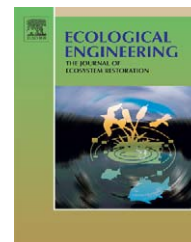


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Effects of long-term municipal effluent discharge on the nutrient dynamics, productivity, and benthic community structure of a tidal freshwater forested wetland in Louisiana

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ABSTRACT

Nutrient dynamics, net aboveground primary productivity (NPP), and benthic macroinvertebrates were measured in a Louisiana tidal, freshwater forested wetland that received secondary treated effluent for 27 years. NO₃, NH₄, TKN, PO₄, TP concentrations were measured at treatment and control sites. TKN (2.0–4.0 mg/L), NH₄-N (0.4–1.0 mg/L) and NO₃-N accounted for almost 75%, 25% and less than 1% of TN, respectively. PO₄ (0.1–0.9 mg/L) was about 50% of TP. TN and TP were reduced by 79% and 88%, respectively, as water flowed through the wetland, which is consistent with low loading rates of 9.4 g N and 1.2 g P/m²/yr. Litterfall was significantly greater in the treatment site (717 g/m²/yr) than one of the control sites (412 g/m²/yr). Stem growth (302–776 g/m²/yr) was not statistically different among the sites. Total NPP was highest at treatment sites (1467 and 1442 g/m²/yr) which were statistically higher than one of the control sites (714 g/m²/yr). Total individuals, total species, and species richness of macroinvertebrates were greatest near the outfall and declined away from the discharge. In summary, long-term addition of secondarily treated municipal effluent resulted in a high level of nutrient retention, enhanced forest productivity, and minimal impact on benthic community structure.

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1. Introduction

Wetlands are widely used for assimilation of treated municipal wastewater and numerous studies have shown that both natural and constructed wetlands can be effective tertiary pro-

cessors of municipal wastewater effluent (Kadlec and Knight, 1996; Richardson and Davis, 1987; Conner et al., 1989; Day et al., 2004). Wetlands are efficient at removing excess nutrients and pollutants by physical settling and filtration, chemical precipitation and adsorption, and biological processes that result

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in burial, storage in vegetation, and denitrification (Hammer, 1989; Conner et al., 1989; Kadlec and Alvord, 1989; Faulkner and Richardson, 1989; Richardson, 1999).

In the deteriorating wetlands of the Mississippi delta, discharge of secondarily treated municipal effluent results in a number of benefits including improved water quality, increased accretion rates, increased productivity of vegetation, and financial and energy savings (Breux, 1992; Breux and Day, 1994; Ko et al., 2004; Day et al., 2004). The high rate of burial due to subsidence, and high rates of denitrification due to warm temperatures provide permanent uptake pathways for nutrients (Rybczyk et al., 2002; Day et al., 2004). Increasing vegetative productivity is especially crucial in many parts of Louisiana where coastal subsidence in the Mississippi delta results in a relative sea-level rise nearly 10 times greater than eustatic sea-level rise (Conner and Day, 1988; Penland et al., 1988). Increased productivity in wetlands receiving secondarily treated effluent (Hesse et al., 1998; Brantley, 2005) leads to higher organic soil formation that can enhance the accretion necessary to offset subsidence (Rybczyk et al., 2002; Brantley, 2005). Thus, treated effluent can serve as a restoration tool for coastal wetlands impacted by sea-level rise.

Nutrient retention in wetlands is related to loading rate, with higher retention at low loading rates (Richardson and Nichols, 1985; Faulkner and Richardson, 1989; Richardson, 1999; Mitsch et al., 2001; Mitsch and Jorgensen, 2004; Fisher and Acreman, 2004). Potential permanent nutrient sinks in wetlands are denitrification, burial, and plant uptake (Day et al., 2004). There are several cities in south Louisiana where natural wetlands are used to assimilate secondarily treated municipal effluents. Because wetland areas are generally large, loading rates are low and there is a high burial capacity, there is generally high nutrient retention (Blahnik and Day, 2000; Zhang et al., 2000; Day et al., 2004).

In this study, we investigated nutrient dynamics, net primary productivity, and benthic community structure in a forested swamp at Amelia, Louisiana, which has received secondarily treated municipal effluent from an oxidation pond for 27 years. The objectives of this study were to determine the effects of long-term discharge of treated municipal effluent on a tidal, freshwater forested wetland with respect to

- (1) nutrient reductions within the forested wetland,
- (2) net aboveground primary productivity of the forested wetland,
- (3) benthic community structure.

We hypothesized that there would be a significant reduction in nutrient concentrations of the effluent, a stimulation of aboveground net primary productivity of the wetland, and a decrease in the diversity and an increase in the density of the benthic community near the effluent outfall.

1.1. Study area description

The study site, Ramos Swamp, is a continuously flooded tidal freshwater forested wetland located south of Lake Palourde approximately 2 km north of Amelia, Louisiana (Fig. 1). The area has a mild climate reflecting the subtropical location and proximity to the Gulf of Mexico. The mean annual air tem-

perature is 20.6°C, ranging from 13.0°C in January to 27.5°C in July. Because the site is over 40 km from the coast, daily water level fluctuations are less than a few centimeters. The swamp area directly affected by the effluent flow was estimated at 77 ha based on analysis of aerial photographs and the field inspection of hydrologic flow patterns. This area is within a larger forested wetland area of over 1000 ha. The swamp is largely a needle-leaved deciduous and broad-leaved forested wetland (Cowardin et al., 1979) characterized by bald cypress (*Taxodium distichum*), water tupelo (*Nyssa aquatica*), black willow (*Salix nigra*), red maple (*Acer rubrum*), and green ash (*Fraxinus pennsylvanica*). Floating aquatic vegetation (FAV) is dominated by mosquito fern (*Salvinia minima*), duckweed (*Lemna minor*), water-meal (*Wolffia* sp.), pennywort (*Hydrocotyl ranunculoides*), and waterlettuce (*Pistia stratiotes*). Submerged aquatic vegetation (SAV) is predominantly hornwort (*Ceratophyllum* sp.). The flooded soils in this forest consist of well-consolidated riverine clay layers overlain by high organic mucky clays and topped by a poorly consolidated 30–60 cm layer of plant detritus (Lytle et al., 1959). The detritus originates from litterfall and submerged and floating aquatic vegetation.

The wetland has received treated effluent since 1973 into approximately the same area of the swamp. The treatment system presently serves approximately 2500 people. An average of 3.0 million liters per day (0.8 million gallons per day) are discharged from three oxidation ponds that cover 14 ha.

The study area was divided into five sites for net primary productivity measurements with two additional sites for water nutrient analysis (Fig. 1). The treatment site (T) was located within 100 m of effluent discharge between the ponds and the lake. Two control sites (C1 and C2) were located on the southern edge of the oxidation ponds. Two sites were established in the swamp near the shore of Lake Palourde (Edge 1 and Edge 2, or E1 and E2 sites) and two additional sites (L1 and L2, for water sampling only) were established about 200 m from shore in Lake Palourde. The T, C1, and C2 sites will also be referred to as interior swamp sites.

Benthic sampling was conducted at a single effluent-free reference station (R) and four monitoring stations 5, 30, 175, and 670 m from the point of effluent discharge along the main direction of effluent flow (Fig. 1). The 5 m station was cleared of trees and had higher mineral content sediment. The 30 m station was at the forest edge while the remaining stations were in the forest.

2. Methods

2.1. Hydrology and water budget

Water depth measurements were taken during all field trips whenever sampling occurred at litter boxes and water quality stations. These data were averaged for each sampling period to obtain seasonal patterns of water level variation. Stage data were obtained from the nearest water level gage to estimate daily and seasonal water level variations (USACOE at Bayou Bouef; southwest of the study area).

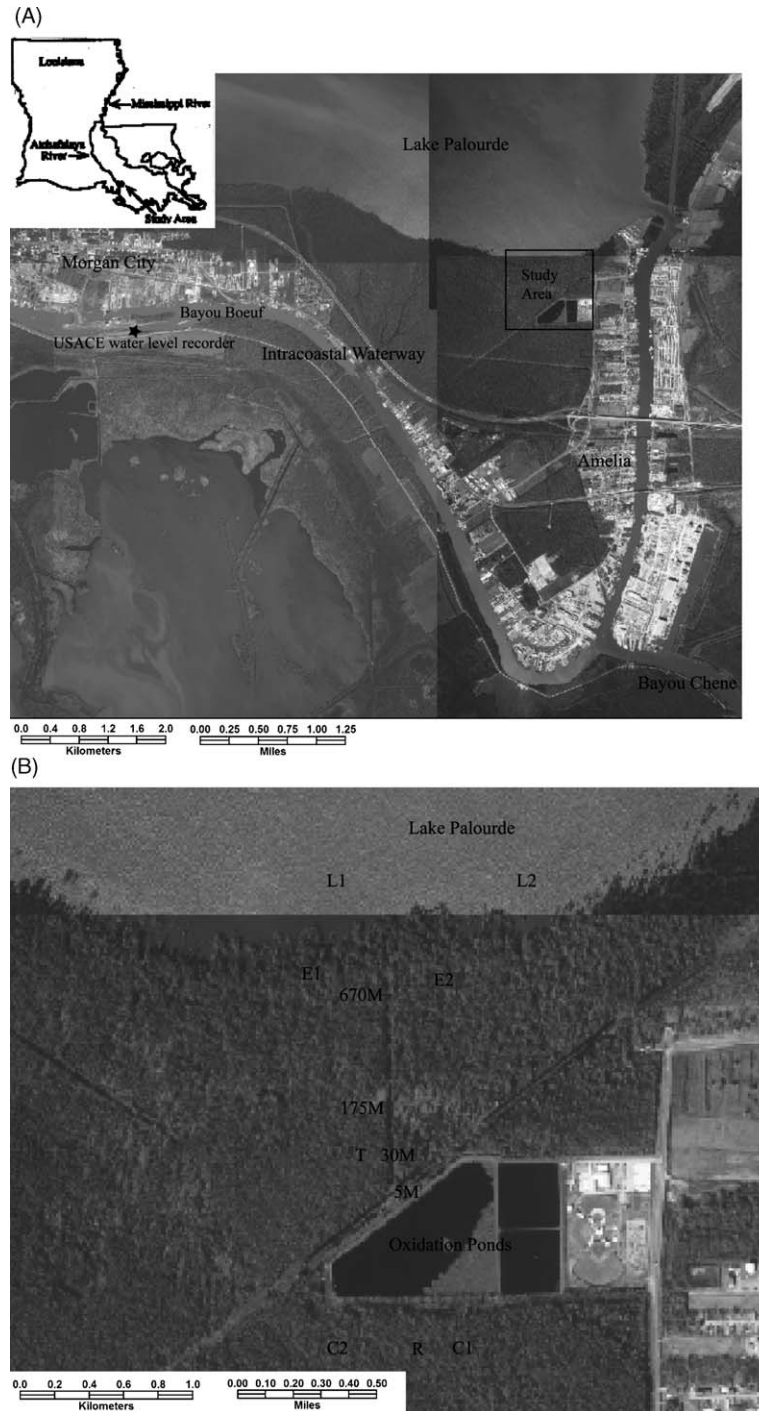


Fig. 1 – Ramos Swamp study area with C1, C2, T, Edge 1 (E1), Edge 2 (E2), Lake 1(L) and Lake 2 (L2) sampling sites for nutrient analysis and net primary productivity. R (reference), 5, 30, 175 and 670 m labels show locations of benthic sample collection in Amelia, St. Mary Parish, Louisiana.

A water budget was calculated for the study area. Monthly precipitation (PPN) and temperature data were obtained from the National Climatic Data Center (NCDC) for Morgan City, the closest weather stations to the study area (<http://www.ncdc.noaa.gov>). Thornwaite's equation was used to calculate monthly potential evapotranspiration (PET) for Amelia (McCabe et al., 1985). The effluent was added to the water budget.

2.2. Water nutrients

Water samples were collected quarterly at each sampling station on 27 July 1995, 6 October 1995, 11 January 1996, and 4 and 12 April 1996. Samples were collected in 500 mL acid-washed polyethylene bottles, stored on ice, and transported to the laboratory for the analyses of NO_3 , NH_4 , PO_4 , TKN, and TP. In the laboratory, portions of the samples were filtered through 0.45-

μm glass-fiber filters and frozen for analysis of nitrate + nitrite (NO_x ; EPA, 1979 method 353.2), NH_4 (EPA, 1979 method 350.1), and PO_4 (EPA, 1979 method 365.2). An unfiltered portion of the sample was frozen for analysis of TKN (EPA, 1979 method 351.3) and TP (EPA, 1979 method 365.4).

Water nutrient data were log transformed and statistical analyses and tests of significance were based on the log transformed data. However, the results are presented graphically as means in units of mg/L. All statistics were done with SAS (SAS Institute Inc., 1987, Procedure GLM).

A two-factor ANOVA model was used with the two factors of month and site. In this model, the month factor has four levels (July, October, January, and April) and the site factor has five levels (T, C1, C2, Edge, and Lake sites). All factors, including interactions, are considered fixed effects. Since a significant interaction may interfere with the conclusions of main effects, main effects were only interpreted if the interaction was non-significant. Since four water samples were taken at each of the T, C1, and C2 sites, but only two samples were taken at the Lake and Edge sites, this was an unbalanced design, and type III sum of squares were used in the analyses.

The R-square statistics, which give the percentage of total variability explained by the model, were 0.48, 0.75, 0.72, 0.71, and 0.64 for NO_3 , NH_4 , PO_4 , TKN, and TP, respectively. For NO_3 , NH_4 , PO_4 , and TP, the Shapiro–Wilks statistic was non-significant, thus normality was not rejected. For TKN, the Shapiro–Wilks statistic was significant, and normality was rejected. However, the residuals for TKN were symmetric and bell-shaped with a few outliers. The F-test is relatively robust from these departures of assumptions (Milliken and Johnson, 1984).

Since there were significant interaction effects for NO_3 , NH_4 , and PO_4 ($p < 0.08$, 0.001, and 0.0005, respectively), differences among sites were investigated by looking at differences in each month. These determinations were made by using Fisher's least significant difference on site \times month least squares means. Only effects that were significant using Bonferroni adjusted p -values are presented. Since there were 10 pairs of sites, these adjustments were made by site using a p -value of 0.05/10 or 0.005. Those differences with p -values of 0.005 or less were considered statistically significant while differences with p -values between 0.005 and 0.05 were noted as moderate differences. Since there were no significant site \times month interactions for TKN and TP, Tukey's post-ANOVA comparisons were used, and all pair-wise comparisons are controlled for an experimental-wise error of 5%.

2.3. Productivity

Sixteen 16 m by 16 m vegetation plots were established in the study area, four located at each of sites C1, C2, and T1 and two vegetation plots located at each E1 and E2 site. Within each vegetation plot, all trees with a diameter at breast height (dbh) > 2.5 cm were marked with an aluminum identification tag and the species recorded. Measurements of dbh were taken on 11 January 1996, and 24 January 1997. The standing biomass of all tree species was calculated in kg/m^2 using dbh versus tree biomass regression equations reported by Clarke

et al. (1985) for similar forests in the southeastern United States.

Litterfall was collected from three, 0.25 m^2 boxes with 1 mm mesh bottoms, placed randomly in each vegetation plot for 12 total litter boxes each at the C1, C2, and T sites and six total litter boxes at each E1 and E2 site. Litterfall was collected monthly from October 1995 to September 1996, separated into leaves and woody material, dried at 60 °C for 48 h, and weighed.

Three variables were analyzed to determine differences in aboveground tree productivity among the C1, C2, T, E1, and E2 sites. These variables include average yearly leaf litterfall and average tree biomass increase (wood production). The sum of these two variables allowed the analysis of a third variable: total aboveground net primary productivity (NPP). The monthly leaf litterfall data from each 0.25 m^2 litterbox were summed over 1 year to yield values in $\text{g}/\text{m}^2/\text{yr}$. The three litterbox values in each vegetation plot were averaged to get one data point for each vegetation plot. Therefore, there were four data points for each of the C1, C2, and T sites. There were two data points for each of the E1 and E2 sites since each site had only two vegetation plots, and therefore this was an unbalanced design.

For the tree growth data, there were also four data points each for the C1, C2, and T sites, and two data points for the E1 and E2 sites. Using the difference in dbh from January 1996 to January 1997, the increase in tree biomass in kilogram was calculated and converted to $\text{g}/\text{m}^2/\text{yr}$. All statistics were done with SAS (SAS Institute Inc., 1987, Procedure GLM).

A one-factor ANOVA model was used. In this model, site was the treatment effect for the five sites (C1, C2, T, E1, and E2). Additionally, Tukey pair-wise comparisons were used to protect experiment-wise error for pair-wise comparisons. The R-square statistics, which give the percentage of total variability explained by the model were 0.63, 0.42, and 0.69 for average leaf litterfall, average tree biomass, and NPP, respectively. For all three variables, the Shapiro–Wilks statistic was non-significant, indicating normal residuals.

2.4. Benthic macroinvertebrates

Sampling at each station was carried out at random points along a 16 m transect at each station. The transects were located away from other research activity in areas of homogeneous mud and at least 1 m from trees. Sampling occurred in the fall of 1996 (November 9 and 15) and the spring of 1997 (May 23 and June 4). Macroinvertebrates were sampled with six replicate 10 cm long stainless steel cores (43 cm^2 area) modified from Miller and Binham (1987). Cores were double sieved at 500 μm and the contents preserved in a buffered 10% formalin solution and stained with rose Bengal. All macroinvertebrates were hand picked under 12 \times magnification and identified to lowest possible taxonomic level. Sediment parameters were sampled by two replicate 30 cm long cores of the same design taken at each of the stations for the fall sampling and three replicates in the spring. Cores were sealed, kept upright, and frozen. Surface water temperature, dissolved oxygen (YSI model 55), pH, and Eh (platinum probe at 10 cm into the sediment with a calomel reference electrode) were measured in the field.

The upper 10 cm of the frozen cores were sectioned for analysis. The sediments were dried to constant weight (100 °C for 24–48 h), milled to a uniform particle size, and analyzed for bulk density (Klute, 1986), percent organic matter, TKN, and organic carbon (C), Bray phosphorous (P), and exchangeable cations (Mg, K, Na, and Ca). Percent organic matter was determined by ignition at 550 °C for 2 h (Plumb, 1981). Exchangeable cations (Mg, K, Na, and Ca) were extracted with 1M NH₄OAc (Thomas, 1982), and Bray P was extracted with 0.003 M NH₄F, 0.1 M HCl (Byrnside and Sturgis, 1958). Extracts were analyzed by inductively coupled plasma spectrometer (ICP). Total N and total C were determined by direct combustion with a C–H–N analyzer (Nelson and Sommers, 1982). Total metals in fall samples were extracted by HNO₃ (Plumb, 1981) and analyzed by ICP. Analysis for total Fe, Al, Ni, Cd, Zn, Cr, and Cu were conducted on subsamples of the dried sediment samples used in the nutrient analysis.

For descriptive purposes, the environmental data was used without transformation and correlations among variables calculated. Significant between-station differences were tested using a completely randomized design (CRD) analysis of variance (ANOVA) in conjunction with an a posteriori Tukey–Kramer test (SAS Institute Inc., 1987). Traditional univariate community parameters were calculated from faunal data: species richness (S), Shannon–Wiener diversity (H'), Evenness (Pielou's, J), and Simpson's dominance indices (SI). Between-station differences in these parameters were then examined by the same CRD, ANOVA with a posteriori Tukey–Kramer test as applied to the environmental data. Ordination of faunal data was carried out using the Plymouth Routines In Multivariate Ecological Research (PRIMER) computer programs (Clarke and Warwick, 1994). Rare taxa with less than four specimens in a sample were removed and data were fourth-root transformed to minimize the effect of dominant species. Non-parametric multi-dimensional scaling (MDS, Clarke and Green, 1988) and hierarchic agglomerative clustering on percent similarity (Clarke and Warwick, 1994) were calculated. Hypothesis tests for differences between stations were conducted using analysis of similarity (ANOSIM, Clarke, 1993) without adjustment for multiple comparisons. A ranked similarity matrix was constructed using Bray–Curtis similarity and group-average sorting for the community data. The relationship between multivariate environmental variables and multivariate community structure were analyzed using the BIOENV (Clarke and Ainsworth, 1993). This procedure calculates rank correlations between the benthic community similarity matrix and matrices calculated from the environmental variables, thereby defining variables or groups of variables that best explain the community structure.

3. Results

3.1. Hydrology and water budget

The interior wetland sites were flooded continuously during the study period, often at depths greater than 40 cm over the forest floor (Fig. 2). Mean water depths for the E2 edge site was about 20 cm while the E1 site was without standing water for much of the study period due to its higher elevation (Fig. 2).

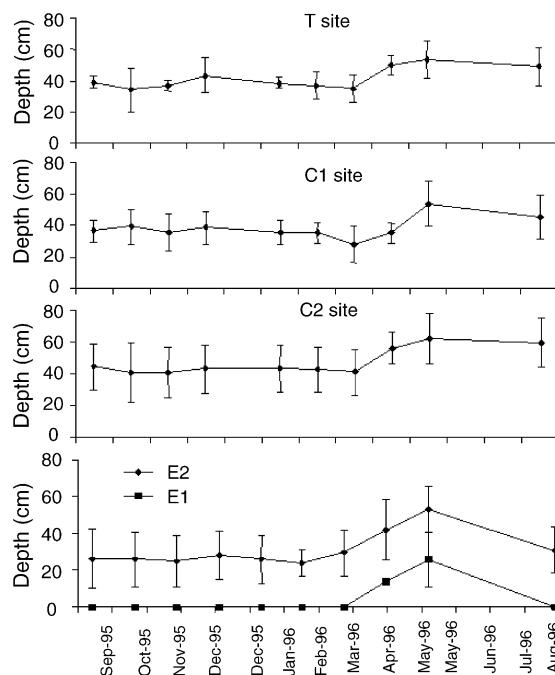


Fig. 2 – Mean water depths (±S.D.) for the T, C1, C2 sites (interior wetland sites), and for the lake Edge sites (E1 and E2) in Ramos Swamp. Data are missing for January 1996, June 1996, and July 1996 for T, C1 and C2. Data are missing for June and July 1996 for E1 and E2 sites.

The continuous flooding at most sites is a reflection of rapid subsidence in this Mississippi delta site (e.g., Conner and Day, 1988). The average water depths were relatively constant with an increase in April and May 1996. Water levels at the Bayou Bouef gauge indicated that there was a stronger seasonal variation compared to daily fluctuations (Fig. 3). Bayou Bouef is affected by both the Gulf of Mexico and the Atchafalaya River. This is reflected in a seasonal variation of approximately 90 cm with a high in the spring caused by high river levels and average daily water level variations of less than 10 cm caused by the tide (Fig. 3). Seasonal and daily water level variations in the wetlands south of Lake Palourde are much less than those at the Bayou Bouef gauge reflecting the 14 km distance

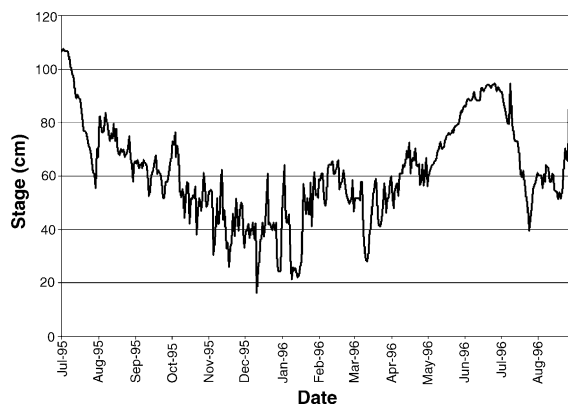


Fig. 3 – Stage at Bayou Boeuf East Lock (see Fig. 1 for gauge location). (Source USACE. www.mvn.usace.gov).

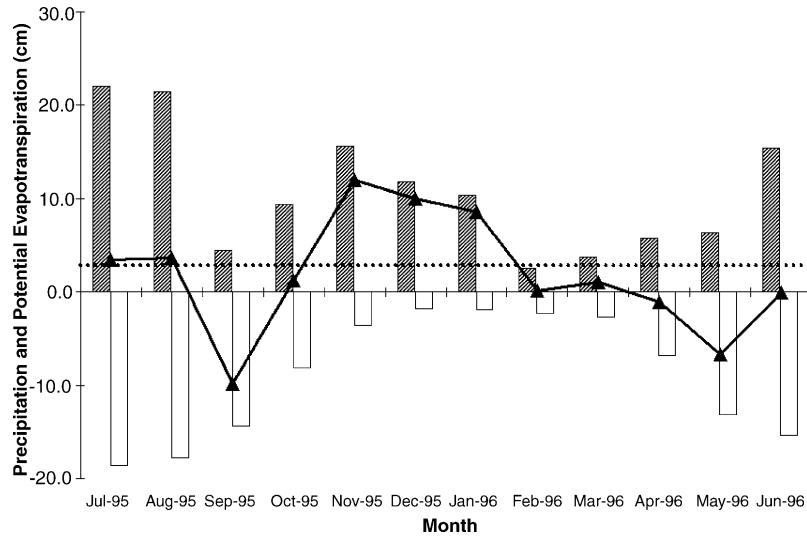


Fig. 4 – Water Budget for Amelia, LA. Precipitation in shaded bars, potential evapotranspiration in white bars, triangles indicates precipitation minus potential evapotranspiration. The dotted line indicates daily discharge from the municipal treatment plant.

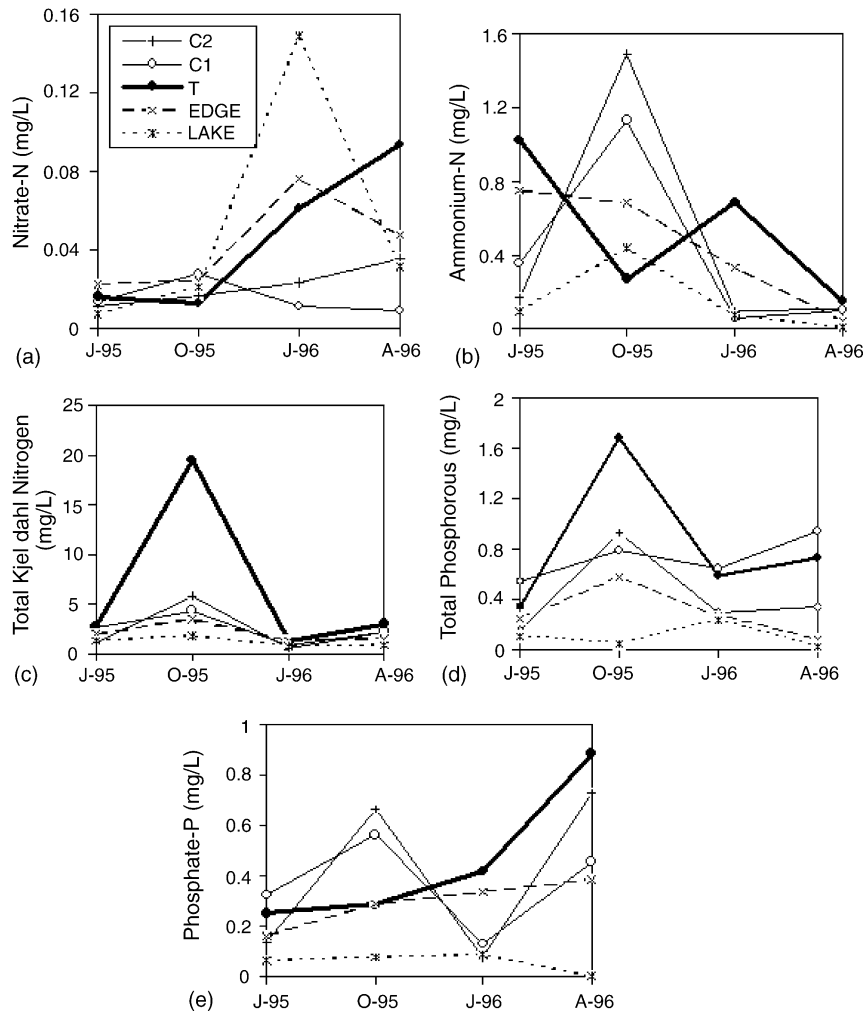


Fig. 5 – Nutrient concentrations at the different sampling stations over the duration of the study.

between the two sites and the high friction of the dense wetland vegetation. There was never a noticeable daily water level variation inside the wetlands during field sampling. However, the seasonal affect of the Atchafalaya River is reflected in a spring water level increase at all wetland sites of about 20 cm.

The low variability of water levels in the interior forested swamp contributes to a long residence time of water in the swamp. Residence time of the effluent in the swamp based on a 40 cm mean water depth and 800,000 L/d discharge was approximately 38 days.

The water budget calculations yielded an annual PPN and PET of 128.9 and 106.6 cm/yr, respectively, for the study period of July 1995–June 1996. The annual surplus of PPN minus PET was 22.3 cm/yr. PPN was highest in July and August 1995 and lowest in February 1996. PET exceeded precipitation in September 1995 and April 1996–June 1996 (Fig. 4). Daily discharge from the municipal treatment plant was 3.1 cm/mo (Fig. 4). Thus, the effluent input to the 77 ha site was about 23% of precipitation input.

3.2. Nutrients

With the exception of nitrate, all nutrients generally decreased from the treatment area to the lake.

3.2.1. Nitrate–nitrogen

NO₃ concentrations were very low in the effluent and wetland throughout the study period, and higher values occurred at the Lake site in January (Fig. 5a). Mean values ranged from a low of 0.007 mg/L to a high of 0.15 mg/L at the Lake site in July and January, respectively. NO₃ concentrations were uniformly low at the C1 and C2 sites with values between 0.01 and 0.02 mg/L, and relatively low at all sites during July and October. In the wetland system, NO₃ generally represented less than 1% of total nitrogen. Considered individually, there were no significant differences across sites during July, October, and April. The only difference during the above months was in April, when the T Site was moderately greater than the C1 site ($p=0.03$). However, in January there were statistically significant differences with the Lake site significantly higher than both the C1 and the C2 sites ($p<0.002$ in both cases). Also in January, the T site was moderately greater than the C1 site ($p<0.02$).

3.2.2. Ammonium–nitrogen

Mean NH₄ concentrations were generally between 0.1 and 1.0 mg/L and represented 25% or less of total nitrogen (Fig. 5b). In October, the highest concentrations of the study period occurred at the C1 and C2 sites (1.13 and 1.5 mg/L, respectively). The lowest values generally occurred at the lake sites and the lowest values occurred in April for all sites (0.007–0.15 mg/L). Considered individually, there was one significant difference across sites during July, with the T site greater than the Lake site ($p=0.008$). In October, the T site was moderately lower than the C1 site ($p=0.008$) and significantly lower than the C2 site ($p=0.001$). In January, the C1 and C2 sites were significantly higher than the T site ($p=0.0005$ and 0.004, respectively) and moderately higher than the Lake site ($p=0.007$). In April, the Lake site was moderately lower than

the C1 and C2 sites ($p=0.007$ and 0.009, respectively) and significantly lower than the T site ($p=0.004$).

3.2.3. Total Kjeldahl nitrogen

TKN was the dominant form of nitrogen representing almost 75% of TN, and concentrations were generally between 2.0 and 4.0 mg/L (Fig. 5c). The values at every site peaked in October, most notably at the T site (19.5 mg/L) with all other sites below 6.0 mg/L. Concentrations were generally low at the Lake site (<1.0 mg/L). Two-way ANOVA results showed statistical differences among sites ($p=0.002$) and months ($p=0.0001$). Tukey post-ANOVA comparisons showed that October and January were different from all other months, with October higher and January lower than all other months (July and April were not significantly different). Statistically, concentrations of TKN at the T site were higher than both the Lake site and the C2 site, indicating enrichment from the oxidation ponds.

3.2.4. Phosphate–phosphorus

PO₄ was generally higher at the wetland sites than at the Lake site suggesting P retention in the wetlands (Fig. 5e). Lake levels ranged from 0.1 to 0.9 mg/L and averaged 0.4 mg/L. Roughly 50% of TP was PO₄. The C1 and C2 sites had the highest levels in October while the T site was considerably lower. PO₄ was also high at the C2 and T sites in April. Considered individually, the C1 site was moderately greater than the Lake site ($p=0.028$). In October, the Lake site was lower than the C1 and C2 sites ($p=0.027$ and 0.024, respectively). In January, the T site was greater than the C1, C2, and Lake sites ($p=0.03$, 0.01, and 0.05, respectively). In April, all other sites were significantly greater than the Lake site (all $p<0.0001$).

3.2.5. Total phosphorus

TP concentrations in the swamp were higher than Lake site concentrations indicating retention of TP in the swamp (Fig. 5d). Lake site TP concentrations were lower than 0.2 mg/L, but ranged from 0.2 to 1.8 mg/L in the swamp. The highest TP concentration was at the T site in October, when TP was roughly twice PO₄. In July, January, and April, TP concentrations were mostly all PO₄ at the C1 site. Two-way ANOVA results showed statistical differences among sites ($p=0.0001$) and months ($p<0.01$). Tukey post-ANOVA comparisons showed that concentrations in October were higher than those in July. The Lake site was significantly lower than all other sites, except the Edge site. Also, the T site was statistically higher than the Edge site indicating a reduction in TP as the effluent flowed through the swamp.

3.3. Productivity

Mean leaf litterfall values ranged from 412 g/m²/yr at the C2 site to 716 g/m²/yr at the T site (Table 1), with values at the C1, E2 and E1 sites of 622, 666, and 546 g/m²/yr, respectively (Table 1). Overall site main effects were significant ($p=0.0187$). Tukey comparisons showed that the T site was statistically greater than the C2 site ($p=0.0128$). Mean tree wood production values ranged from 302 g/m²/yr at the C2 site to 776 g/m²/yr at the E1 site (Table 1). There were no statistically significant differences among sites ($p=0.17$). Average total productivity values ranged from 715 g/m²/yr at the C2 site to

Table 1 – Litterfall, stem growth, and total net aboveground primary productivity (NPP) from October 1995 to September 1996 at sites C1, C2, T, E1, and E2

Site	Litterfall (g/m ² /yr ± S.E.)	Stem growth (g/m ² /yr ± S.E.)	Total NPP (g/m ² /yr ± S.E.)
C1	621.5 ± 50.5 a,b	439.5 ± 92.0 a	1061.0 ± 73.5 a,b
C2	412.4 ± 48.4 b	302.4 ± 110.9 a	714.8 ± 78.2 b
T	716.7 ± 62.2 a	750.8 ± 128.7 a	1467.5 ± 93.7 a
E1	666.4 ± 83.7 a,b	776.0 ± 194.8 a	1442.4 ± 278.5 a
E2	546.1 ± 44.3 a,b	638.5 ± 367.8 a	1184.5 ± 323.5 a,b

Means with different letters are statistically different at $\alpha=0.05$. Note: all sites were continuously flooded with the exception of E1 which was flooded for less than half the year.

1467 g/m²/yr at the T site (Table 1). Overall, there were significant differences among sites ($p=0.007$). Tukey pair-wise comparisons showed significantly higher average total productivity values at the T and E1 sites compared to the C2 site, with p -values of 0.006 and 0.029, respectively.

3.4. Benthic macroinvertebrates

3.4.1. Environmental parameters

Interstation differences in environmental parameters were clear and statistically significant for most variables. Of the 13 variables, 9 were significantly correlated with distance from discharge (Table 2). There were no clear trends in potassium, Bray phosphorus, Eh, and pH. Sediment bulk density decreased by as much as a factor of 3 across the stations, being higher at the 5 m station (Fig. 6). This was associated with a three-fold increase in percent organic matter with distance from the treatment discharge. Like organic matter, total carbon and total nitrogen in the soil increased with distance from the discharge (Fig. 6). The much lower Bray Phosphorous levels showed a weak maximum at the 30 m station. Ca and Mg increased away from the discharge; iron and potassium exhibited no clear trends. Dissolved oxygen decreased away from the discharge, being lowest at 175 m in the spring sampling. pH decreased slightly with distance, and Eh showed a strong minimum at the 30 m station.

3.4.2. Faunal data

A total of 8871 organisms were counted and classified into 109 taxa (for a detailed list see Pratt, 1998). Station counts were highest at the 5 m station and decreased away from the discharge. As reflected in the MDS ordination (Figs. 7 and 8) distinct associations of taxa existed at the 5 and 670 m stations in fall and spring. Intermediate associations at 175 and 30 m shifted seasonally. The near-discharge fauna was dominated by chironomid larvae (*Nimboecera limnectica*, *Einfeldia natchitocae*, *Chironimus ochreatus*, *Micropsectra* sp. and *Chironomus stigmaterus*), gastropods (*Physella* sp.), and oligochaetes (Naididae). The 670 m fauna was dominated by amphipods (*Hyallela azteca*), oligochaete lumbriculidae, chironimids (*Glyptotendipes* sp. and *Tanytus carinatus*), and daphnids (*Ceriodophania laticadata*). Consistent with this pattern of distinct assemblages at the end stations, BIOENV analysis found distance and a strong correlate of distance (total carbon) most closely associated with the faunal change (Table 3).

Univariate community parameters were distinct and statistically significant at each station. Total individuals, total species, and species richness were greatest at the 5 and 30 m station and then declined away from the discharge. Parameters based on proportional composition showed less distinct spatial patterns (Fig. 9). The Shannon–Weiner index had a slight maximum at 30 m, Evenness at 175 m, and Simpson’s dominance was maximum at the far 670 m station.

Table 2 – Correlation between environmental variables

	Dist	Eh	DO	pH	BD	% O	TC	TN	BP	Mg	Na	Ca	K	Fe
Dist	****													
Eh	0.16	****												
DO	-0.61	0.07	****											
pH	-0.52	-0.5	0.19	****										
BD	-0.48	-0.3	0.56	0.36	****									
% O	0.66	0.15	-0.75	-0.21	-0.63	****								
TC	0.61	0.18	-0.75	-0.23	-0.64	0.99	****							
TN	0.51	0.17	-0.71	-0.15	-0.65	0.96	0.98	****						
BP	-0.18	0.16	0.22	0.11	-0.4	0.16	0.16	0.23	****					
Mg	0.68	0.05	-0.61	-0.36	-0.6	0.53	0.49	0.4	-0.04	****				
Na	0.5	0.26	0.03	-0.52	-0.46	0.43	0.4	0.34	0.35	0.56	****			
Ca	0.62	0.05	-0.65	-0.26	-0.75	0.79	0.77	0.72	0.25	0.84	0.51	****		
K	0.07	-0.1	0.11	0.41	-0.02	-0.15	-0.19	-0.2	0.25	0.23	-0.15	0.11	****	
Fe	-0.31	-0.1	0.32	0.02	0.24	-0.76	-0.78	-0.8	-0.31	-0.02	-0.24	-0.39	0.21	****

Bold typed values are significantly correlated (>0.05). Dist, distance from discharge; temp, temperature; DO, dissolved oxygen; BD, bulk density; %O, percent organic matter; TC, total carbon; TN, total nitrogen; BP, Bray phosphorus.

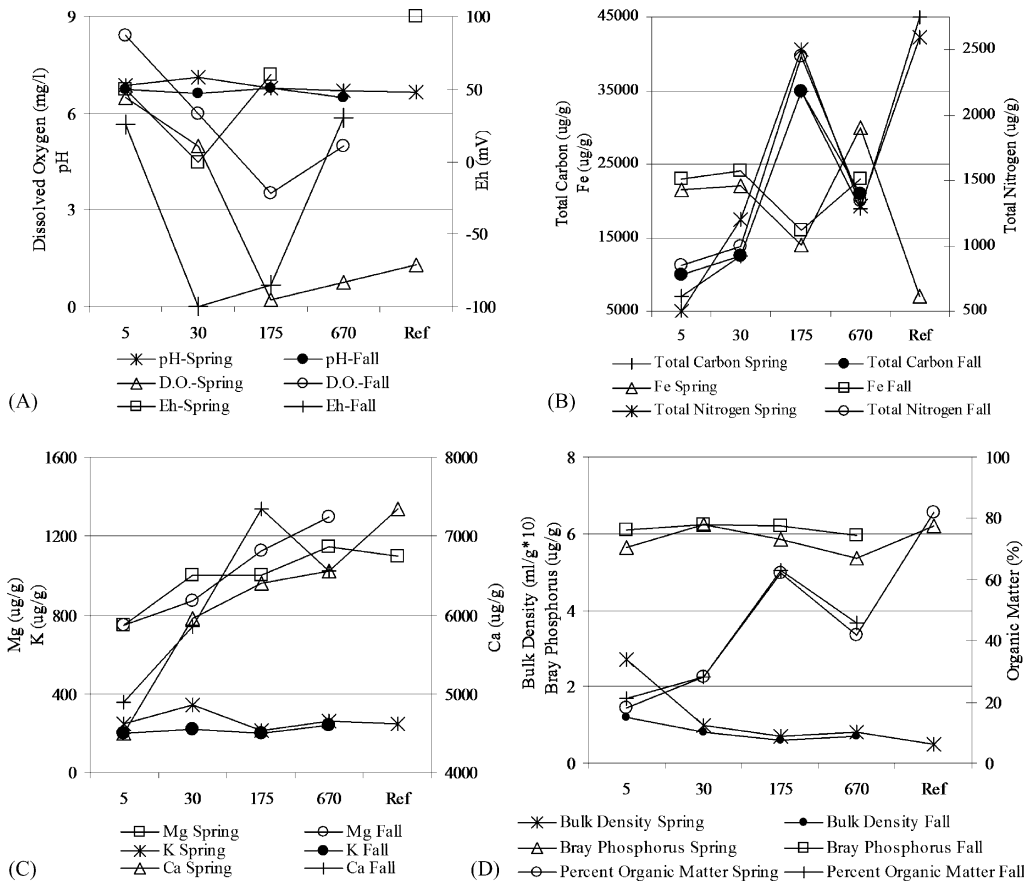


Fig. 6 – Environmental variables least squared means for stations sampled in the fall and spring for bulk density, % organic matter, Bray P, total C, total N, Fe, Ca, Mg, K, surface oxygen and pH. Ref. = reference station.

4. Discussion

The hydrological results of the study provide insights into the hydro-dynamics of this freshwater tidal wetland. Seasonal

water level fluctuations are greater than daily fluctuations, the area is continuously flooded, the hydrologic loading rate is low and the residence time is high. These factors allow time and volume for strong nutrient transformations.

Even though the site has a direct and free connection with the Gulf of Mexico, daily water level fluctuations are very small. This is mainly due to the long distance to the coast (approximately 60 km) and also to the high friction of the dense vegetation. The astronomical tidal range at the coast is about 30 cm. By the time the tide reaches the Bayou Boeuf gauge, the daily water level fluctuation is reduced to less than 10 cm. The study site is located about 15 km further inland from the gauging station and daily water level inside the wetland are imperceptible. There are seasonal water level changes in the study area due to Atchafalaya River discharge and seasonal changes in the mean water level of the Gulf of Mexico (Baumann, 1987). Other studies in Louisiana have shown that tidal, freshwater forested wetlands are affected by seasonal coastal water levels but that daily water level fluctuations are generally very small (Conner and Day, 1988; Swenson and Turner, 1987). In our study, the spring rise in the Atchafalaya River increased water levels at our site by about 20 cm.

One of the major influences of Gulf water levels in this subsiding wetland is that there is continuous flooding because accretion in the wetland is not keeping pace with a high relative water level rise due to a combination of high subsidence

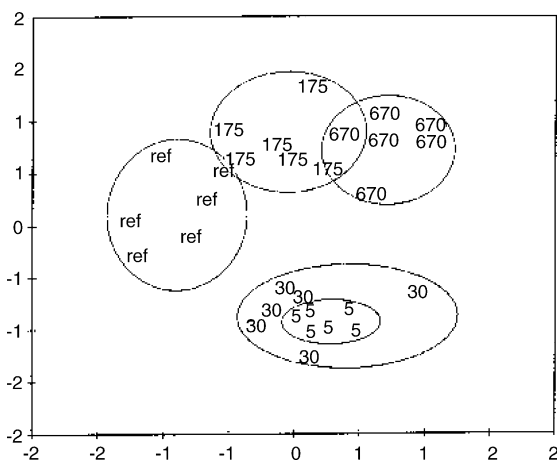


Fig. 7 – MDS ordination of the fourth-root transformed benthic community data for the spring 1997 sampling (stress = 0.18). Each number represents a sampling station replicate.

Table 4 – Mean soil nutrient data ± S.E. for Ramos Swamp taken from two locations at each site (from Day et al., 1997)

Site	NO ₃ (ppm)	Total N (%)	PO ₄ (ppm)	Total P (ppm)	Bray P (ppm)
C1	5.70 ± 3.64	0.89 ± 0.25	BDL ^a	1135.5 ± 352	145.27 ± 52.7
C1	BDL ^a	1.28 ± 0.16	0.79 ± 0.79	904.5 ± 2.5	141.20 ± 18.4
C2	3.66 ± 0.25	0.63 ± 0.30	1.16 ± 1.16	743.0 ± 3.0	247.35 ± 10.2
C2	BDL ^a	1.72 ± 0.10	32.62 ± 5.20	1046.5 ± 32.5	131.05 ± 7.5
T	2.10 ± 0.99	0.99 ± 0.20	45.45 ± 31.5	1098.5 ± 19.5	503.45 ± 38.4
T	1.13 ± 1.13	1.05 ± 0.47	54.80 ± 54.8	845.5 ± 219	206.85 ± 5.7
E1	2.20 ± 0.91	0.96 ± 0.06	BDL ^a	699.5 ± 30.5	184.15 ± 21.6
E2	4.09 ± 0.06	0.75 ± 0.08	BDL ^a	518.5 ± 11.5	104.05 ± 3.9

^a Below detection limit.

loading rate–assimilation relationships at Amelia are similar to those reported for systems outside of Louisiana (Richardson and Nichols, 1985; Faulkner and Richardson, 1989; Kadlec and Knight, 1996; Mitsch et al., 2001; Richardson, 1999).

The effectiveness of treatment by wetland systems varies with factors such as the loading rate of effluent, residence time, soils, plant communities, temperature, and season (Faulkner and Richardson, 1989). With loading rates of 9.4 g/m²/yr for total nitrogen and 1.2 g/m²/yr for total phosphorus, the rates for the Amelia system are similar to other municipal systems which have been evaluated and permitted by LADEQ and EPA to discharge to swamp forests in Louisiana (Breux and Day, 1994; Rybczyk et al., 1996; Day et al., 2004) and lower than many treatment wetlands (Richardson and Nichols, 1985). Wetlands with loading rates up to 20–30 g/m²/yr nitrogen and 5.0 g/m²/yr phosphorus can continue to support removal efficiencies of approximately 70% for both nutrients for many years (Faulkner and Richardson, 1989).

A recent review of phosphorus cycling by Richardson (1999) suggests that the “one gram rule” of 1.0 g/m²/yr of phosphorus loading results in a high assimilation of TP in wetlands. Data collected over several years in different types of freshwater wetlands have shown that the assimilative capacity of freshwater wetlands decreases after this threshold level of phosphorus input (Knight et al., 1994). While a wetland can assimilate additional phosphorus past this level, it is likely to be in the short term or at the expense of increased phosphorus loading to output waters (Richardson, 1999). The Amelia system wetland falls close to this range. It is likely, however, that the wetland at Amelia can continue to assimilate phosphorus. The high subsidence rate in the Mississippi delta means that considerable phosphorus can be buried, providing long-term storage (Day et al., 2004). The fact that the system still assimilates phosphorus without apparent release to surface waters after 27 years is indicative of this. Soil data from the Ramos Swamp taken as part of a larger study (Day et al., 1997) indicate that there were higher mean values for certain parameters in the area of immediate discharge at the T site (Table 4). This was most pronounced for PO₄. Our results are consistent with findings of many studies that have shown that swamp forests chemically, physically, and biologically remove pollutants, sediments and nutrients from water in contact with the vegetation and soils of the system (Wharton, 1970; Boyt, 1976; Nessel, 1978; Yarbrow, 1979; Nessel and Bayley, 1984; Kuenzler, 1987; Zhang et al., 2000; Blahnik and Day, 2000; Kadlec and Knight, 1996; Mitsch and Jorgensen, 2004).

Nutrient concentrations at the Amelia swamp were similar to other freshwater forested wetlands in Louisiana. Phosphate values at other assimilation sites in Louisiana range from 0.05 to 0.44 mg/L (Kemp and Day, 1984; Day et al., 2004) and from 0.49 to 1.01 mg/L at the outlet of the Thibodaux treatment wetland from 1992 to 1996 (Zhang et al., 2000). The values for TP were similar to those reported for the Thibodaux treatment wetland outlet (0.49–0.98 mg/L) and for the Thibodaux control wetland (0.24–0.46 mg/L, Zhang et al., 2000). Kemp and Day (1984) reported TP values of 0.15–0.66 mg/L for three swamp sites in Louisiana. The concentrations of NO₃ are within the range (0.01–0.13 mg/L) reported for other Louisiana swamp sites (Kemp and Day, 1984) and for the outlet of other treatment wetlands in Louisiana (Day et al., 2004). NH₄ concentrations are similar to the outlet values at the Thibodaux, Louisiana wetland treatment system (Zhang et al., 2000), where mean values for NH₄ ranged from 0.11 to 0.90 mg/L at the outlet, while those in the control swamp ranged from 0.02 to 0.59 mg/L (Zhang et al., 2000). TKN means at the interior wetland sites tended to be higher than those reported for the outlet of the Thibodaux treatment wetland (0.6–1.6 mg/L).

In general, total inorganic nitrogen (TIN) was low compared to total inorganic phosphorus (TIP) relative to the Redfield N:P ratio of 16:1 (Redfield, 1958). The ratio of TIN:TIP was usually less than 5.0, with several exceptions at the Lake site (Fig. 10). In the interior wetland sites, TKN was low compared to total phosphorus (Redfield, 1958) with the TKN:TP ratio generally less than 16.0, with several exceptions at the T site and Lake site (Fig. 10). This indicates that nitrogen is the likely limiting nutrient necessary for production.

4.2. Productivity

Net primary productivity data for the Ramos Swamp forest at Amelia indicate that the forest is productive and the values are similar to or greater than those of other continuously flooded forested wetlands in Louisiana (Table 5). It appears that 27 years of wastewater effluent discharge have stimulated the productivity of the trees in the study area. Litterfall at the T site (716.7 g/m²/yr) was generally higher than all other site means (Table 1) but was only statistically higher than the C2 site (412.4 g/m²/yr). It is likely that the wastewater effluent has contributed to the higher litterfall values at the T site, but the differences were not pronounced across all sites due to the open nature of this system and to the relatively high litterfall means at both Edge (E1 and E2) sites that are better drained than the interior sites. Reports from the lit-

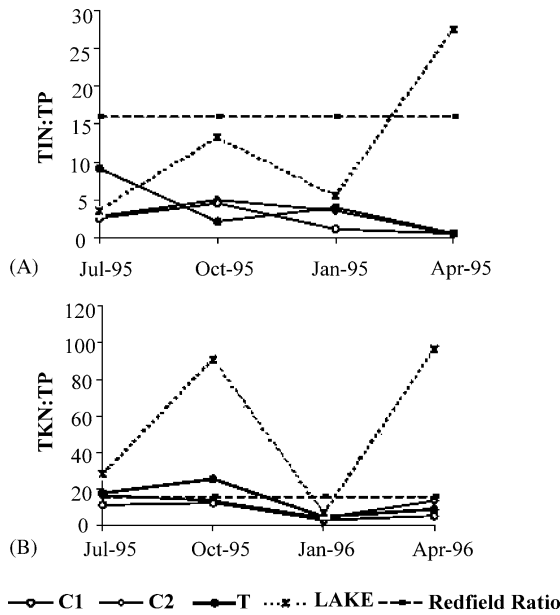


Fig. 10 – Ratio of (A) total inorganic nitrogen (TIN) to total inorganic phosphorus (TIP) and (B) total Kjeldahl nitrogen (TKN) to total phosphorus (TP) at C1, C2, T, and Lake sites within Ramos Swamp study area. The Redfield ratio (---) is shown for comparative purposes.

erature show that litterfall is either increased or unaffected by wastewater effluent. Brown (1981) showed that a cypress dome in Florida receiving sewage discharge had a significant increase in net primary productivity, litterfall, and biomass. A

significant litterfall increase was also observed for a sewage enriched cypress forest in Florida (Nessel, 1978). However, other studies have shown no increase in litterfall (Deghi et al., 1980). Seasonally flooded wetlands are generally more productive than those that are continuously flooded (Table 5). This is due to the pulsing of water and nutrients during seasonal flooding which is typically followed by a dry period and released nutrients (Taylor et al., 1990; Mitsch and Gosselink, 1993).

Stem growth (Table 1) was highest at the E1 (776.0 g/m²/yr) and T sites (750.8 g/m²/yr) but there were no statistical differences among sites. These values are similar to those found in other continuously flooded forested wetlands (Table 5). Since this study compares only 1 year of tree growth, it is possible that an analysis of the long-term history of tree growth at this site would show differences among sites. Using tree ring analysis in a Louisiana forested wetland, Hesse et al. (1998) showed a significant increase in baldcypress tree productivity which began after the addition of secondarily treated wastewater and continued for nearly 50 years. Brown (1981) also found a significant increase in wood production at a cypress dome receiving wastewater discharge compared with untreated cypress domes.

NPP was highest at the T and E1 sites (1467.5 and 1442.4 g/m²/yr, respectively), both of which were significantly higher than the C2 site. The values at all sites are comparable to those of other studies, and are relatively high for continuously flooded forested wetlands (Table 5). The high total productivity at both Edge sites is likely due in part to the higher elevation ridge at the edge of Lake Palourde. The Edge sites, E1 in particular, were inundated less frequently and less and for lower depths than the interior swamp sites

Table 5 – Comparative values for litterfall, stem growth, and total aboveground net primary productivity reported for continuously flooded and seasonally flooded forested wetlands

Forest Type	Location (state)	Litterfall (g/m ² /yr)	Stem Growth (g/m ² /yr)	Total NPP (g/m ² /yr)	Reference
Continuously flooded					
Forested wetland (wastewater treatment site)	LA	717	751	1467	This study
Forested wetland (wastewater control sites)	LA	622/412	440/302	1061/715	This study
Forested wetland (wastewater treatment site)	LA	434	890	1324	Delgado (1995)
Impounded swamp	LA	328	558	886	Conner et al. (1989)
Forested wetland (wastewater treatment site)	LA	245	226	473	Day et al. (1994)
Forested wetland (wastewater control site)	LA	383	603	990	Day et al. (1994)
Cypress stand (sewage treatment site)	FL	650	640	1290	Nessel (1978)
Mature stand dome	FL	415	541	956	Brown (1981)
Sewage dome	FL	734	1060	1794	Brown (1981)
Seasonally flooded					
Forested wetland (wastewater reference site)	LA	541	722	1263	Delgado (1995)
Cypress-Tupelo	LA	620	500	1120	Conner and Day (1976)
Bottomland hardwood	LA	574	800	1374	Conner and Day (1976)
Managed hardwood	LA	549	1231	1780	Conner et al. (1989)
Cypress floodplain	FL	521	1086	1607	Brown (1981)

(Fig. 2). This is consistent with the general hypothesis that the productivity of freshwater forested wetlands is greatest for seasonally (or periodically) flooded ecosystems, intermediate for those with slow-flowing floods of long duration, and lowest for systems with continuous stillwater flooding (Conner and Day, 1976; Odum, 1979). The relatively lower productivity of forests with continuous flooding results from the physiological stress of the waterlogged conditions. Megonigal et al. (1997) tested a more specific hypothesis that periodically flooded forests have higher aboveground net primary productivity (NPP) than other types of forests in the Southeastern U.S. After examining 32 forested sites in Louisiana and South Carolina, they classified them as “wet”, “dry”, and “intermediate” based on their flooding regime. They concluded that the NPP of the wet sites was significantly lower than the NPP of the intermediate and dry sites. However, there was no significant difference between the intermediate and dry sites, and the intermediate sites were not significantly different from other upland forests. They acknowledged, “it is possible that periodic floods create the potential for high rates of NPP, but that negative influences on tree growth typically prevent high rates from being realized” (Megonigal et al., 1997). The high productivity of the T site, despite continuous flooding, is likely related to the enrichment of this site by nutrients in the effluent.

Baldcypress and tupelo in the study area show little promise for future regeneration as no seedlings or saplings were observed in the interior swamp sites. Since cypress and tupelo seeds will not germinate under flooded conditions, a drawdown period is required for regeneration (Mitsch and Gosselink, 1993). Survival of cypress and tupelo seedlings depends largely on their ability to stay alive during possible flooding in the growing season (Conner et al., 1989). The constant flooding at the Ramos Swamp wetland, especially in the interior sites, is restricting regeneration. While cypress-tupelo swamp forests are adapted to continuous flooding (Mitsch and Gosselink, 1993) and already established trees can survive for many years under such conditions, in the long-term this forest will likely convert to some combination of open water, emergent, and floating aquatic habitats as the tree canopy thins due to tree mortality and lack of regeneration. This eventual change in the habitat of the area may affect the assimilation of nutrients since the storage of these parameters in tree biomass will no longer occur. Periodic plantings could lead to a sustainable forest in the area. But eventually, sediment introduction into the area will be necessary if this forest is to survive. River diversions are planned as part of the plan for restoration of the Mississippi delta (Day et al., 2000, 2004). The close proximity of the Atchafalaya River makes this an option for this site.

4.3. Benthic community

There was increased abundance of microbenthic organisms at the station nearest the effluent discharge. This is consistent with reports of enhanced abundance of benthic organisms in other effluent-impacted forested wetlands (Brightman, 1976, 1984; Kadlec and Alvord, 1989). However, the level of replication and the variability in the sedimentary environment make it difficult to attribute this spatial pattern only to dis-

charge. Indeed, the chironomid and oligochaete-dominated near fauna is typical of unimpacted benthos in Louisiana forested wetlands (Beck, 1977; Loden, 1978; Ziser, 1978; Sklar, 1983).

On a gradient away from effluent discharge, two contrasting patterns of sedimentary nutrient occurred. Sedimentary nutrients can decrease away from the source reflecting dilution and utilization, or there can be an increase away from the source as nutrients are progressively sequestered in the sediments and productivity is increased (Zhang, 1995; Kadlec and Knight, 1996; Day et al., 1994). For example, Rybczyk et al. (2002) reported that the buildup of organic carbon was stimulated by a factor of 3 in another treatment wetland in Louisiana. In this study total carbon, total nitrogen, calcium, and magnesium all increased with distance. These gradients may be more reflective of spatial heterogeneity of sediment composition than effluent effects. At the 5 and 30 m stations, the sediments had a much higher mineral fraction and lower organic detritus content. However, there was considerable heterogeneity in sediment physical and chemical characteristics. Most environmental parameters may simply reflect this heterogeneity. Thus, there is the possibility that between-station differences in the benthic community composition sedimentary were due to underlying heterogeneity of the environment and this limits interpretation of the benthic community results.

If the 5–670 m faunal differences do reflect a gradient in effluent impact, then the species diversity patterns are the opposite of what is normally reported. Typically, high abundance is associated with a low number of species and high dominance by a few species (Pearson and Rosenberg, 1978). We found the reverse; both the number of species and overall abundance were highest at the –5 m station. This could be due to two reasons. First, there is an effluent-associated increase in food for the benthos, but it is not sufficient to lead to dominance as predicted by the Pearson and Rosenberg (1978) model (Lenat, 1983). Second, abundance and diversity may be suppressed away from the effluent discharge by additional factors, dissolved oxygen being the most likely. The oxygen decrease away from the discharge may be due to slow utilization of nutrients downstream, or it could be due to factors not considered in the design. A floating aquatic vegetation (FAV) was persistent at the 175 and 650 m stations possibly contributing to very low oxygen levels (Brightman, 1984; Price, 1975). Similar mats were often removed from the higher oxygen 5 and 30 m stations by stronger flow and winds. Overall, we conclude that the impacts on the benthic fauna at the site due to the effluent were subtle. The low loading rate of effluent at the site likely contributed to the lack of a distinct impact on the benthic fauna.

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