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Decomposition patterns and nitrogen dynamics of black mangrove (*Avicennia germinans*) leaf litter in disturbed estuaries linked to the Lower Laguna Madre, Texas

Mario Alberto Marquez

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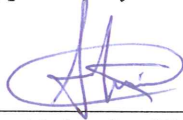
DECOMPOSITION PATTERNS AND NITROGEN DYNAMICS OF BLACK
MANGROVE (*Avicennia germinans*) LEAF LITTER IN DISTURBED ESTUARIES
LINKED TO THE LOWER LAGUNA MADRE, TEXAS

BY

MARIO ALBERTO MARQUEZ

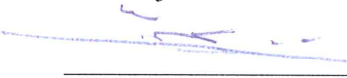
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MATHEMATICS AND TECHNOLOGY IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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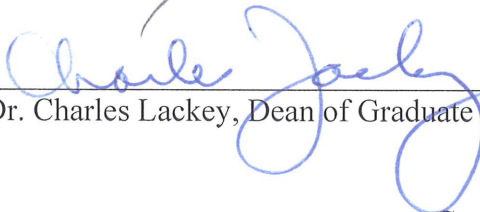
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November 2013

Decomposition patterns and nitrogen dynamics of black mangrove
(*Avicennia germinans*) leaf litter in disturbed estuaries linked to the Lower
Laguna Madre, Texas

A Thesis Presented to the Faculty of the College of Science, Mathematics
and Technology

University of Texas at Brownsville

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
In the field of Biology

by

Mario Alberto Marquez

November 2013

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By

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Abstract

Estuaries are among the most productive aquatic systems in the world but are subject to both anthropogenic and natural disturbances. With increasing environmental concerns, efforts have become commonplace in assessing the status and trends of environmental conditions. In this study, an assessment of ecosystem status of various estuaries affected by different disturbances, was attempted through the examination of key functional processes such as leaf litter decomposition and nutrient dynamics during decay. Three estuaries located along the Brownsville Ship Channel near the southern terminus of the Lower Laguna Madre in Texas were studied. The overlying goal of this study was to compare the functional state of these estuaries, utilizing leaf litter decomposition rates and nitrogen dynamics. Black mangrove (*Avicennia germinans*) leaves were used as the decomposition substrate since it is endogenous to the Lower Laguna Madre system. Several aims were addressed in this investigation: 1) determine decomposition patterns (decay rates, half-lives, recalcitrant pool sizes); 2) compare N content changes in decomposing mangrove leaves; 3) evaluate N immobilization/release during decay processes; and 4) appraise the value of decomposition process and N dynamics measurements as functional indicators in estuaries linked to LLM. Metrics derived from the decomposition process and concurrent N dynamics of leaf litter did discriminate among sites with different known disturbance histories. The ranking of the studied sites based in decomposition patterns did not fully correspond to the ranking obtained through the variables of N dynamics.

While these processes are linked by the activity of the decomposer community, they should be looked at separately to further classify the stability and ecological status of this type of estuarine systems in terms of ecosystem function.

Table of Contents

	PAGE
Acknowledgements.....	iii
Abstract.....	iv
Table of Contents.....	vi
List of Figures.....	vii
List of Tables.....	viii
Introduction	
Estuarine Overview.....	1
Ecological Indicators.....	3
Litter Breakdown	4
Study Purpose and Hypotheses.....	6
Materials and Methods	
Site Description.....	7
Decomposition Assays.....	8
Elemental and Isotopic Analyses.....	10
Water Parameters.....	11
Decay Rates and Constants.....	12
Nitrogen Dynamics.....	13
Water Parameter Analyses.....	15
Results	
Decomposition Patterns.....	16
Nitrogen Immobilization.....	17
Nitrogen Release.....	18
Isotopic Ratios.....	18
Water Parameters.....	19
Discussion.....	20
References.....	39

List of Figures

FIGURE	PAGE
1. Study Site Locations.....	26
2. Bahia Grande Study Site.....	27
3. San Martin Lake Study Site.....	28
4. South Bay Study Site.....	29
5. Mass Remaining Single-Exponential Model.....	30
6. Biomass Remaining as a Function of Nitrogen Concentration.....	31
7. Absolute Nitrogen Remaining as a Function of Time.....	32
8. Changes in $\delta^{15}\text{N}$ in decomposing leaf litter	33
9. Mid-Water Column Principle Component Analysis.....	34

List of Tables

TABLE	PAGE
1. Single-component model	35
2. Repeated measures ANOVA mass remaining pairwise comparison.....	35
3. Variables of N immobilization	36
4. Repeated measures ANOVA nitrogen loss pairwise comparison.....	36
5. Nitrogen release rates.....	36
6. Mangrove litter initial characteristics.....	37
7. PCA eigenvalues and eigenvectors.....	37
8. Water Parameters.....	38

Introduction

Estuarine and coastal lagoon ecosystems are among the most productive aquatic environments in the world and are important both ecologically and economically (Tunnel and Judd, 2002; Mouillot et al., 2006). They are usually characterized by an abundance of nutrients which are rapidly recycled; constant tidal water movement; and a wide array of primary producers, including seagrasses, benthic algae, phytoplankton, and halophytic vascular vegetation like mangroves in tropical and subtropical latitudes (Day et al., 1989). They can also vary drastically in their salinity regime and flow, at times being characterized as negative flowing estuaries due to the lack of freshwater input (Britton and Morton, 1989). These transitional wetlands serve as large nurseries to many marine and estuarine species and provide food for a variety of consumers, therefore linking various trophic webs. They also constitute a barrier that protects coastal zones from natural disturbing events and processes such as hurricanes, flooding, and erosion (Britton and Morton, 1989). While estuaries vary in their characteristics and can be classified differently (i.e. drowned river valleys, fjord-type estuaries, bar-built estuaries, and estuaries produced by tectonic processes), these water bodies can be considered as estuaries by meeting two conditions defined by Pritchard (1967): 1) being a semi-closed coastal body of water, 2) and maintains a free connection with the open sea.

A driving force in the development of any estuary is the physical environment (i.e. climate, geomorphology, water movement, chemical cycling, and

physical structure), highly controlling the structure of biota by the levels of change within the system (Day et al., 1989). Day et al. (1989) and Fairweather (1999) indicate that while such ecosystems are in a constant state of transition, their function and structure is maintained. Many of the important energetic pathways in coastal aquatic systems begin in estuaries, due to their high productivity and rapid nutrient cycling, which is typically greater than that in the ocean or freshwater systems (Fourqurean and Schrlau, 2003; Schelske and Odum, 1962). Major energy flows to higher trophic levels originates in detrital pathways in most estuaries (Day et al., 1989; Fourqurean and Schrlau, 2003). Because they are linked to upstream terrestrial and aquatic systems, estuaries and coastal lagoons are frequently under intense anthropogenic stress and have a higher degree of vulnerability (Mouillot et al., 2006). In addition, natural disturbances in estuaries are a normal phenomenon because of greater exposure to environmental changes and cycles than other aquatic systems. A disturbance can best be defined as an event, agent, or process either natural or anthropogenic that changes ecosystem structure and/or function (Greipsson, 2010; Rykiel, 1985). Perturbation and stress are effects (responses) of a disturbance. While some natural disturbances are routine such as winds, tidal currents, and waves; anthropogenic disturbances cause the greatest and most devastating effects (Young et al., 2004). Anthropogenic disturbances imposed to estuaries include pollution, land-use change, altered hydrological and salinity regimes, atmospheric nitrogen deposition, and climate change (Gessner and Chauvet, 2002). While restoration efforts are increasingly common, the success of such efforts remains unclear (Fairweather 1999). Thus, studies that assess estuarine

ecosystem status, both under natural and restored conditions, are imperative to detect degrading or recovering trends (Fano et al., 2003).

With increasing environmental concerns, efforts have become commonplace in assessing the status and trends in environmental conditions. While environmental monitoring begins with obvious measures such water quality parameters, it soon becomes evident that studies should also examine ecological receptors (known effects) because they can express unknown disturbances in the system (Jackson et al., 2000). Ecological receptors in the ecosystem help characterize ecosystem condition through the use of ecological indicators (Mouillot et al., 2006). Ecological indicators, either structural or functional, are signals that relay complex messages in a simple and useful manner and can reveal a biological, chemical, or physical feature within the system (Jackson et al., 2000). Structural indicators are commonly used examining patterns and assemblages of various biological communities, such as benthic and fishes (Engle and Summers, 1999; Whitfield and Elliott, 2002). While structural indicators may be adequate in many situations, transitional water bodies such as estuaries can be difficult to monitor due to the daily extreme physicochemical variability coupled with its position to the land-ocean interface (Fano et al., 2003). Dissimilarity of species among sites, long distances between sites, a tendency to underestimate true taxonomic richness due to sampling effort, and anthropogenic constraints further complicate structural assessments of ecological status (Young et al., 2004; Mouillot et al., 2006).

Assessing the habitat health and condition can most accurately come from examining ecosystem processes (Fairweather, 1999; Fano et al., 2003; Gessner and

Chauvet, 2002). An assessment of the function (i.e. measuring rates of processes) of an ecosystem can integrate environmental conditions over time (Young et al., 2004). When assessing ecosystem status, functional indicators are often more consistent and sensitive than structural indicators alone (Young et al., 2004). Gessner and Chauvet (2002) have shown that using functional measurements are relatively effective in tracing the sources of the disturbances. Measurements of ecosystem processes can indicate its functional state, since they are primarily driven by abiotic variables (Day et al., 1989; Statzner et al., 2001). Rates of organic matter (OM) decomposition, nutrient transformations, and primary productivity are useful functional indicators, as they play pivotal roles in ecosystem functioning (Dangles et al., 2004).

Understanding rates of leaf litter decomposition can be a starting point for developing a diagnostic tool for assessing the integrity of a given system, both aquatic or terrestrial (Gessner and Chauvet, 2002; Knacker et al., 2003). Leaf litter decomposition rates link vegetation characteristics with activities of both macro- and microorganisms within the system. Leaf litter decomposition rates are affected by natural and anthropogenic variation of environmental factors (Young et al., 2004). The leaf litter decomposition approach typically use leaves from surrounding areas to maximize accuracy measurements of natural litter decay within the ecosystem (Boulton and Boon, 1991). Decomposition patterns are altered by availability of N and P, either from endogenous or exogenous origin (Fierro et al., 2000). Several other factors such as litter quality, exogenous nutrient supply,

temperature, and oxygen availability have been shown to affect decomposition patterns (Gessner and Chauvet, 2002; Melillo et al., 1984; Young, 2004).

Measurements of OM decomposition can be effectively complemented by measures of stable isotope ratios to provide additional information regarding ecosystem function. Stable isotope ratios for carbon can indicate differences in decomposer communities present at different sites. For instance, preferential use of either ^{12}C or ^{13}C by decomposers is reflected in different trends of $\delta^{13}\text{C}$ among sites (Osono et al., 2006). Stable isotope ratios for nitrogen can be used to determine if N is being incorporated into decomposing material from surrounding media (e.g., soil, water or sediments), therefore assessing N immobilization, and/or the onset of N release (Osono et al., 2006).

Southern Florida estuaries have a litter fall of 4-8 tons of mangrove leaf litter per hectare year⁻¹, indicating that mangrove leaf litter is a significant input of organic matter into the aquatic system (Fourqurean and Schrlau, 2003). Litter fall and its decomposition are thus significant processes in the functioning of estuarine systems with mangrove stands.

The overlying goal of this study is to compare the functional state of three disturbed estuaries along the Lower Laguna Madre (LLM) in south Texas, utilizing leaf litter decomposition rates and N dynamics.

The LLM main lagoon and secondary bays are very productive estuaries with abundant marine life, seagrass meadows, and mangrove stands (Tunnel and Judd, 2002). In this study, three estuaries (Figure 1) within the LLM were examined, all located along the Brownsville Ship Channel (BSC). The sites have physical

similarities like their shallowness, and one narrow passage into the ship channel; but have clear differences in their ecological status due to different disturbances . I hypothesized that black mangrove (*Avicennia germinans* (L. 1759)) leaf litter decomposition rates and half-lives, as well as their N content, immobilization potential and duration, and release rates differ among estuaries due to their various disturbance histories and current ecological status. Specifically, this study tried to 1) determine decomposition patterns (decay rates, half-lives, recalcitrant pool sizes); 2) compare N content changes in decomposing mangrove leaves; 3) evaluate N immobilization/release during decay processes; and 4) appraise the value of decomposition process and N dynamics measurements as functional indicators in estuaries linked to LLM.

Materials & Methods

Site Description

The LLM extends south from Port Mansfield to Brazos Santiago Pass, TX and is part of the largest hypersaline estuarine system in the world, encompassing 1,364 km² with a mean depth of 1.4 m (Britton and Morton, 1989; Tunnel and Judd, 2002). The LLM is a semi-closed coastal body of water with no permanently flowing river into the lagoon, leading to frequent hypersaline conditions (Britton and Morton, 1989). This study took place in three estuaries (Bahia Grande, San Martin Lake, and South Bay) located along the BSC at the southern tip of the LLM (Figure 1). The largest is Bahia Grande, which for the purposes of this study was considered as two distinct systems separated into north and south sections by an old railroad trestle, but also by more importantly differences in environmental conditions such as salinity and dissolved oxygen (Cornejo, 2009). The other two study sites are San Martin Lake and South Bay (Figure 1). San Martin Lake and Bahia Grande are 1.97 km from one another, and South Bay is 6.17 km from Bahia Grande.

Bahia Grande [26°05'05.91"N and 26°00'43.10"N, 97°16'50.70"W and 97°19'45.04"W] is a 16.5 km² (Figure 2), hypersaline shallow basin estuary once part of the LLM (Figure 2). In the 1930's, construction of the BSC cut off water exchange between this estuary and the LLM, converting this aquatic ecosystem into a dust bowl for 70 years (Hiney, 2002) (Figure 2). In July 2005, a channel was dredged, opening the estuary to the ship channel and allowing water to flood the area and connect it to the LLM (Cornejo, 2009). The goal of the inundation was to restore the Bahia Grande into a functioning wetland ecosystem through the Bahia

Grande Restoration Project, which is managed by U.S. Fish and Wildlife Laguna Atascosa National Wildlife Refuge. This system is therefore in the process of being restored.

San Martin Lake [26°00'28.15"N and 26°00'11.18"N, 97°17'59.48"W and 97°20'20.33"W] (Figure 3) is the smallest of the estuaries (2.3 km²). It receives freshwater inflow from the city of Brownsville, Texas and surrounding areas, implicating potential runoff pollution. The area is a popular fishing site with soft sediment bottom, oyster beds along the fringes, and dense black mangrove stands around the shoreline.

South Bay [26°03'10.00"N and 26°00'24.80"N, 97°10'04.23"W and 97°12'28.58"W] is the southernmost estuary in the LLM near Brazos-Santiago Pass encompassing 11.3 km² (Figure 4). This estuary, which is a popular fishing spot for fishermen, has productive marine life, extensive seagrass meadows and black mangrove stands surrounding most of its shoreline (Britton and Morton, 1989). South Bay is connected to the BSC and the Gulf of Mexico through a relatively narrow channel, and was considered the reference system in terms of ecosystem condition for the purpose of this study because of its apparent good ecological state and fewer disturbances.

Decomposition Assays

To compare decomposition patterns among the three estuaries, litterbags were deployed March 2012 at each study site and retrieved after 14, 30, 60, 190, and 320 (d). Eight stations within every site (Figures 2-4) were selected using a

computer random number generator from 30 possible locations in each site: separated from one and another by about 200-500 m. A water depth of at least 0.75 m was considered a prerequisite to ensure complete and constant submersion of all litterbags, based on the area's mean tidal movement (Fourqurean and Schrlau, 2003). Mature leaves of black mangrove were used as the decomposition substrate, and were collected from one local mangrove stand, to minimize variability in the decomposing substrate (Boulton and Boon, 1991; Young et al., 2004). After collection, fresh leaves were washed with distilled water to remove excess salt from their surface. The leaves were then dried at 55 °C for 72 h, and thoroughly mixed to obtain a homogenous pool (Dick and Streever, 2001). Litterbags were 15 cm x 15 cm nylon mesh square envelopes, with a 1 mm pore size to minimize loss of litter due to handling while allowing access to microflora and microfauna decomposers (Fierro et al., 2000). Bags were filled with a known mass of dried leaves (around 10 g). Identification labels were placed in each bag, which were closed using rustproof (T50 monel) staples.

One 5 cm diameter x 300 cm long PVC pipe was staked 180 cm into the sediment at each of the eight stations in all sites. Ten litterbags were tied to each PVC stake suspended in the water column around 23 cm from the sediment. Two replicate bags were collected randomly at each retrieval date. The litterbag's exterior was carefully rinsed to remove excess sediment and biofouling (i.e. barnacle shell). Density fractionation using saline water (~60 psu) was used when needed to further separate the litter from exogenous materials (e.g., algae, amphipods, polychaetes, and barnacles). Litterbag contents were dried for 72 h to

constant weight at a temperature of 55 °C to prevent non-enzymic browning reaction (Van Soest, 1965). Litterbags were then opened and litter gently cleaned of all remaining biofouling material and weighed to record the dry mass remaining. The leaf litter collected was ground to a powder and homogenized using an (IKA A11, Germany) analytical mill and stored in sealed vials. To obtain ash content needed to account for sediment infiltration into the litterbags, subsamples were incinerated in a muffle furnace (500 °C, 6 h) (Fierro et al., 2000).

Elemental and Isotopic Analyses

Total C and N content subsamples of ground initial and decomposing material were analyzed in the Department of Biological Sciences at The University of Texas at Brownsville. Samples were combusted using an elemental analyzer (Costech ECS 4010, Valencia, CA). Standard blank assessment and correction procedures were applied. Standardization is based on atropine for elemental concentration; C = 70.56%, and N = 4.84%.

Stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of ground subsample leaf materials were analyzed at the Stable Isotope Laboratory at The University of Texas Marine Science Institute, using a continuous-flow gas-ratio mass spectrometer (FinniganMat Delta Plus, Waltham, CA) coupled to an element analyzer (Carlos Erba NC 2500). Samples were combusted in the elemental analyzer. Standard blank assessment and correction procedures were applied. Standardization is based on casein for elemental concentration; $\delta^{13}\text{C}$ = -26.95 and 46.5‰, and $\delta^{15}\text{N}$ = 5.94 and 13.32‰. Isotopic composition was expressed in permil (‰). Deviations of samples

materials were derived from atmospheric nitrogen (AIR, N₂) and Pee Dee Belemnite (PDB) international standards. The samples' isotopic ratios were reported using the following equations (Fourqurean and Schrlau, 2003; Manchás et al., 2006; Osono et al., 2006):

$$\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}}/({}^{13}\text{C}/{}^{12}\text{C})_{\text{standard}} - 1] \times 1000$$

$$\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}}/({}^{15}\text{N}/{}^{14}\text{N})_{\text{standard}} - 1] \times 1000$$

Carbon-13 samples of leaf litter were not included in the analyses due to the presence of calcium carbonates (CaCO₃) from barnacle infiltration.

Water Parameters

Water-column parameters were measured at each station at the time of litterbag deployment (d 0) and at all retrieval dates. Parameters measured in situ included dissolved oxygen ($\pm 0.02 \text{ mg L}^{-1}$), salinity ($\pm 0.01 \text{ psu}$), temperature ($\pm 0.03^\circ\text{C}$), (multimetric sondes YSI Pro2030, Hach HQ40d), total depth, Secchi depth, and *in vivo* chlorophyll 'a' content ($\pm 0.03 \mu\text{g L}^{-1}$) (Turner Aquafluor). In addition, water samples were collected for laboratory analysis, which included total Kjeldahl Nitrogen (TKN) (SM 4500-NH₃D Ammonia-Selective Electrode Method; $\pm 0.038 \text{ mg NH}_3\text{-N L}^{-1}$), total suspended solids (TSS) (SM 2540D Total Suspended Solids Dried at 103-105°C; $\pm 5.2 \text{ mg L}^{-1}$), total organic carbon (SM 5310B Total Organic Carbon; $\pm 2 \text{ mg L}^{-1}$) (composite samples combining the two closest stations) were analyzed at A&B Environmental Services, Inc. in Houston, Texas.

Decay Rates and Constants

Decomposition rates and decay constants were calculated by fitting a single-component exponential model to time series data:

$$M_r = M_0 e^{-kt}$$

where M_r is the fraction of initial ash free dry weight (AFDW) remaining at time (t) relative to the start of the experiment (%), M_0 is the initial AFDW at time 0 (defined as 100%), k corresponds to the decay constant (d^{-1}), and t (d) to the time in days elapsed since the beginning of the experiment (Chimney and Pietro, 2006; Fourqurean and Schrlau, 2003; Manchás et al., 2006). Equation constants were obtained using a non-linear exponential decay (single, 2 parameters) model (SigmaPlot v12). A two-component exponential model was also fitted if it better described mass loss of labile and recalcitrant pools:

$$M_r = M_1 e^{-k_1 t} + M_2 e^{-k_2 t}$$

where M_r is the mass of leaf litter AFDW remaining (%), M_1 and M_2 are the sizes of the two compartments (labile and recalcitrant material, %), k_1 and k_2 are the decay constants of each compartment (d^{-1}), and t is cumulative days from deployment (Fierro et al., 2000). The *a priori* null hypothesis of no differences in mass loss (AFDW) among sites was analyzed using a One-Way Repeated Measures Analysis of Variance (ANOVA) with sites as the fixed effect (Fourqurean and Schrlau, 2003). A post hoc (Tukey Test) all pairwise comparison procedure was used to compare sites. A natural log transformation was used to linearize AFDW data to adjust for exponential decay did not correct for heteroscedasticity (Sokal and Röhlf 1995). The

ANOVA was still performed because it is robust enough to allow for deviations from homoscedasticity (Underwood, 1997).

Half-life, or the time (d) required for 50% of leaf litter to decompose from the deployment date, was calculated using the following equation, with value of the decay constant (k) per site calculated above (Chimney and Pietro, 2006):

$$t_{50} = \log_e(2)/k$$

Mean residence times (MRT) which is the time in days that the litter persisted within the system before completely decomposed, was calculated using the inverse value of the decay constant (k) per site:

$$\text{MRT} = 1/k$$

Nitrogen Dynamics

Metrics describing N dynamics were calculated using the slope and y-intercept from the regression equation of the inverse linear relationship between mass remaining (AFDW %) and N concentration (%) based on the approach of Aber and Melillo (1980).

Immobilization potential (N_{\max}) of mangrove leaf litter, which is the maximum amount of exogenous N incorporated into decomposing tissue before net N mineralization began, was calculated using the following equation (Aber and Melillo, 1980; Melillo et al., 1983):

$$N_{\max} = [(I^2/(-4xS)) - 100xN_0]0.1$$

where N_{\max} is mg of N immobilized g^{-1} initial leaf litter, N_0 is the initial N concentration at deployment (d 0), I is the y-intercept and S is the slope of the regression described above.

The amount of organic leaf litter (AFDW %) remaining at the immobilization potential can be calculated using the regression intercept calculated above in the following equation (Melillo et al., 1984):

$$ADFW_{\max} = \text{Intercept} \times 0.5$$

The duration of immobilization phase, which is the time required to reach the immobilization potential, was calculated using the following equation (Melillo et al., 1984; Osono et al., 2006):

$$T = \log_e[(\text{Intercept} \times 0.5) \times 0.01] / -k$$

Where the intercept is from the regression equation calculated above, (k) is the decay constant calculated above, and T is the duration of the immobilization phase (d).

Nitrogen net release rates after immobilization were calculated using the slope (release rate d^{-1}) from the regression equation of the inverse linear relationship between absolute N (mg N g^{-1} leaf litter) and time from deployment (d) (Fierro et al., 2000).

The *a priori* null hypothesis of no differences in the absolute amount of N (mg N g⁻¹ litter) among sites was analyzed using a One-Way Repeated Measures Analysis of Variance (ANOVA) with sites as the fixed effect. A *post hoc* (Tukey Test) all pairwise comparison procedure was used to compare sites.

Water Parameter Analyses

A principle component analysis (PCA) was used to compare environmental variables to identify the principle abiotic components that drove the variance among sites using PRIMER-6 software package (Clarke and Warwick, 2001). A Draftsman's Plot was run to analyze and check for data symmetry and variable correlations, since having redundant parameters could misrepresent the actual results (Clarke and Warwick, 2001). Environmental parameters were normalized to create unit-less values for equal comparison to identify the principle components that drove the variance.

Results

Decomposition Patterns

The double-component exponential model failed to show significant changes in the *in situ* litter compartmentalization of labile and recalcitrant pools, yielding the same decay constants (k), therefore a single-component model was used to express the decomposition patterns among study sites. The *in situ* leaf litter decomposition was well described by the single exponential model when time (d) was considered as the independent variable (Figure 5). Initial composition of black mangrove leaf litter used in this study (C:N, 27:1) (is within the range of decomposition leaf litter used in other studies (C:N, 19-87:1) (Table 6) either with the same or different species of mangrove. For the duration of the study, decay patterns were significantly different among all sites ($F_{0.05(3,35)} = 38.6, p < 0.001$), except for the reference site (South Bay) and Bahia Grande South which showed no significant difference ($P = 0.96$) (Table 2). San Martin Lake exhibited the fastest decomposition rate ($k = 0.023$), while leaf litter in Bahia Grande North decomposed slowest ($k = 0.0097$). Bahia Grande South had the same decay rate ($k = 0.0141$) as the reference site (South Bay). On average, decomposition in all sites occurred rapidly within the first 50 d, during which litter lost about half of its initial mass and reached a phase of slower decomposition. The half-life and MRT of leaf litter in Bahia Grande North ($T_{50} = 71.5$ d; MRT = 103 d) were more than twice as long as that of San Martin Lake ($T_{50} = 30.1$ d; MRT = 43 d). Decomposing litter in Bahia Grande South and South Bay had the same half-life and residence times ($T_{50} = 49.2$ d; MRT = 71 d) (Table 1).

Nitrogen Immobilization

Total *in situ* N dynamics in the decaying leaf litter followed a two-phase pattern of immobilization and mineralization varying among sites. A net accumulation of N occurred during the first week after deployment, which was followed by a net release of N throughout the remaining of the study (Table 3). The inverse linear function between mass loss and N concentrations in the remaining leaf litter was used to determine the variables of N immobilization. Nitrogen immobilization variables were calculated in all sites except San Martin Lake, which appeared to show a direct relationship between mass loss and N concentration (Figure 6). The maximum amount of exogenous N accumulated per unit of initial leaf litter (N_{\max}) before net mineralization began, varied from 4.15 to 6.89 mg N g⁻¹ leaf litter among study sites (Table 3). Although Bahia Grande South and South Bay displayed the same decay constants, their N dynamics behaved differently. South Bay had the highest immobilization potential (N_{\max}) of 6.89 (mg N g⁻¹ leaf litter) with the longest time required to reach immobilization begin N net release of 6.6 d, while Bahia Grande South had the smallest immobilization potential of 4.15 (mg N g⁻¹ leaf litter) with the shortest time of 3.6 d before N mineralization (Table 3). Bahia Grande North having the slowest decay constant had an intermediate immobilization potential of 4.38 (mg N g⁻¹ leaf litter) and an immobilization time of 3.7 d. The onset of mineralization (at N_{\max}) occurred when leaf litter remaining was between 96% and 91% of the original mass (AFDW) (Table 3).

Nitrogen Release

In situ net release of N began quickly, occurring after as little as 3 d after leaf litter deployment. Nitrogen release rates were obtained using the initial 60 d of the decay process, when more than half of the initial litter mass was lost in all sites. The release of N from leaf litter (after immobilization) was adequately described by the inverse linear relationship between the absolute amount of N (mg N g^{-1} leaf litter) and days (Figure 7). During the initial 60 d, N release rates were significantly different among sites ($F_{(3,31)} = 19.515$, $p < 0.001$), except for both study sites within the same estuary (Bahia Grande North and Bahia Grande South), which showed no significant difference ($P = 0.898$) (Table 4). Nitrogen release rates varied from 0.83 to 1.97 $\text{mg N/g leaf litter d}^{-1}$, averaging a loss of 1.27 $\text{mg N/g leaf litter d}^{-1}$ among all four sites. Bahia Grande North and South had no significant differences in their release rates of -1.00 and -1.29 $\text{mg N/g leaf litter d}^{-1}$. South Bay appeared to have had the most conservative release rate of -0.83 $\text{mg N/g leaf litter d}^{-1}$, and San Martin Lake was the least conservative yielding -1.97 $\text{mg N/g leaf litter d}^{-1}$, more than twice as fast as the reference site (Table 5).

Isotopic Ratios

Leaf litter $\delta^{15}\text{N}$ ratios were compared over time at each site (Figure 8). All sites began with an initial $\delta^{15}\text{N}$ leaf litter of $7.9 \pm 0.15\text{‰}$ (Table 6; Figure 8). Among the initial 60 d, Bahia Grande North, Bahia Grande South, and South Bay followed a similar pattern in $\delta^{15}\text{N}$ changes and were consistent with less than a per mil difference. Over the duration of ~ 200 d, there was steady decrease in $\delta^{15}\text{N}$ (Figure 8). Bahia Grande North and Bahia Grande South fell within a 1.0‰ (7.12 ± 0.16 and

6.11 ± 0.20‰, respectively) of difference at ~200 d. Over the course of the incubation, leaf litter in South Bay was most depleted with $\delta^{15}\text{N}$ dropping 3‰ (4.92 ± 0.47‰). San Martin did not follow the same pattern or slow depletion of $\delta^{15}\text{N}$. Within the initial 30 d, San Martin had a steep $\delta^{15}\text{N}$ enrichment of almost 3.9‰ (11.78 ± 0.28‰) compared to the other site locations never exceeding a 0.74‰.

Water Parameters

The principle component analysis indicated that 60.1% of the variation among sites due to physicochemical parameters was driven by TKN, chlorophyll 'a', and dissolved oxygen (Table 7). Eigenvalues showed that 37.3% of the variation was due to TKN and chlorophyll 'a' in the water-column. Both variables showed an indicative gradient from low to high (ranging 0.7-1.1 mg L⁻¹) (Table 8) concentrations among sites as they increased along the PC1-axis (Figure 9; Table 7). Dissolved oxygen accounted for 22.8% of the variability (ranging 6.0-7.8 mg L⁻¹) (Table 8) among sites but failed to show a distinction among sites by clustering them together along the PC2-axis. Salinity ranged from 31.6-45.1 psu throughout the study sites, with San Martin Lake having the lowest and Bahia Grande North having the highest (Table 8).

Discussion

Net decomposition of black mangrove leaf litter among study sites was well described ($r^2 > 0.98$, $p < 0.001$) using the single-component exponential model and not the double component model. This can be explained by the high litter quality of this leaf litter. With black mangrove leaves having a decreased biochemical complexity (less lignins) and high initial N content (Twilley, 1982), compartmentalization of recalcitrant pools was superfluous as observed also by Melillo et al. (1984). Mass loss rates were close to the ranges reported in the literature (Nielson et al., 2004; Twilley, 1982). Black mangrove leaf decay coefficients were near the ranges (0.0025 – 0.0155 k) reported in estuaries in southern Florida (Fourqurean and Schrlau, 2003; Twilley, 1982). Decay coefficients (k) for black mangrove leaves were generally greater than reported for other mangrove species (*Rhizophora sp.*), but comparable to leaves of emergent and floating aquatic macrophytes (e.g. seagrasses) (Nielson et al., 2004).

In this study, mass loss of decomposing leaf litter was significantly different among sites, except for Bahia Grande South and the reference site (South Bay) having the same decay coefficient. In general, the decomposition of black mangrove leaves was relatively fast in the studied sites, with average half-lives being reached within 50 d after deployment. Other study also reported black mangrove leaves reaching their half-life within 44 d (Twilley, 1982). With elevated decay constants, relatively short MRT's were reflected, but considerable differences were observed between study sites, ranging from 43 to 103 d. Bahia Grande North had the longest

MRT (103 d), San Martin Lake had the shortest (43 d), and Bahia Grande South had the same intermediate MRT (71 d) of South Bay.

Leaf litter in Bahia Grande North had the slowest decomposition rate, possibly due to restricted water flow, which may have hindered decomposer communities (Gessner and Chauvet, 2002). On the other hand, decomposition process in San Martin Lake appear to be relatively faster compared to the other sites, possibly pointing at higher availability of exogenous nutrients (Fierro et al., 2000) coming from its influx or runoff and residual waters. However, significantly higher concentrations of total N were not detected in the samples taken in this estuary, presumably due to the sampling during incoming tides across sites. Metrics derived from mass loss appear to discriminate among study sites suggesting, for example, that ecosystem functioning in Bahia Grande South may be getting closer to the reference site. Bahia Grande North may require further intervention to fully restore ecosystem function; whereas San Martin Lake is probably disturbed by nutrient pollution, which may be affecting ecosystem function.

Nitrogen dynamics in the study sites were compared using the residual leaf litter material. Nitrogen dynamics of decomposing litter discriminated sites differently than decomposition patterns. Mass loss alone can sometimes be misleading in evaluating the function of the system, so determination of N immobilization and release allows to further assess the dynamics, which are occurring during litter decay (Nielson et al., 2004). Variables of N immobilization were calculated for all sites except San Martin Lake, which did not follow the inverse linear function between mass loss and N concentrations in the remaining leaf litter

used to determine these variables; a pattern consistently observed in other studies (Aber and Melillo, 1980). Leaf litter in San Martin Lake appeared to decrease its N concentration proportionally with mass loss. Explanations to this anomaly could be due to at least two factors: 1) N loss occurring as fast as leaf mass loss, possibly pointing at other mechanisms of N loss other than microbial mineralization, and/or 2) a lack of accretion of N from the surrounding environment (Melillo et al., 1984).

Results of this study showed that N dynamics in decaying black mangrove litter presented a two-phase pattern of brief immobilization followed by mineralization. Net accumulation of exogenous N occurred within the first week after deployment. The brief immobilization time can be explained by the relatively high quality of black mangrove leaf litter. Critical concentrations of N (expressed in C:N ratios) for mineralization in aquatic systems have been estimated at 16 to 30:1 (Twilley, 1982). Black mangrove leaves used in this study had an initial C:N ratio of 27:1 within the range for quick mineralization (Russell-Hunter, 1970). The maximum amount of exogenous N accumulated per unit of initial leaf litter (N_{max}) before net mineralization began, also known as the immobilization potential (Osono et al., 2006), ranged from 4.15 to 6.89 mg N/g leaf litter, with South Bay exhibiting the highest potential of over a 6 d immobilization period. Bahia Grande North and South had immobilization potentials of 4.38 and 4.15 mg N/g leaf litter over only a 3 d period. Release rates were calculated using data from the initial 60 d post deployment, which corresponds to the most intensive part of the mineralization process. This also produced more accurate values and a more robust depiction due to more complete retrieval sets. Nitrogen release rates were significantly different

among all sites except for Bahia Grande North and South. Mineralization of N over time is not significantly different within the Bahia Grande estuary, even though decomposition patterns in both sections were different and immobilization potentials varied. South Bay, the reference site, was the most conservative in its N cycling with the lowest release rate, which may be indicative of a less disturbed, or more stable ecosystem. This is in accordance to N immobilization pattern observed in South Bay. Leaf litter decomposing in San Martin Lake had an N release rate twice as fast as the reference site (South Bay), which may be considered as evidence of a more disturbed system.

Stable N isotope ratios of incubated leaf litter followed the same overall decreasing pattern observed with elemental N concentrations, except for San Martin Lake (Figure 8). Bahia Grande North and South, and South Bay showed small ($\cong 1\text{‰}$) fluxes in their $\delta^{15}\text{N}$ ratios within their initial 60 d, with and overall decrease in $\delta^{15}\text{N}$ as decomposition progressed. These small fluxes have also been seen observed in similar decomposition studies of *Rhizophora spp.* and seagrasses (Fourqurean and Schrlau, 2003) and could be representative of highly intense N dynamics with short episodes of immobilization and release. This may reflect rapid changes in isotopic composition during both net immobilization and net release phases, possibly due to rapid incorporation and release of N from exogenous sources (Fourqurean and Schrlau, 2003). On the other hand, decomposing litter in San Martin Lake had a steep $\delta^{15}\text{N}$ enrichment of $\sim 3\text{‰}$ within 33 d, showing, once again a completely different N dynamic compared to other study sites.

San Martin Lake showed different trends in its N dynamics and isotope ratios throughout the study. These marked differences could be due to heavy leaching of nitrogenous compounds in the leaf litter. Heavy leaching may be due to strong tidal currents caused by the small size and narrow elongated shape of the basin. Large tidal flow entering and exiting the system can be observed on a daily basis of O₂. The proportional relationship of mass loss and N concentration found for this site, confirms that N is being depleted at a similar rate as leaf litter mass is lost. Further, the increasing $\delta^{15}\text{N}$ signatures observed during study, exhibit that ¹⁴N is being preferentially lost, most likely solubilized, which is also in agreement with the high N release rate observed in San Martin Lake.

The significantly faster decomposition process observed in San Martin Lake may be explained by examining the drivers of this process. The four main factors controlling decomposition rates in aquatic systems are litter quality, temperature, dissolved oxygen, and nutrient availability, mainly nitrogen and sometimes phosphorus (Gessner and Chauvet, 2002; Mellilo et al., 1984; Young et al., 2004). In this study, a homogenous pool of black mangrove leaves was used as decomposition substrate at all sites, thus litter quality was the same. Differences in water temperature (Table 8) were minimal among sites.

While greater tidal influence and turbulence could have increase oxygen availability in San Martin Lake, this was not reflected in measured dissolved oxygen levels (Table 8) being near the maximum saturation concentrations (BGS = 8.15 ± 0.7 BGN = 8.36 ± 0.7 , SML = 8.05 ± 0.5 , SB = 8.11 ± 0.44 mg l⁻¹) for the temperature ranges in all sites (EPA, 2013). Alternatively, while N concentrations in water

appear to have varied only slightly among sites, high N availability is likely the culprit for the faster decomposition observed, compared to the reference site. Water samples for nutrient analyses were taken always during incoming high tide in order to be able to navigate from one sampling station to the next. This water sampling protocol provided only a snap shot indication of N availability within the system, specifically when water from the ship channel was moving in.

In conclusion, metrics derived from the decomposition process and concurrent N dynamics of black mangrove leaf litter did discriminate among sites with different known disturbance histories. While these processes are linked by the activity of the decomposer community, they should be looked at separately to further classify the stability and ecological status of the system in terms of ecosystem function. The ranking of the studied sites based in decomposition patterns did not necessarily correspond to the ranking obtained through the variables of N dynamics. For example, while South Bay and Bahia Grande South displayed identical decomposition patterns, their N dynamics were significantly different. Further, Bahia Grande North and South, although displaying significant differences in their decomposition rates, had similar N dynamics. Isotopic measurements in decomposing leaf litter were suitable to confirm the trends in both detrital mass loss and N dynamics. These isotopic measurements also pointed out the more advanced research hypothesis of intense N dynamics during initial litter decay

Figures



Figure 1: Locations of study sites (A) South Bay (B) Bahia Grande (C) San Martin Lake. Coordinates for entire study area [26°05'35.30"N and 26°00'02.40"N, 97°22'20.00"W and 97°00'02.10"W]

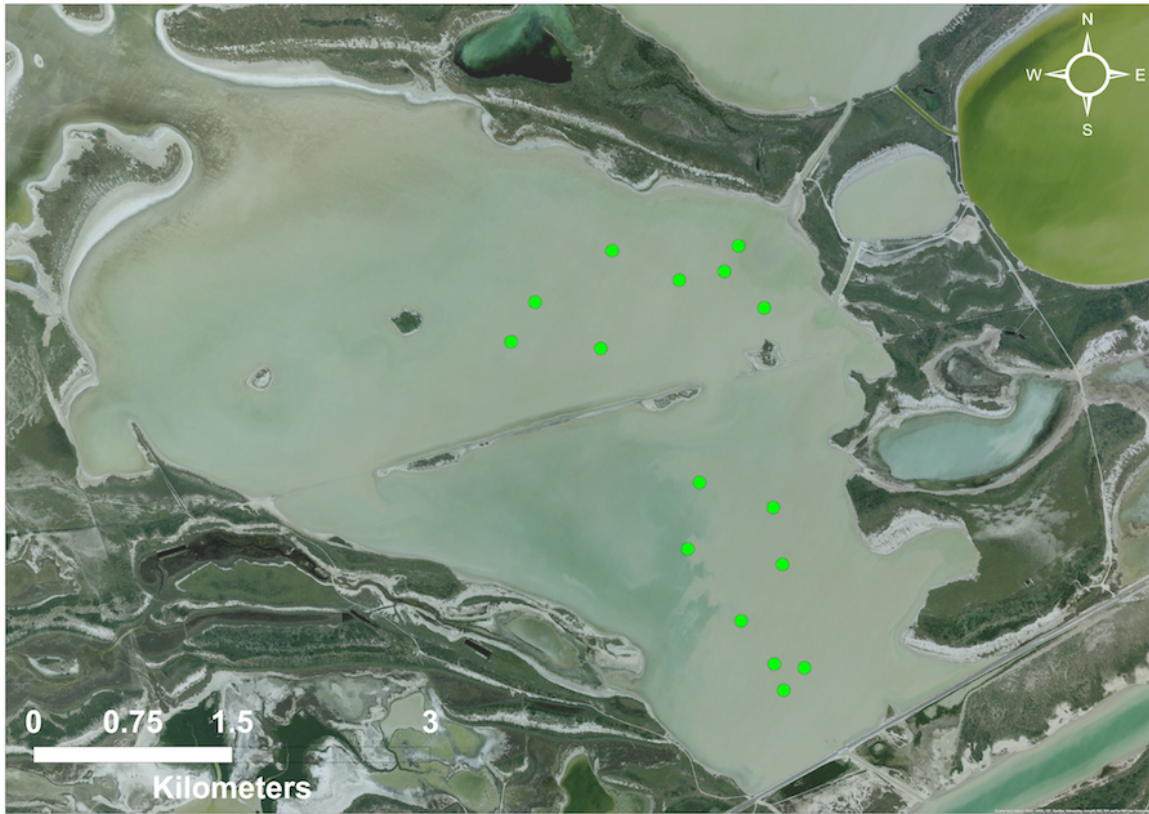


Figure 2: Bahia Grande study site with station locations (Bahia Grande North and Bahia Grande South [26°05'05.91"N and 26°00'43.10"N, 97°16'50.70"W and 97°19'45.04"W])



Figure 3: San Martin Lake study site with station locations [26°00'28.15"N and 26°00'11.18"N, 97°17'59.48"W and 97°20'20.33"W]



Figure 4: South Bay (reference site) with station locations [26°03'10.00"N and 26°00'24.80"N, 97°10'04.23"W and 97°12'28.58"W]

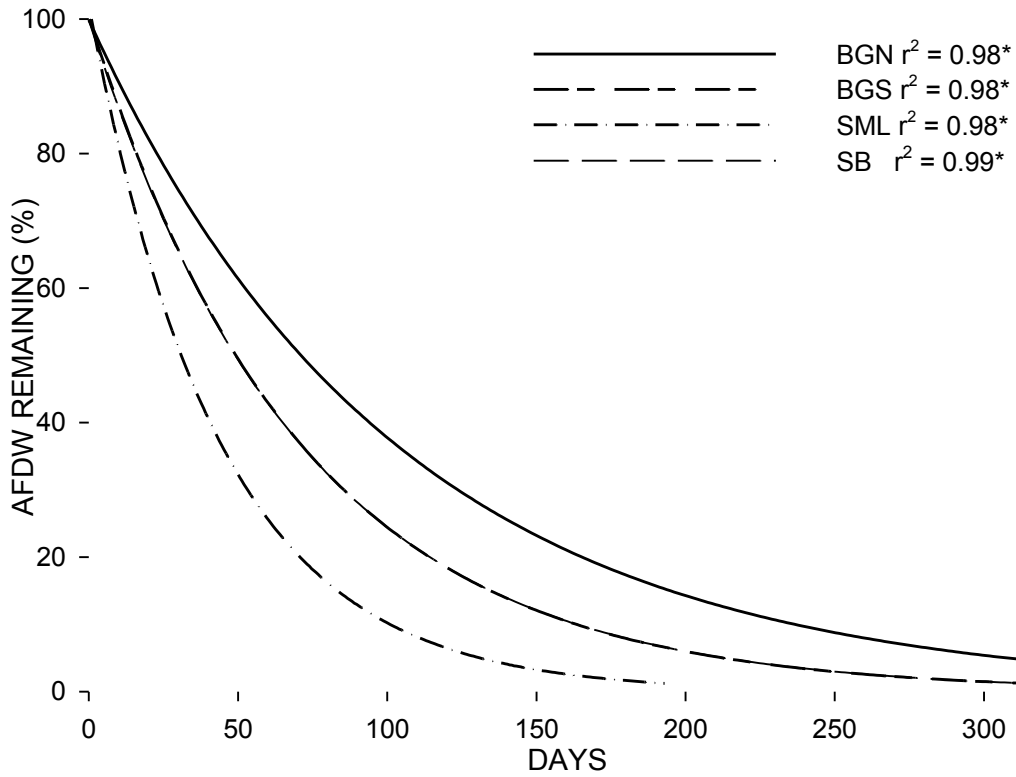


Figure 5: Single-component exponential model of biomass remaining expressed as percent ash-free dry weight (AFDW) of *Avicennia germinans* leaf litter in four study sites in the Lower Laguna Madre, Texas over a 322 day period. Bahia Grande North = BGN, Bahia Grande South = BGS, San Martin Lake = SML, South Bay = SB. Only three lines are visible because SB and BGS overlap having the same decay constant. *: significant at $P < 0.001$

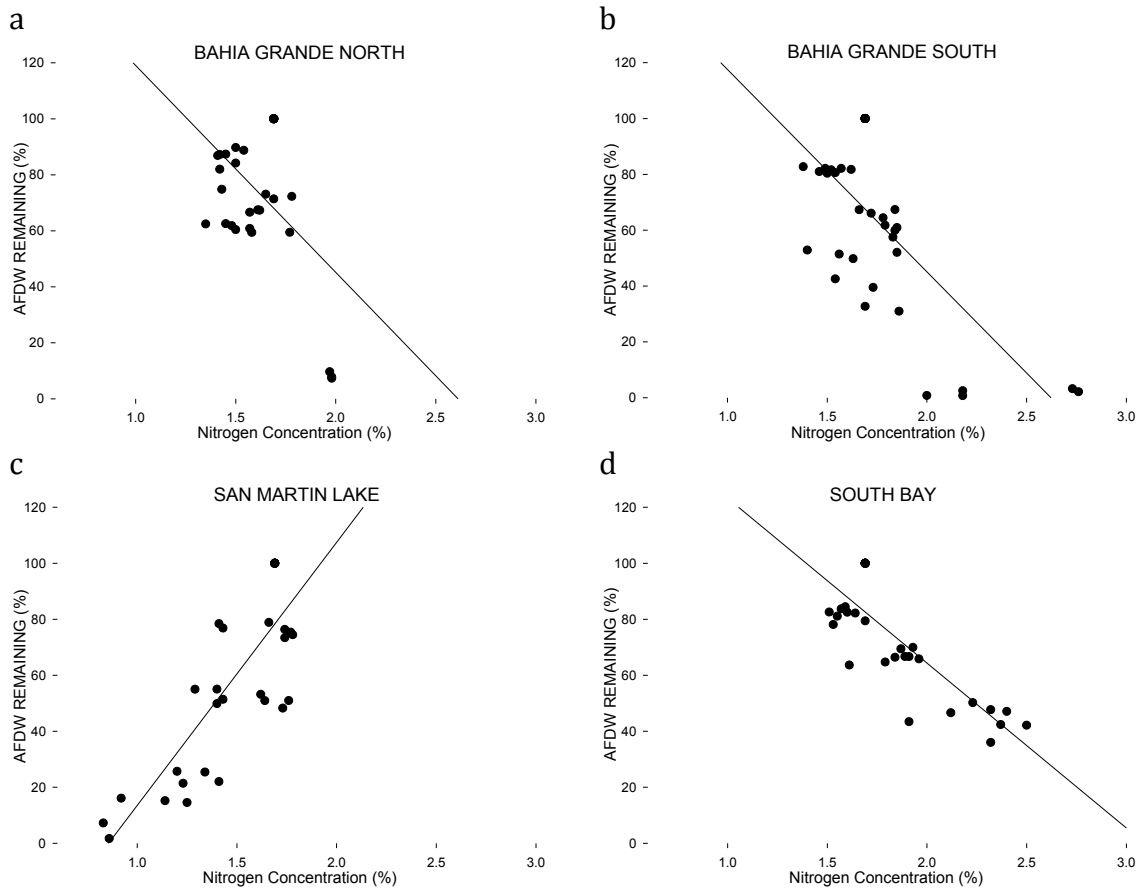


Figure 6: Original biomass remaining expressed as percent ash-free dry weight (AFDW), as a function of nitrogen concentration (%) in the residual leaf litter of *Avicennia germinans* decomposing in four study sites in the Lower Laguna Madre, Texas.

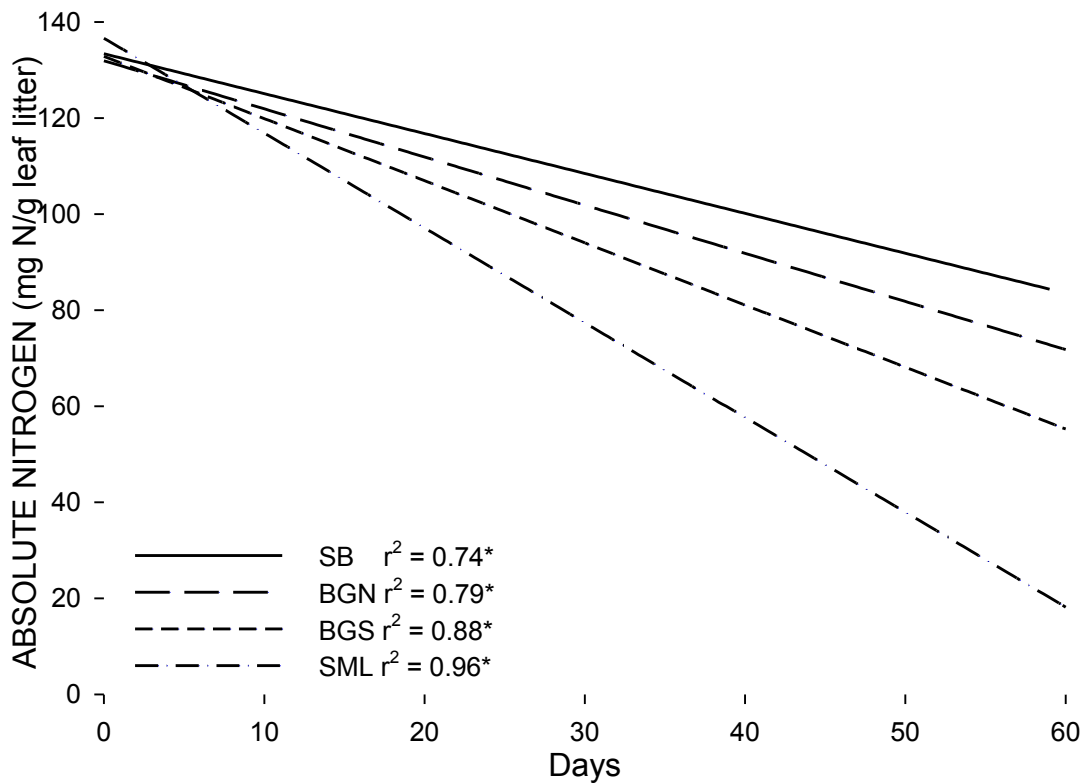


Figure 7: Absolute amount of nitrogen remaining (mg N/g leaf litter) as a function of time in residual leaf litter of *Avicennia germinans* decomposing in four study sites in the Lower Laguna Madre, Texas. Bahia Grande North = BGN, Bahia Grande South = BGS, San Martin Lake = SML, South Bay = SB.

*: significant at $P < 0.001$

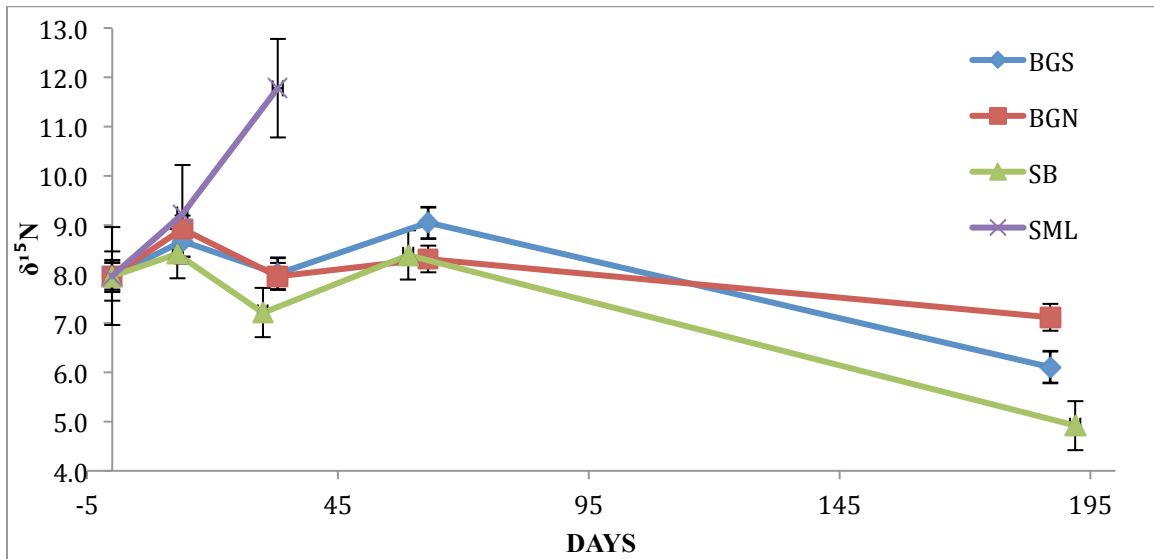


Figure 8: Average changes in $\delta^{15}N$ in decomposing leaf litter (*Avicennia germinans*) in four study sites in the Lower Laguna Madre, Texas. Bahia Grande South = BGS, Bahia Grande North = BGN, South Bay = SB, San Martin Lake = SML. Bars equal standard error.

Water Parameters

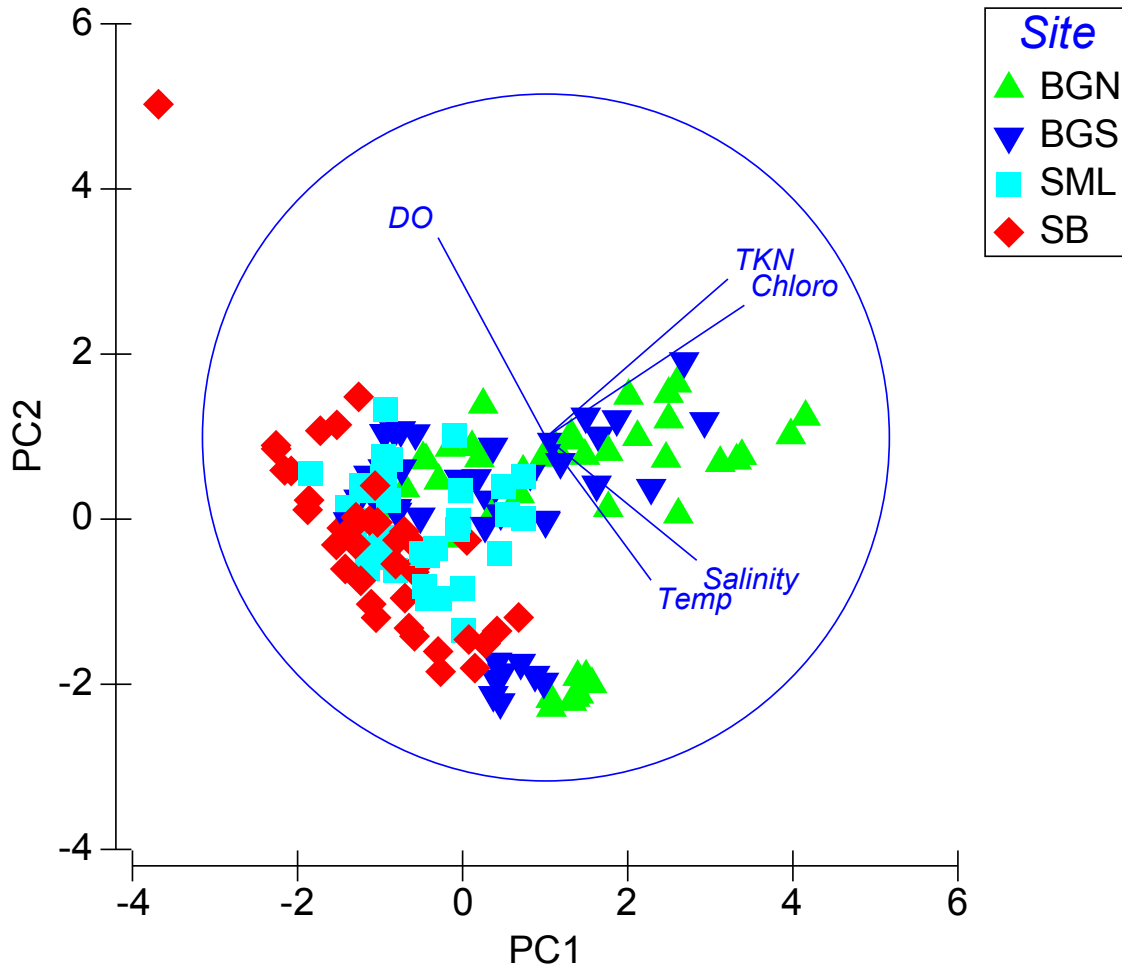


Figure 9: Principle Component Analysis of mid-water column physico-chemical parameters among four sites in the Lower Laguna Madre, Texas. Samples were transformed to normalize data with different units of measure. Bahia Grande North = BGN, Bahia Grande South = BGS, San Martin Lake = SML, South Bay = SB. Temperature = Temp, Chlorophyll 'a' = Chloro, total Kjeldahl Nitrogen = TKN, Dissolved Oxygen = DO.

Tables

Table 1: Decomposition values derived from the single-component model among sites throughout entire study (322 days). Decay constant = $k(d^{-1})$; r^2 = Coefficient of determination; n= Total duplicates retrieved; T_{50} = Half-life; MRT= Mean residence time.

	$k(d^{-1}, \pm SE)$	r^2	n	$T_{50}(d)$	MRT(d)
Bahia Grande North	0.0097±0.0004	0.98*	45	71.5	103
Bahia Grande South	0.0141±0.0006	0.98*	44	49.2	71
San Martin Lake	0.0230±0.0009	0.98*	34	30.1	43
South Bay	0.0141±0.0004	0.99*	39	49.2	71

*: significant at $P < 0.001$

Table 2: Repeated Measures ANOVA mass remaining among sites post hoc (Tukey) test. Bahia Grande North = BGN, Bahia Grande South = BGS, San Martin Lake = SML, South Bay = SB.

Comparison	Difference of means	P
BGN vs. SML	16.643	<0.001
BGN vs. BGS	6.911	<0.001
BGN vs. SB	6.196	<0.001
SML vs. SB	10.447	<0.001
BGS vs. SB	0.715	0.963
BGS vs. SML	9.732	<0.001

Source of variation between sites: d.f. = 3, SS = 5116.388, MS = 1705.429, $F_{(3,45)} = 38.583$, $P < 0.001$

Table 3: Variables of nitrogen immobilization derived from inverse linear functions relating mass loss of leaf litter in percent ash-free dry weight (AFDW) and N concentration (%) in residual leaf litter of *Avicennia germinans*, as affected by four sites in the Lower Laguna Madre, Texas

	Slope	Intercept	r ²	Nmax ^z	AFDW ^y	T ^x
				mg g ⁻¹	%	d ⁻¹
Bahia Grande North	-73.9	192.9	0.22	4.38	96.4	3.7
Bahia Grande South	-72.4	189.9	0.47*	4.15	95.0	3.6
San Martin Lake	93.9	-80.4	0.66*	--	--	--
South Bay	-58.9	182.1	0.65*	6.89	91.1	6.6

^z: maximum amount of N immobilized per gram initial material

^y: amount of organic leaf litter (AFDW %) remaining at the Nmax

^x: time required to reach the immobilization potential

*: significant at P<0.001

--: not able to calculate, positive function

Table 4: Repeated Measures ANOVA absolute amount of N remaining among sites post hoc (Tukey) test. Bahia Grande North = BGN, Bahia Grande South = BGS, San Martin Lake = SML, South Bay = SB.

Comparison	Difference of means	P
BGN vs. SML	16.007	<0.001
BGN vs. BGS	2.335	0.898
BGN vs. SB	8.908	0.049
SML vs. SB	24.985	<0.001
BGS vs. SB	11.243	0.005
BGS vs. SML	13.742	<0.001

Source of variation between sites: d.f. = 3, SS = 9676.78, MS = 3225.593, F_(3,31) = 19.515, P < 0.001

Table 5: Nitrogen release rates (slopes), intercepts, and coefficients of determination from the linear regressions between N remaining and days over the phase of net N mineralization (60 days) among four sites in the Lower Laguna Madre, Texas.

	Slope ^z	Intercept ^y	r ²
Bahia Grande North	-1.00	131.96	0.79*
Bahia Grande South	-1.29	132.85	0.88*
South Bay	-0.83	133.41	0.74*
San Martin Lake	-1.97	136.63	0.96*

^z: N release rate of mg N/g of leaf litter remaining per day

^y: Initial mg N/g

*: significant at P<0.001

Table 6: Mangrove litter initial characteristics, in this and other decomposition studies.

Leaf Litter	C	N	C:N	δC^{13}	δN^{15}	Reference
<i>A. germinans</i>	46.5	1.69	27:1	-25.1	7.9	This study
<i>A. germinans</i>	45.5	1.82	25:1			(Twilley, 1982)
<i>Rhizophora sp.</i>	44.6	0.51	87:1	-28.7	5.6	(Fourqurean and Schrlau, 2003)
<i>A. marina</i>	44.8	2.37	19:1			(Dick and Streever, 2001)

Table 7: (A) Eigenvalues and percent explained variation for each principle component in the PCA analysis. (B) Eigenvectors showing the amount each variable accounted for in each principle component. Temperature = Temp, Chlorophyll 'a' = Chloro, total Kjehldahl Nitrogen = TKN, Dissolved Oxygen = DO.

(A)

PC	Eigenvalues	% Variation	Cum. % Variation
1	1.87	37.3	37.3
2	1.14	22.8	60.1
3	0.90	18.0	78.1
4	0.742	14.8	92.9
5	0.353	7.1	100

(B)

Variables	PC1	PC2	PC3	PC4	PC5
Total Kjehldahl N	0.530	0.461	-0.251	-0.228	0.626
Temperature	0.306	-0.416	-0.813	0.141	-0.228
Salinity	0.439	-0.358	0.383	0.670	0.290
Dissolved O ₂	-0.314	0.582	-0.288	0.692	-0.027
Chlorophyll 'a'	0.578	0.385	0.214	0.001	-0.689

Table 8: Means and Standard Errors of water column-parameters taken during duration of the study of 322 days. Bahia Grande North = BGN, Bahia Grande South = BGS, San Martin Lake = SML, South Bay = SB. (n=48)

Water parameters	BGS	BGN	SML	SB
Total Kjehldahl N (mg l ⁻¹)	0.9 (±0.37)	1.1 (±0.43)	0.8 (±0.22)	0.7 (±0.19)
Salinity (psu)	39.3 (±7.79)	45.1 (±7.65)	31.6 (±5.05)	35.0 (±6.43)
Temperature (°C)	25.7 (±4.60)	24.2 (±4.70)	26.3 (±3.55)	25.5 (±2.91)
Total organic carbon (mg l ⁻¹)	29.0 (±4.97)	31.2 (4.41)	29.6 (±5.40)	23.9 (±3.96)
Dissolved O ₂ (mg l ⁻¹)	6.5 (±0.83)	6.0 (±1.01)	6.4 (±1.12)	7.8 (±5.00)
Total suspended solids (mg l ⁻¹)	280.8 (±25.20)	283.3 (±23.54)	103.8 (±7.42)	98.5(±13.9 7)

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