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Experimental Evaluation of the Halophyte, *Salicornia virginica*, for Biomitigation of Dissolved Nutrients in Effluent from a Recirculating Aquaculture System for Marine Finfish

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Abstract

The ability of the halophyte, *Salicornia virginica*, planted in drainage lysimeters to biomitigate dissolved nutrients in effluent from a recirculating aquaculture system (RAS) for marine finfish was evaluated. Seawater effluent from a RAS producing black sea bass, *Centropristis striata* (filtered to reduce total suspended solids), was used as irrigant. Plant growth and dissolved N and P removal were determined as a function of leachate fraction (LF%) – that is, proportion of irrigant that leaches from the plant-substrate lysimeter. Lysimeters were irrigated weekly to produce 30, 40, and 50% LF. A control (unplanted) lysimeter was included at the 30% LF. Plant growth was excellent in all LF% treatments until Day 141 when salt buildup in the lysimeter substrate inhibited nutrient uptake. Salt accumulation was mitigated at higher LF%, so that plant biomass and net removal (μg) of dissolved N and P by the p-s lysimeter remained higher ($P < 0.05$) at the 40 and 50% than at the 30% LF. On Day 141, percent removal efficiency at the 50% LF was 79.2% for inorganic N and 73.9% for total phosphorus. Through Day 355, substrate salinity was minimized and plant biomass and nutrient removal were maximized at the 50% LF. *S. virginica* is an effective biofilter for dissolved nutrients in effluent from an RAS for marine finfish.

KEYWORDS

dissolved nitrogen and phosphorus, halophyte, integrated multitrophic aquaculture, marine finfish recirculating aquaculture systems, recirculating aquaculture system, *Salicornia virginica*, salt-tolerant plants

Integrated multitrophic aquaculture (IMTA) systems, where wastes from one fed aquatic species (e.g., fish) are used as inputs (fertilizer) for another species (e.g., salt-tolerant plants), can reduce economic losses due to underutilization of feed and increase profitability for the farmer (Shpigel et al. 1993; Wu 1995; Shpigel and Neori 1996; Van Rijn 1996; Schuenhoff et al. 2003; Neori et al. 2004; Shpigel et al. 2013). Through IMTA, food and energy lost in fed monoculture operations are recaptured and transformed into valuable commercial crops, while biomitigation takes place (Chopin et al. 2001). IMTA can lower production costs, conserve water, reduce environmental effects from effluent discharge, and increase social acceptability. Previous studies on IMTA in marine finfish aquaculture have found large reductions of nitrogen (N) and phosphorus (P) using seaweeds (Shpigel and Neori 1996; Chopin et al. 2001; Schuenhoff et al. 2003; Neori et al. 2004; Marinho-Soriano et al. 2009), marine microalgae (Truesdale et al. 2007; Allen and Watanabe 2009), and salt-tolerant plants (halophytes) (e.g., Spartina spp., *Aster tripolium*, *Suaeda esteroa*, and *Salicornia* spp.) (Brown et al. 1999; Brown and Glenn 1999; Sousa et al. 2008, 2011; Webb et al. 2012, 2013; Buhmann and Papenbrock 2013; Shpigel et al. 2013; Quintã et al. 2015).

A major pollutant in the effluent of recirculating aquaculture systems (RASs) is total

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suspended solids (TSSs), consisting of uneaten feed, fish feces, algae, and biofloc sloughed from biological filters (Ebeling et al. 2004, 2005; Summerfelt and Vinci 2008). These biosolids are concentrated as they are sequestered by solids removal components (particle traps, swirl separators, bead filters, microscreen filters, foam fractionators) from the process water (Losordo and Westers 1994; Cripps and Bergheim 2000; Ebeling et al. 2005), producing a high-strength low-volume effluent that requires significant treatment before discharge (Losordo and Westers 1994). Traditionally used in the construction and excavation industries to remove solids from runoff, geotextile fabric tube (Geotube) technology has been adopted by aquaculture facilities as a method of solids management and mitigation (Sharrer et al. 2009). Chemical polymers are used to bind suspended solid particles in the effluent for greater retention by the Geotube. Recent studies in our laboratory (Gibson 2014; Watanabe et al. 2015) have demonstrated that Geotube filtration systems are highly effective at removing suspended solids from intensive, marine RAS discharge and can aid production facilities in meeting government discharge compliance as well as reducing the impact of aquaculture on local waters. In contrast to suspended solids, polymer treatment did not remove dissolved N and P from the effluent (Gibson 2014), and effective methods for reducing these dissolved components are important for the discharge of the Geotube filtrate to the environment, or for its reuse in the RAS.

Salicornia is a genus of succulent halophyte plants (glasswort, pickleweed, and sea asparagus) that grow in salt marshes and on beaches in the Northern hemisphere, South Africa, and South Asia and are important candidates as food for humans and livestock and as feedstock for biofuels (Boer 2006; Ventura et al. 2011). While *Salicornia* is valuable as a food (cooked, atop a salad, or as a garnish in seafood dishes) (Shimabukuro 2006; Ventura et al. 2011; Buhmann and Papenbrock 2013), some species (*Salicornia bigelovii*) produce seeds with high levels of protein (35%) and highly unsaturated fatty acids (30%, mainly linoleic acid) similar to soybeans (Glenn et al. 1991). The oil is readily extracted (Glenn et al. 1991, 1998), making *Salicornia* candidates for livestock feed (Masters et al. 2007) and for biofuels (Glenn et al. 1998; Rozema and Flowers 2008; Buhmann and Papenbrock 2013). Because they can be grown in saltwater, they are readily adaptable to coastal land where conventional crops cannot be grown (Rozema and Flowers 2008) and are attractive candidates for IMTA with marine finfish RASs (Christiansen 2008; Dickerson 2008; Shpigel et al. 2013; Webb et al. 2013; Quintã et al. 2015). Field trials in Mexico showed that *Salicornia* produced an average of 1.7 kg/m^2 of total biomass and 0.2 kg/m^2 of oilseed per year, comparable to the yields of soybeans (Glenn et al. 1998; Brown et al. 1999; Rozema and Flowers 2008). Studies are needed to evaluate production of *Salicornia* spp. for biomitigation of marine RAS effluent wastes and as a valuable forage crop (for humans, livestock, and fish) and for biofuels. The objective of this study was to evaluate, under controlled conditions, the ability of the native halophyte, *Salicornia virginica*, to serve as a biofilter for treatment of dissolved nutrients in effluent discharge from an intensive RAS for marine finfish.

Materials and Methods

Pilot-scale Intensive RAS for Marine Finfish

This study was conducted at the University of North Carolina at Wilmington Center for Marine Science (UNCW-CMS) Aquaculture Facility (Wrightsville Beach, NC, USA). Wastewater for irrigation experiments was generated by a near-commercial-scale RAS for marine finfish (Carroll et al. 2005). The pilot scale growout system comprised six 16.7-m^3 tanks (4.57×1 m = diameter × depth) tanks supported by a RAS that was supplied with natural seawater (32–35 g/L) pumped from the Atlantic Intracoastal Waterway adjacent to the laboratory and then sand- and UV-filtered before use. A total of six tanks were arranged in three groups of two tanks, each supported by independent RAS components, including double drain and particle trap, microscreen drum filter, a biosump containing air-circulated biomedia (Kaldnes, AnoxKaldnes Company, Lund, Sweden) for biofiltration, and a foam fractionator (RK75PE, RK2 Systems, San Diego, CA, USA) to remove fine particulates. Water temperature was controlled using a heater/chiller (4 HP), and water was treated with a ultraviolet (UV) sterilizer to eliminate pathogens. After temperature adjustment and UV treatment, the inflow water passed through an oxygen cone where liquid oxygen was injected into the water before it was returned to the fish tank. Two of the three RASs had identical filtration components, while the third had a propeller-wash bead filter (10 ft^3) on the influent line for added mechanical and biological filtration.

Tanks were stocked with black sea bass, *Centropristis striata*, at a biomass loading of 20–25 kg/m^3. The fish were fed a commercial diet containing 50% protein and 12% fat at approximately 1% biomass daily. Concentrated effluent discharged from the RAS (ca. 10% system volume/d), containing feces, feed, and biofloc from filter backwashing and continuous exchange, was collected in a 4.6-m^3 raw effluent sump and was used for IMTA experiments below. To simulate the filtering of RAS effluent through a geotextile bag the raw effluent was pumped to 4.6-m^3 settling tank where large particulate matter was allowed to settle. The supernatant was then filtered through a 100-µm nitex screen to remove small particulates before use as an irrigant for *S. virginica*.

Experimental Plants and System

S. virginica collected from salt marshes on Wrightsville Beach and on Kure Beach, NC, USA, were transported to the UNCW-CMS Aquaculture Facility. The experimental units consisted of 20-L drainage lysimeters made from a plastic 15-L planter inserted into a 30-L container, creating a void space at the bottom of the container of approximately 15-L, where leachate was collected after irrigation. The external 30-L container provided a firm base for the lysimeter, which fit snugly inside the container, minimizing evaporative losses of leachate. The perforated bottom of the lysimeter was lined with geotextile fabric screen and covered with pea gravel and then filled with 10 kg (dry weight) of substrate, consisting of a mixture of sand and potting soil in a 1:1 ratio. For uniformity of composition, this mixture was selected as the experimental substrate in replacement of natural marsh substrate. The geotextile screen retained the substrate but allowed water (leachate) to drain through the lysimeter and into the outer container. Six lysimeters were housed in each of four 800-L outdoor concrete tanks ($1.82 \times 0.91 \times 0.48$ m = length × width × depth) (24 total experimental units) enclosed in greenhousing. Greenhouse panels were opened or closed depending on weather conditions.

Each of 24 lysimeters was planted with a rooted *Salicornia* cutting. Natural substrate was removed from the roots by rinsing with seawater before planting into the lysimeters containing the prepared substrate. The mean initial weights (shoot plus root) for the 30, 40, and 50% LF treatments at the time of planting into the lysimeters were 15.1 ± 0.02 g for all treatments, with no significant differences. During a 6-wk pre-experimental acclimation period, all lysimeters were irrigated at a rate of approximately 1000 mL of natural seawater per week. Plants regained vigor and became established in the experimental lysimeters before the prescribed irrigation treatments with RAS effluent were started on December 4, 2012. Plants were irrigated weekly with effluent from the marine finfish RAS and the leachate from each lysimeter was collected according to the experimental design described below.

Experimental Design

When halophytes are irrigated with high-salinity water, inadequate irrigation can lead to a buildup of salts in the substrate, which inhibit plant growth, while excessive irrigation can result in inefficient absorption of nutrients by the plant-substrate (p-s) system and poor removal efficiency (Brown et al. 1999; Brown and Glenn 1999; Radulovich et al. 2017). Leachate fraction (LF% = leachate volume (L)/total irrigation volume (L) × 100) is the proportion of water applied that leaches from the lysimeter after an irrigation event. Halophytes require a LF of 30–50% to flush excess salts and optimize growth (Miyamoto et al. 1996; Brown et al. 1999; Brown and Glenn 1999). The experimental objectives were to evaluate plant biomass yield and nutrient (N and P) removal capacity from marine RAS effluent as a function of LF%.

To determine the effect of LF% on plant growth and removal of N and P, lysimeters were irrigated weekly with RAS effluent to replace evapotranspiration losses and to produce 30, 40, and 50% LF (Miyamoto et al. 1996; Brown et al. 1999; Brown and Glenn 1999). Control lysimeters (substrate without plants) were included at the 30% LF treatment to determine the effect of the substrate alone on nutrient removal. Six replicate lysimeters were assigned per LF% treatment. Lysimeters planted with *Salicornia* are hereafter referred to as p-s lysimeters because nutrient removal is attributable to the plant and the substrate. However, nutrient removal from unplanted (control) lysimeters is attributable to the substrate alone. The experiment was run for 356 d under ambient light and temperature conditions. Air temperatures at the study site were recorded daily using a maximum and minimum thermometer.

To monitor *Salicornia* growth, each plant was photographed at approximately 6–8 wk intervals from three different perspectives, with a 10-cm ruler (1-mm hatch marks) placed on the surface of the substrate as a calibration reference. Image analysis (ImageJ, Co., Bethesda, MD, USA) was used to quantify changes in shoot length. The combined total shoot length of each plant was determined from the photographs by image analysis. Aboveground biomass of each plant was then estimated from a linear regression relationship between shoot length (x) and weight (y): $y = 0.0066x$, $R^2 = 0.9906$, $n = 362$, $P < 0.001$, which was determined by weighing *S. virginica* shoot cuttings of different lengths. Monitoring of plant growth by image analysis was completed on Day 356. Nine days later (Day 365), plants were harvested from their lysimeters and the roots were separated from aboveground shoots and were weighed separately. Roots and shoots of each plant were dried in a laboratory oven at 60 C to constant weight and then reweighed to obtain dry weight. Net aboveground biomass produced was obtained by subtracting the mean initial seedling dry weight (corrected for root weight) from the final dry weight of shoots.

Water and Plant Nutrient Analyses

Leachate volumes varied weekly and seasonally depending on temperature conditions in the greenhousing. To achieve the nominal LF%, all lysimeters within a treatment were irrigated with a prescribed volume of irrigant, which was increased or decreased based on the mean LF% achieved during the previous week. Irrigant volumes were the same for all lysimeters within a LF% treatment.

With seasonally declining temperatures in the fall and the winter, greenhouse panels were closed throughout the day to retain heat. However, as air temperatures increased during spring and summer (May and June 2013), greenhouse panels were opened to increase ventilation, but higher rates of evaporation of the substrate in the lysimeters required higher irrigant volumes to maintain the targeted LF%.

On each day of irrigation, raw effluent was sampled before irrigation, and the leachate from the lysimeter was collected 4–6 h postirrigation to maximize the LF%. Leachate was poured from the collecting container into a 1000-mL graduated cylinder to measure leachate volume and then transferred to a Nalgene bottle and stored at 0 C until nutrient analysis. Irrigant (i.e., RAS effluent) and p-s lysimeter leachate samples were analyzed for pH, conductivity, total Kjeldahl nitrogen (TKN), P, NO_3-N, and NH_4-N (Grasshoff et al. 2002) by the North Carolina Department of Agriculture and Consumer Services (Plant, Waste, Solution and Media Section, Agronomic Division Laboratory, Raleigh, NC, USA).

Analytical Methods

The amount of nutrients removed by the p-s system in each lysimeter was calculated on three dates of the experiment: Day 141 (April 24, 2013), Day 260 (August 21, 2013), and day 350 (November 19, 2013). Total quantities of a nutrient in the irrigant and in the leachate from each lysimeter were calculated as the product

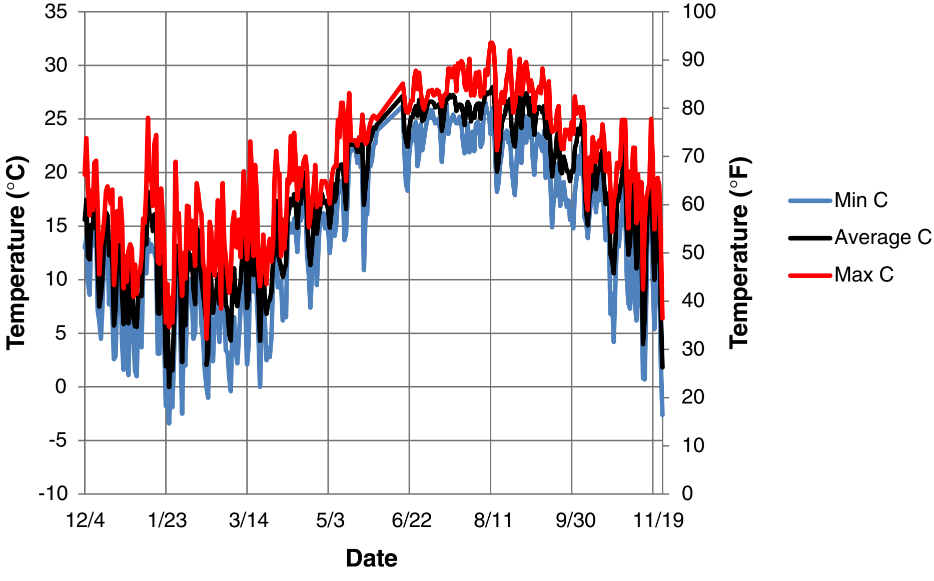

FIGURE 1. *Daily maximum, minimum, and mean air temperatures during the study period (December 2012 to November 2013).*

of the nutrient concentration (µg/mL) and the volume (mL) of the irrigant or the leachate. The quantity of a nutrient removed (net removal, µg) by the plant substrate lysimeter was calculated by subtracting the quantity of the nutrient in the leachate from the quantity that was added in the irrigant. The percentage of a nutrient removed (% removal efficiency) from the RAS irrigant was also calculated from these quantities by the formula:

%Nutrient removed = (Nutrient [irrigant] – nutrient [leachate]/nutrient [irrigant]) × 100

For both net and percent removal of a nutrient, a negative value indicated that the quantity of the nutrient in the leachate was greater than in the irrigant.

Statistical Analysis

Treatment means were compared by one-way ANOVA (JMP, version 12.0, SAS Institute, Cary, NC, USA). Homogeneity of variances was tested using O'Brien's test. Significant differences between means were evaluated by Tukey–Kramer test (Kramer 1956), or by nonparametric multiple comparisons (Wilcoxon test). Probabilities of $P < 0.05$ were considered significant.

Results

Leachate Fractions

During the 356-d study period, ambient temperature conditions varied seasonally from as low as −3.4 C in winter (January 25) to as high as 32.1 C in summer (August 12), with significant diurnal variation (Fig. 1). Seasonal and daily variations in climatic conditions required the adjustment of irrigant volumes from week to week to achieve the prescribed LF treatments. Over the 356-d experiment, mean irrigant volumes (ranges) for the 30% (control), 30, 40, and 50% LF were 361 (250–600), 361 (250–600), 468 (325–700), and 574 (400–800) mL, respectively. Based on the volumes of all leachate samples collected weekly from each lysimeter over the course of the 356-d experiment, mean LF% (±SE of the mean = SEM, $n = 48$) were 35 ± 2.2, 36 ± 2.5, 44 ± 2.3, and 53 + 2.0% for the prescribed treatment LF of 30% control (unplanted), 30, 40, and 50%, respectively.

Salicornia *Growth*

On Day 1, mean aboveground shoot weights for the 30, 40, and 50% LF treatments were 11.2, 10.2, and 12.1 g, respectively, with no

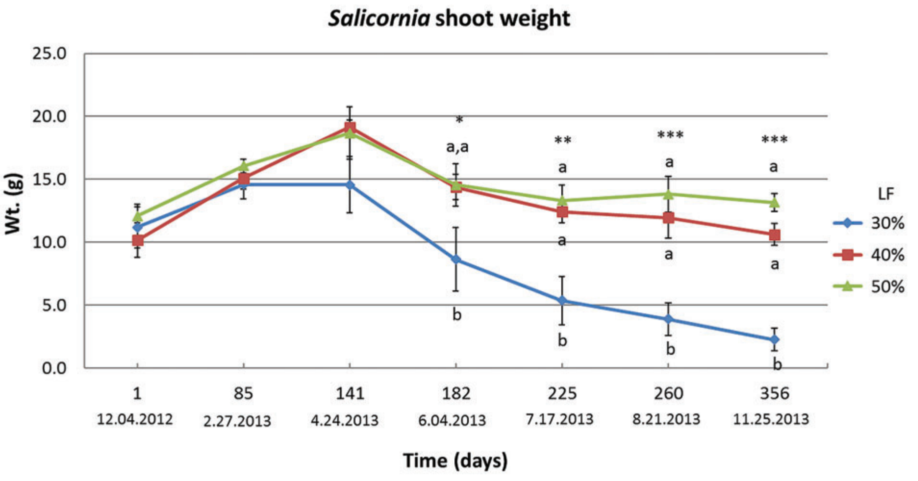

FIGURE 2. *Aboveground (shoot) biomass of* Salicornia virginica *raised in plant-substrate (p-s) lysimeters irrigated with recirculating aquaculture system effluent to produce leachate fractions (LF) of 30, 40, and 50%. Values represent means ± SEM* (n = 6). *Asterisks indicate the probability level of the overall ANOVA (*P < 0.10, **P < 0.05, ***P < 0.01). Means not sharing a letter in common are significantly different (Tukey-Kramer or Wilcoxon test).*

significant differences (Fig. 2). On Day 85, aboveground shoot weights ranged 14.6–16.1 g among treatments, with no significant differences. By Day 141, aboveground shoot weights appeared higher in the 40% (19.1 g) and 50% (18.6 g) LF treatments than in the 30% LF treatment (14.6 g), with differences between the 40 and 50% LF treatments approaching a level of significance ($P < 0.093$) (Figs. 2, 3). By Day 182, plant biomass was reduced in all LF% treatment but was significantly higher in the 40% LF (14.4 g) and 50% LF (14.6 g) treatments than at 30% LF (8.64 g). By Day 225, biomass stabilized in the 50% LF treatment (13.3 g), declined slightly in the 40% LF treatment (12.4 g), but continued to decline in the 30% treatment (5.36 g), which showed a significantly lower biomass than in the 40 and 50% LF treatments. This trend continued on Day 260 when biomass in the 50% (13.8 g) and 40% LF (11.9 g) LF treatments were significantly higher than the 30% LF (3.88 g) (Fig. 2). By Day 356, the same trend toward higher aboveground biomass with higher LF% was observed, from a minimum in the 30% LF (2.27 g) to an intermediate level at the 40% LF (10.6 g) and to a maximum at the 50% LF (13.2 g) (Fig. 2).

At harvest on Day 365 (December 4, 2013), total plant wet biomass (wet weight of shoots plus roots) was significantly higher at the 40 and 50% LF treatments (22.4–27.2 g) than at the 30% LF (9.92 g) (Table 1). Wet shoot weights were significantly higher in the 40 and 50% LF treatments (15.8–19.9 g) than at 30% LF (4.28 g). Wet root weights (5.39–6.64 g) were not significantly different among treatments. Roots represented a significantly higher percentage of total wet plant weight in the 30% LF (56.4%) than in the 40 or 50% LF (27.8–24.6%).

On Day 365 (December 4, 2013), total plant dry biomass (shoots plus roots) was significantly higher at the 40 and 50% LF treatments (7.49–9.28 g) than at 30% (4.58 g) (Table 1). Shoot weights (dry) were significantly higher in the 40 and 50% LF treatments (3.98–5.30 g) than at 30% (1.55 g). Dry root weights (3.02–3.98 g) were not significantly different among treatments. Roots represented a significantly higher percentage of total dry plant weight in the 30% LF (65.5%) than in the 40 or 50% LF (47.0–43.0%).

On Day 1, plant aboveground biomass (mean ± SEM, $n = 6$) in the 30, 40, and 50% LF treatments were 11.2 ± 1.63, 10.2 ± 1.38, and 12.1 ± 0.97 g, respectively, with no significant differences. On Day 141, shoot biomass was 14.55 ± 2.23, 19.14 ± 0.55, and 18.67 ± 2.07 g, respectively. Plant aboveground biomass yields

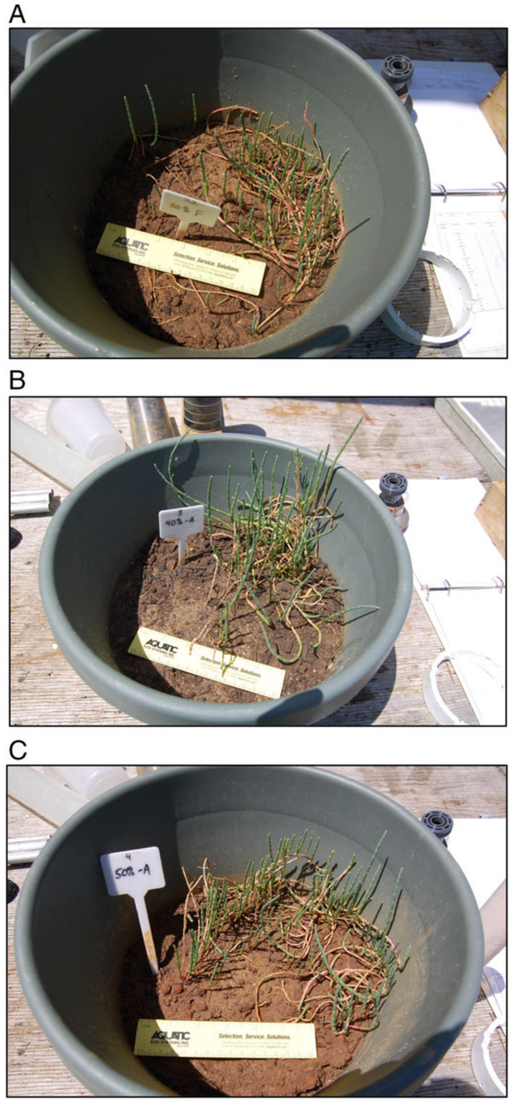

FIGURE 3. *Photos of* Salicornia virginica *on April 24, 2013 (Day 141) raised in plant-substrate (p-s) lysimeters irrigated with recirculating aquaculture system effluent to produce leachate fractions (LF) of 30% (A), 40% (B), and 50% (C).*

for this 141-d study period were 3.38 ± 3.08, 8.99 ± 1.71, and 6.59 ± 2.92 g, for the 30, 40, and 50% LF, respectively.

Nutrient Removal by Lysimeter

On Day 141 (April 24, 2013), nutrient concentrations in the irrigant (RAS effluent) (Table 2) were as follows: salinity (29.2 g/L), pH (7.97), total ammonia nitrogen (TAN = 10.1 μg/mL), nitrate (NO_3-N = 0.33 μg/mL), inorganic N (10.4 μg/mL), organic N (79.5 μg/mL), TKN (89.5 μg/mL), and total phosphorus (TP = 6.53 μg/mL). On Day 141, nutrient concentrations (treatment ranges) in the lysimeter leachates were as follows: salinity (54.4–59.0 g/L), pH (7.58–7.73), TAN (2.82–4.11 μg/mL), NO_3-N (0.528–2.28 μg/mL), inorganic N (3.35–5.61 μg/mL), organic N (66.0–68.4 μg/mL), TKN (69.9–71.9 μg/mL), and TP (3.46–4.47 μg/mL) (Table 2). No significant differences in salinity (g/L) or in nutrient concentrations (μg/mL) among LF% treatments (30% control, 30, 40, or 50% LF) were observed on Day 141.

On Day 141, significant treatment differences in net removal (μg) of nutrients by the p-s lysimeters were observed for some nutrients (Table 3). TAN removed by the p-s lysimeters was not significantly different in the 30% control (unplanted) and 30% LF treatments (3218–3128 μg) but increased significantly in the 40% and 50% LF treatments (3393–4150 μg). Compared to TAN, quantities of NO_3-N removed by the p-s lysimeters were relatively small among treatments, ranging from negative (−) 81.5 to positive 74.2 μg, with no significant differences. Consisting primarily of TAN, inorganic N removed was not significantly different in the 30% control (unplanted) and 30% LF treatments (3126 μg) but increased to 3995 μg at 40% LF and 4119 μg at 50% LF. Organic N removed showed the same trend, with no significant differences between the 30% control (unplanted) and 30% LF treatments (21,620–21,651 μg) but increased to 25,183 μg at 40% LF and to 25,905 μg at 50% LF. TKN (sum of organic N and TAN) correspondingly showed no significant difference between the 30% control (unplanted) and 30% LF treatments (24,802–24,745 μg) but increased to 29,075 at 40% LF and to 30,007 μg at 50% LF (Table 3). TP removed also showed the same trend, with no significant difference between the 30% control (unplanted) and 30% LF treatments (1873–1868 μg), but increased to 2333 at 40% LF and to 2413 μg at 50% LF.

TABLE 1. *Harvest weights (wet and dry) on Day 365 of* Salicornia virginica *raised in plant-substrate (p-s) lysimeters irrigated with recirculating aquaculture system effluent under three leachate fractions: 30, 40, and 50%.*[1]

	Leachate fraction (%)			
	30	40	50	*P* value
Plant component				
Total wet (g)	9.92 ± 1.55 b	22.4 ± 1.39 a	27.2 ± 1.88 a	*
Wet shoot (g)	4.28 ± 0.98 b	15.8 ± 1.21 a	19.9 ± 1.57 a	*
Wet root (g)	5.39 ± 0.84 a	6.23 ± 0.69 a	6.64 ± 0.64 a	NS
Wet root (% of total)	56.4 ± 4.62 a	27.8 ± 2.71 b	24.6 ± 2.19 b	*
Total dry (g)	4.58 ± 0.67 b	7.49 ± 0.44 a	9.28 ± 0.42 a	*
Dry shoot (g)	1.55 ± 0.25 b	3.98 ± 0.42 a	5.30 ± 0.33 a	*
Dry root (g)	3.02 ± 0.51 a	3.51 ± 0.37 a	3.98 ± 0.24 a	NS
Dry root (% of total)	65.5 ± 3.50 a	47.0 ± 4.23 b	43.0 ± 2.14 b	*

NS = not significant.

*Probability level for overall ANOVA: < 0.001.

[1]Weights of aboveground (shoots), roots, and total (shoots plus roots) biomass are provided. Values represent means ± SEM ($n = 6$). Means in a row not sharing a letter in common are significantly ($P < 0.05$) different.

On Day 141, the percentage of nutrients removed (i.e., nutrient removal efficiency) from the irrigant (i.e., RAS effluent) by the p-s lysimeter did not differ significantly among LF% treatments for the following parameters: TAN (82.2–91.6%), inorganic N (85.9–90.4%), and TP (73.9–84.1%) (Table 4). However, percent NO_3-N removed was not significantly different in the 30% control (unplanted), 30% LF, and 40% LF treatments (79.2–76.5%) but decreased to 67.1% in the 50% LF treatment. Percent organic N removed showed a similar trend, with no significant differences between the 30% control (unplanted), 30 and 40% LF treatments (77.8–74.5%), but decreased to 65.2% at the 50% LF. Percent TKN removed was also not significantly different between the 30% control (unplanted), 30, and 40% treatments (79.2–76.5%) but decreased to 67.1% in the 50% LF treatment (Table 4).

On Day 260 (August 21, 2013), nutrient concentrations in the irrigant (RAS effluent) (Table 2) were as follows: salinity (32.1 g/L), pH (7.81), TAN (6.53 μg/mL), NO_3-N (0.41 μg/mL), inorganic N (6.94 μg/mL), organic N (67.4 μg/mL), TKN (73.9 μg/mL), and TP (7.56 μg/mL) (Table 2). On Day 260, pH (7.34–7.53), organic N (63.5–71.8 μg/mL), TKN (70.9–85.7 μg/mL), and TP (3.16–3.93 μg/mL) concentrations in the p-s lysimeter leachates were not significantly different. However, significant concentration differences were evident for the other parameters (Table 2). Leachate salinity decreased with increasing LF% treatment from 88.2–87.1 g/L in the 30% control (unplanted) and 30% LF treatments to 73.3 g/L at 40% LF and to 59.3 g/L at 50% LF. Leachate TAN concentrations also clearly decreased with increasing LF% from 22.3 μg/mL in the 30% control (unplanted) and 13.7 μg/mL in the 30% LF to 6.69 μg/mL at 40% LF and 3.05 μg/mL at 50% LF. Leachate NO_3-N concentrations also showed a clear decline with increasing LF from 17.5–12.9 μg/mL in the 30% control (unplanted) and the 30% LF treatments to 9.53 μg/mL at 40% LF and to 6.14 μg/mL at 50% LF. Leachate inorganic N concentrations reflected the sums of TAN and NO_3-N, with no significant difference in the 30% control (unplanted) and 30% LF (39.8–26.6 μg/mL), decreasing to 16.2 μg/mL at 40% LF and to 9.2 μg/mL at 50% LF.

On Day 260 (August 21, 2013), net removal of organic N (range = 7332–12,200 μg), TKN (8183–11,110 μg), and TP (2595–3110 μg) by the p-s lysimeters was not significantly different among LF% treatments (Table 3). TAN removed was negative and not significantly different between the 30% control (unplanted) and 30% LF treatments (− 4022 to −1361 μg) but increased to 787 μg at 40% LF and to 2873 μg at 50% LF. NO_3-N removed on Day

TABLE 2. *Salinity, pH, and concentrations (µg/mL or ppm) of total ammonia nitrogen (TAN), nitrate nitrogen (NO_3-N), inorganic nitrogen, organic N, total Kjeldahl nitrogen (TKN), and total phosphorus (TP) in leachate fractions of plant-substrate (p-s) lysimeters planted with* Salicornia virginica *and irrigated with recirculating aquaculture system (RAS) effluent at different leachate fractions (30, 40, and 50%).*[1]

Day of experiment, date (2013)	Sample or treatment (LF%)	Salinity (g/L)	pH	TAN	NO_3-N	Inorganic N (µg/mL)	Organic N (µg/mL)	TKN (µg/mL)	TP (µg/mL)
141, April 24	RAS Eff	29.2	7.97	10.1	0.33	10.4	79.5	89.5	6.53
	Control (30%)	54.4 ± 3.2 a	7.73 ± 0.07 a	3.32 ± 0.47 a	2.28 ± 0.89 a	5.61 ± 0.70 a	67.4 ± 3.01 a	70.7 ± 3.12 a	4.47 ± 0.30 a
	30%	59.0 ± 3.7 a	7.62 ± 0.05 a	3.82 ± 0.66 a	1.12 ± 0.23 a	4.94 ± 0.74 a	66.0 ± 3.72 a	69.9 ± 3.6 a	4.23 ± 0.32 a
	40%	55.1 ± 2.3 a	7.64 ± 0.07 a	2.82 ± 0.66 a	0.528 ± 0.131 a	3.35 ± 0.61 a	68.4 ± 2.5 a	71.3 ± 2.2 a	3.46 ± 0.40 a
	50%	53.9 ± 4.1 a	7.58 ± 0.09 a	4.11 ± 0.96 a	0.817 ± 0.239 a	4.93 ± 1.10 a	67.8 ± 1.8 a	71.9 ± 1.8 a	3.97 ± 0.58 a
260, August 21	RAS Eff	32.1	7.81	6.53	0.41	6.94	67.4	73.9	7.56
	Control (30%)	88.2 ± 6.3 a	7.34 ± 0.09 a	22.3 ± 4.7 a	17.53 ± 2.52 a	39.8 ± 7.0 a	63.5 ± 2.1 a	85.7 ± 5.8 a	3.49 ± 0.31 a
	30%	87.1 ± 3.2 a	7.36 ± 0.07 a	13.7 ± 2.42 ab	12.9 ± 0.74 a	26.6 ± 2.4 a	64.5 ± 3.5 a	78.1 ± 3.2 a	3.21 ± 0.17 a
	40%	73.3 ± 2.4 ab	7.37 ± 0.06 a	6.69 ± 1.62 b	9.53 ± 1.03 ab	16.2 ± 2.6 b	64.2 ± 1.3 a	70.9 ± 1.7 a	3.16 ± 0.12 a
	50%	59.3 ± 3.0 b	7.53 ± 0.05 a	3.05 ± 0.33 b	6.14 ± 0.82 c	9.20 ± 1.12 c	71.8 ± 3.3 a	74.8 ± 3.3 a	3.93 ± 0.48 a
350, November 19	RAS Eff	38.6	7.85	0.62	0.45	1.07	69.5	70.1	5.24
	Control (30%)	81.5 ± 4.2 a	7.49 ± 0.05 a	2.11 ± 0.95 a	12.3 ± 3.22 a	14.4 ± 3.95 a	76.7 ± 1.71 a	78.8 ± 1.2 a	3.07 ± 0.23 a
	30%	75.1 ± 2.8 a	7.51 ± 0.04 a	1.00 ± 0.34 a	5.51 ± 1.20 a	6.52 ± 1.48 a	76.4 ± 3.1 a	77.4 ± 3.2 a	3.39 ± 0.29 a
	40%	69.9 ± 1.8 ab	7.51 ± 0.05 a	0.207 ± 0.039 b	1.40 ± 0.90 b	1.60 ± 0.93 b	75.9 ± 2.2 a	72.8 ± 3.6 a	3.01 ± 0.26 a
	50%	58.8 ± 3.5 b	7.60 ± 0.03 a	0.163 ± 0.020 b	0.418 ± 0.296 b	0.582 ± 0.309 b	72.4 ± 5.4 a	72.6 ± 5.4 a	3.63 ± 0.24 a

[1]Control lysimeters (30% F) were not planted. Values are means ± SEM ($n = 6$), except for RAS effluent ($n = 1$).
For each sampling date, parameter means not sharing a letter in common are significantly ($P < 0.05$) different.

TABLE 3. *Net removal of nutrients (μg) by plant-substrate (p-s) lysimeters planted with* Salicornia virginica *and irrigated with recirculating aquaculture effluent at different leachate fractions (LF): 30, 40, and 50%.*[1]

Day, date (2013)	Sample or treatment (LF %)	TAN removed by p–s lysimeter (μg)	NO_3–N removed by p-s lysimeter (μg)	Inorganic N removed by p-s lysimeter (μg)	Organic N removed by p-s lysimeter (μg)	TKN removed by p-s lysimeter (μg)	TP removed by p-s lysimeter (μg)
141, April 24	Control (30%)	3,218 ± 61 b	−81.5± 73.9 a	3126 ± 68 b	21620 ± 569 b	24802 ± 618 b	1873 ± 39 b
	30%	3,128 ± 118 b	7.88 ± 32.2 a	3126 ± 137 b	21651 ± 888 b	24745 ± 987 b	1868 ± 91 b
	40%	3,393 ± 94 a	74.2 ± 17.7 a	3995 ± 88 a	25183 ± 639 ab	29075 ± 640 a	2333 ± 69 a
	50%	4,150 ± 264 a	−16.7 ± 60.7 a	4119 ± 306 a	25905 ± 1414 a	30007 ± 1614 a	2413 ± 183 a
260, August 21	Control (30%)	−4022 ± 1337 c	−5583 ± 627 b	−9600 ± 1884 c	12200 ± 989 a	8183 ± 1189 a	2595 ± 115 a
	30%	−1361 ± 692 bc	−4249 ± 304 ab	−5616 ± 644 bc	11310 ± 1785 a	9929 ± 1555 a	2666 ± 89 a
	40%	787 ± 726 ab	−4210 ± 486 ab	−3424 ± 1170 ab	10340 ± 1202 a	11110 ± 1348 a	3064 ± 34 a
	50%	2873 ± 203 a	−3136 ± 502 a	−268 ± 694 a	7332 ± 2540 a	10168 ± 2588 a	3110 ± 293 a
350, November 19	Control (30%)	−55.8 ± 101.4 b	−1059 ± 296 b	−1116 ± 384b	9915 ± 286 a	9854 ± 294 a	1006 ± 34 b
	30%	44.3 ± 40.8 ab	−476 ± 133 ab	−432 ± 168 ab	9204 ± 501 a	9246 ± 520 a	953 ± 24 b
	40%	169 ± 7 a	−78.2 ± 144 a	90.8 ± 149 a	10920 ± 371b	11636 ± 347 b	1233 ± 58 a
	50%	210 ± 5 a	73.8 ± 78.8 a	284 ± 83 a	10750 ± 1607 ab	10951 ± 1607 ab	1238 ± 84 a

NO_3-N = nitrate; TAN = total ammonia nitrogen; TKN = total Kjeldahl nitrogen; TP = total phosphorus.
[1]Control lysimeters (30% LF) were not planted. Values are means ± SEM ($n = 6$).
For each sampling date, parameter means not sharing a letter in common are significantly ($P < 0.05$) different.

TABLE 4. *Removal efficiency (%) of nutrients by the plant-substrate (p-s) lysimeters planted with* Salicornia virginica *and irrigated with recirculating aquaculture effluent at different leachate fractions (LF): 30, 40, and 50%.*[1]

Day of experiment, date (2013)	Sample or treatment (LF%)	TAN removed by p-s lysimeter (%)	NO_3-N removed by p-s lysimeter (%)	Inorganic N removed by p-s lysimeter (%)	Organic N removed by p-s lysimeter (%)	TKN removed by p-s lysimeter (%)	TP removed by p-s lysimeter (%)
141, April 24	Control (30%)	91.0 ± 1.7 a	79.2 ± 2.0 a	85.9 ± 1.9 a	77.7 ± 2.0 a	79.2 ± 2.0 a	82.0 ± 1.7 a
	30%	88.5 ± 3.3 a	79.0 ± 3.2 a	85.9 ± 3.8 a	77.8 ± 3.2 a	79.0 ± 3.2 a	81.7 ± 4.0 a
	40%	91.6 ± 2.2 a	76.5 ± 1.7 ab	90.4 ± 2.0 a	74.5 ± 1.9 ab	76.5 ± 1.7 ab	84.1 ± 2.5 a
	50%	82.2 ± 5.2 a	67.1 ± 3.6 b	79.2 ± 5.9 a	65.2 ± 3.6 b	67.1 ± 3.6 b	73.9 ± 5.6 a
260, August 21	Control (30%)	−123 ± 41 c	−2723 ± 306 c	−277 ± 54.3 c	36.2 ± 2.9 a	22.1 ± 3.2 a	68.7 ± 3.0 a
	30%	−41.7 ± 21.2 bc	−2073 ± 149 bc	−162 ± 18.6 c	33.6 ± 5.3 a	26.9 ± 4.2 a	70.5 ± 2.4 a
	40%	20.1 ± 18.5 ab	−1711 ± 197 ab	−82.2 ± 28.1 b	25.6 ± 3.0 ab	25.1 ± 3.0 a	67.6 ± 0.8 a
	50%	62.9 ± 4.4 a	−1093 ± 175 a	−5.52 ± 14.3 a	15.5 ± 5.4 b	19.7 ± 5.0 a	58.8 ± 5.5 a
350, November 19	Control (30%)	−36.0 ± 65.4 a	−942 ± 263 b	−417 ± 144 b	57.1 ± 1.6 a	56.2 ± 1.7 a	76.9 ± 2.6 a
	30%	28.6 ± 26.3 ab	−423 ± 118 ab	−161 ± 63 ab	53.0 ± 2.9 a	52.8 ± 3.0 ab	72.7 ± 1.9 a
	40%	83.9 ± 3.4 b	−53.5 ± 98.2 a	26.1 ± 42.8 a	48.3 ± 1.6 ab	51.1 ± 1.5 ab	72.4 ± 3.4 a
	50%	84.5 ± 5.1 b	41.0 ± 43.8 a	66.2 ± 19.4 a	38.7 ± 5.8 b	39.1 ± 5.7 b	59.1 ± 4.0 b

NO_3-N = nitrate; TAN = total ammonia nitrogen; TKN = total Kjeldahl nitrogen; TP = total phosphorus.

[1]Control lysimeters (30% LF) were not planted. Values are means ± SEM ($n = 6$).

For each parameter, means not sharing a letter in common are significantly ($P < 0.05$) different.

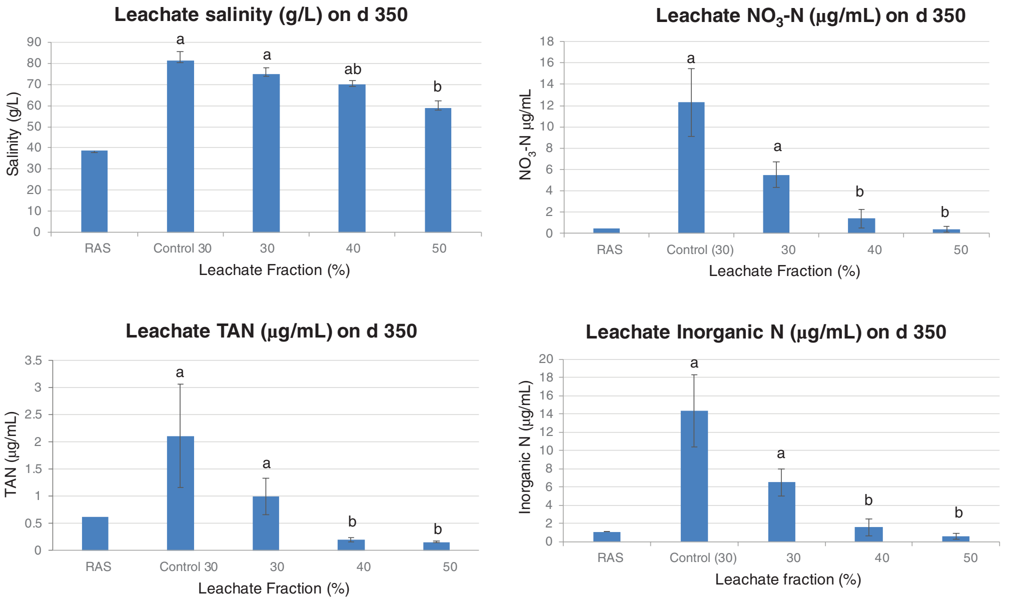

FIGURE 4. *Concentrations (µg/mL) of nutrients (total ammonia nitrogen [TAN], nitrate nitrogen [NO_3-N], inorganic N, organic N, total Kjeldahl nitrogen [TKN], and total phosphorus [TP]) on Day 350 in leachate fractions (LFs) of plant-substrate (p-s) lysimeters planted with* Salicornia virginica *and irrigated with recirculating aquaculture (RAS) effluent at different LF): 30, 40, and 50%. Control lysimeters (30% LF) were not planted. Values are means ± SEM* (n = 6)*, except for RAS effluent* (n = 1)*. Means not sharing a common letter are significantly different.*

260 was negative in all treatments and not significantly different in the 30% control (unplanted) (−5583 µg), 30% LF (−4249 µg), or 40% LF (−4210 µg) treatments. NO_3-N removed was significantly higher at the 50% LF (−3136 µg) than in the 30% control (unplanted) (−5583 µg) (Table 3). Accordingly, inorganic N removed increased from −9600 µg in the 30% control (unplanted) and −5616 µg in the 30% LF treatments to −3424 µg at 40% LF and to −268 µg at 50% LF.

On Day 260 (August 21, 2013), the percentage of nutrients removed from the irrigant by the p-s lysimeter was not significantly different among treatments for TKN (19.7–26.9%) and TP (58.8–70.5%) (Table 4). Significant treatment differences were observed for the other components (Table 4). Percent TAN removed increased from −123% in the 30% control (unplanted) and −41.7% in the 30% LF treatments to 20.1% at 40% LF and 62.9% at 50% LF. Percent NO_3-N removed was not significantly different in the 30% control (unplanted) and 30% LF treatments (−2723 to −2073%) but increased to −1711% at 40% LF and to −1093% at 50% LF (Table 4). Percent inorganic N removed by the p-s lysimeter was also not significantly different in the 30% control (unplanted) (−277%) and 30% LF treatments (−162%) but increased to −82.2% at 40% LF and to −5.52% at 50% LF. Percent organic N removed by the p-s lysimeter showed an opposite trend, with no significant differences in the 30% control (unplanted) and 30% LF treatments (36.2–33.6%), but decreasing to 25.6% at 40% LF and to 15.5% at 50% LF (Table 4).

On Day 350 (November 19, 2013), nutrient concentrations in the irrigant (RAS effluent) were as follows: salinity (38.6 g/L), pH (7.85), TAN (0.62 µg/mL), NO_3-N (0.45 µg/mL), inorganic N (1.07 µg/mL), organic N (69.5 µg/mL), TKN (70.1 µg/mL), and TP (5.24 µg/mL) (Table 2). On Day 350, pH (7.49–7.60) and leachate concentrations of organic N (72.4–76.7 µg/mL), TKN (72.6–78.8 µg/mL), and TP (3.01–3.63 µg/mL) were not significantly different among treatments (Table 2). Leachate salinity decreased with increasing LF treatment from 81.5–75.1 g/L in the 30%

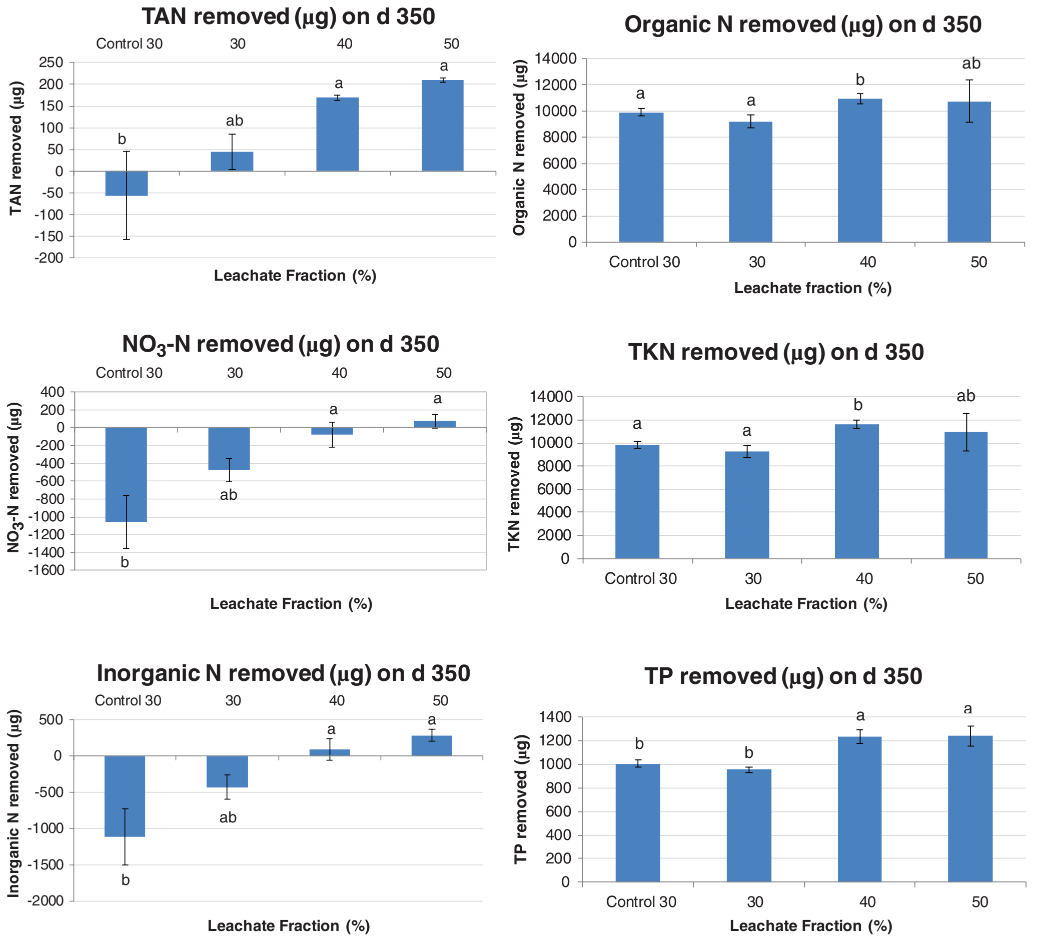

FIGURE 5. *Net removal (µg) of nutrients (total ammonia nitrogen [TAN], nitrate nitrogen [NO_3-N], inorganic N, organic N, total Kjeldahl nitrogen[TKN], and total phosphorus [TP]) on Day 350 in leachate fractions (LFs) of plant-substrate (p-s) lysimeters planted with* Salicornia virginica *and irrigated with recirculating aquaculture effluent at different LFs: 30, 40, and 50%. Control lysimeters (30% LF) were not planted. Values are means ± SEM* (n = 6)*. Means not sharing a common letter are significantly different.*

control (unplanted) and 30% LF treatments to 69.9 g/L at 40% LF and to 58.8 g/L at 50% LF (Table 2, Fig. 6). Leachate TAN concentration was not significantly different in the 30% control (unplanted) and the 30% LF (2.11–1.00 µg/mL) treatments but decreased at the 40% LF (0.207 µg/mL) and at the 50% (0.163 µg/mL) LF. Leachate NO_3-N concentrations were not significantly different in the 30% control (unplanted) and 30% LF treatments (12.3–5.51 µg/mL) but decreased at 40% LF (1.40 µg/mL) and at 50% LF (0.418 µg/mL). Leachate inorganic N concentrations reflected the sums of TAN and NO_3-N, with no significant differences in the 30% control (unplanted) and 30% LF treatments (14.4–6.52 µg/mL), but decreased at the 40% LF (1.60 µg/mL) and the 50% LF (0.582 µg/mL) (Table 2, Fig. 6).

On Day 350, the net quantities of nutrients removed by the p-s lysimeter varied significantly among LF treatments (Table 3, Fig. 5). TAN removed increased with LF from −55.8 µg in the 30% control (unplanted) to 44.3 µg in the 30% LF and to 169.1 µg at 40% LF and 210 µg at 50% LF. NO_3-N removed also increased with LF from −1059 to −476 µg in the 30% control (unplanted) and the 30% LF to −78.2 μg at 40% LF and to 73.8 μg at 50% LF. Inorganic N removed on Day 350 reflected the sums of TAN and NO_3-N, increasing from −1116 to −432 μg in the 30% control (unplanted) and 30% LF to 90.8 μg at 40% LF and to 284 μg at 50% LF (Table 3, Fig. 5). Organic N removed also increased with LF, with no significant difference in the 30% control (unplanted) and 30% LF (9915−9204 μg), but increased to 10,920−10,750 μg at the 40% and 50% LF. Reflecting its inorganic and organic constituents, TKN removed by the p-s lysimeter was not significantly different in the 30% control (unplanted) and 30% LF treatments (9854−9246 μg) but increased to 11,636 and 10,951 μg at the 40% and 50% LF treatments (Table 3, Fig. 5). TP removed showed a similar trend, with no significant differences in the 30% control (unplanted) and 30% treatments (1006−953 μg), but increased to 1233−1238 μg at the 40% and 50% LF.

On Day 350 (November 19, 2013), percent TAN removed by the p-s lysimeter was not significantly different in the 30% control (unplanted) and 30% LF treatments (−36 to 28.6%) but was higher in the 40% and 50% LF treatments (83.9−84.5%) (Table 4, Fig. 6). Percent NO_3-N removed was not significantly different in the 30% control (unplanted) (−942%) and the 30% LF (−423%) but increased to −53.5% at 40% LF and to 41.0% at 50% LF. Percent inorganic N removed was also not significantly different in the 30% control (unplanted) and 30% LF treatments (−417 to −161%) but increased to 26.1% at 40% LF and to 66.2% at 50% LF. Percent organic N removed showed an opposite trend, with no significant differences between the 30% control (unplanted) and 30% LF (57.1−53.0%), but decreasing 48.3% at 40% LF and to 38.7% at 50% LF (Table 4, Fig. 6). Percent TKN removed reflected its main constituent (organic N), with no significant differences between the 30% control (unplanted) and 30% LF treatments (56.2−52.8%), decreasing to 51.1% at 40% LF and to 39.1% at 50% LF. Percent TP removed showed a similar trend, with no significant differences between the 30% control (unplanted), 30 and 40% LF treatments (76.9−72.4%), decreasing to 59.1% at 50% LF.

Discussion

Although mean LF% for the duration of the experimental period were 35−36, 44, and 53%, slightly above the prescribed LF of 30, 40, and 50%, respectively, relative differences in LF% among treatments and the integrity of the experimental design were maintained. A greenhouse building may have moderated diurnal and seasonal temperature regimes and improved *Salicornia* growth throughout the study period.

Plant vigor and growth (aboveground biomass) were excellent among all LF% treatments over the first 85 d of the experiment, with no treatment effects observed (Fig. 2). However, by Day 141 a departure in growth was apparent, with biomass reaching a plateau at the 30% LF, but continuing to increase at the 40 and 50% LF. After this, biomass declined in all treatments but remained more stable at the 40 and 50% LF than at the 30% LF, which showed a more rapid decline. Reduced plant vigor and biomass in all treatments after Day 141 were associated with high maximum daytime temperatures, which reached 30 C by Day 205 (June 26, 2013) (Fig. 1). Dehydrating conditions in mid-summer probably contributed to salt buildup in the lysimeter substrate, which was mitigated by greater irrigant volumes at the higher LF of 40 and 50% (Table 2). From Day 225, plant vigor and biomass increased with increasing LF from a minimum at 30% LF to a maximum at 50% LF.

The contribution of roots to total plant biomass under the different LF treatments was determined on Day 365 (Table 1). Despite clear treatment differences in aboveground biomass, root biomass (dry and wet) was similar among treatments and therefore represented a relatively high percentage (56.4%) of total plant biomass at the lowest (30%) LF treatment in which aboveground biomass was severely degraded. As observed for shoot biomass, total (shoot + root) biomass (wet and dry) on Day 365 showed a progressive increase with increasing LF% from lowest biomass at 30% LF to highest biomass at 50% LF. This suggests that a LF > 50% may have further improved growth of *S. virginica* in this study.

Maximum aboveground biomass under all LF treatments was observed on Day 141 (Fig. 2). Based on the surface area of the p-s lysimeters (0.046 m^2), aboveground biomass yields at the 30, 40, and 50% LF were estimated as 0.524, 1.39, and 1.02 g per m^2/d, respectively, or 0.191, 0.507, and 0.372 kg/m^2yr. In comparison, biomass yields of 1.7 kg/m^2yr were reported for *S. bigelovii* in actual field trials (Glenn et al. 1998). Although the data reported in the present study are derived from a laboratory-scale experiment, they indicate that comparable yields to *S. bigelovii* are attainable with *S. virginica* given further optimization of growing conditions.

Irrigant pH (7.81–7.97) and leachate pH (7.51–7.73) were comparable, and leachate pH remained stable among LF% treatments during this study (Table 2). Whereas the irrigant (RAS effluent) salinity ranged from 29.2 to 38.6 g/L, and the leachate salinity in all LF% treatments increased to 53.9–58.9 g/L by Day 141 and to 59.3–88.2 g/L by Day 260 (Table 2). Therefore, the reduction of plant biomass observed after Day 141 (April 24, 2013) in all treatments was likely due to excessive salt buildup in the root zone inhibiting plant nutrient uptake and growth. A major constraint to the cultivation of halophytes is that irrigating with seawater leads to the accumulation of salt in the soil (Brown et al. 1999; Brown and Glenn 1999; Radulovich et al. 2017). In the present study, salt buildup and growth inhibition were mitigated at the higher LF of 40% and 50%. This suggests that LF > 50% may have further minimized the accumulation of salt in the substrate, improving nutrient uptake by the plants and promoting *Salicornia* growth.

Nutrient Removal by P-S Lysimeter: Concentration, Net Removal, and Removal Efficiency

Removal of nutrients from the irrigant (RAS effluent) by the p-s lysimeter was analyzed on Day 141 when plants attained maximum biomass and on Days 260 and 350 when clear departures in aboveground biomass among LF treatments were evident. Data were expressed and analyzed as irrigant (RAS effluent) and leachate nutrient concentrations (µg/mL), net removal of a nutrient from the irrigant (µg) by the p-s lysimeter, and removal efficiency (%) of a nutrient from the irrigant by the p-s lysimeter.

When maximum plant biomass was reached on Day 141 (April 24, 2013), no differences in leachate nutrient concentrations (ppm or µg/mL) among LF treatments were evident (Table 2). However, leachate concentrations are indirect measures of the biofiltering effects of the p-s lysimeters on a nutrient because the irrigant volumes (and nutrient inputs) were not equivalent across LF% treatments – that is, they were 34.6% and 59.1% higher at the 40% and 50% LF, respectively, than at the 30% LF. Given that LF of 30–50% were tested, substantial losses of irrigant from the lysimeters due to evapotranspiration (i.e., the sum of evaporation and plant transpiration) were inherent in the experimental design and were expected to lower leachate volumes below the irrigant volumes. Furthermore, because the substrate adsorbs irrigant, a significant net removal of irrigant water (and dissolved nutrients) by the p-s lysimeter substrate was expected, even in control (unplanted) lysimeters. Hence, the nutrient concentrations in the lysimeter leachate were affected by irrigant volume, evapotranspiration, removal by the substrate and the *Salicornia* (i.e., the p-s lysimeter), and treatment comparisons of these leachate nutrient concentrations therefore require careful interpretation.

In contrast to leachate nutrient concentrations, net removal (µg) of a nutrient from the irrigant by the p-s lysimeter is a direct measure of the biofiltering effects of the p-s lysimeters. When maximum plant biomass was reached on Day 141, positive net removal (µg) of nutrients from the irrigant by the p-s lysimeter was observed for TAN, inorganic N, organic N, TKN, and TP (Table 3). At the 30% LF, a significant net removal of these nutrients in the control (unplanted) lysimeters indicates that the substrate played an important role in removing these nutrients from the irrigant. This is similar to what was reported in small-scale constructed wetlands irrigated with wastewater discharged from a land-based RAS growing marine shrimp, where control (unplanted) beds removed significantly less influent dissolved inorganic N than beds planted with *Salicornia europaea* (Webb et al. 2013). In the present study, net removal of these nutrients was not different between the 30% control (unplanted) and the 30% LF (planted with *Salicornia*) lysimeters, consistent with zero or low net removal by *Salicornia* and a slowing of growth observed on Day 141 at the lowest 30% LF (Fig. 2). In contrast, the p-s lysimeters irrigated at higher LF of 40 and 50% on Day 141 showed greater net removal of inorganic N and TP from the irrigant (Table 3) and continued plant growth (Fig. 2).

Greater removal of TAN by the p-s lysimeter at the higher 40 and 50% LF is consistent with the significant concentrations of TAN in the irrigant (RAS effluent) and its rapid uptake by the *Salicornia*. Although NO_3-N was also readily absorbed by *Salicornia*, low net removal of NO_3-N among treatments on Day 141 was due to relatively low NO_3-N concentrations in the irrigant (Table 2). The negative net removal values for NO_3-N, such as in the 30% control (unplanted) lysimeters, indicates that the quantities of NO_3-N in the leachate were greater than those added in the irrigant, probably due to leaching of residual NO_3-N from the p-s lysimeter. While counterintuitive, this excess NO_3-N probably originated from previous irrigation events as organic N (e.g., proteins and amino acids) retained by the substrate where it was ammonified and nitrified by substrate-living bacteria (Buhmann and Papenbrock 2013; Webb et al. 2013). Such excess NO_3-N was present in all LF% treatments but was effectively removed by the *Salicornia*, producing near-zero or positive net removal in the planted lysimeters (Table 3). Quintã et al. (2015) found that *S. europaea* was able to utilize both TAN and NO_3-N in simulated aquaculture wastewater experiments and predicted high N removal and plant production in IMTA systems.

Because irrigant volumes (and nutrient inputs) differed across LF% treatments, percent removal efficiency is also an indirect measure of the biofiltering effects of the p-s lysimeters on a nutrient. For example, percent removal efficiency declines as irrigant volume (and LF%) is increased if the quantity of nutrient removed by the p-s lysimeter remains constant. When maximum biomass was reached on Day 141, higher irrigant volumes and LF% resulted in greater net removal of TAN, inorganic N and TP from the irrigant by the p-s lysimeters (Table 3), without a reduction in removal efficiency (Table 4), which ranged from 79.2 to 90.4% for inorganic N and from 73.9 to 84.1% for TP. Excessive irrigation of halophytes planted in drainage lysimeters results in poor nutrient removal efficiency (Brown and Glenn 1999). In the present study, the relatively high removal efficiency of inorganic N (79.2%), TKN (67.1%), and TP (73.9%) at the highest 50% LF indicates that the irrigation volumes were not excessive.

On Day 260 (August 21, 2013), when a clear departure in plant biomass among LF treatments was observed (Fig. 2), leachate TAN, NO_3-N, and inorganic N concentrations (ppm or μg/mL) clearly decreased with increasing LF to minimum concentrations at 50% LF (Table 2). This is consistent with rapid uptake of TAN and NO_3-N, much greater net removal of these inorganic N components from the irrigant by the p-s lysimeters, and with greater plant biomass and vigor at the highest 50% LF. Accordingly, the net removal (μg) of TAN and inorganic N from the irrigant by the p-s lysimeter on Day 260 clearly increased at higher LF% (Table 3). The negative net removal values for TAN, NO_3-N, and inorganic N on Day 260 indicate a leaching of these components (derived from decomposing organic N) from the lysimeters, especially in the 30% control (unplanted) lysimeter and emphasizes the importance of the *Salicornia* in reducing nutrient leaching from the p-s system. The clear trend toward greater net removal of these inorganic N components at higher LF (Table 3) is consistent with more efficient removal of these nutrients by the *Salicornia* at the higher 40% and 50% LF. In agreement with net nutrient removal data, percent removal efficiency by the p-s lysimeter on Day 260 (Table 4) for TAN,

NO_3-N, and inorganic N was lowest at the 30% LF and increased at higher LF of 40 and 50%.

The lack of significant treatment differences in net removal (µg) of TKN (organic N + TAN) on Day 260 was probably related to the leaching of residual TAN from the lysimeters, especially at the lowest 30% LF and to the inability of *Salicornia* to directly utilize organic N, the main component of TKN in the irrigant. Hence, percent removal efficiency of organic N decreased with increasing LF%, while percent removal efficiencies of TKN and TP were not significantly different among LF% (Table 4), reflecting a limited capacity of *Salicornia* to absorb organically bound N or P.

On Day 350 (November 19, 2013), leachate TAN, NO_3-N, and inorganic N concentrations (ppm or µg/mL) decreased markedly at higher LF% (Table 2, Fig. 4). Leachate organic N, TKN, and TP concentrations (ppm or µg/mL), however, showed no significant differences (Table 2). In accord, net removal (µg) of TAN, NO_3-N, and inorganic N by the p-s lysimeter (Table 3, Fig. 5) markedly increased with LF% to a maximum at 50% LF, consistent with higher nutrient uptake and aboveground biomass at higher LF%. Negative net removal of TAN, NO_3-N, and inorganic N in the 30% control (unplanted), 30%, and 40% LFs (Fig. 5) was attributable to leaching of these components (derived from decomposing residual organic N) from the substrate, especially in the 30% control (unplanted) lysimeter. On Day 350, net removal (µg) of organic N, TKN, and TP showed more modest increases with LF% to a maximum at 50% LF (Fig. 5), indicating that these constituents were removed with lower efficiency. In accord with these trends, percent removal efficiencies of TAN, NO_3-N, and inorganic N by the p-s lysimeter on Day 350 were negative in the control 30% (unplanted) and 30% LF but increased markedly with higher LF to 84.5, 41.0, and 66.2%, respectively, at 50% LF (Table 4, Fig. 6). For leachate organic N, TKN, and TP, percent removal efficiencies showed an opposite trend of decrease at higher LF% (Table 4, Fig. 6). This downward trend with increasing LF% was related to organic N being the main component of TKN in the irrigant and a limited capacity of *Salicornia* to remove organically bound N and P. The data on nutrient removal by the p-s lysimeter over three sampling periods are consistent with the direct removal of dissolved inorganic N and P from the irrigant by *Salicornia* in the p-s lysimeter or their accumulation in the substrate before removal or discharge in leachate. In contrast, organically bound N and P are not directly removed by *Salicornia* and may accumulate in the substrate where they are converted by microbiota into inorganic N and P, which are then removed by *Salicornia* or discharged in leachate.

A practical method for removing TSS in wastewater from commercial land-based RASs producing marine finfish is to pass the raw wastewater through a geotextile fabric bag (Geotube) (Gibson 2014, Watanabe et al. 2015). The results of the present study suggest that IMTA using salt-tolerant *Salicornia* may be a practical means for removing dissolved nutrients from the Geotube filtrate before release to the environment or reuse in the RAS.

Summary and Conclusions

The salt-tolerant *S. virginica* planted in drainage lysimeters was an effective biofilter for dissolved N and P in effluent from a RAS for marine finfish. When plants were irrigated with effluent (prefiltered to reduce TSS) to produce LF of 30, 40, and 50%, plant biomass, vigor, and nutrient removal were maximized at the highest LF of 50%. Nutrient uptake and growth of *Salicornia* in all LF% after Day 141 were associated with a buildup of salt in the lysimeter substrate, and this buildup was mitigated at higher LF%, suggesting that LF higher than 50% may have further optimized *Salicornia* growth. Consistent with trends in plant biomass, net removal of TKN (organic N + TAN), NO_3-N, and TP was increased at the higher LF of 40 and 50% compared to 30%. On Day 141 when maximum biomass was attained, removal efficiencies by the p-s lysimeters for TAN, NO_3-N, total inorganic N, and TP were 82.2, 67.1, 79.2, and 73.9%, respectively (Table 4). To increase biomass yield and nutrient removal, studies are

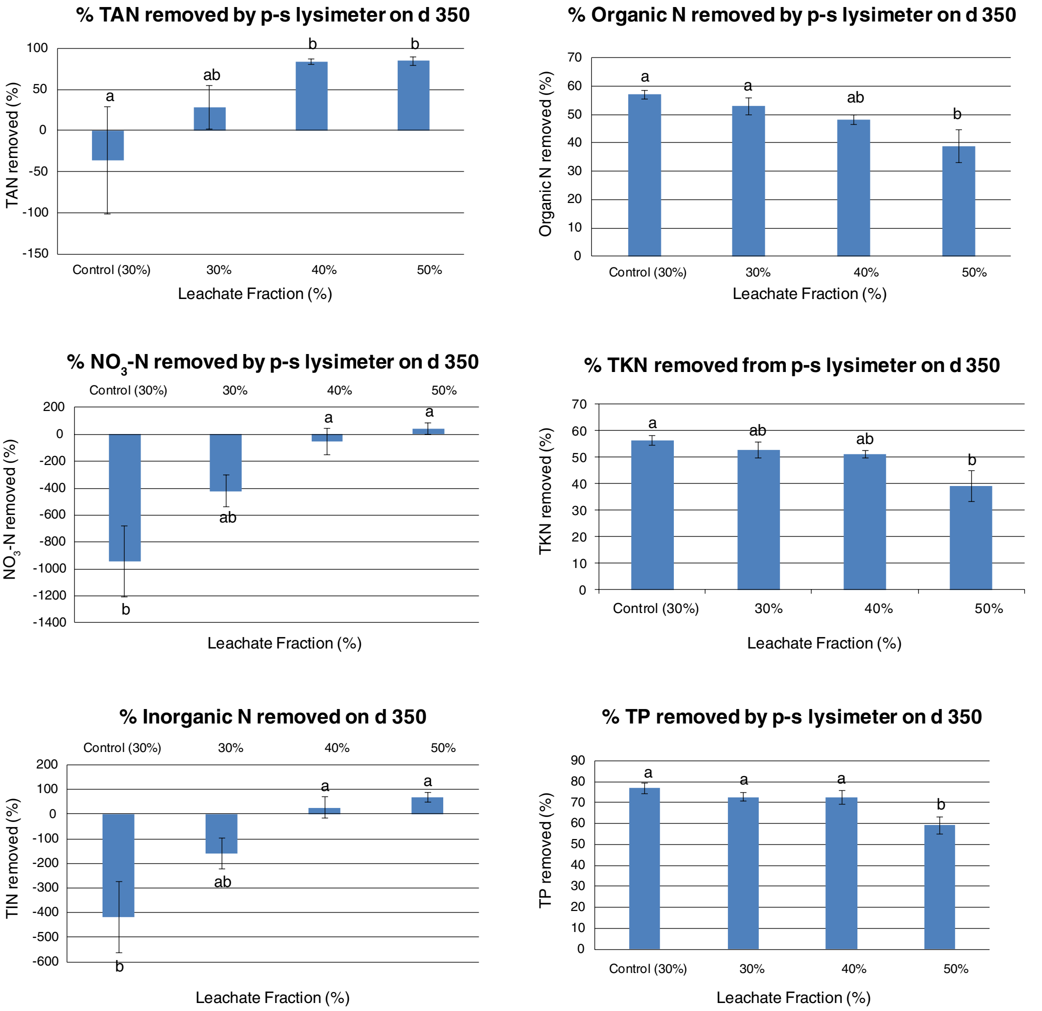

FIGURE 6. *Removal efficiency (%) of nutrients (total ammonia nitrogen [TAN], nitrate nitrogen [NO_3-N], total Kjeldahl nitrogen [TKN], and total phosphorus [TP]) removed on Day 350 by the plant-substrate (p-s) lysimeters planted with* Salicornia virginica *and irrigated with recirculating aquaculture effluent at different leachate fractions (LFs): 30, 40, and 50%. Control lysimeters (30% LF) were not planted. Values are means ± SEM* (n = 6)*. Means not sharing a common letter are significantly different.*

needed to minimize salt buildup in the lysimeter substrate by increasing LF% and frequency of irrigation. Recycling of leachate through the p-s lysimeter, or passing it through a series of p-s lysimeters in sequence will enable more complete nutrient removal from the effluent.

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