Research Article

Use of ^{15}N Natural Abundance and N Species Concentrations to Assess N-Cycling in Constructed and Natural Coastal Wetlands

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Natural abundance of N stable isotopes used in combination with concentrations may be useful indicators of N-cycling in wetlands. Concentrations and ^{15}N signatures of NO_3^-, NH_4^+, and sediment organic nitrogen (SON) were measured in two impacted coastal golf course retention ponds and two natural marshes. Limited NO_3^- was detected in natural site surface water or pore water, but both isotopic signature and concentrations of NO_3^- in surface water of impacted sites indicated anthropogenic inputs. In natural sites, NH_4^+ concentrations were greatest in deeper pore water and least in surface water, suggesting diffusion predominates. The natural sites had greater %SON, and ^{15}N indicated that the natural sites also had greater NH_4^+ released from SON mineralization than impacted sites. In NO_3^--limited systems, neither concentrations nor ^{15}N natural abundance was able to provide information on N-cycling, while processes associated with NH_4^+ were better elucidated by using both concentrations and ^{15}N natural abundance.

1. Introduction

Salt marsh estuaries are important coastal environments in South Carolina both environmentally and economically. This type of estuary serves as a nursery for many marine species [1] including those of commercial importance [2], dampens the effect of storm surges on coastal areas [3], and provides a removal mechanism for nutrient pollutants before they reach the greater ocean [4]. As development along the South Carolina coast continues to increase, it is important to understand the effects that increasing anthropogenic nutrient pollution may have on nutrient cycling in salt marsh estuaries, and thereby their beneficial functions. For example, excess nitrogenous pollution can lead to eutrophication and algal blooms, including blooms of potentially toxic species. In the southeastern USA fish kills have been associated with the genus *Pfiesteria* in eutrophic coastal waters. Harmful algal blooms are also linked to the deaths of other species including oysters and blue crabs [5].

Nitrogenous pollution can be removed from salt marsh estuaries through the process of denitrification. Nitrogenous pollution also can be maintained in the system and be converted between the various forms of nitrogen (N) through the processes of dissimilatory nitrate reduction to ammonia (DNRA) and nitrification. Inorganic N can be added to the system naturally through the processes of mineralization and N fixation. The amounts and types of nitrogenous pollution entering coastal ecosystems may affect the partitioning of these various N-associated processes, which in turn affects the concentrations and species of N present. The overall objective of this research was to examine N processes in both constructed and natural coastal environments by using N stable isotopes and measured N concentrations in laboratory microcosm experiments.

There are two ways that ^{15}N can be used to track the flow of N through a system. The first method is the ^{15}N tracer method, which involves adding an inorganic N source that has been enriched in ^{15}N to the system and
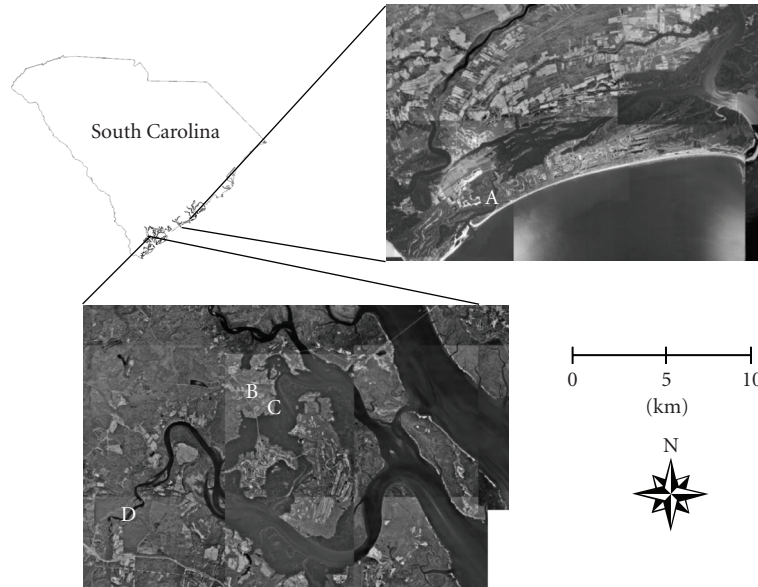
FIGURE 1: Sampling locations include the impacted (A) Oyster Rake and (B) Chechessee Creek Club golf course retention pond and the unimpacted (C) Chechessee Marsh and (D) Graves' Dock.

subsequently tracing the ^{15}N through N pools over time. Tracer methods can be expensive and have the disadvantage when used *in situ* of adding a previously absent ^{15}N source to the environment. The second method is the natural abundance (δ^{15}N) method, which relies on natural variations in isotopic ratios between N pools to trace N sources and possible transformation processes through the system [6]. Natural abundance methods are difficult because they require significant differences in isotopic signatures between N pools to identify possible sources and follow N pathways. In addition mixing and fractionation effects, and N transformations, must be well understood to identify predominant N-cycle processes [7]. Despite these challenges, the δ^{15}N method has been successfully used for more than a decade in nitrate (NO_3^-) source characterization in N-contaminated environments including rivers, watersheds, and groundwater [8–12].

The δ^{15}N method was used in this research to examine potential N-cycle processes in two constructed and two natural coastal environments in South Carolina, USA. The two natural sampling sites were located directly within salt marsh estuaries. The two man-made sampling locations were located in coastal golf course retention ponds used as best management practices to abate anthropogenic N and pesticide inputs, and both drain into the salt marsh estuaries. One constructed site received fertilizers and irrigation from treated wastewater. The second site received only fertilizer applications. In addition to environmental measurements, laboratory experiments also were carried out to measure N-cycle processes, primarily denitrification and DNRA in aquatic sediments. Comparing the ability of man-made and naturally occurring coastal systems to convert N inputs could provide insight into the effects of anthropogenic N sources on N-cycle processes and potential accumulation of N in these different systems.

2. Experimental

2.1. Study Sites. The two constructed sites were Oyster Rake pond and the Chechessee Creek Club golf course pond (Figure 1). Oyster Rake is located on Kiawah Island, SC, a barrier island south of Charleston. Oyster Rake is a shallow, freshwater, constructed retention pond located on a golf course green. The golf course receives both ammonia- (NH_3) based fertilizers and treated wastewater for irrigation. Chechessee Creek Club golf course is located in Beaufort County, SC, and also receives fertilizer but not treated wastewater. Both retention ponds are used as a best management practice to process runoff from the course before it can enter the respective marshes. Samples from both ponds were collected ∼2 m from the pond's edge at a depth of <1 m.

The two naturally occurring sites were Grave's Dock marsh and the Chechessee Marsh (Figure 1). Both are located in the Okatee River estuary in Beaufort County, SC. Grave's Dock marsh is a completely undeveloped and tidally influenced *Spartina*-dominated salt marsh. The Chechessee marsh site is located next to the Chechessee Creek Club golf course along the Chechessee creek and receives runoff from the golf course. This site is also a *Spartina*-dominated salt marsh. All samples at these locations were collected at low tide in a shallow salt marsh creek.

2.2. Sampling Procedure and Preparation. Sediment cores were collected in 40-cm acrylic cylinders (7.5 cm id). One sediment core in January, two cores in April, and three cores in October were collected from both Grave's Dock marsh and Chechessee Marsh. Samples from individual cores were analyzed separately, and the mean results are reported. One sediment core was collected in Oyster Rake pond in October, and no sediment samples were collected from the Chechessee

Creek Club golf course pond. Sealed cores were kept on ice during transport and were divided into 10-cm sections upon return to the laboratory. Sediment sections were immediately extracted with 1 M KCl at a ratio of 150 mL of KCl for every 40 g (wet weight) of sediment by placing samples in 250 mL Nalgene bottles on a shaker table at room temperature for 1 hour. Extracts were filtered through a $0.45 \mu m$ Selectron membrane filter (Schleicher & Schuell, Inc.), acidified to a pH of ~2 using 12 N H_2SO_4, and stored at 4°C until analysis.

Water samples were collected in 1-L Nalgene bottles and stored on ice during transport. Upon return to the laboratory water samples were immediately filtered through a $0.45 \mu m$ Selectron membrane filter (Schleicher & Schuell, Inc.) and acidified to a pH of ~2 using 12 N H_2SO_4. Water samples were stored at 4°C until analysis.

Water samples and extracts were analyzed for nitrate plus nitrite ($NO_3^- + NO_2^-$) and NH_4^+ using a Lachat 8000 series QuikChem Flow Injection Analyzer (FIA) using the cadmium reduction (USEPA Method 353.2) and phenolate method (USEPA Method 350.1), respectively, (detection limit 0.01 mg N l^{-1}) [13]. Samples were preserved with 12 N H_2SO_4 and sent to the Colorado Plateau Stable Isotope Laboratory (CPSIL) at Northern Arizona University for ^{15}N analysis of ($NO_3^- + NO_2^-$) and NH_4^+ in surface water and pore water. A diffusion method adapted by CPSIL from Khan et al. [14], Sigman et al. [15], and Holmes et al. [16] was used to concentrate ($NO_3^- + NO_2^-$) and NH_4^+, and the ^{15}N was measured using isotope ratio mass spectrometry. A minimum of 20–40 μg of N was necessary for ^{15}N analysis. Standard deviations of diffused standards were ≤ ±0.35‰ for δ^{15}N-NO_3^- and ≤ ±0.25‰ for δ^{15}N-NH_4^+. Sediment was dried at 90°C for 48 hours and grounded with a mortar and pestle before being sent to CPSIL for sediment organic N (SON) and C concentrations, and ^{15}N and ^{13}C analysis. A minimum of 60 mg of N was necessary for ^{15}N analysis. Because C is found in higher concentrations in soil than N, no samples fell below the minimum for ^{13}C analysis. External precision on the working National Institute of Standards and Technology (NIST) standard peach leaves (NIST 1547) was ≤ ±0.20‰ for δ^{15}N and ≤ ±0.10‰ for δ^{13}C.

2.3. Laboratory Experimental Design. Sediment cores and water samples were collected from Oyster Rake for microcosm studies using the same method as that used for δ^{15}N analysis. The acetylene block technique was used to determine potential denitrification rates. Sediment slurry was prepared using a 3 : 1 sediment-to-site water ratio from the 0–10 cm sediment core section. Approximately 10 g of sediment was added to each 150 mL microcosm. A subset of microcosms was autoclaved and used as an abiotic control. Solutions of 1400 μg NO_3-N mL^{-1} and 1000 μg NH_4-N mL^{-1} were prepared using DI water and KNO_3 or NH_4Cl, respectively. Abiotic NO_3^- controls were amended with 1 mL of 1400 μg NO_3-N mL^{-1} solution (total NO_3-N concentration of 156 μg mL^{-1}, 350 μg g^{-1} dry wt) and 2 mL of 6 M H_2SO_4 after autoclaving. One set of experimental microcosms was amended with 1 mL of 1400 μg NO_3-N mL^{-1} solution and 2 mL of site water, and a second set was amended with 1 mL of 1400 μg NO_3-N mL^{-1} solution

(final concentrations of 156 μg mL^{-1} and 350 μg g^{-1} dry wt), 1 mL of 1000 μg NH_4-N mL^{-1} solution (final concentrations 111 μg mL^{-1} and 275 μg g^{-1} dry wt), and 1 mL of site water. A live control was amended with 3 mL of site water only, to quantify any changes in NO_3^- and NH_4^+ occurring at background concentrations.

Oxyrase (Oxyrase Inc.) (0.5 mL) was added to all microcosms to remove oxygen from the slurry and each microcosm was flushed with helium for ~2 minutes to remove oxygen from the headspace. Microcosms were capped with mininert valves to allow gas sampling while maintaining anaerobic conditions. Fifteen mL of headspace were removed from each microcosm and replaced with 15 mL of acetylene [13, 17].

Headspace from three microcosms from each live treatment was sampled for N_2O at times 0, 12, 18, 24, 30, 36, 48, and 72 hours. Abiotic treatments were sampled in triplicate every 24 hours. Nitrous oxide (N_2O) was analyzed using a Varian 3700 gas chromatograph (GC) equipped with an electron capture detector (ECD). Oven temperature was isothermal at 80°C, injector and detector temperatures were 200 and 300°C, respectively. N_2O dissolved in microcosm liquid was accounted for using Henry's constant adjusted for temperature and salinity. Headspace and dissolved N_2O concentrations were summed to determine the total N_2O produced. In order to analyze for ($NO_3^- + NO_2^-$) and NH_4^+ in each experimental set, and two additional microcosms of each treatment were destructively sampled at each time point by the addition of 2 mL of 6 M H_2SO_4 and subsequently extracted with 1 M KCl.

2.4. Statistical Analysis. Statistical analysis was conducted using SPSS statistical software [18]. Model 1 ANOVA was used to compare N species concentrations and isotopic values both between months and between sediment/water fractions within months. A Randomized Complete Block (RCB) ANOVA design was used in instances where the effect of the blocking factor time of sampling was deemed not important and the effects of this factor were accounted for by the test itself in order to avoid interaction effects between factors. RCB ANOVA was also used to compare changes in NH_4^+ concentration after 36–72 hours between treatments during the laboratory experiments. The Bonferroni Test was used to determine which month or which fraction had a significantly different concentration or isotopic value and which treatment had a significantly different change in NH_4^+ concentration. A paired t-test was used to compare SON isotopic values with corresponding sediment pore water NH_4^+ isotopic values. Denitrification rates were calculated after any apparent lag phase, using the least squares linear regression of N_2O versus time in each treatment using a minimum of 4 time points. The Mann-Whitney U-test was used to compare denitrification rates between treatments. The significance level was $\alpha = 0.05$ for all comparisons.

3. Results and Discussion

3.1. Constructed Sites. Laboratory experiments conducted using sediments and site water from the Oyster Rake golf course retention pond indicated that when provided with
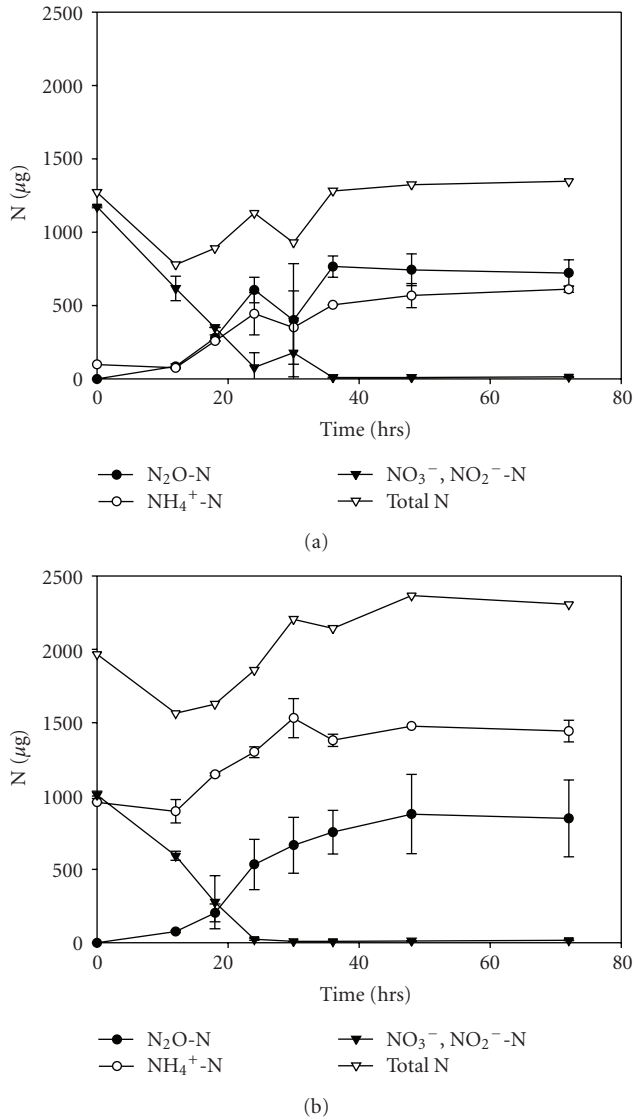
(a)

(b)

FIGURE 2: Concentrations of nitrogen species over time (μg N per vial) in Oyster Rake retention pond sediment microcosms amended with (a) 1400 μg NO_3-N per vial (NT) and (b) 1400 μg NO_3-N$^-$ + 1000 μg NH_4-N per vial (NA). Equivalent concentrations are 350 μg N per gram dry weight for NO_3^- and 275 μg N per gram dry weight NH_4^+.

an external source of NO_3^-, the sediment microbes quickly converted NO_3^- to both N_2O and NH_4^+ under anaerobic conditions, suggesting both denitrifying and DNRA capabilities (Figure 2). Average denitrification rates calculated from N_2O production, 4.84 μg N_2O-N g dry weight^{-1} (s.d. = 1.50, $n = 3$) and 5.13 μg N_2O-N g dry weight^{-1} (s.d. = 0.58, $n = 3$) for NO_3^- and NO_3^- + NH_4^+ amendments, respectively, were not significantly different ($n = 3$, $P > .200$). No significant difference was observed between the mean increase in NH_4^+ concentration between treatments NO_3^-, and NO_3^- + NH_4^+, and between treatment live controls and abiotic NO_3^- controls, but significantly more NH_4^+ was evolved in the first group than in the second ($P < .001$) suggesting the occurrence of DNRA in live
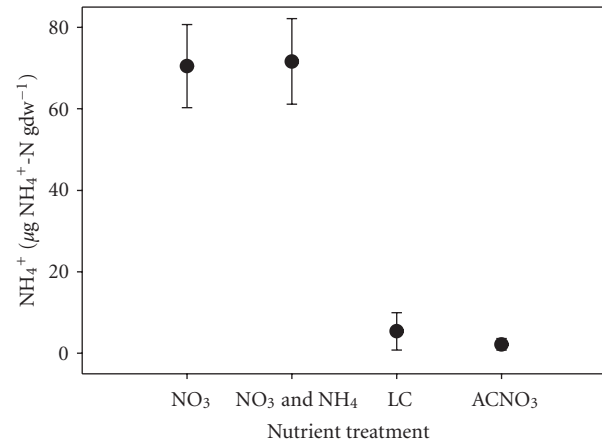

FIGURE 3: Average increase in NH_4^+ concentration (μg NH_4-N per gram dry weight) in Oyster Rake retention pond sediment microcosms (calculated by subtracting NH_4^+ concentration at time 0 from NH_4^+ concentration at time of sampling) between sampling times 36 hours and 72 hours for 4 different nutrient treatments (1400 μg NO_3-N per vial addition (NO_3); 1400 μg NO_3-N$^-$ + 1000 μg NH_4-N per vial addition (NO_3 and NH_4); live control with no NO_3^- or NH_4^+ addition (LC); abiotic control with 1400 μg NO_3-N$^-$ addition and no NH_4^+ addition (ACNO$_3$)). Error bars represent one standard deviation.

microcosms receiving NO_3^- inputs (Figure 3). The final average NH_4^+ concentration in live control and abiotic NO_3^- control treatments after 72 hours was 25.2 μg g^{-1} dry weight ($n = 2$) and 13.0 μg g^{-1} dry weight ($n = 2$), respectively, (data not shown).

Surface water NO_3^- concentrations at Oyster Rake were higher (4.6 mg L^{-1}) than pore water concentrations, and higher than any NO_3^- concentrations in surface water of any of the other sites. NO_3^- concentrations were low in sediment pore water collected at both depths in the Oyster Rake golf course retention pond. Surface water NO_3^- had a δ^{15}N value of 3.65‰. All other NO_3^- concentrations were too low to measure a δ^{15}N value. NH_4^+ concentrations followed an opposite trend to those of NO_3^-; they were lowest in surface water and highest in sediment pore waters. Surface water NH_4^+ concentrations were too low to measure δ^{15}N, but the $NH_4^+\delta^{15}$N value of surface sediment pore water was 4.93‰ and the δ^{15}N value of 20–30 cm sediment pore water was 1.7‰ (Table 1). The % SON of Oyster Rake was low reflecting the fill material used to construct these artificial ponds. The δ^{15}N of SON was heavier in the surface sediments than in deeper sediments (Table 2), similar to results of δ^{15}N NH_4^+ in the pore water.

Added NO_3^- was completely removed after short time periods in laboratory experiments and *in situ* pore water NO_3^--N concentrations in Oyster Rake were low, suggesting that sediment microbial processes effectively consumed NO_3^- in pore water. Previous studies have demonstrated that similarly impacted sediments have greater potential denitrification rates than those in natural systems [17, 19]. NO_3^- appeared to drive the reactions, as the addition of both NO_3^- and NH_4^+ in laboratory microcosms neither stimulated nor suppressed the production of N_2O and NH_4^+

TABLE 1: Nitrate and ammonium data for surface and pore water in Grave's Dock (GD), Chechessee March (CM), Chechessee Creek Club retention pond (CP), and Oyster Rake (OR).

Site	Month	Depth (cm)	NO_3-N (mg L^{-1})	$\delta^{15}NO_3^-$ (‰)	NH_4-N (mg L^{-1})	$\delta^{15}NH_4^+$ (‰)
GD	Jan	Surface Water	BDL	ND	0.05	3.3
		Sediment 0–10	BDL	ND	6.05	4.2
		Sediment 30–40	BDL	ND	13.16	3.3
	Apr	Surface Water	BDL	ND	0.06	ND
		Sediment 0–10	BDL	ND	2.36	2.3
		Sediment 30–40	BDL	ND	17.07	3.5
	Oct	Surface Water	BDL	ND	0.01	−4.7
		Sediment 0–10	BDL	ND	11.43	3.2
		Sediment 30–40	BDL	ND	15.70	3.3
CM	Jan	Surface Water	BDL	ND	0.42	5.9
		Sediment 0–10	BDL	ND	3.24	2.7
		Sediment 30–40	BDL	ND	9.00	2.7
	Apr	Surface Water	BDL	ND	BDL	ND
		Sediment 0–10	BDL	ND	1.67	5.8
		Sediment 30–40	BDL	ND	3.13	1.9
	Oct	Surface Water	BDL	ND	BDL	ND
		Sediment 0–10	BDL	ND	3.01	6.0
		Sediment 30–40	BDL	ND	4.88	4.4
CP	Jan	Surface Water	1.73	5.0	BDL	ND
	Apr	Surface Water	1.58	3.7	0.13	5.7
	Oct	Surface Water	0.33	−1.2	0.11	7.7
OR	Oct	Surface Water	4.60	3.7	0.08	ND
		Sediment 0–10	0.16	ND	3.67	4.9
		Sediment 20–30	0.18	ND	2.58	1.7
		Treated Wastewater	3.15	4.9	1.74	26.4

TABLE 2: Characteristics of sediment organic matter for Grave's Dock (GD), Chechessee Marsh (CM), and Oyster Rake (OR).

Site	Month	Depth (cm)	N (%)	$\delta^{15}N$ (‰)	C (%)	$\delta^{13}C$ (‰)	C:N
GD	Jan	0–10	0.22	3.6	2.52	−19.7	11.76
		30–40	0.18	3.8	2.33	−19.5	12.66
	Apr	0–10	0.25	3.7	2.68	−20.1	10.87
		30–40	0.20	4.0	2.48	−20.5	12.16
	Oct	0–10	0.20	4.2	2.42	−20.4	12.14
		30–40	0.21	4.6	2.49	−19.9	12.08
CM	Jan	0–10	0.09	3.6	1.14	−19.4	12.81
		30–40	0.11	4.1	1.47	−19.5	13.67
	Apr	0–10	0.17	4.0	2.36	−18.5	13.66
		30–40	0.15	3.7	2.15	−19.8	14.78
	Oct	0–10	0.16	4.2	1.82	−19.3	11.34
		30–40	0.11	4.7	1.49	−19.9	13.38
OR	Oct	0–10	0.01	2.8	0.13	−25.8	15.06
		20–30	0.02	0.6	0.44	−26.4	19.30

compared to the addition of NO_3^- alone. Nitrification, the conversion of NH_4^+ to NO_3^-, could not occur in laboratory experiments because the systems were anaerobic, although nitrification may occur in the aerobic site surface waters.

NO_3^- concentrations in the surface waters of Chechessee Creek Club golf course retention pond were detected in each month sampled, and were lower than those in the Oyster Rake golf course retention pond. The $\delta^{15}N$ of retention pond surface water NO_3^- ranged from a high of 5.0‰ in January corresponding to the high NO_3^- concentration, to a low of −1.17‰ in October corresponding to the low NO_3^- concentration. NH_4^+ concentrations were lower than NO_3^- concentrations with no detectable NH_4^+ measured in January and concentrations of ~0.1 mg L^{-1} measured in April and October. The ^{15}N of the Chechessee golf course retention pond surface water NH_4^+ was more enriched in October than in April, and more enriched than the ^{15}N of NO_3^- (Table 1). Chechessee Creek Club retention pond sediment and pore water were not collected for NH_4^+ and $\delta^{15}N$ SON analyses.

A possible source of NO_3^- to the surface water of Oyster Rake is the treated wastewater used in combination with well water for irrigation of the golf course. Treated wastewater was collected from the wastewater treatment facility after undergoing all treatment procedures. NO_3^- concentrations measured 3.15 mg L^{-1} with a $\delta^{15}N$ of 4.93‰, and NH_4^+ concentrations measured 1.74 mg L^{-1} with a $\delta^{15}N$ of 26.37‰ (Table 1). This high $\delta^{15}N$ value of wastewater NH_4^+ was consistent with those reported by other

researchers [20]. The N concentrations of irrigation water reaching the golf course may vary throughout the year as treated waste water is mixed with well water in proportions that vary based on needs of the turf on the golf course. NO_3^- in irrigation water may enter Oyster Rake directly through storm drains on the golf course that pipe into the pond or irrigation water may infiltrate the soil and carry its nutrients to the pond as part of the shallow groundwater. A previous hydrologic assessment on Kiawah Island found that nutrient exchange between groundwater and retention ponds can occur through both fluctuating vertical groundwater movement and lateral movement [21]. In addition, the ground-water NO_3^- concentration increased while the NH_4^+ concentration decreased along the path of flow in one of the retention pond drainage areas studied [21]. Nitrification may be a second source of NO_3^- entering Oyster Rake. The relatively high NO_3-N concentration in Oyster Rake surface water ($4.6 \, mg \, L^{-1}$) and depleted $\delta^{15}N$ value compared to the irrigation water could be the result of a combination of NO_3-N from irrigation water and NO_3-N generated through nitrification.

The Chechessee Creek Club golf course retention pond is not irrigated with wastewater, but is impacted by fertilizer from the golf course entering via surface water runoff during rain events or shallow groundwater. Samples of four fertilizers used on the Chechessee Creek Club golf course were analyzed for $\delta^{15}N$: Green and Tees (1.46‰), Greens Grade (0.13‰), Tee Time (1.47‰) and Plant Marvel (2.50‰). Most of the fertilizers are urea-based, but Plant Marvel is composed of 13% NO_3-N and is a possible source of NO_3^- to the retention pond. NO_3^- is expected to be more readily transported through sediment with water flow than the N in ammonia-based urea fertilizers. Plant Marvel $\delta^{15}N$ value was lighter than the values measured for NO_3^- in the Chechessee Creek Club golf course retention pond water in January and April, and heavier than that measured in October. The enriched isotopic values of NO_3^- in the retention pond during January and April suggest the occurrence of microbial processes like denitrification or assimilation that preferentially use the lighter isotope and thereby enrich the NO_3^- pool. In October the isotopic signature of NO_3^- was very different, suggesting that the NO_3^- was a product rather than a reactant of a reaction. Nitrification of surface water NH_4^+ is the likely source of the NO_3^-, and this is supported by the lower NO_3^- concentration and higher $NH_4^+ \delta^{15}N$ value of surface water in October than at other sampling times. The NO_3^- concentrations in the surface water of Oyster Rake were higher than those measured at any time in the Chechessee Creek Club retention pond, indicating that the combination of treated wastewater irrigation and fertilizer has a greater impact on surface water NO_3^- concentrations and N contamination than that of fertilizer alone.

Based on laboratory experiments, mineralization of SON was likely a minor process in the Oyster Rake golf course retention pond, and not contributing N to surface or pore water concentrations. If mineralization were occurring, N, in the form of NH_4^+, would be expected to increase in experimental microcosms over time. After 72 hours, mass balance calculations for treatments NO_3^- only, and NO_3^-

$+ \, NH_4^+$ found average N recoveries of 106% and 117% of the initial N amounts, respectively. In contrast, Ma and Aelion [13] described similar laboratory experiments conducted with 0–10 cm sediment from the Grave's Dock and Chechessee Marsh sites with N recoveries of ∼150% of the initial N amounts after similar time periods and NO_3^- additions. Low % SON conditions exist at Oyster Rake due to removal of native soils during construction of the golf courses, which may limit mineralization. Alternatively, the limited NH_4^+ concentrations measured could be indicative of a higher rate of microbial NH_4^+ assimilation masking the effect of SON mineralization.

In situ concentrations of NO_3^- in the surface water of the man-made systems Oyster Rake and the Chechessee Creek Club golf course retention pond were high compared to those of the natural sites even though NO_3^- may be effectively consumed in pore water. These results suggest that although denitrification may occur in Oyster Rake sediments, anthropogenic inputs of NO_3^- may exceed removal capacity in the surface water and have the potential to negatively impact the water quality of the retention pond. The concentration of NO_3^- in Oyster Rake surface water and in the Chechessee Creek Club golf course retention pond surface water in January and April exceeded the recommended level of N to avoid algal blooms in estuaries ($1 \, mg \, L^{-1}$) [22].

NH_4^+ concentrations in the constructed sites were less affected by anthropogenic inputs than NO_3^- concentrations and were often lower than the natural sites. Oyster Rake had higher NH_4^+ concentrations in pore water than surface water, the opposite of Oyster Rake NO_3^- results. Based on data from our laboratory experiments, the $\delta^{15}N$ value of surface sediment pore water NH_4^+ (4.9‰) compared to that of SON (2.8‰), and the low % SON (0.01%) of the Oyster Rake surface sediments, it is unlikely that SON mineralization is a significant contributor to surface sediment NH_4^+. NH_4^+ in the treated wastewater used for irrigation entering the sediments in the form of shallow groundwater is a possible source for heavy surface sediment NH_4^+, however the $NH_4^+ \delta^{15}N$ of treated wastewater was 26.4‰, considerably enriched compared to the $NH_4^+ \delta^{15}N$ of surface sediment (4.9‰). Another possible source of surface sediment NH_4^+ is NO_3^- in the shallow groundwater (likely originating from the treated wastewater used for irrigation) being converted to NH_4^+ through the process of DNRA. Our laboratory experiments found that significant rates of DNRA are possible in anaerobic Oyster Rake sediments. In addition the $\delta^{15}N$ value of surface sediment NH_4^+ is the same as the $\delta^{15}N$ value measured in treated wastewater NO_3^-. NO_3^- in irrigation water is more likely to affect the retention pond than NH_4^+ because NO_3^- was present in higher concentrations than NH_4^+ in the irrigation water, NO_3^- is more mobile in the environment than NH_4^+, and NO_3^- concentrations may increase over the course of ground-water flow due to nitrification [21].

3.2. Natural Sites. NO_3^- concentrations were below detection limit at the Grave's Dock site in all sampling events in both the surface water and the sediment pore water. NH_4^+ concentrations were significantly greatest in the

deepest sediment fraction (30–40 cm) pore water, decreased upwards to the surface sediments pore water (0–10 cm), and were significantly lowest in the surface water ($P < .001$) across all sampling events. Surface water NH_4^+ collected in October was anomalously depleted with a $\delta^{15}N$ value of -4.66 compared to the average $NH_4^+ \delta^{15}N$ values of 3.21‰ (s.d. = 0.56, $n = 13$) (excluding -4.66) of the other pore water and surface water $NH_4^+ \delta^{15}N$ samples. No significant differences in pore water $NH_4^+ \delta^{15}N$ values were found between sediment fractions ($P = .323$) (Table 1).

The percentage of N in soil was approximately 0.2 at both sediment depths across all months at Grave's Dock. SON $\delta^{15}N$ values were significantly greater ($P = .002$) in October than in January or April, and significantly greater ($P = .021$) in the deep sediments than in surface sediments. Measured SON $\delta^{15}N$ values were significantly enriched compared to pore water $NH_4^+ \delta^{15}N$ values ($P < .01$) for all samples combined (Table 2).

Similar to the natural Graves Dock site, the natural Chechessee Marsh estuary site contained no detectable NO_3^- in any of the three months in either the surface water or the sediment pore water. NH_4^+ was measured in surface water only in January and at a concentration lower than that of sediment pore water. NH_4^+ in sediment pore water was significantly greater in concentration at the deeper depth (30–40 cm) than the shallower depth (0–10 cm) ($P = .014$), and not different by month sampled ($P = .055$). No significant differences were measured in $\delta^{15}N$ of pore water NH_4^+ across depths ($P = .128$), but NH_4^+ values had a greater range (1.9 to 6.0‰) than those from the Grave's Dock site (Table 1).

The percentage of N in soil was similar at both sediment depths and across months at the Chechessee Marsh site averaging 0.14% (s.d. = 0.03, $n = 12$) for all sediment data combined. No significant difference in the $\delta^{15}N$ of SON was measured between sediment depths ($P = .186$) (Table 2). Measured $\delta^{15}N$-ON values were not significantly different than pore water $\delta^{15}N$-NH_4^+ values ($P = .738$) for all Chechessee golf course data combined.

NO_3^- was not detected in any of the Grave's Dock or Chechessee Marsh surface water or sediment pore water samples reflecting less NO_3^- input compared to our man-made sites or alternately, rapid microbial utilization of NO_3^-. Laboratory experiments conducted by Ma and Aelion [13] found that both Grave's Dock and Chechessee Marsh sediments had high potential denitrification rates and potential DNRA, processes which could quickly remove NO_3^- from the sediment system.

It is generally believed that diffusion processes are the dominant form of solute transport in estuarine systems with bioturbation playing a role near the sediment-water interface. At both undeveloped sites, NH_4^+ concentrations were always highest in the deepest sediment layer pore water, followed by the shallower sediment pore water, and lowest in the surface water, a concentration gradient that is consistent with diffusion. No significant differences in pore water $NH_4^+ \delta^{15}N$ were found between sediment depths, results also consistent with diffusion. The range of pore water $NH_4^+ \delta^{15}N$ values at Grave's Dock was similar to the range

of SON $\delta^{15}N$ values suggesting that mineralization in the deeper sediment layer may be the main source of pore water NH_4^+. One anomalous result is the $\delta^{15}N$ value measured in surface water NH_4^+ at the Grave's Dock site in October, which was depleted compared to the NH_4^+ isotopic values in the sediment pore water. The depleted value suggests that this surface water NH_4^+ is a product of a reaction such as nitrification and has a different, unknown, source than the NH_4^+ in the sediment pore water.

At Chechessee Marsh, sediment pore water NH_4^+ concentrations were lower and $\delta^{15}N$ values were more variable than at Grave's Dock so while the pattern of NH_4^+ concentrations is consistent with diffusion, the isotopic data suggest a more complicated system and the potential contribution of shallow groundwater. Moore et al. [23] used radium isotopes to estimate submarine ground-water discharge (SGD) into the Okatee estuary in our study area, and concluded that SGD is a significant source of nutrients to the system. Weston et al. [24] used pore water equilibration samplers to take inventories of pore water nutrients from several sites in the Okatee system and similar nearby estuaries, and while diffusion was determined to be dominant, there was evidence of advection in some samples. The variable pore water $\delta^{15}N$ values at Chechessee Marsh may be the result of ground-water NH_4^+ interacting with NH_4^+ produced in the sediments through mineralization. The effects of ground-water NH_4^+ on pore water $NH_4^+ \delta^{15}N$ values may be more noticeable at Chechessee Marsh than Grave's Dock because of the lower overall NH_4^+ concentration at Chechessee Marsh. Chechessee Marsh sediment organic matter had higher C : N ratios and lower % N than sediment organic matter from Grave's Dock which may lead to less N being released through mineralization and the lower NH_4^+ concentrations of sediment pore water.

4. Conclusion

In situ N species ^{15}N data and concentrations in laboratory studies provided insight into the dominant N-cycle processes occurring in both constructed and natural coastal systems. In the constructed systems, measurable concentrations of NO_3^- were present *in situ*. Although sediment microbes effectively consumed the added NO_3^- in laboratory experiments via denitrification and DNRA as measured via N_2O and NH_4^+ production, respectively, N from irrigation water and/or NO_3^--based fertilizer may be entering the system faster than it can be removed regardless of the potential for sediment denitrification and DNRA, and has the potential to negatively affect surface water quality. The particularly high concentration of NO_3^- in Oyster Rake surface water, and its similar ^{15}N signature to that of ^{15}N of NO_3^- in treated waste water, suggests that NO_3^- from waste water irrigation has a larger effect on water quality than golf course fertilizers alone, as occurs at the Chechessee Creek Club golf course. In the natural systems, no NO_3^- was detected in surface water and pore water samples, a finding consistent with low NO_3^- inputs compared to our constructed sites, and/or rapid microbial utilization of any NO_3^- inputs to the system. Thus NO_3^- does not dominate the unimpacted areas.

In situ NH_4^+ concentrations were generally low in surface water, and pore water concentrations were greater than those in the surface water at all the sites. At the constructed sites based on both laboratory experiments and $\delta^{15}N$ data, it appears that irrigation water may be entering the retention pond via shallow groundwater discharge and increased NH_4^+ is from anthropogenic sources. Little evidence of Oyster Rake sediment mineralization, which could add N to pore water, was found in laboratory experiments, and may be due to the low concentrations of SON in constructed golf course retention ponds. Sources of NH_4^+ to the unimpacted sites based on isotopic signatures appear to be mineralization of the sediment organic matter. In addition, the range of $\delta^{15}N$ data, particularly at the Chechessee Marsh unimpacted site suggests that shallow sediment is more microbially-active than the deeper sediment, and that this enhanced microbial activity in addition to soil mineralization, may have a significant effect on N availability in the unimpacted marsh. The dominant N-cycle process at the natural sites appears to be diffusion of microbially-released NH_4^+ (via mineralization) from deep sediment layers to surface water.

N concentrations and isotopic signatures were useful in identifying the different N sources and potential N-cycling processes occurring in the constructed and unimpacted sites. From the ecological stand point, in the impacted areas anthropogenic sources of NO_3^-, and in the unimpacted sites natural sources of NH_4^+ dominated the N profile of the areas, respectively. Enhanced microbial activity was not able to compensate for anthropogenic N addition in the constructed areas, suggesting best management practices are needed to protect these surface waters from nutrient degradation.
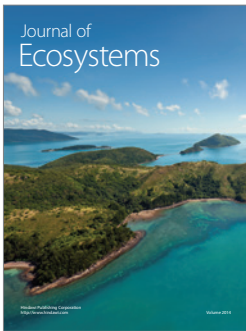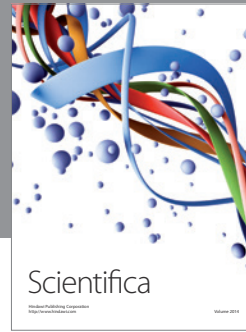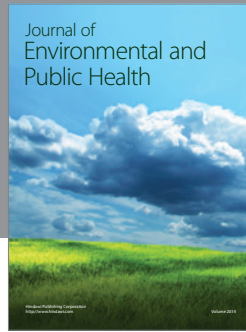
Acknowledgments

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