

Wetlands and Aquatic Processes

Particulates, Not Plants, Dominate Nitrogen Processing in a Septage-Treating Aerated Pond System

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ABSTRACT

In pond and wetland systems for wastewater treatment, plants are often thought to enhance the removal of ammonium and nitrogen through the activities of root-associated bacteria. In this study, we examined the role of plant roots in an aerated pond system with floating plants designed to treat high-strength septage wastewater. We performed both laboratory and full-scale experiments to test the effect of different plant root to septage ratios on nitrification and denitrification, and measured the abundances of nitrifying bacteria associated with roots and septage particulates. Root-associated nitrifying bacteria did not play a significant role in ammonium and total nitrogen removal. Investigations of nitrifier populations showed that only 10% were associated with water hyacinth [*Eichhornia crassipes* (Mart.) Solms] roots (at standard facility plant densities equivalent to 2.2 wet g roots L⁻¹ septage); instead, nitrifiers were found almost entirely (90%) associated with suspended septage particulates. The role of root-associated nitrifiers in nitrification was examined in laboratory batch experiments where high plant root concentrations (7.4 wet g L⁻¹, representing a 38% net increase in total nitrifier populations over plant-free controls) yielded a corresponding increase (55%) in the non-substrate-limited nitrification rate (V_{max}). However, within the full-scale septage-treating pond system, nitrification and denitrification rates remained unchanged when plant root concentrations were increased to 7.1 g roots L⁻¹ (achieved by increasing the surface area available for plants while maintaining the same tank volume). Under normal facility operating conditions, nitrification was limited by ammonium concentration, not nitrifier availability. Maximizing plant root concentrations was found to be an inefficient mechanism for increasing nitrification in organic particulate-rich wastewaters such as septage.

NITROGEN (N) REMOVAL from wastewater is mandated in most jurisdictions because of the toxicity of ammonium (NH₄) to aquatic fauna and the contribution of N compounds (including nitrite + nitrate [NO_x]) to the eutrophication of aquatic ecosystems. In pond and gravel bed wastewater treatment systems engineered for N removal, aquatic macrophytes are thought to contribute to N removal through a variety of mechanisms (Orth and Sapkota, 1988; Peterson and Teal, 1996; Weisner et al., 1994). Early research on plant roles in wastewater N removal focused on N uptake through biomass production (Reddy and Sutton, 1984). Aquatic plants may also promote microbial nitrification of NH₄

to NO_x via oxygen (O₂) loss from roots (Reddy et al., 1989). Plants are sources of organic carbon that can support the denitrification of NO_x to nitrogen gas (N₂) by providing carbon substrate and/or creating anaerobic microsites (Gersberg et al., 1986; Hamersley and Howes, 2002). Microenvironments surrounding submerged roots and leaves may also indirectly affect N cycling by supporting the activities of nitrifying and/or denitrifying bacteria. Plant roots create fixed attachment sites for N-processing bacteria, potentially increasing populations (Kaplan, 1983).

Direct N uptake by plants is proportional to biomass production rate and plant N content. Water hyacinth has frequently been used for wastewater N removal because of its growth rates (<42 dry g m⁻² d⁻¹) and N uptake (<19.7 mg N m⁻² h⁻¹) (Reddy and Sutton, 1984). Nitrogen removal by plant uptake requires regular biomass harvest, or else biomass N storage reaches steady state, where uptake is balanced by the return of N into the wastewater through senescence, leaching, and decay. The benefits of N removal through plant uptake are also limited by the potentially costly composting and drying procedures required for biomass disposal (Bagnall et al., 1987; DeBusk and Reddy, 1987). Regardless, plant uptake is only a partial solution to the problem of N disposal, since N is merely transferred from inorganic to organic forms, which can subsequently re-release inorganic N to the environment on decay.

Although plant uptake can remove NH₄ from wastewater, oxidation to NO_x by nitrifying bacteria is the dominant removal mechanism in most biological treatment systems. The contribution of O₂ lost through wetland plant roots (0.02–9.6 g m⁻² d⁻¹) to wastewater aeration may be small relative to O₂ diffusion from the atmosphere (Moorhead and Reddy, 1988; Howes and Teal, 1994), and unaerated constructed wetlands typically have low dissolved oxygen (DO) concentrations (approximately 0.5 mg L⁻¹). As a result, long retention times are required to reduce NH₄ concentrations to levels that allow discharge to aquatic environments (Dinges and Doersam, 1987; Reed and Brown, 1995). Although O₂ translocation is critical to aquatic plant survival, in practice it does not seem to contribute significantly to nitrification within wetland treatment systems (Watson et al., 1990; Reed and Brown, 1995). The high O₂ de-

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Abbreviations: DO, dissolved oxygen; DON, dissolved organic nitrogen; K_M , half-saturation (Michaelis) constant; MPN, most probable number; ON, organic nitrogen; PON, particulate organic nitrogen; TN, total nitrogen; TSS, total suspended solids; V_{max} , maximum (non-substrate-limited) nitrification rate.

mand of wastewaters may therefore require costly mechanical aeration to support adequate nitrification.

Wastewater NO_x removal through denitrification to N_2 requires an organic carbon substrate to proceed (Focht and Chang, 1975). However, in most aerobic biological wastewater treatment systems, organic carbon oxidation and nitrification occur simultaneously, resulting in an effluent high in NO_x but low in organic carbon. To support denitrification in these wastewaters, soluble organic carbon may be added to the nitrified process stream in the form of acetate, methanol, or food processing wastes (Isaacs and Henze, 1995; Halling-Sørensen and Jørgensen, 1993). Similarly, harvested wetland plant material has been successfully used to stimulate denitrification in constructed wetlands (Gersberg et al. 1986; Weisner et al., 1994; Hamersley and Howes, 2002). However, it is not clear whether organic carbon losses from living plants growing within the process stream contribute significantly to denitrification in wastewater.

The availability of attachment sites for nitrifying bacteria can limit nitrification rates when they are not limited by substrate (NH_4) and DO availability (Kaplan, 1983). In suspended-growth biological nutrient removal systems, suspended organic particles provide a substrate for bacterial biofilm growth, while in attached-growth systems, a fixed, high-surface-area mechanical support fills the role (Horan, 2002). Biofilms on fixed substrates

guard against washout of suspended bacteria, and plant root systems may provide a high-surface-area fixed biofilm substrate (40–200 times the plant cover area; Kinsinger et al., 1991). Although nitrifiers have been found attached to aquatic plant roots (Matulewich and Finstein, 1978; Ottová et al., 1997), the contribution of plants to total nitrifier populations in wastewater-treating ponds and wetlands has not yet been quantified.

In this study, we investigate the role of plants in nutrient uptake and in supporting nitrification and denitrification within a septage-treating aerated pond system. Septage is the waste solids periodically pumped from septic tanks and its disposal is an important problem in rural areas because of its high concentrations of recalcitrant organic matter and ammonium (Hamersley et al., 2001). The aerated pond system we studied was part of an engineered pond–wetland designed to treat septage to nutrient removal standards (Teal and Peterson, 1991, 1993; Teal et al., 1994; Peterson and Teal, 1996). In earlier publications (Hamersley et al., 2001; Hamersley and Howes, 2002) we constructed nitrogen and carbon budgets for the septage-treating pond–wetland system and its components, measured rates of N mineralization, nitrification, and denitrification reactions, and studied the role of septage particulate organic carbon in supporting denitrification in this highly particle-loaded system. During a 6-mo study period, the influent septage had a high organic matter and N content, with total suspended solids (TSS) concentrations averaging 45 times (7460 vs. 165 mg L^{-1}) and ammonium N ($\text{NH}_4\text{-N}$) more than three times (32.8 vs. 10.1 mg L^{-1}) local sewage levels (Hamersley et al., 2001). Raw septage was first treated by preliminary biological oxidation and sedimentation; further oxidation and nitrification took place in an aerated pond system containing floating tanks (Fig. 1). After secondary sedimentation, it flowed into a gravel wetland bed for final solids polishing and denitrification. Ninety-nine percent of the influent N was removed during treatment, primarily by sedimentation (58%) and sequential mineralization–nitrification–denitrification (41%). The total nitrogen (TN) concentration of the septage-treating wetland's effluent averaged 6.1 mg L^{-1} , well below that of a nearby septage-treating rotating biological contactor (52.6 mg L^{-1}). Effluent inorganic N concentrations were also low, with $\text{NH}_4\text{-N}$ averaging 0.6 mg L^{-1} and nitrate + nitrite N ($\text{NO}_x\text{-N}$) averaging 1.7 mg L^{-1} .

In the present study, we chose the aerated pond system portion of the septage-treating wetland to study the effects of plants on nitrogen processing and removal, since it was the site of rapid nitrification (0.61 $\text{mg L}^{-1} \text{h}^{-1}$) and the effects of plants as attachment sites for nitrifying bacteria could be studied separately from any oxygen-transporting capabilities. This system consisted of two trains of nine aerated tanks (0.75 m in diameter \times 1.5 m high; 640 L) each containing a mixture of floating plants (see Materials and Methods, below), and later, water hyacinth (Fig. 1). The influent to the tanks was septage that had been subjected to preliminary biological oxidation and sedimentation; however, organic nitrogen (ON) still accounted for 84% of TN, mostly (91%)

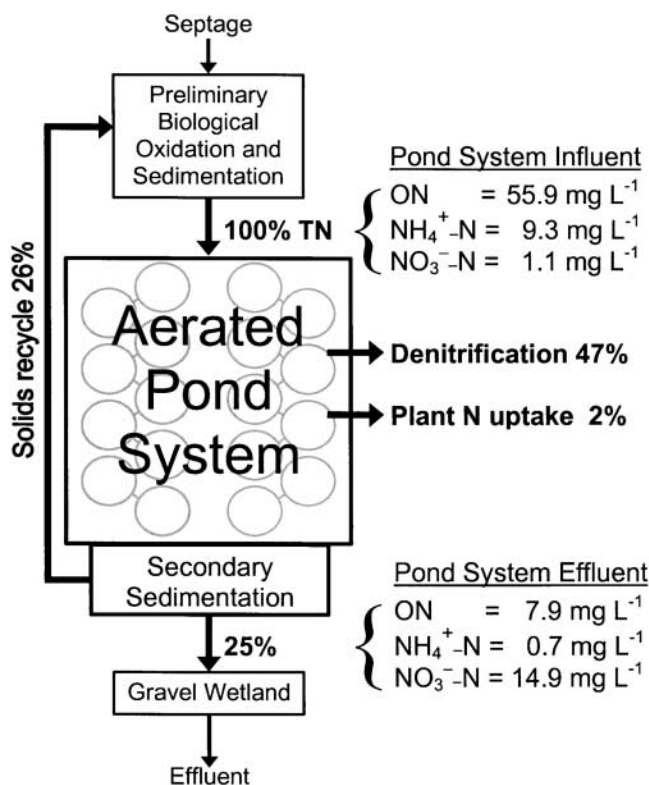


Fig. 1. Schematic of the Marion, MA septage-treating pond–wetland system (not to scale). Percentages indicate fate of total nitrogen (TN) flowing into the aerated pond system containing floating plants over a 6-mo period (flow-weighted mass balance; Hamersley et al., 2001). Concentrations indicate composition of N species in pond influent and effluent (flow-weighted average during 6 mo).

as particulates (Hamersley et al., 2001). Influent NH_4 and NO_x levels were similar to local sewage, but ON levels exceeded typical sewage levels by more than five-fold. During the 4.8-d retention time, 47% the influent TN was removed by denitrification, 26% as settled solids, and 2% by direct plant N uptake (Fig. 1). In this study, we quantified the distribution of nitrifying bacteria and their activity throughout the septage-treating pond system and in plant root material. We manipulated the abundance of rhizospheric nitrifiers both within the process stream and in laboratory experiments and measured the effect of these manipulations on nitrification and denitrification rates under different levels of substrate (NH_4) availability during septage treatment.

MATERIALS AND METHODS

Site Description

The study system was a 2200 L d^{-1} demonstration scale septage-treating artificial wetland in Marion, MA (designed and operated by Ecological Engineering Associates, Weston, MA) described in detail in an earlier study (Fig. 1; Hamersley et al., 2001). Our study focused on mechanisms of treatment in the aerated pond system of nine interconnected planted tanks. Each tank maintained a mixture of floating plants including willows (*Salix nigra* Marshall), water hyacinth, pennywort (*Hydrocotyle umbellata* L.), primula (*Primula veris* L.), and mint (*Mentha arvensis* L.). To study the role of plants in N removal within the aerated pond system, plant root to septage ratios were manipulated in three different experiments: (i) a batch experiment using laboratory microcosms (the "laboratory batch" experiment), (ii) an in situ experiment conducted under batch conditions with septage flow stopped and the tanks isolated (the "in situ batch" experiment), and (iii) an in situ experiment under normal operating conditions (the "in situ flow" experiment). We have expressed the different experimental treatment levels of plant roots and "root concentrations" in units of wet plant root weight per liter of septage. A concentration (L^{-1}) rather than a density (m^{-2}) measurement was chosen since in these well-mixed tanks, the plant roots came in contact with all of the septage in the tank. We chose wet root weight rather than dry weight or total biomass since we felt that this measure best expressed the physical capability of the plants to serve as attachment sites for bacteria. Although dry biomass density is the most common expression for plant density reported in the literature, comparisons with our values would be misleading, since the root to leaf ratios and organic matter composition of septage-grown plants differ from plants grown in other environments.

Nitrifying Bacteria

We determined the populations of nitrifying bacteria in raw septage, during preliminary biological oxidation (Fig. 1), in pond system influent, within the tanks of the aerated pond system, and in water hyacinth roots, using the most probable number (MPN) method (American Public Health Association, 1999). Septage and hyacinth roots from Tanks 1 through 9 were sampled on two dates. All samples were stored on ice for less than 24 h. The samples were then homogenized (VirTis [Gardiner, NY] blender) for 4 min (empirically derived time required to maximize MPN results). Test tubes containing sterilized ammonia oxidizer medium (Schmidt and Belser, 1982) mixed with phenol red indicator were inoculated with serial 10-fold dilutions of root and septage homogenates.

Laboratory Batch Experiment

To examine N processing in septage treated with and without root biomass, laboratory batch experiments were conducted with an N-Serve (2-chloro-6-[trichloromethyl] pyridine; Dow AgroSciences LLC, Indianapolis, IN) treatment added where nitrification was blocked. N-Serve inhibition of nitrification allowed an accurate determination of N mineralization rates from NH_4 -N accumulation. Twenty microcosms (4 L each) were filled with septage from Tank 2 ($266 \pm 11 \text{ mg L}^{-1}$ particulate organic carbon, $47.1 \pm 1.8 \text{ mg L}^{-1}$ ON, $0.23 \pm 0.01 \text{ mg L}^{-1}$ NH_4 -N). All microcosms were adjusted with NH_4Cl to bring the initial NH_4 -N concentration from the atypically low value to 6.65 mg L^{-1} (typical for this part of the treatment train; Hamersley et al., 2001) and to release nitrification from substrate limitation. Ten microcosms were stocked with water hyacinth from the tanks to a mean root concentration of 7.4 wet g L^{-1} ; the other ten were left without plants. A subsequent experiment was conducted with root concentrations of 12.4 g L^{-1} . N-Serve was added to half of the plant and half of the control microcosms, to a final concentration of 10 mg L^{-1} . The microcosms were aerated at levels equivalent to those used in the full-scale facility and incubated in daylight for 12 h, with 15-mL samples withdrawn at time zero and every 2 h thereafter. Samples were immediately cooled on ice, filtered ($0.45 \mu\text{m}$; Millipore, Bedford, MA), and analyzed for NH_4 within 1 h (see Analytical Methods, below). Samples were also collected at the beginning and end of the experiment for determination of NO_x and particulate organic nitrogen (PON) concentrations. Water volume in the microcosms was measured at the beginning and end of the incubations, and measured concentrations were corrected for evapotranspirative water losses. Nitrogen mineralization rates were calculated as the slope of a linear regression of the interval of linear NH_4 -N increase in the N-Serve treatment. The mineralization rate was subtracted from the NH_4 loss rate in the non-N-Serve-amended microcosms to obtain the nitrification rate during each 2-h sampling interval. The inhibition of nitrification by N-Serve was cross-checked by measuring the PON change from the beginning to the end of the experiment. Since the decline in PON equals N mineralization (dissolved organic nitrogen [DON] remains constant; Hamersley et al., 2001), it provided an independent estimate that was only 12% lower than the mineralization rate derived from NH_4 production under N-Serve. The NH_4 -N concentrations in control microcosms did not decline to nitrification-limiting levels (only one point of $<1 \text{ mg L}^{-1}$) during the first experiment, so the experiment was repeated with control microcosms with a lower initial NH_4 -N concentration and data from both experiments were pooled for analysis. In both the in situ and laboratory batch experiments, nitrification rates were determined at 2-h intervals while NH_4 -N levels decreased, which permitted a nonlinear fit of the Michaelis-Menten equation (Stryer, 1988):

$$R_N = \frac{V_{\max} \times [\text{NH}_4]}{K_M + [\text{NH}_4]} \quad [1]$$

where R_N is the nitrification rate, V_{\max} is the maximum (non-substrate-limited) nitrification rate, and K_M is the half-saturation constant. The V_{\max} thus estimated was used to separate substrate limitation of nitrification from the effects of the experimental manipulations of the plant root-nitrifier complex.

In Situ Experiments

To study the effect of plants in situ in the full-scale aerated pond system, we redirected two-thirds of the flow from Tank 2 in each of the two tank trains to an experimental tank system,

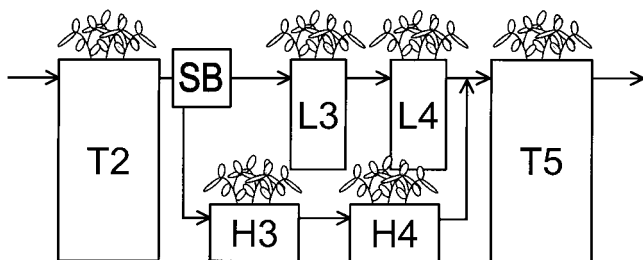


Fig. 2. Schematic of experimental tank system with varying root concentrations. Plant root concentrations were manipulated by changing the proportions of the tanks; tank volumes and hydraulic retention times were the same across treatments. One such experimental tank system was inserted into each of the two pond system tank trains. T2 and T5, aerated Tanks 2 and 5; SB, flow splitter box; L3 and L4, low root concentration treatment (2.5 g L^{-1} septage); H3 and H4, high root concentration treatment (7.1 g L^{-1} septage).

after which it returned to Tank 5 (Fig. 2). We selected Tanks 3 and 4 for the experimental manipulations because NH_4 levels here were less likely to be limiting to nitrification, permitting the experiments to focus on the role of plants (Hamersley et al., 2001). In addition, since the essential processes controlling N transformation in all the tanks are independent of location within the train, temporal variation in waste stream characteristics allowed study of conditions similar to those found at locations throughout the treatment train through long-term measurement of these experimental tanks. Two parallel subtrains of two tanks each were inserted in series into each of the two main treatment trains for a total of eight experimental tanks (Fig. 2). The experimental tanks were stocked with hyacinth at the same areal density as the standard tanks. However, although all of the tanks had the same volume (186 L, one-third of the standard tank volume), the tanks of one subtrain were shallow (0.4 m), providing nearly three times the standard root concentration (7.1 vs. 2.2 wet g root L^{-1} septage). The tanks of the other subtrain had a depth (1.2 m) similar to the standard tanks (1.4 m), hence a similar root concentration (2.5 g root L^{-1} septage). The distribution of flow to the subtrains was periodically checked using graduated flasks and a stopwatch, and adjusted to maintain equal flow across all treatments. Chloride tracer studies (unpublished data, 2002) during system operation showed that flow in both the standard and the experimental tanks conformed to the tanks-in-series model (Ruzicka and Hansen, 1988). The model showed that the effective mixed volume of the experimental tanks averaged 72% of the total tank volume, for a mean hydraulic retention time of 4.2 h per tank. The hydraulic retention times of each subtrain were the same; only the depth and therefore the root concentration were altered.

For the in situ batch experiment, after two weeks of equilibration we temporarily halted septage flow, and prevented flow between the tanks (Fig. 2). During the subsequent incubation, variation in septage characteristics and flow rate was eliminated, and all transformations and losses of N resulted from in situ processes alone. The batch experiment lasted 12 h with sampling at 2-h intervals. Initial NH_4 -N concentrations varied from tank to tank because of their respective positions in the treatment train, permitting study of the effects of both plant root concentration and substrate (NH_4) limitation on nitrification. For the in situ flow experiment, we sampled septage from Tank 2 and the experimental tanks in both trains eight times during 22 d of normal flow and operating conditions.

During both of the in situ experiments, septage samples for nutrient analysis were analyzed for particulate organic

nitrogen (PON), dissolved organic nitrogen (DON), NH_4 -N, and NO_x -N (see Analytical Methods, below). We determined N transformation rates in the in situ batch experiment from temporal changes within a single tank. Rates during the in situ flow experiment were determined from changes in N species concentrations between two adjacent tanks, divided by the tank hydraulic retention time (method detailed in Hamersley et al., 2001). Nitrogen mineralization rates were determined from changes in organic nitrogen ($\text{ON} = \text{PON} + \text{DON}$) concentrations, nitrification rates (including all net NH_4 -oxidizing processes) from changes in ON and NH_4 -N, and denitrification rates (including all gaseous N losses) by difference from the changes in TN (ON , NH_4 -N, and NO_x -N). Losses through sorption and sedimentation were ruled out, and the only other potential N loss pathway was by plant uptake, which was known to be $<4\%$ of nitrification-denitrification losses (Hamersley et al., 2001). Temperature, flow rate, and DO (calibrated YSI [Yellow Springs, OH] oxygen meter) were measured concurrently with water sampling. Mechanical aeration (as in the standard tanks) averaged $1.8 \text{ m}^3 \text{ m}^{-3}$ septage h^{-1} , and was adjusted to maintain a DO level of $>5.0 \text{ mg L}^{-1}$ (mean $7.2 \pm 0.2 \text{ mg L}^{-1}$) in all tanks. Aeration was kept high to study the interaction between the availability of root-associated nitrifiers and substrate on nitrification rates without limitation by aeration.

Plant Growth

During the in situ experiments, the experimental tanks were planted with a mixture of plants (water hyacinth, willow, pennywort, primula, and mint). During this period, plants were harvested only as needed, and were not managed for maximum growth rates. The weight of all plants harvested was recorded, and subsamples taken for N content determination. In the following year of study, the tanks were planted with water hyacinth only, and these plants were used for the laboratory batch experiments, MPN measurements of nitrifiers, and quantification of maximum plant N uptake rates. To determine maximum plant N uptake and study the effect of root concentration on uptake rates, half the hyacinths in each experimental tank were harvested every two weeks to maintain exponential growth. Growth rates in each of the eight tanks were determined by summing the final plant biomass with the weight of plants harvested during a 69-d growth period (September–November), and subtracting the initial plant biomass. Plant roots were gently rinsed to remove excess septage particulates. Tops and roots were weighed separately and dried at 60°C to constant weight before a subsample was ground for C and N content analysis on an elemental analyzer.

Analytical Methods

All septage samples collected during the in situ flow and in situ batch experiments were collected in duplicate and immediately transported (45 min) to the laboratory on ice. Upon reaching the laboratory, the first sample was pressure-filtered ($0.22 \mu\text{m}$) and the filtrate assayed for NH_4 -N, NO_x -N, and DON. The NH_4 -N was analyzed immediately (to prevent ammonia volatilization) by a colorimetric indophenol method (Scheiner, 1976). The NO_x -N was analyzed with azo dye after cadmium reduction using a Lachat (Milwaukee, WI) automated ion analyzer (Wood et al., 1967). Total dissolved N was measured as NO_3 following persulfate oxidation (D'Elia et al., 1977). Dissolved organic N was measured as the difference between total dissolved N and NH_4 -N and NO_x -N. The second sample was vacuum-filtered through tared precombusted Whatman (Maidstone, UK) GFF filters and dried to constant

weight (60°C) to collect solids for TSS, particulate organic carbon, and PON content determination on a PerkinElmer (Wellesley, MA) Model 2400 CHN elemental analyzer. All reported values are means \pm standard error (SE).

RESULTS

The abundance of nitrifying bacteria associated with septage increased from 0.8×10^8 MPN L⁻¹ in raw septage to 1.4×10^8 MPN L⁻¹ during preliminary biological oxidation, in part because of the influx of activated solids from secondary sedimentation (Table 1, Fig. 1). Nitrifier levels in the pond system influent (2.0×10^8 MPN L⁻¹) were 2.5 times higher than in the raw septage, and reached their peak in passage through the aerated pond system by Tank 2 (6.0×10^8 MPN L⁻¹). Nitrifier concentrations declined (along with NH₄ concentrations; Hamersley et al., 2001) to 1.6×10^8 MPN L⁻¹ by Tank 9, and the average concentration within the pond system was 3.7×10^8 MPN L⁻¹. A 4-min sample homogenization was required to maximize nitrifier recovery, suggesting that nitrifiers within the septage are associated with organic particulates, rather than existing free in the water, since disturbance was required to dissociate nitrifiers from the particles. Further, the highest nitrifier levels in the facility were found within the solids generated by secondary sedimentation (5.0×10^8 MPN L⁻¹), while the supernatant (pond system effluent) had the lowest nitrifier levels (0.008×10^8 MPN L⁻¹). When nitrifier abundances were normalized to particulate concentrations (TSS), patterns of change in particle-associated nitrifiers can be seen independently of sedimentation effects. The TSS-specific nitrifier concentration increased until the pond system tanks, but by secondary sedimentation, both the recycled solids and the supernatant (pond system effluent) solids had lower concentrations than in other parts of the facility (Table 1). However, the TSS-specific nitrifier concentration was the same in both the solids and the supernatant (pond system effluent), indicating that partitioning of nitrifiers by sedimentation matched the partitioning of solids, further support for the dominance of particulate-associated nitrifiers in this system. Nitrifiers were found associated with hyacinth roots at 0.19×10^8 MPN wet g⁻¹. In a standard tank in the aerated pond system (2.2 g roots L⁻¹), the standing stock of nitrifiers found on plant roots therefore represents only an 11% enhancement over the number of septage particulate-associated nitrifiers present in the tank.

The laboratory batch experiment demonstrated that the presence of plants could affect non-substrate-limited nitrification rates. Initially, we observed some sorption of added NH₄ onto septage particulates in N-Serve-amended treatments (Fig. 3a). The NH₄-N concentration subsequently increased as a result of N mineralization unbalanced by nitrification. In the plant-containing N-Serve-amended treatments, NH₄-N levels began to decline after 10 h, probably due to N-Serve degradation and the resulting release of nitrification from inhibition. The linear portion of the time course was therefore used to calculate mineralization rates. The mean rate

Table 1. Most probable number (MPN) of nitrifying bacteria in water from various sources in the septage-treating wetland and on plant roots from the aerated pond system. Column showing nitrifying bacteria in septage normalized to total suspended solids (TSS) illustrates changes in particle-associated nitrifier concentrations independent of sedimentation effects. Only MPN tank⁻¹ values allow comparison of water and plant root nitrifier abundances. Values are means, with standard errors in parentheses. Dashes indicate value not applicable (no tank volume).

Source [†]	Water			Plant roots (2.2 wet g L ⁻¹)	
	MPN L ⁻¹	MPN L ⁻¹ mg ⁻¹ TSS [‡]	MPN tank ⁻¹	MPN g ⁻¹	MPN tank ⁻¹
Influent raw septage	0.8×10^8	1.1×10^4	—	—	no plants
Preliminary biological oxidation	1.4×10^8	1.9×10^4	—	—	no plants
Pond system influent	2.0×10^8	2.1×10^4	—	—	no plants
Pond system tanks [‡]	3.7×10^8 (0.5×10^8)	82×10^4 (3.4×10^4)	2.3×10^{11} (0.3×10^{11})	0.19×10^8 (0.06×10^8)	0.26×10^{11} (0.08×10^{11})
Pond system effluent	0.008×10^8	0.59×10^4	—	—	no plants
Recycled solids	5.0×10^8	0.58×10^4	—	—	no plants

[†] See Fig. 1 for sample locations.

[‡] Tanks 1–9, Water, $n = 15$; plants, $n = 13$.

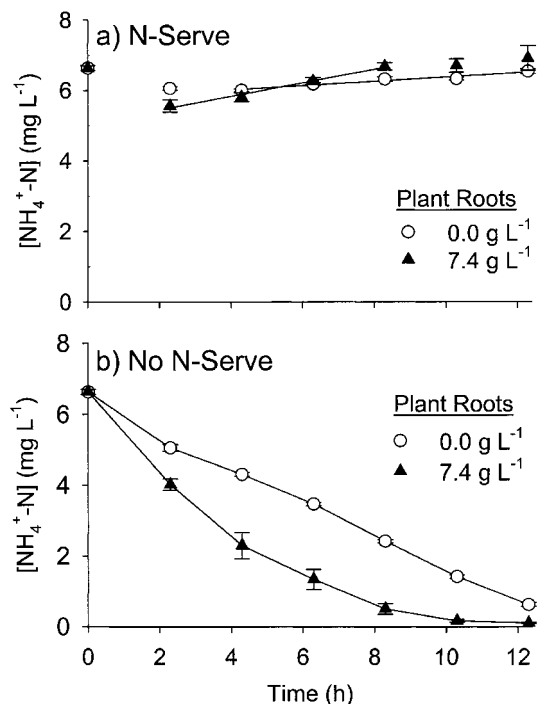


Fig. 3. Change in $\text{NH}_4\text{-N}$ concentration with time during laboratory batch incubations of septage. Rates of change for particulate organic nitrogen (PON) and $\text{NO}_x\text{-N}$ are reported in the text. Values are means \pm SE ($n = 5$); error bars are often smaller than points. (a) Increase in $\text{NH}_4\text{-N}$ concentration due to N mineralization in treatments where nitrification was inhibited by addition of N-Serve. Initially, $\text{NH}_4\text{-N}$ concentration declined due to sorption onto septage particulates. In treatments containing plant roots, N-Serve inhibition of nitrification decreased after 8 h. Nitrogen mineralization rates were calculated from the slopes (\pm SE) of linear regressions (lines shown) of unaveraged $\text{NH}_4\text{-N}$ concentrations during the period of linear $\text{NH}_4\text{-N}$ increase. Nitrogen mineralization was threefold higher with plant roots present ($0.190 \pm 0.031 \text{ mg N L}^{-1} \text{ h}^{-1}$, $n = 20$, $P < 0.001$), versus absent ($0.0605 \pm 0.0089 \text{ mg N L}^{-1} \text{ h}^{-1}$, $n = 25$, $P < 0.001$). (b) Decrease in $\text{NH}_4\text{-N}$ concentration, due to nitrification in excess of N mineralization in treatments containing no plants or 7.1 g plant roots L^{-1} . Earlier work showed that plant uptake was $<4\%$ of denitrification losses (Hamersley et al., 2001).

of mineralization in the control microcosms was $0.061 \pm 0.008 \text{ mg N L}^{-1} \text{ h}^{-1}$, compared with $0.190 \pm 0.015 \text{ mg N L}^{-1} \text{ h}^{-1}$ in the plant-containing microcosms ($7.4 \text{ g roots L}^{-1}$), probably due to mineralization of particulate organic matter trapped on the roots. The large continuous decline in $\text{NH}_4\text{-N}$ concentrations in the non-N-Serve-amended treatments indicates nitrification rates in excess of N mineralization rates (Fig. 3b). The decrease in $\text{NH}_4\text{-N}$ levels reflects the sum of $\text{NH}_4\text{-N}$ -producing mineralization and $\text{NH}_4\text{-N}$ -consuming nitrification reactions. The rate of net $\text{NH}_4\text{-N}$ decrease was initially greater in plant-containing versus control treatments, but net $\text{NH}_4\text{-N}$ change decreased during the experiment, as declining NH_4 levels limited nitrification rates.

Nitrogen mineralization rates (determined from the N-Serve treatments) were added to the net $\text{NH}_4\text{-N}$ change in non-N-Serve treatments to determine the nitrification rate during each 2-h period (Fig. 4). Both plant and control treatments showed substrate-limited

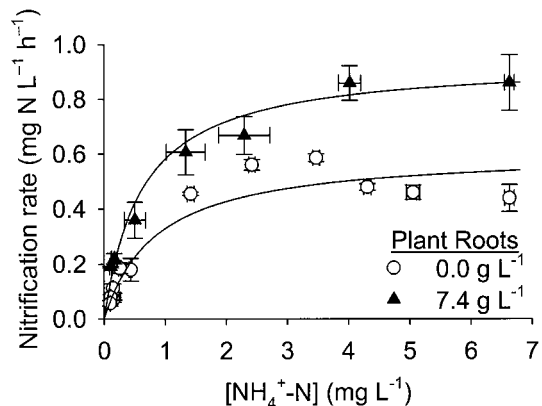


Fig. 4. Substrate limitation of nitrification in laboratory batch experiment differs between treatments containing plants versus no plants. Values are means \pm SE ($n = 5$). Lines are nonlinear Michaelis–Menten fits to unaveraged data points for calculation of maximum (non-substrate-limited) nitrification rate (V_{\max}) and half-saturation (Michaelis) constant (K_M). No plant roots treatment (0.0 g L^{-1}): $V_{\max} = 0.608 \pm 0.031 \text{ mg N L}^{-1} \text{ h}^{-1}$, $K_M = 0.85 \pm 0.14 \text{ mg N L}^{-1}$, $n = 70$, $R^2 = 0.85$, $P < 0.001$. Plant-containing treatment ($7.4 \text{ g roots L}^{-1}$): $V_{\max} = 0.942 \pm 0.047 \text{ mg N L}^{-1} \text{ h}^{-1}$, $K_M = 0.62 \pm 0.12 \text{ mg N L}^{-1}$, $n = 35$, $R^2 = 0.88$, $P < 0.001$. The V_{\max} of nitrification in treatments containing plants was significantly higher than that of treatments containing no plants ($P < 0.001$), but no significant difference was found for K_M .

kinetics, and there was a significant difference between the responses of the two treatments. The V_{\max} of nitrification in control microcosms ($0.608 \pm 0.031 \text{ mg L}^{-1} \text{ h}^{-1}$; nonlinear Michaelis–Menten fit) was 35% lower than that of the plant-containing treatment ($0.942 \pm 0.047 \text{ mg L}^{-1} \text{ h}^{-1}$) (Fig. 4; one-tailed t test, $P < 0.001$), though no significant difference was found in K_M ($P > 0.05$). Denitrification rates (measured as $\text{NO}_x\text{-N}$ loss under N-Serve amendment) in control and plant-containing microcosms in this experiment did not significantly differ (mean $0.13 \pm 0.01 \text{ mg N L}^{-1} \text{ h}^{-1}$; two-tailed t test, $P > 0.10$), but a significant increase (to $0.27 \pm 0.03 \text{ mg N L}^{-1} \text{ h}^{-1}$; two-tailed t test, $P = 0.004$) was observed at the highest tested plant root concentration (12.4 g L^{-1}).

The in situ batch experiment indicated that nitrification within the aerated pond system was controlled by $\text{NH}_4\text{-N}$ concentration rather than plant root concentration (Fig. 5). Nitrification rates were highest at the start of the experiment when $\text{NH}_4\text{-N}$ concentrations were high, but declined during the course of the experiment as $\text{NH}_4\text{-N}$ concentration declined (as a result of nitrification in excess of N mineralization rates). No significant difference was found between Michaelis–Menten constants (V_{\max} and K_M) derived from separate nonlinear fits to data from high and low plant treatments (two-tailed t test, $P > 0.01$). When all treatments were pooled, nitrification rates followed substrate-limited kinetics with a V_{\max} of $0.811 \pm 0.056 \text{ mg NH}_4\text{-N L}^{-1} \text{ h}^{-1}$ and a K_M of $0.75 \pm 0.13 \text{ mg NH}_4\text{-N L}^{-1}$ ($P < 0.001$, $R^2 = 0.76$).

Likewise, under normal facility operating conditions during the 22 d of the in situ flow experiment, root concentration did not significantly affect mean N mineralization, nitrification, or denitrification rates within the experimental tanks (Table 2, Fig. 2) ($2.5 \text{ g roots L}^{-1}$ vs. $7.1 \text{ g roots L}^{-1}$; two-tailed t test, $P > 0.50$). In both

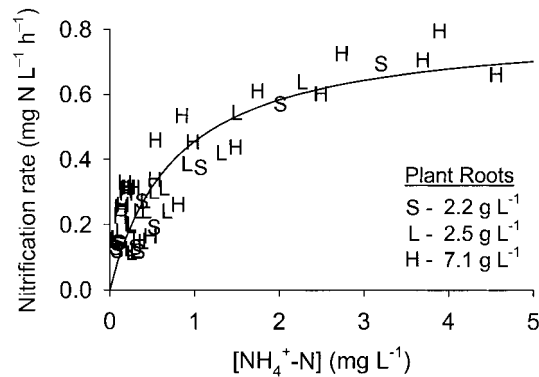


Fig. 5. Substrate limitation of nitrification by $\text{NH}_4\text{-N}$ concentration follows Michaelis–Menten-type kinetics in eight experimental and two standard tanks during the in situ batch experiment (Fig. 2). Each point represents conditions in a single tank over a 2-h period, with measurements repeated every 2 h for 12 h total. Initial $\text{NH}_4\text{-N}$ concentrations varied from tank to tank due to differences in in situ conditions at start of experiment when flow between tanks was stopped. No significant differences were found between the parameters maximum (non-substrate-limited) nitrification rate (V_{\max}) and half-saturation (Michaelis) constant (K_M) of nonlinear Michaelis–Menten fits to high and low (including standard) plant root treatments ($P > 0.10$). Line shows the Michaelis–Menten fit to the pooled data set. $V_{\max} = 0.811 \pm 0.059 \text{ mg N L}^{-1} \text{ h}^{-1}$, ($P < 0.001$), $K_M = 0.78 \pm 0.13 \text{ mg N L}^{-1}$, ($P < 0.001$), $n = 60$, $R^2 = 0.76$.

treatments, nitrification was the most active N transformation process, followed by N mineralization and denitrification. Similarly, root concentrations did not significantly affect concentrations of ON, $\text{NH}_4\text{-N}$, or $\text{NO}_x\text{-N}$, neither within the experimental tanks nor in their effluent (two-tailed t test, $P > 0.50$). During the 8.4-h retention time of the experimental tanks, ON decreased from 30.2 to 22.2 mg L^{-1} , and $\text{NH}_4\text{-N}$ decreased from 6.7 to 3.5 mg L^{-1} . These changes in the reduced N pools were due to net oxidative processes, as confirmed by the increase in NO_x from 1.9 to 10.1 mg L^{-1} during treatment resulting from more rapid nitrification than denitrification within the experimental tanks.

When hyacinth N uptake rates were maximized by frequent harvest, plants at standard concentrations (2.5 g roots L^{-1}) grew at $29.6 \pm 2.5 \text{ g biomass m}^{-2} \text{ d}^{-1}$, while plants at high concentrations (7.1 g roots L^{-1}) grew less than half as fast, $13.3 \pm 4.1 \text{ g m}^{-2} \text{ d}^{-1}$ (Table 3). Nitrogen uptake rates expressed on an areal basis showed the same pattern as biomass, with a rate of $1.69 \pm 0.14 \text{ g N m}^{-2} \text{ d}^{-1}$ at the standard root concentration, and $0.76 \pm 0.23 \text{ g N m}^{-2} \text{ d}^{-1}$ at the high root concentration. How-

Table 2. Rates of major N transformations and concentrations of selected N species during the in situ flow experiment where the experimental tank system (see Fig. 2) contained different plant root concentrations. Values are means, with standard errors in parentheses.

	Plant root concentration (g L^{-1})	
	2.5†	7.1‡
	Rate, $\text{mg N L}^{-1} \text{ h}^{-1}$ §	
N mineralization	0.47 (0.06)	0.56 (0.09)
Nitrification	0.72 (0.10)	0.76 (0.12)
Denitrification	0.26 (0.07)	0.29 (0.10)
	Concentrations within the experimental tanks, mg L^{-1} §	
Organic N (ON)‡	24.3 (2.0)	23.8 (1.8)
$\text{NH}_4\text{-N}$	4.3 (0.7)	5.3 (0.9)
$\text{NO}_x\text{-N}$	8.1 (1.3)	7.4 (1.2)
	Concentrations in influent and effluent of experimental tanks (Fig. 2; retention time 8.4 h), mg L^{-1} §	
	Influent¶	Effluent
ON#	30.2 (2.7)	22.1 (2.9)
$\text{NH}_4\text{-N}$	6.7 (1.2)	3.0 (0.5)
$\text{NO}_x\text{-N}$	1.9 (1.0)	10.4 (2.0)

† Sample size = 13.

‡ Sample size = 12.

§ No significant differences between root concentration treatments (two-tailed t test, $P > 0.50$).

¶ Sample size = 14.

Particulate ON + dissolved ON. Mean dissolved ON was 4.95 (0.12) mg L^{-1} throughout the aerated pond system.

ever, the pattern was reversed when N uptake was normalized for tank volume, with a lower rate found at the standard root concentration ($0.076 \pm 0.006 \text{ mg N L}^{-1} \text{ h}^{-1}$) than at the high root concentration ($0.113 \pm 0.034 \text{ mg N L}^{-1} \text{ h}^{-1}$). Hyacinth biomass was $5.2 \pm 0.04\%$ of wet weight and the N content of dry hyacinth biomass was 5.7%. Roots accounted for 18% of the total wet weight of the plants.

DISCUSSION

Under normal operating conditions in the septage-treating aerated pond system, direct plant uptake played little role in N removal. An N mass balance showed that during 6 mo of operation, plant uptake accounted for <2% of fate of N (Fig. 1). Sedimentation played an insignificant role (until secondary sedimentation), and the balance of the N removal was through denitrification (47%) (Hamersley et al., 2001). Taking the septage-treating wetland facility as a whole, plants played an even smaller role, removing only 0.5% of the influent

Table 3. Plant growth in the aerated pond system during normal operation compared with plant N uptake maximization experiment. Values are means, with standard errors in parentheses.

Plant growth	Plant root concentration (wet g L^{-1})†		
	Normal operation‡	2.5	7.1
Dry $\text{g biomass m}^{-2} \text{ d}^{-1}$	6.3	29.6 (2.5)	13.3 (4.1)
$\text{g N m}^{-2} \text{ d}^{-1}$	0.36	1.69 (0.14)	0.76 (0.23)
$\text{mg N L}^{-1} \text{ h}^{-1}$	0.016	0.076 (0.006)	0.113 (0.034)
Plant N uptake as % of total N removal	2	13	19

† 1 $\text{g wet root} = 0.29 \text{ mg dry total plant biomass}$.

‡ Data from mass balance study of 6 mo of normal system operation using a mixture of plant species (described in Materials and Methods) (recalculated from Hamersley et al., 2001).

§ Water hyacinth.

TN as plant biomass (Hamersley et al., 2001). The effects of plants on N processing appeared to be indirect, primarily through the activities of root-associated nitrifying bacteria. However, at the standard root concentrations found in the aerated pond system, the enhancement of the total population of nitrifiers by plant root-associated nitrifiers was only 11% because of the high numbers of nitrifiers present in the particulate-rich septage (Table 1). Under conditions of no substrate (NH_4) limitation in laboratory batch experiments, the presence of plant roots at three times the standard concentration (a 38% enhancement of nitrifier populations over septage alone) increased the V_{max} of nitrification by 55% over control microcosms (Fig. 4). Nevertheless, under normal operation (the in situ flow experiment), when NH_4 availability rather than nitrifier abundance frequently limits nitrification, there was no significant change in nitrification or denitrification rates associated with a threefold increase in root concentrations over standard concentrations.

Nitrification

Plants play both direct and indirect roles within the septage-treating aerated pond system. The direct role of the plants was through plant N uptake, which was low and probably undesirable. Their primary indirect role was via enhancement of the nitrifying community within the aerated pond system. When substrate (NH_4) or DO are not limiting, nitrification can be controlled by the abundance of active nitrifying bacteria present in biofilms in the tank environment (Kaplan, 1983). Under these conditions, the contribution of plant root-associated nitrifiers could play a role in promoting nitrification. The potential influence of nitrifier concentration on nitrification (when not limited by other factors) is supported by the laboratory batch experiments, where the increase in V_{max} of nitrification in plant-containing versus unplanted treatments was consistent with the increase in the total nitrifier population (Fig. 4).

Hyacinth roots from the septage-treating pond system held high numbers of nitrifiers (0.19×10^8 MPN g^{-1} roots). In contrast, in an unaerated constructed gravel wetland treating pretreated high- NH_4 sewage, nitrifier abundances were two to four orders of magnitude lower (1–100 MPN mg^{-1} ; *Glyceria* spp.) (Ottová et al., 1997). Despite the high nitrifier levels we found, the abundance of root-associated nitrifiers per dry root weight was less than half that of septage particulates (3.3×10^5 MPN mg^{-1} roots vs. 8.2×10^5 MPN mg^{-1} TSS). Further, at the root concentration used in the aerated pond system, only 10% of the nitrifiers were root-associated, with the balance found on organic septage particulates.

Plant root-associated nitrifier populations in the aerated pond system could be increased by choosing plants with longer root systems, by increasing plant areal densities, or by increasing root concentrations (by reducing the water depth, as in the experimental tanks [Fig. 2]). In the nutrient-rich medium of the aerated pond system, water hyacinth roots did not appear to be well-developed and extended about 10 to 20 cm below the water

surface. Selecting plant species with longer root systems might well increase nitrifier concentration, but as with other methods of increasing root concentration, the resulting decline in the effectively mixed volume of the tanks (from the normal value of 72%) would decrease the hydraulic retention time and probably offset any gains in nitrification rates. Reducing harvest frequency would result in a tightly packed root mass, which would efficiently reduce undesirable plant N uptake as well. Nevertheless, the maximum observed root concentration in the standard tanks (3.6 g L^{-1}) resulting from unharvested growth corresponds to a total tank nitrifier concentration only 6% above normal, unlikely to significantly increase nitrification rates. A tightly packed root system would also have a lowered hydraulic conductivity, presenting a lower surface area for contact with NH_4 -rich wastewater. Finally, as in the in situ experiments, shallower tanks could be used to significantly increase total nitrifier concentrations; however, at the high root concentrations used in the in situ experiments (7.1 g L^{-1}), equivalent to a tank depth of only 0.4 m, nitrifier concentration could be increased by only 23%, while land use would increase nearly 3.5 times.

In practice, moreover, increasing nitrifier abundances through manipulating root concentration did not affect nitrification rates. The threefold root concentration increase of the in situ experiments raised the nitrifier abundance by 23% (calculated from Table 1), but resulted in no significant change in the mean nitrification rate (Fig. 5, Table 2). The absence of significant differences between treatments in these experiments is not surprising since the increase in total nitrifier populations above standard levels (23%) was similar to the coefficient of variation of the nitrification rates (14–16%) observed during the experiment. However, the absence of an effect of plant roots on nitrification in these experiments primarily reflects the dominance of controls other than nitrifier abundance on nitrification under in situ conditions in the septage-treating pond system. Earlier work showed that nitrification was limited primarily by oxygen availability in the first half of the treatment tank train, and by NH_4 availability in the last half (Hamersley et al., 2001). Despite high DO levels in the bulk fluid of the first five tanks, the high rates of organic matter degradation lowered biofilm O_2 concentrations, and caused competition for O_2 between nitrifiers and heterotrophs (van Loosdrecht et al., 1995). As respiration rates and NH_4 concentrations decreased through the treatment process train, NH_4 became limiting to nitrification (Fig. 5; $K_M = 0.78 \text{ mg NH}_4\text{-N L}^{-1}$).

Under the highly particle-loaded conditions of septage treatment, plants appear to add little to nitrifier concentrations, and methods of increasing plant contributions may lead to unwanted side effects such as increased plant N uptake and hydraulic interference. In any case, nitrification in the pond system was usually limited by factors other than nitrifier concentration. Attempts to increase root concentrations, as with plant N uptake, are instead likely to be detrimental to efficient N removal in the septage-treating pond system.

Denitrification

In a previous study in the septage-treating aerated pond system, denitrification was stimulated under normal facility operating conditions by the addition of dried plant material in mesh bags placed into Tanks 7 through 9 (Hamersley and Howes, 2002). The stimulation of denitrification was shown to be due to the formation of anaerobic microsites within and around particulate plant material, since sufficient organic carbon to support denitrification was already available from the degradation of septage solids. Leaching of additional dissolved organic carbon from living plant roots into the tanks is therefore unlikely to further stimulate denitrification. In addition, although *particulate* plant detritus from senescing plants could potentially enhance denitrification, no such enhancement was observed in either of the in situ experiments of the present study (Table 2). Enhancement of denitrification rates by plants was only observed in one laboratory batch experiment when root concentrations were increased to 12.4 g L^{-1} , more than five times the standard concentration. We attributed this increase to the formation of anoxic zones resulting from obstruction of aeration by plant roots that would have interfered with hydraulic conductivity in a full-scale facility. Evidence for a stimulating role for plants on denitrification, particularly in highly carbon- and particle-loaded septage, is inconclusive. Plants are more likely to have an effect in wastewaters with low organic carbon availability, where plant detritus can provide supplemental carbon to support denitrification.

Plant Growth in Septage

The growth rates of water hyacinth observed in this study ($6.3\text{--}29.6 \text{ g dry wt. m}^{-2} \text{ d}^{-1}$) were at the low end of the range reported for sewage facilities primarily in warm climates ($21\text{--}65 \text{ g dry wt. m}^{-2} \text{ d}^{-1}$; reviewed by Reddy and Sutton, 1984 and Kawai et al., 1987) (Table 3). The lower production rates are probably due to the season (fall) and high latitude (42° N) in which the study was conducted. In contrast, the N removal rates in our study ($0.36\text{--}1.69 \text{ g N m}^{-2} \text{ d}^{-1}$) exceeded the range reported for sewage systems ($0.33\text{--}0.47 \text{ g N m}^{-2} \text{ d}^{-1}$) and growth in nutrient solutions ($0.69\text{--}1.47 \text{ g N m}^{-2} \text{ d}^{-1}$) (Reddy and Sutton, 1984). The mean N content of hyacinth grown in septage was also high (5.7% dry wt.) compared with the N content reported for sewage-grown hyacinth (2.9–3.7%) (Reddy and Sutton, 1984; Kawai et al., 1987). Higher septage nutrient content probably contributed to the high biomass N content and uptake rates, and the relatively slow biomass growth rate may have permitted luxury N uptake. The high N content of septage-grown hyacinth was not an artifact associated with septage solids attached to plant roots, since root N content (4.9%) was lower than that of shoots (5.9%).

Under the exponential growth conditions of the plant growth maximization experiment, plant N uptake in the aerated pond system could potentially be made to account for as much as 19% of TN removal (Table 3). Nevertheless, taking the septage-treating wetland fac-

ity as a whole, we do not believe that maximizing plant N uptake is either efficient or economical. With plants unmanaged for growth, plant N uptake averaged only 0.5% of the total facility N input (Hamersley et al., 2001). Even without significant plant N uptake, only 1% of the influent N remained in the effluent ($\text{TN} = 6.1 \text{ mg L}^{-1}$), while the remainder was removed through the microbial processes of mineralization of labile organic N, nitrification–denitrification, or the sedimentation of recalcitrant organic N. Since only $0.56 \text{ mg L}^{-1} \text{ NH}_4\text{-N}$ and $1.7 \text{ mg L}^{-1} \text{ NO}_x\text{-N}$ remained in the final effluent, nearly all of the dissolved inorganic N mineralized during treatment was nitrified and denitrified. Greater plant uptake would only remove dissolved inorganic N from the pool available for nitrification–denitrification. Since denitrification represents a true removal (to N_2) of biologically available N (in contrast to plant uptake, which merely transfers it to another form: biomass), denitrification may be preferable for protecting environmental health. In addition, denitrification produces no waste, whereas plant uptake produces organic N-containing biomass, which presently must be treated as solid waste, creating disposal costs. For these reasons, plant N uptake in wastewater-treating wetland and pond systems must be considered a liability rather than a benefit, and plants should be managed to minimize growth and N uptake.

CONCLUSIONS

The role that plants can play in the septage-treating aerated pond system is not clear. Plant N uptake is an inefficient way to remove N from wastewater, and creates costs in the harvesting and disposal of biomass. Nor is the contribution of plant root-associated nitrifiers likely to stimulate N removal through nitrification. Under standard plant densities, only 10% of the total nitrifiers in the system were found associated with plant roots, while most were associated with organic septage particulates. In highly particle-loaded wastewaters such as septage, the contribution of plant roots to total nitrifier populations is likely to be small relative to the populations sustained on suspended particulate matter. Although small increases in nitrification rates might be attained by increasing plant root to wastewater ratios, any benefit is likely to be offset by decreases in hydraulic conductivity, increases in the required wetland area, and plant biomass disposal costs. Moreover, any increase in nitrifier populations is unlikely to have an effect in a system where nitrification is more often limited by substrate or DO availability. Our results suggest that similar investigations should be made of the role of plant roots in other wastewater-treating wetlands and ponds. Plant roots may play a greater role in systems treating wastewaters with low solids content, where the substrate provided by plant roots might increase nitrifier populations. Plants may significantly boost nitrifier abundance even in gravel wetlands with high inert substrate availability, if oxygen leakage through roots supports a significant nitrifier population. However, nitrification rates in these systems may still be limited by NH_4 availability rather

than nitrifier abundance, and experimental work will be required to determine the role of plants in these systems. If plants are retained in wastewater-treating ponds and wetlands for these reasons, biomass production should be minimized to reduce the associated costs.

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REFERENCES

- American Public Health Association. 1999. Standard methods for the examination of water and wastewater. 20th ed. APHA, Washington, DC.
- Bagnall, L.O., C.E. Schertz, and D.R. Dubbe. 1987. Harvesting and handling of biomass. p. 599–620. *In* K.R. Reddy and W.H. Smith (ed.) Aquatic plants for water treatment and resource recovery. Proc. of the Conf. on Res. and Applications of Aquatic Plants for Water Treatment and Resource Recovery, Orlando, FL. 20–24 June 1986. Magnolia Publ., Orlando, FL.
- DeBusk, T.A., and K.R. Reddy. 1987. Wastewater treatment using floating aquatic macrophytes: Contaminant removal processes and management strategies. p. 643–656. *In* K.R. Reddy and W.H. Smith (ed.) Aquatic plants for water treatment and resource recovery. Proc. of the Conf. on Res. and Applications of Aquatic Plants for Water Treatment and Resource Recovery, Orlando, FL. 20–24 June 1986. Magnolia Publ., Orlando, FL.
- D'Elia, C.F., P.A. Steudler, and N. Corwin. 1977. Determination of total N in aqueous samples using persulfate digestion. *Limnol. Oceanogr.* 22:760–764.
- Dinges, R., and J. Doersam. 1987. The Hornsby Bend hyacinth facility in Austin, Texas. *Water Sci. Technol.* 19:41–49.
- Focht, D.D., and A.C. Chang. 1975. Nitrification and denitrification processes related to waste water treatment. *Adv. Appl. Microbiol.* 19:153–186.
- Gersberg, R.M., B.V. Elkins, S.R. Lyon, and C.R. Goldman. 1986. Role of aquatic plants in wastewater treatment by artificial wetlands. *Water Res.* 20:363–368.
- Halling-Sørensen, B., and S.E. Jørgensen. 1993. The removal of nitrogen compounds from wastewater. Elsevier, Amsterdam.
- Hamersley, M.R., and B.L. Howes. 2002. Control of denitrification in a septage-treating artificial wetland: The dual role of particulate organic carbon. *Water Res.* 36:4415–4427.
- Hamersley, M.R., B.L. Howes, D.S. White, S. Johnke, D. Young, S.B. Peterson, and J.M. Teal. 2001. Nitrogen balance and cycling in an ecologically-engineered septage treatment system. *Ecol. Eng.* 18: 61–75.
- Horan, N.J. 2002. Biological wastewater treatment systems: Theory and operation. 2nd ed. John Wiley & Sons, Chichester, UK.
- Howes, B.L., and J.M. Teal. 1994. Oxygen loss from *Spartina alterniflora* and its relationship to salt marsh oxygen balance. *Oecologia* 97:431–438.
- Isaacs, S.J., and M. Henze. 1995. Controlled carbon source addition to an alternating nitrification–denitrification wastewater treatment process including biological P removal. *Water Res.* 29:77–89.
- Kaplan, W.A. 1983. Nitrification. p. 139–190. *In* E.J. Carpenter and D.G. Capone (ed.) Nitrogen in the marine environment. Academic Press, New York.
- Kawai, H., M.Y. Uehara, J.A. Gomes, M.C. Jahnel, R. Rossetto, S.P. Alem, M.D. Ribeiro, P.R. Tinel, and V.M. Grieco. 1987. Pilot-scale experiments in water hyacinth lagoons for wastewater treatment. *Water Sci. Technol.* 19:129–173.
- Kinsinger, W.C., P.S. Mankiewicz, and J.A. Mankiewicz. 1991. A living symbol of participatory ecology: Wastewater, groundwater, and air purification schematics for the René Dubos Bioshelter, planned south transept of the Cathedral of St. John the Divine in New York City. p. 327–330. *In* C. Etnier and B. Guterstam. (ed.) Ecological engineering for wastewater treatment. Proc. of the Int. Conf. at Stensund Folk College, Sweden. 24–28 Mar. 1991. Bokskogen, Gothenburg, Sweden.
- Matulewicz, V.A., and M.S. Finstein. 1978. Distribution of autotrophic nitrifying bacteria in a polluted river (the Passaic). *Appl. Environ. Microbiol.* 35:67–71.
- Moorhead, K.K., and R.R. Reddy. 1988. Oxygen transport through selected aquatic macrophytes. *J. Environ. Qual.* 17:138–142.
- Orth, H.M., and D.P. Sapkota. 1988. Upgrading a facultative pond by implanting water hyacinth. *Water Res.* 22:1503–1511.
- Ottová, V., J. Balcarová, and J. Vymazal. 1997. Microbial characteristics of constructed wetlands. *Water Sci. Technol.* 35:117–123.
- Peterson, S.B., and J.M. Teal. 1996. The role of plants in ecologically engineered wastewater treatment systems. *Ecol. Eng.* 6:137–148.
- Reddy, K.R., W.H. Patrick, Jr., and C.W. Lindau. 1989. Nitrification–denitrification at the plant root–sediment interface in wetlands. *Limnol. Oceanogr.* 34:1004–1013.
- Reddy, K.R., and D.L. Sutton. 1984. Water hyacinths for water quality improvement and biomass production. *J. Environ. Qual.* 13:1–8.
- Reed, S.C., and D. Brown. 1995. Subsurface flow wetlands—A performance evaluation. *Water Environ. Res.* 67:244–248.
- Ruzicka, J., and E.H. Hansen. 1988. Flow injection analysis. 2nd ed. John Wiley & Sons, New York.
- Scheiner, D. 1976. Determination of ammonia and Kjeldahl N by indophenol method. *Water Res.* 10:31–36.
- Schmidt, E.L., and L.W. Belser. 1982. Nitrifying bacteria. p. 1029–1031. *In* A.L. Page, R.H. Miller, and D.R. Keeney (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.
- Stryer, L. 1988. Biochemistry. W.H. Freeman, New York.
- Teal, J.M., B.L. Howes, S.B. Peterson, J.E. Petersen, and A. Armstrong. 1994. Nutrient processing in an artificial wetland engineered for high loading: A septage treatment example. p. 421–428. *In* W.J. Mitsch. (ed.) Global wetlands: Old world and new. Elsevier, New York.
- Teal, J.M., and S.B. Peterson. 1991. The next generation of septage treatment. *Res. J. Water Pollut. Control Fed.* 63:84–89.
- Teal, J.M., and S.B. Peterson. 1993. A solar aquatic system septage treatment plant. *Environ. Sci. Technol.* 27:34–37.
- Van Loosdrecht, M.C.M., L. Tjihuis, A.M.S. Wijdicks, and J.J. Heijnen. 1995. Population distribution in aerobic biofilms on small suspended particles. *Water Sci. Technol.* 31:163–171.
- Watson, J.T., K.D. Choate, and G.R. Steiner. 1990. Performance of constructed wetland treatment systems at Benton, Hardin, and Pembroke, Kentucky during the early vegetation establishment phase. p. 171. *In* P.F. Cooper and B.C. Findlater (ed.) Constructed wetlands in water pollution control. Pergamon, Oxford.
- Weisner, S.E.B., P.G. Eriksson, W. Granéle, and L. Leonardson. 1994. Influence of macrophytes on nitrate removal in wetlands. *Ambio* 23:363–366.
- Wood, E., F. Armstrong, and F. Richards. 1967. Determination of nitrate in sea water by cadmium copper reduction to nitrite. *J. Mar. Biol. Assoc. U. K.* 47:23–31.