel Sorption

The bottle experiments performed on washed and autoclaved gravel taken from a *Carex* column indicated that the gravel was capable of quickly removing a large fraction of ammonium from solution. Within 12 hours, $69 \pm 5\%$ of

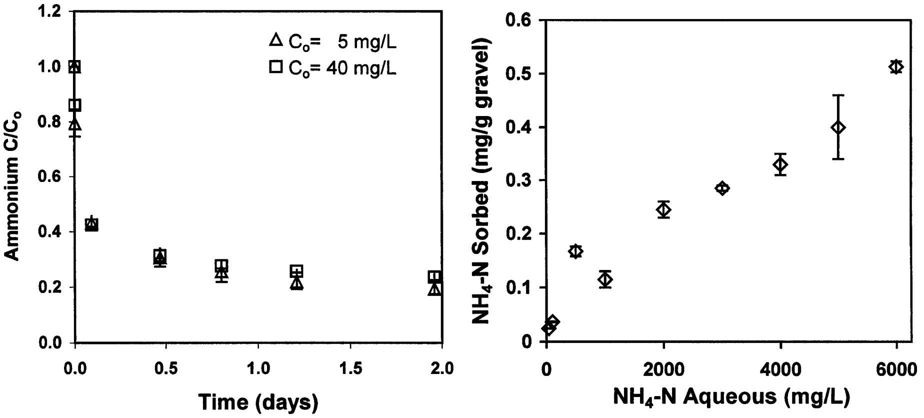

Figure 2: Results of ammonium sorption experiments performed on washed and autoclaved gravel taken from a *Carex* column. Left panel shows the change in concentration for 100 mL solution at given initial concentration continuously shaken with 100 g of gravel. Symbols represent mean of four replicates ± one standard error. Right panel shows mass of ammonium sorbed versus aqueous phase concentration for 100 mL solution continuously shaken for two days with 100 g of gravel. Symbols represent mean of two replicates ± one standard error.

applied ammonium concentrations were removed from the aqueous solution, after which little change occurred (Fig. 2a). Initial concentration had no effect on the removal rate over the range evaluated (5–40 mg/L). The second set of experiments revealed an approximately linear relationship between mass sorption ratio and aqueous NH_4-N concentration for initial aqueous concentrations up to 6000 mg/L (Fig. 2b). Therefore, the gravel was not saturated, even at the highest value, indicating a strong sorption potential.

DISCUSSION

Nitrogen removal measured in operating SSFs has tended to decrease in winter.[4,22,23] However, only *Carex* (*NC*) columns showed a statistically significant decrease in ammonium removal in winter; mean *Carex* (*C*) removal increased in winter. When averaged over all treatments, more nitrate was measured in winter than in summer. Therefore, our results lend credence to other observations[17,18,24,25] that COD and ammonium removal need not be limited by winter conditions.

Apparent variation in COD degradation during the one-day temperature equilibration period caused influent COD for the *Carex* (*C*) treatment, and ranged from 64–189 mg/L for a particular incubation. This unintended variability did not statistically compromise overall treatment comparisons previously described, and was advantageously used to demonstrate the seasonal variation of the influence of COD on ammonium removal (Fig. 3). During the summer incubations, NH_4-N removal decreased with an increase in influent COD. Conversely, NH_4-N removal consistently increased with increasing influent COD

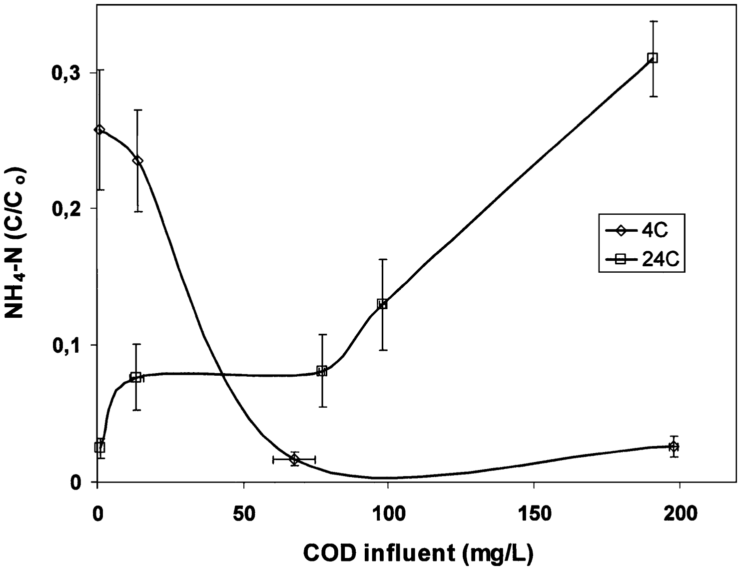

Figure 3: *Carex* ammonium removal ratio (average concentration in each column from day 1 to day 10 divided by influent concentration) versus average influent COD at 4 and 24°C. Symbols represent the arithmetic mean of replicates within a specific incubation ± one standard error. A vertical line at approximately 50 mg/L effectively separates *Carex* (*NC*) (left) from *Carex* (*C*) treatments (right).

levels in winter incubations. This seasonal effect indicates simple C-N balance relationships cannot explain ammonium removal.

The implicated reason for plant treatment and seasonal variation in performance in our results is increased plant-mediated oxygen availability in winter versus summer. There is a growing body of evidence that some wetland species, including *C. rostrata*, can enhance the oxygen available for microbial processes in winter over summer.[15,18,26,27] In our study, all planted treatments (but not controls) had significantly more sulfate in winter than in summer.[19] The only source of sulfate in our experiment was from oxidation of previously reduced and sequestered sulfide.[17,19] Results from the previous experiment in the same columns[17] and a concurrent study with like columns[16] correlated higher sulfate concentrations in planted treatments during the winter to higher measured redox values. In those studies the effect was more pronounced at COD concentrations <100 mg/L, as used in this study.

Reduced winter nitrogen removal has been attributed to decreased plant uptake during the dormant season and dramatically decreased microbial metabolism at colder temperatures.[23] Though the presence of plants positively influenced NH_4-N removal compared to unplanted controls, the performance of *Typha* was not seasonally dependent. Seasonal variation in *Carex* depended on organic carbon load. Other experiments have ascertained that plant assimilation is not an important nitrogen removal mechanism under typical wastewater nitrogen and carbon loads and stand establishment.[28–30] Therefore, plant uptake probably plays a minor role in seasonal variation of ammonium removal.

The temperature dependency of microbial metabolism likely influences overall wetland performance; however, the potential for certain species to increase oxygen availability in winter may mitigate the effect of reduced microbial metabolic rates at lower temperatures by favoring faster aerobic metabolic pathways.[25] Though our evidence is circumstantial, similar lines of reasoning explain increased winter nitrate levels and the seasonality of the relationship between ammonium removal and influent COD load. In summer, when oxygen is limiting, higher organic carbon load decreases the competitiveness of nitrifiers versus heterotrophs for available oxygen, limiting ammonium removal. In winter, when oxygen is more available in *C. rostrata* wetlands, increased organic carbon levels may be less inhibitory to nitrifiers while simultaneously stimulating denitrification. Though the importance of the nitrification/denitrification process in wetlands is debated as previously discussed, it is reasonable to expect seasonal variation in oxygen flux to the root rhizosphere to influence its effect.

The similarity in the ammonium depletion curves observed in the wetland columns (Fig. 1) and our ex-situ bottle experiments (Fig. 2) indicate that the consistently rapid initial ($<$24 h) ammonium removal is attributable to sorption, similar to findings of Sikora et al.[8] Even though sorption can only be considered a temporary storage mechanism, not a removal mechanism,[31] it may sequester ammonium making it available to subsequent microbial immobilization and/or nitrification/denitrfication. Temporal redox variation may also enhance this temporary storage mechanism. Batch or drain-fill operated SSF wetlands periodically introduce oxygen[25,32,33] rejuvenating redox dependent sorption sites.

Sorption may indirectly promote biofilm immobilization by accumulating nutrients at the gravel surface. An increasing number of studies suggest that SSF wetlands operate as anaerobic fixed-film reactors that may sequester nitrogen species and organic carbon within biofilms.[1,33] The 10-day drain-and-fill batch operation of our columns may have enhanced nitrogen removal through immobilization, as observed at higher drain-fill frequencies by Tanner et al.[32] The apparent assimilation of nitrogen by heterotrophs coupled with the stimulation of heterotrophic activity in winter due to higher redox levels also explains the seasonally-dependent relationship between carbon load and ammonium removal. During winter, *Carex* (*C*) columns displayed a rapid decrease in COD, correlating with significantly better NH_4-N removal compared to *Carex* (*NC*). Conversely, COD removal in *Carex* (*C*) columns was less rapid in summer and compounded by higher internal organic carbon production (Fig. 1), correlating with less rapid NH_4-N removal. Allen et al. and Hook et al.[17,18] attributed more rapid *Carex* COD removal in winter to higher redox levels.

CONCLUSIONS

Our results indicate that high levels of ammonium nitrogen removal are possible year-round in batch operated subsurface wetland systems. Though sorption of ammonium to the gravel was likely an important initial removal mechanism throughout our study, all evidence points to increased root-zone oxidation, especially for *C. rostrata* in winter, as the underlying reason for sustained differences in ammonium removal between species and season. This evidence includes: (1) better overall ammonium removal in planted treatments; (2) elevated nitrate and sulfate levels in winter; (3) better removal of exogenous organic carbon in winter; (4) higher measured redox in the same *Carex* columns in winter during a previous study;[17] and (5) the positive influence of increased carbon load on ammonium removal in winter but not summer. Increased root zone oxidation may have enhanced ammonium removal by increasing not only direct nitrification but also gravel sorption and heterotrophic immobilization, especially early in the batch incubation. Increased heterotrophic activity for *Carex* (*C*) columns in winter would have presumably increased microbial ammonium uptake. Though our results shed light on potential seasonal and plant effects on ammonium removal mechanisms in operational SSF wetlands, we recognize important differences between typical operational systems and our experimental units. The small size of our columns likely enhanced the effect of plants on all root zone processes, especially oxygenation. We deliberately excluded yearly variation in environmental factors such temperature and precipitation, and our "winter" did not include real factors such as snowfall and ice formation. We employed batch hydraulics, which permits intermittent (though infrequent) oxygenation, and eliminated all influent organic carbon for most treatments. The small size, relatively young age, hydraulic conditions, and substratum characteristics of the columns may have emphasized the temporary storage capabilities for nitrogen. Nonetheless, many of these characteristics can be incorporated into operational SSF wetlands, and our goal was not to mimic operational systems, but to elucidate potential seasonal and species effects on removal mechanisms and stimulate further research.

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