Can ecosystem functional recovery be traced to decomposition and nitrogen dynamics in estuaries of the Lower Laguna Madre, Texas?

Running head: Tracing functional recovery in estuaries

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Author contributions

MAM, AFC, CECB conceived and designed the research; MAM performed the experiments; MAM, AFC, CECB analyzed the data; MAM, AFC, CECB wrote and edited the manuscript.

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Abstract

The biggest incentive to attempt the restoration and protection of estuaries is their widely acknowledged ecological and economic importance. Assessing estuary health and recovery can most accurately come from examining ecosystem processes. The purpose of this study was to explore the potential of mass loss and nitrogen (N) dynamics during leaf litter decomposition, to detect signs of functional recovery in two estuarine systems in south Texas. Submerged litterbags with black mangrove (*Avicennia germinans)* leaves were retrieved at various dates over 320 days. Decomposition was about 50% slower in one of the recovering systems compared to a reference site. Nitrogen immobilization and release from decaying leaf litter also discriminated among sites. Nitrogen immobilization potentials ranged from 4.15 to 6.89 mg N g^{-1} leaf litter, with the reference site exhibiting the highest value and thus the highest potential to conserve N during litter decomposition. The reference site also had a N immobilization time twice as long as the recovering sites, and a slower net release after the immobilization, appearing again as the most conservative in this part of the N cycling, possibly pointing to a less disturbed, or more stable ecosystem. Overall, N dynamics during decomposition of mangrove leaf litter were

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similar in both recovering sites, whereas the reference site had a more conservative nutrient dynamics with more N being retained for longer in decomposing litter, coupled with a slower net release. Metrics derived from N dynamics may provide a finer resolution assessment of functional recovery, than only decomposition metrics.

Keywords

Avicennia germinans, mangrove, restoration, stable isotopes, ecological indicators, leaf litter

Implications for practice

- Metrics derived from decomposition and concurrent N dynamics have the potential to be effective indicators of functional recovery in estuaries.
- Nitrogen immobilization potential, immobilization time, and release rates may be more accurate tracers of estuarine system recovery than mass loss rates.
- Decomposition and nutrient net immobilization and release are processes that can be quantified with relative ease and could be included in assessments of restoration efforts.
- Stable isotope analyses can detect more subtle differences in N dynamics during litter decay.

Introduction

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Estuarine systems are currently under intense anthropogenic pressure as human population density in coastal zones increases (Mouillot et al. 2006; Borja et al. 2010). Impairment of these systems can be caused by alterations of their hydrological and saline regimes (e.g. draining, filling, damming, and deviating), toxic and nutrient pollution, and devastation of aquatic and riparian vegetation (Borja et al. 2010). These stressors usually result in hypoxia, turbidity, excessive sedimentation, osmotic stress and/or simplified biotic communities and altered ecosystem processes including organic turnover and nutrient cycling (Adams 2005).

While restoration efforts are increasingly common, their success frequently remains unclear (Fairweather 1999; Vovides et al. 2011). Over the last two decades, significant assessment and monitoring efforts in estuarine and coastal marine systems have been based on community measures and indices derived from benthic invertebrate, fish, macrophyte, and planktonic assemblages (Gibson et al. 2000). Assessments of the ecosystem status of Bahia Grande (estuarine system under restoration, the focus of this study) have been attempted examining fish and macrobenthic assemblages, but high temporal variation was observed in these communities (Cornejo 2009; Tamez 2014), pointing at the need to complement these measurements with other ecological indicators.

Many taxonomic-based metrics and related community structural variables such as species richness, abundances, and derived evenness indices have been proposed as indicators of change for transitional water systems (Borja et al. 2000; Gibson et al. 2000; Rosenberg et al. 2004). However, there are several concerns for their use in these

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environments. It is recognized that estuarine biotic communities reflect high spatial and temporal variability as an adaptation to natural stress in a highly variable environment (Elliott & Quintino 2007). The ability to withstand natural stress also confers high tolerance to anthropogenic stress, thus such structural metrics alone are not sufficient to detect anthropogenically-induced change in estuaries. This has been named the "Estuarine Quality Paradox" (Elliott & Quintino 2007). In addition, taxonomic-based metrics present inherent difficulties for reliable multisite comparisons. Common concerns include underestimations of true richness, identification errors in sibling or cryptic species, and the frequent occurrence of highest diversity being observed under intermediate levels of disturbance (Mouillot et al. 2006).

It has been suggested that functional characteristics are potentially more adequate than community structural variables to detect anthropogenic stress in estuarine systems (Fairweather 1999; Elliott & Quintino 2007), as they are more robust than structural ones (Odum 1985), but some variability due to the abiotic environment can be expected. Ecosystem processes such as primary production, community respiration, nutrient transformations and litter decomposition respond to anthropogenic stress and can therefore be good indicators of functional impairment (Dangles et al. 2004). For example, litter decomposition rates can decrease when water acidity is a stressor, or increase under higher nutrient concentrations (Dangles et al. 2004; Niyogi et al. 2013), as detritivores and decomposer communities are affected. This is partially explained by decomposer

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bacterial biomass being directly correlated with decomposition rates (Rejmáková & Houdková 2006).

Measuring rates of leaf litter decay can be the basis for developing a diagnostic tool for assessing the functioning of an aquatic or terrestrial system (Gessner & Chauvet 2002; Niyogi et al. 2013; Silva-Junior et al. 2014). Decomposition patterns not always respond to availability of N and P from either endogenous or exogenous sources (Fierro et al. 2000). Litter quality, temperature, pH and oxygen availability also affect decomposition patterns (Melillo et al. 1984; Gessner & Chauvet 2002; Young et al. 2004; Niyogi et al. 2013). This approach for a functional indicator typically uses plant litter from an abundant producer in the system (Boulton & Boon 1991). In the case of estuaries, the plant litter to be considered could come from seagrasses, marsh plants or mangroves. Mangroves in southern Florida have a litter fall of 4 to 8 tons ha^{-1} yr^{-1} (Twilley et al. 1986), which represents a considerable source of organic matter in tropical and subtropical estuaries. Both litter mass loss and N dynamics in mangrove forests may be controlled by N availability, hydrology of the estuary, temperature and the amount of recalcitrant compounds of the litter (Keuskamp et al. 2015). Mangrove litter breakdown integrates nutrient recycling and energy transfer, which are key ecosystem processes in estuarine systems with mangrove stands (Twilley et al. 1986).

Nitrogen dynamics during decay of organic substances are dominated by the transformations between organic and inorganic forms. Immobilization and mineralization constitute these transformations in a delicate balance (Swift et al. 1979). Microbial

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immobilization of inorganic N (preferentially NH_4^+) supports decomposition (Joye & Anderson 2008), while net mineralization/release provides for most of the needs of primary producers (Parton et al. 2007). Nitrogen fluxes in marine ecosystems are frequently the largest between inorganic and organic pools (O'Neil & Capone 2008). Conditions that alter either of these transformations have a considerable effect on the N budget of the system (Boyer & Howart 2008). Immobilization and release of N from decomposing litter of Black (*Avicennia germinans*) and Red (*Rhizophora mangle*) mangrove were differentially affected by increased availability of N and P (Keuskamp et al. 2015). Most human-caused disturbances have the potential to alter N dynamics, especially so in estuaries and other ecosystems at the lower end of watersheds. Measures derived from these N transformations can thus be good indicators of functional degradation or recovery.

Determinations of stable isotope ratios for N ($^{15}N/^{14}N = ′ ^{15}N$) during litter decay have the potential to refine and complement concurrent determinations of the decomposition process, and confirm indications on ecosystem functioning. For example, N immobilization and the onset of N release can be directly assessed with $′ ^{15}N$ determinations, which also allow tracing N flow and sources within estuaries (Fourqurean & Schrlau 2003; Sharp 2006). Variation in $′ ^{15}N$ during decomposition can point at shifts in the source of N consumed by the microbial decomposer community from endogenous to exogenous (Machás et al. 2006).

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The use of indicators derived from decomposition processes (i.e., decay constants, recalcitrant fraction, final mass remaining, final/initial nutrient ratios), has been explored in lotic freshwater systems in recent years with encouraging results (Young et al. 2008; Niyogi et al. 2013; Silva-Junior et al. 2014). However, in estuarine systems functional indicator approaches are rare, despite the advantages they may have over structural measurements in a transitional environment context. The purpose of this study was to explore the potential of a suite of metrics derived from mass loss and N dynamics during litter decomposition, to detect signs of functional recovery in two estuarine systems in south Texas where their rehabilitation into functional systems is being attempted. The restored systems were compared to a reference site assumed to be in a good functional status with more efficient nutrient recycling and organic turnover. We hypothesize that the restored sites have functionally recovered and present similar decay constants, mean residence times, N immobilization potentials and times, as well as similar rates of N release compared to the reference site. Specific objectives include: 1) to determine decomposition patterns in both restoring and reference systems; 2) compare N content changes in decomposing Black mangrove leaves; and 3) estimate N immobilization/release during decay of mangrove leaf litter.

Methods

Study sites

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The present study took place in the Bahia Grande (BG) and South Bay (SB) estuaries, located along the Brownsville Ship Channel (BSC) at the southern tip of the Lower Laguna Madre (LLM; Fig. 1). The LLM is part of the largest hypersaline estuarine system in the world, with a climate in the interface of hot semiarid and humid subtropical (Koppen-Geiger classification Cfa).

One of the largest estuarine restoration efforts in the US is the BG Project in south Texas. In the 1930's, construction of the BSC, and later a state highway, cut off tidal exchange between the estuary and the LLM, converting it into a dust bowl for more than 70 years (Hiney 2002). Today, the estuary is in the process of being restored following its reinundation in 2005, when a narrow channel was dredged, flooding the basin and connecting it to the LLM and the Gulf of Mexico (U.S. Fish & Wildlife Service 2013). Bahia Grande is a 16.5 km^2 (Fig. 1), hypersaline shallow basin estuary. Its shorelines are still void of vegetation possibly due to wave erosion and hypersaline mist, as is the bottom of the basin with the exception of few small patches of Shoalgrass (*Halodule wrightii*) close to the mouth of the restoration channel. For the purposes of this study, the BG basin was considered as two distinct systems separated into north and south sections by an abandoned historical railroad trestle; which restricts tidal flows into the northern part. The north section or Bahia Grande North (BGN) is farthest from the connection with the ship channel, whereas the south section or Bahia Grande South (BGS) is directly connected through the restoration channel (Fig. 1). Thus, BGS benefits from a better tidal water exchange, comparable to the reference site (described below), which may favor the

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establishment of the Shoalgrass observed. Average water depths measured at the sampling stations are 56 and 64 cm for BGN and BGS, respectively. Anecdotal reports from residents indicate BG was a flourishing estuary before the construction of the ship channel and the highway. During the mid-1920s, approximately 10,000 gulls and terns were reported nesting on an island within the basin. Waterfowl were also abundant, especially Redhead Ducks, *Aythia americana*, for which the adjacent Redhead Ridge is named. Furthermore, Blue crabs *Callinectes sapidus*, flounder and shrimp were harvested in BG. Scattered pilings (remnants from the 1800s railroad) and fence posts across the basin show evidence of former thriving populations of barnacles and oysters.

South Bay is the southernmost estuary in the LLM encompassing 11.3 km^2. It is a shallow basin estuary (85 cm average depth at sampling stations) connected to the ship channel and the Gulf of Mexico through a relatively narrow channel (Fig. 1). South Bay is popular for recreational fishing, has extensive seagrass meadows and fringe Black mangrove stands (Britton & Morton 1989). This estuary is considered the reference system for the purpose of the study, and is the endpoint or goal of the restoration program in BG.

Water parameters

To better characterize study sites, water-column parameters were measured at each station at the time of litterbag deployment (day 0) and at every retrieval date (see next section). Parameters measured in situ included salinity (± 0.01 psu), temperature (± 0.03°C), and dissolved oxygen (± 0.02 mg l^{-1}), using multimetric sondes (YSI Pro2030 or

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Hach HQ40d); with few exceptions because of instrument malfunction while recording temperature and dissolved oxygen. In addition, and to reduce analytical costs, composite water samples combining the two closest stations were collected for analyses of total Kjehldahl Nitrogen (TKN) (SM 4500-NH_3D Ammonia-Selective Electrode Method; ± 0.038 mg NH_3-N l^{-1}). In several instances, TKN values were below reading limits (0.5 mg l^{-1}).

Decomposition assays

To compare decomposition patterns among the three study sites, 240 litterbags were deployed March 2012 and retrieved after 14, 30, 60, 190, and 320 days (± 2 days). Eight sampling stations within every site were selected using a computer random number generator that met the prerequisite of at least 50 cm mean water depth to ensure complete and constant submersion of all litterbags. Mature leaves of Black mangrove were used as the decomposition substrate, and were collected from a single local mangrove stand to minimize variability in the substrate (Boulton & Boon 1991; Young et al. 2004). After collection, fresh leaves were washed with deionized water to remove salts from their surface. The leaves were then dried at 55°C for 72 h or until constant mass, and thoroughly mixed to obtain a homogenous pool (Dick & Streever 2001). Litterbags (15 x 15 cm envelopes) were made of nylon mesh with a 1 mm mesh size to minimize loss of litter due to handling while allowing access to microbial decomposers (Fierro et al. 2000). Bags were filled with a known mass of dried leaves (10 g). Identification labels were placed in each bag, which were closed using rustproof staples (T50 monel). Ten

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additional subsamples of dried leaves were used to determine initial chemistry of leaf litter.

One 5 cm diameter x 300 cm long PVC pipe was driven into the sediment at each of the eight stations in all study sites. Ten litterbags were tied to each PVC stake suspended in the water column ca. 20 cm from the bottom. Two bags were collected randomly at each of the five retrieval dates; the purpose of a second bag was to cover for potential loss of a bag during the study, minimizing missing data. When both bags were recovered, their average was used as the experimental unit's value. The litterbag's exterior was carefully rinsed to remove sediment and biofouling. Litterbag contents were dried as before (72 h at 55°C). Litterbags were then opened and litter gently cleaned of all remaining biofouling material. Density fractionation using saline water (~ 60 psu) was done when needed to further separate the litter from heavier exogenous materials (e.g., amphipods, polychaetes, and barnacle shells). The remaining clean material was weighed to record the dry mass remaining, ground to a powder and homogenized using an analytical mill (IKA A11, Germany), 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 (6 h at 500°C) (Fierro et al. 2000).

Elemental and isotopic analyses

For total C and N content, subsamples of pulverized leaf litter were analyzed using an elemental analyzer (Costech ECS 4010, Valencia, CA). Standard blank assessment and

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correction procedures were applied. Standardization was based on atropine for elemental concentration: C = 70.56%, and N = 4.84%.

Stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of ground subsamples of leaf materials were analyzed at the Stable Isotope Laboratory at The University of Texas Marine Science Institute, with a continuous-flow gas-ratio mass spectrometer (FinniganMat Delta Plus, Waltham, CA) coupled to an element analyzer (Carlos Erba NC 2500). Standard blank assessment and correction procedures were applied. Standardization is based on casein for elemental concentration: $\delta^{13}C$ = - 26.95 and 46.5%, and $\delta^{15}N$ = 5.94 and 13.32%. Isotopic composition was expressed in permil (‰). Precision was better than ± 0.1 for $\delta^{13}C$ and ± 0.2 for $\delta^{15}N$, based on repeated internal standards. Deviations of sample materials were derived from atmospheric nitrogen (AIR, N_2) and Pee Dee belemnite (PDB, C) international standards. The isotopic ratios are reported using the equations described in Appendix S1. Results from $\delta^{13}C$ and total C determinations are not reported due to the presence of calcium carbonates from sediment infiltration into the bags.

Data analyses

Site characterization was based on water parameters with salinity and TKN data being analyzed with a one-way repeated measures ANOVA, and a post hoc (Tukey test, Tukey hereafter) all pairwise comparison procedure when significant differences were detected. Water temperature and dissolved oxygen were not recorded in two out of the five

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retrieval dates because of the aforementioned instrument malfunctioning, thus only descriptive statistics (mean $\pm$ SE) were used.

Decomposition rates and decay constants were obtained by fitting a single-component exponential model (SigmaPlot v12) to time series data. Mean residence time (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. Half-life (T_{50}), or the time required for 50% of leaf litter to decompose from the deployment date, was calculated using the equation described in Appendix S1.

Differences in mass remaining (AFDW) among sites were analyzed using a generalized linear mixed model due the unbalanced design resulting from loss of two litter bags at the last retrieval. A post hoc (Tukey) all pairwise comparison procedure was used to compare sites. A natural log transformation was used to linearize AFDW data as an attempt to correct for heteroscedasticity (Sokal & Röhlf 1995), which was not achieved. The mixed model analysis was still performed with untransformed data because it is robust enough to allow for deviations from homoscedasticity (Underwood 1997).

Metrics describing N dynamics were derived from the regression equation relating mass remaining and N concentration based on the approach of Aber & Melillo (1982) (Appendix S1). This well established inverse linear relationship reflects faster C loss than N loss, as well as some N immobilization (Melillo et al. 1984).

Nitrogen net release rates following the immobilization phase were calculated using the slope from the regression equation of the inverse linear relationship between N

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remaining in decomposing litter (% of initial amount) and days from deployment (Fierro et al. 2000).

Differences in the absolute amount of N (mg N g^{-1} litter) and N remaining among sites were analyzed for the initial 60 d, when decomposing leaf litter had reached their half-life in all sites. A one-way repeated measures ANOVA with sites as the fixed effect was used for this analysis, followed by a post hoc (Tukey) all pairwise comparison procedure. The same statistical approach was used for the $\delta^{15}N$ data to assess differences among sites. The last retrieval date was not included in the $\delta^{15}N$ analysis because very little organic material was left, and this recalcitrant material is presumed very stable with minimal N dynamics (Swift et al. 1979) compared to actively decomposing material, thus not justifying additional analytical costs for those samples. The disturbance antecedents and geographic context of this study did not allow for replication at the site level (restored, reference). The eight sampling stations within each site were used as replicates. Thus, conclusions based on our findings for sites outside the geographic area of this study should be used with caution.

Results

Water parameters

Salinity ranged from 29 to 66 psu and was significantly different among the three sites (Fig. 2A). Throughout the study, it was considerably higher at BGN ($p < 0.001$) where continuous hypersaline conditions were observed (Fig. 2A). The lowest salinities were

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recorded at the reference site with BGS averaging intermediate values. Water temperature had a similar pattern among sites over the study period, showing an average difference of about 10°C between summer and winter seasons (Fig. 2B). Water temperature recorded at retrieval dates ranged from 16 to 31°C. Nitrogen availability measured as TKN was low to moderate ranging from less than 0.5 to 2.4 mg l^{-1}. Concentrations were significantly different among sites (Fig. 2C) with the reference site presenting the lowest concentrations of this nutrient as per Tukey. Nitrogen concentrations in both restored sites were higher but not significantly different, presenting a comparable pattern. No anoxic conditions were detected during the study period in any of the sites, as expected for these shallow and relatively windy estuarine basins. Dissolved oxygen readings at retrieval dates ranged from 4 to 11 mg l^{-1} (Fig. 2D).

Decomposition patterns

Black mangrove leaf litter decomposed rapidly in these estuaries. Approximately half of the initial mass was lost within the first 50 d. In situ decomposition pattern of mangrove leaf litter was well described by the single exponential model (Fig. 3). For the duration of the study (320 d), decay patterns were significantly different among sites ($F_{0.05\,(2,\,123)} = 34.33$, $p < 0.0001$). The slowest decay rate and highest mass remaining was observed at BGN as per Tukey, while BGS and SB (reference site) had rates about 45% faster (Table 1). The MRT of leaf litter in BGN was 103 d, compared to the 71 d observed for decomposing litter in both BGS and SB (Table 1). Litter half-lives ranged

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from 49.2 d in the reference site to 71.5 d in BGN (Table 1). Mangrove leaf litter decomposed in a nearly identical pattern in both SB and BGS (Fig. 3).

Nitrogen immobilization

As leaf litter decomposed, the concentration of N in the remaining material increased (Fig. 4). This inverse linear function between mass loss and N concentration in the remaining leaf litter allowed the determination of N immobilization parameters summarized in Table 2. Leaf litter appeared to immobilize exogenous N for a brief period of time (4 to 7 d) in all sites. Net accumulation of N occurred during the first week after deployment of litterbags, and was followed by a net release of N. The immobilization potential (N_{max}), or maximum amount of exogenous N accumulated per gram of leaf litter (initial material) before net release began, ranged from 4.15 to 6.89 mg N g^{-1} leaf litter. Although leaf litter decomposed at the same rate in BGS and SB, its concurrent N dynamics differed. The reference site (SB) had an N_{max} about 60% higher and an immobilization time about two times longer than both restored sites (Table 2). The onset of N release (at N_{max}) occurred when leaf litter remaining was still relatively high, above 90% of original mass in all sites.

Nitrogen release

Net release of N began in the fourth day after deployment for litter decomposing in the restored sites, and in the seventh day in the reference site (Table 2). Nitrogen release rates were estimated during 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

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immobilization) was described by the inverse linear relationship between N remaining in the litter and time. Nitrogen release rates averaged 0.007 d^{-1} among all three sites. During the net release phase, remaining N in the leaf litter was significantly different among sites ($F_{(2, 29)} = 25.94$, $p < 0.001$), being highest at the reference site (Tukey), followed by BGS and then BGN. The fastest rate of N release happened in BGS while leaf litter in SB had the slowest release rate (Table 2).

Isotopic ratios

Overall, there was a decrease in $\delta^{15}N$ during leaf litter decomposition (Fig. 5), which had an initial value of 7.9‰ (Table 3). During the initial 60 d, $\delta^{15}N$ changes were consistent with less than a per mil difference. Slight increases in $\delta^{15}N$ occurred at 14 and 60 d post deployment, followed by a decrease to near initial values in the two restored sites, but experiencing a further ~ 1.0‰ decrease in the reference site (Fig. 5). These trends reflect the overall observed pattern for net accumulation of N followed by net release. Over the course of the incubation, leaf litter $\delta^{15}N$ ratios for BGN and BGS fell about 1.0‰ (final values of 7.12 ± 0.16‰ and 6.11 ± 0.20‰, respectively), whereas leaf litter in SB was most depleted with $\delta^{15}N$ dropping 3‰ (final value of 4.92 ± 0.47‰). Between the last two dates, depletion of $\delta^{15}N$ was more pronounced (~3‰) in SB and BGS, and less pronounced in BGN. The differences among sites were significant ($F_{0.05\,(2,\,39)} = 12.8$, $p = 0.001$), separating SB from the other sites as per Tukey, and resulting in a consistently lower isotopic signal compared to the restored sites.

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Discussion

Decomposition patterns and nitrogen dynamics

In this study we documented organic turnover in subtropical estuarine systems, a pivotal ecosystem process in carbon and nutrient cycling (Swift et al. 1979). Mangroves and other wetlands that maintain high primary production, generate large amounts of plant tissues that largely enter the detrital pathways (Rejmáková & Houdková 2006). Decomposition and nutrient mineralization are thus critical processes for sustaining foodwebs in these systems. Overall, measured leaf litter mass loss rates were close to those reported in comparable estuarine systems. Black mangrove leaf decay constants determined in this study, were within the ranges (0.0025 to 0.0155 d^{-1}) reported for estuaries in southern Florida (Twilley 1982; Fourqurean & Schrlau 2003), and were generally greater than reported for other mangrove species like *Rizophora mangle* with higher initial C:N ratios but comparable to leaves of emergent and floating estuarine macrophytes (Nielson et al. 2004). Decay of Black mangrove leaves was relatively fast in the reference site and BGS, with half-lives comparable to the 44 d half-life of similar leaf litter decomposing in Florida estuaries (Twilley 1982).

Considerable differences were observed in MRT among study sites. Mean residence time in BGN was the longest, reflecting a slower decomposition rate, possibly due to restricted tidal water flow and overall higher salinities, which may have hindered decomposer communities (Gessner & Chauvet 2002). The main factors controlling decomposition rates and concurrent nutrient dynamics in aquatic systems are litter

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quality, temperature, dissolved oxygen, hydrology of the system, and nutrient availability, mainly N and sometimes P (Melillo et al. 1984; Gessner & Chauvet 2002; Young et al. 2004; Keuskamp et al. 2015). In this study, a homogenous pool of Black mangrove leaves was used as the decomposition substrate at all sites, thus litter quality was the same. Water temperature was similar among sites, and levels of dissolved oxygen were adequate to sustain aquatic life including aerobic decomposer organisms. Nitrogen availability was slightly higher in BGN, and a faster decay rate was expected instead of the observed slowest rate. The continuous hypersaline conditions observed in BGN may have partially accounted for the slower decomposition process. Higher salinity at BGN is the consequence of longer water residence times as it is farthest from the channel connecting to the Laguna Madre. Also, its extension and shallowness promote intense evaporation under the local warm and windy conditions. The effects of salinity increases in soil have resulted in reduced decomposition and mineralization processes due to a less efficient microbial community under increased osmotic pressure (Wichern et al. 2006). More restricted tidal flows and water movement in BGN could have also translated in reduced mass loss through leaching when compared to BGS and the reference site, which have a direct connection with the LLM. Further experiments, preferably under controlled conditions (i.e., microcosm), might be necessary to determine the causal factors of the observed differences among sites in mass loss and also in N dynamics as discussed below.

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Determinations of N immobilization and release may allow a finer assessment of litter decomposition process in estuarine systems (Nielson et al. 2004). Values of N immobilization presented here were derived from the inverse linear function between mass loss and N concentrations in the remaining leaf litter; a pattern consistently observed in both terrestrial and aquatic systems (Aber & Melillo 1982; Melillo et al. 1984; Fierro et al. 2000), and considered to be an ecosystem characteristic (Seastedt et al. 1992). Results of this study suggest that N dynamics in decaying leaf litter presented a two-phase pattern of a brief net immobilization followed by net release. The short immobilization time (4 to 7 d) can be explained by the relatively high quality of Black mangrove leaf litter compared to substrates with higher C:N ratios presenting longer immobilization times (Twilley et al. 1986; Fierro et al. 2000). Black mangrove leaf litter has higher initial N content than other studied leaf litters (Melillo et al. 1984; Seastedt et al. 1992; Fourqurean & Schrlau 2003; Osono et al. 2006). Critical C:N ratios for N mineralization in aquatic systems have been estimated at 16 to 30:1 (Twilley 1982). Leaf litter used in this study had an initial C:N ratio of 27:1, within the range for rapid onset of N mineralization (Russell-Hunter 1970). The relatively high immobilization potentials and early onset of N release obtained in this study, are consistent with the microbial ecology hypothesis which states that easily decomposable substrates tend to immobilize more N than substrates with larger contents of recalcitrant compounds such as lignin (Keuskamp et al. 2015).

Tracers of functional recovery

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Both restored sites (BGN and BGS) were inundated at the same time as an attempt to reconstruct the estuarine systems that once existed. The southern section (BGS) is directly connected to the restoration channel, and thus it benefits from a comparable tidal influence and water quality as the reference site. Therefore, it may be reasonable to anticipate that BGS could recover faster and better than BGN. Other studies in these estuaries have showed trends in this direction. For example, logistic growth constants of *Balanus eburneus* were consistently similar in BGS and the reference site, but different from BGN (Martinez 2015). Salinity and water clarity, affected by tidal flow, were potential drivers of this difference, as well as the relative abundance of food sources for these sessile organisms.

Metrics derived from decomposition of leaf litter appear to discriminate among study sites. They indicate that after eight years of the inundation, this critical ecosystem process in BGS was much more similar to the reference site than to BGN which is farther from the restoration channel. It also suggests that BGN may require additional intervention (i.e., widening of existing channel, and/or opening other channels in the northern section) to fully restore ecosystem function through an amelioration of its hydrology and the saline regime. Similarly, rates of leaf litter breakdown in streams affected by acidification in northeastern France and in New Zealand clearly discriminated sites (Dangles et al. 2004; Niyogi et al. 2013). Along with leaf decomposition, other ecosystem processes, like community respiration and primary productivity, were also affected by different land uses of the riparian zone in tropical streams (Silva-Junior et al. 2014).

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Leaf litter decomposing in SB had the highest immobilization potential which persisted for the longest time, suggesting that a more conservative N cycling characterizes the reference site. Immobilization of exogenous N in decomposing litter of *Spartina alterniflora*, is a significant mechanism for conserving N in salt marshes (Hopkinson & Giblin 2008). The immobilization potentials in both recovering sites were considerably lower than observed at the reference site, with a much shorter immobilization time.

Release rates of N from decaying leaf litter were obtained using data from the initial 60 d post deployment, which corresponds to the most intensive and dynamic part of the decomposition process. Net release of N was more intense in BGS, followed by BGN, whereas the slowest N release was observed in the reference site. Higher N mineralization rates have been observed at increased salinities but only up to moderate levels (i.e., seawater level) (Chen & Twilley 1999; Gao et al. 2014). At higher salinities, net N mineralized declines as the microbial-mediated ammonification and nitrification are inhibited (Pathak & Rao 1998). Thus, it is possible that N release was differentially enhanced by the higher salinities in both restoring sites compared to the reference site, but not as intense under the highly hypersaline conditions in BGN. These observations point at the need for further research, perhaps under controlled conditions, on the effect of salinity and other water column variables on litter mass loss and N dynamics. Overall, N dynamics during decomposition of mangrove leaf litter were similar in both sections of the restoring BG system. On the other hand, the reference site may have more nutrient

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conservative dynamics with more N being retained in decomposing litter and for longer, coupled with a slower net release. Interestingly, variables of N dynamics did discriminate the reference site from BGS, whereas mass loss did not. Metrics derived from N dynamics may thus provide a finer assessment of functional recovery, than only using mass loss metrics. Other N transformations occurring in estuaries also have the potential for development of functional indicators. For example, in estuaries of northwestern Mexico, fixation of atmospheric N in sediments was clearly reduced in impaired mangroves compared to preserved and restored mangroves, possibly indicating functional recovery (Vovides et al. 2011).

Determinations of decomposition rates and concurrent N transformation rates can be effectively complemented by measures of stable N and C isotope ratios to provide a clearer picture of ecosystem function. For instance stable N isotope ratios can be used to confirm that N is being incorporated into decomposing material from surrounding media (e.g., soil, water or sediments), therefore more accurately assessing N immobilization (Zieman et al. 1984). In this study, δ^{15}N ratios of decaying leaf litter confirmed the observed decreasing pattern of elemental N concentrations over time. In all sites, small (~ 10) fluxes in δ^{15}N ratios within the first two months of decomposition were observed. A release of N was detected by day 14, followed by immobilization and a second release around day 60. These small fluxes were not detected with elemental N concentrations, but have been observed during the decomposition of *R. mangle* and seagrass tissues using isotopic ratios (Fourqurean & Schrlau 2003), and could be indicative of intense N

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dynamics with several short episodes of net immobilization and release beyond the initial week after deployment. The isotopic signal in the reference site was consistently lower than the restored sites and may be reflecting competition for N by the extensive seagrass beds present there and not in the restored sites. It has been reported that N in leaf tissue of Turtle grass (*Thalassia testudinum*) increases as it consumes N from its environment (Sánchez et al. 2013). Furthermore, *Thalassia hemprichii*, *Halodule uninervis*, and *Cymodocea rotundata* efficiently used N released from litterbags in their surroundings (Vonk & Stapel 2008). Interestingly, for the last two dates depletion of the isotopic signal was more pronounced in the reference site and BGS (~3‰) compared to BGN, suggesting consumption of ^{15}N enriched material by the microbial community possibly due to diminished resources available to decomposers. This possibility is supported by the faster decay process and less litter mass remaining in these two sites (SB and BGS).

According to Vonk & Stapel (2008) main processes responsible for changes in $'^{15}N$ during litter decomposition include: 1) input of non-litter N (i.e., microbial immobilization), 2) isotopic fractionation (by physicochemical processes), 3) consumption of ^{15}N enriched material by the microbial community, and 4) unequal labeling of the leaves or components (i.e., litter from various sources). In the context of the present study, the latter process can be discarded given that all *Avicennia germinans* litter was from a homogeneous pool and no N was added. During long-term decay, the microbial community is known to switch to the exogenous use of N (Machás et al. 2006), therefore an input of non-litter N (immobilization), along with consumption of N

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enriched material may have accounted for the 3‰ drop observed in SB and BGS. The use of N isotopic ratios appears to support the site discriminations pointed by mass loss and the other N dynamics metrics, and provided additional evidence of the accumulation and release of N beyond the first week after deployment suggesting the potential use of $\delta^{15}N$ to enhance the monitoring of ecosystem functioning in estuaries.

To the extent of our knowledge, this study is the first to explore the potential of using both mass loss and concurrent N dynamics in decomposing leaf litter, as functional indicators in estuarine systems. These processes were studied using Black mangrove leaf litter, and obtained metrics did discriminate sites with different disturbance histories and different stages of recovery. These processes are linked to the activity of the decomposer community, and they should be analyzed complementary to further assess recovery of ecosystem function. Leaf litter mass loss and nutrient net immobilization and release can be quantified with relative ease using the same samples. The ranking of the studied sites based on decomposition patterns did not precisely correspond to the ranking obtained from the variables of N dynamics. For example, while SB and BGS displayed identical decomposition patterns, their N dynamics were different. Although both restored sites (BGN and BGS) displayed significant differences in decomposition rates, they had less conservative N dynamics than the reference site. Metrics from mass loss (i.e., decay constants, half-lives or MRTs) have been proposed as reliable functional indicators (Niyogi et al. 2013; Silva-Junior et al. 2014) however, metrics from N dynamics (i.e., N_{max}, immobilization time and release rates) may be more accurate tracers of functional

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recovery. Results from this study should motivate experiments under controlled conditions, as well as larger scale studies involving more estuarine systems with replications of both restoring and undisturbed sites, in order to calibrate and validate these potential indicators. Isotopic measurements in decomposing leaf litter confirmed the observed trends in N dynamics, and provided additional details. Once calibrated and validated, the indicators of functional recovery suggested here have the potential to become a component of monitoring and assessment efforts in estuarine systems, complementing standard structural metrics.

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Tables

Table 1: Decomposition values derived from the single-component model, fitted to data from eight sampling stations at every site throughout entire study (320 d). Decay constant = k; standard errors = SE; ¤$_{50}$ = half-life; mean residence time = MRT; * = significant at $p < 0.01$.

Study site	k (d^{-1}, ± SE)	r^2	¤$_{50}$ (d)	MRT (d)
Bahia Grande North	0.0097 ± 0.0004	0.98*	71.5	103
Bahia Grande South	0.0141 ± 0.0006	0.98*	49.2	71
South Bay	0.0141 ± 0.0004	0.99*	49.2	71

Table 2: Parameters of N dynamics during the decomposition of Black mangrove (*Avicennia germinans*) leaf litter. Metrics for N immobilization were derived from inverse linear functions relating mass loss and N concentration in the remaining material. Release rates were derived from inverse linear functions relating N remaining (% of initial amount) and time. Bahia Grande North = BGN, Bahia Grande South = BGS. Nitrogen immobilization potential = N_{max}; ash free dry weight =AFDW.

Study site	N_{max} (mg N g^{-1} initial litter)	AFDW at N_{max} (%)	N Immobilization time (d)	N release rate (d^{-1})

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BGN	4.38	96	3.7	0.0067
BGS	4.15	95	3.6	0.0083
South Bay	6.89	91	6.6	0.0060

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Table 3: Mangrove litter initial characteristics, in this and other decomposition studies.

Leaf litter	**C** (mg/g)	**N** (mg/g)	**C:N**	**´ N^{15}** (‰)	**Reference**
Avicennia germinans	46.5	1.69	27:1	7.9	This study
A. germinans	45.5	1.82	25:1		(Twilley 1982)
Rhizophora mangle	44.6	0.51	87:1	5.6	(Fourqurean & Schrlau 2003)
A. marina	44.8	2.37	19:1		(Dick & Streever 2001)

Figures

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Figure 1: Locations of study sites Bahia Grande North (BGN), Bahia Grande (BGS), South Bay (SB). 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]

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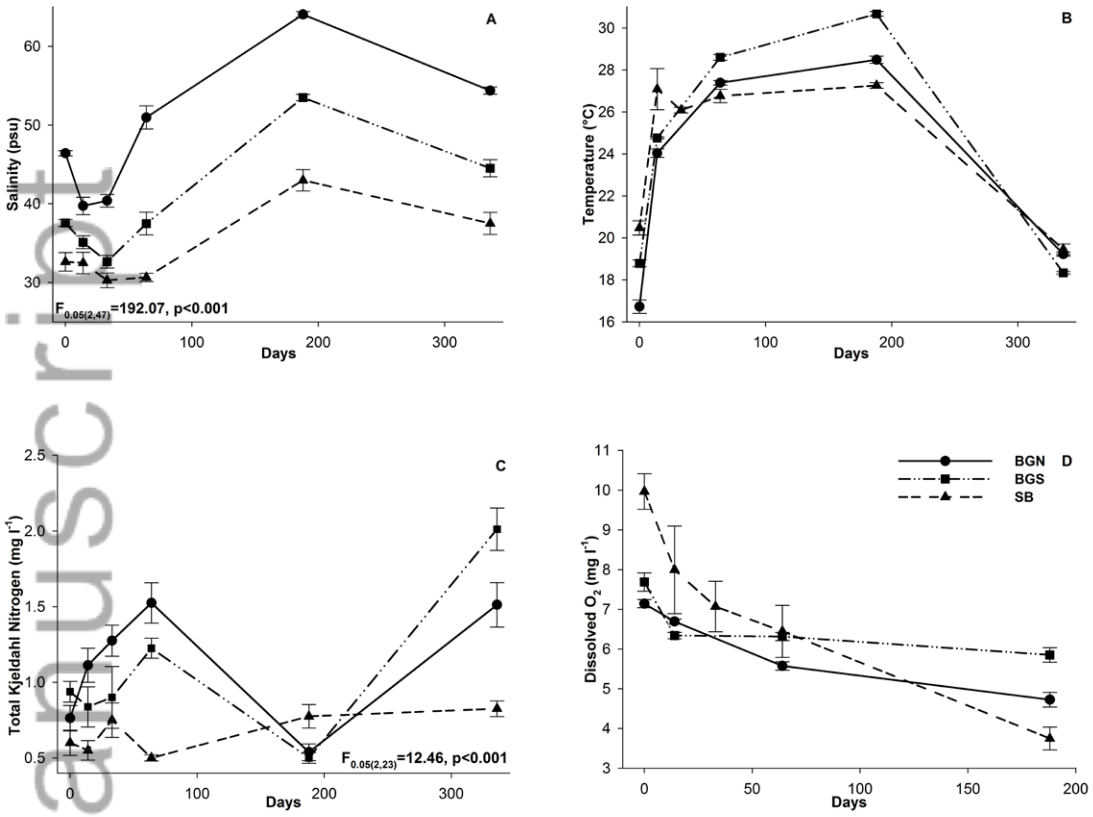

Figure 2: Water column physicochemical parameters of the three sites recorded during the decomposition study of Black mangrove (*Avicennia germinans*) in the Lower Laguna Madre, Texas. A) Salinity, B) temperature, C) total Kjeldahl nitrogen, D) dissolved oxygen. Symbols indicate mean values (± SE) (n = 8 per site per date). Bahia Grande North = BGN, Bahia Grande South = BGS, South Bay = SB. Temperature and dissolved oxygen were not recorded at every date in all sites (see text for details).

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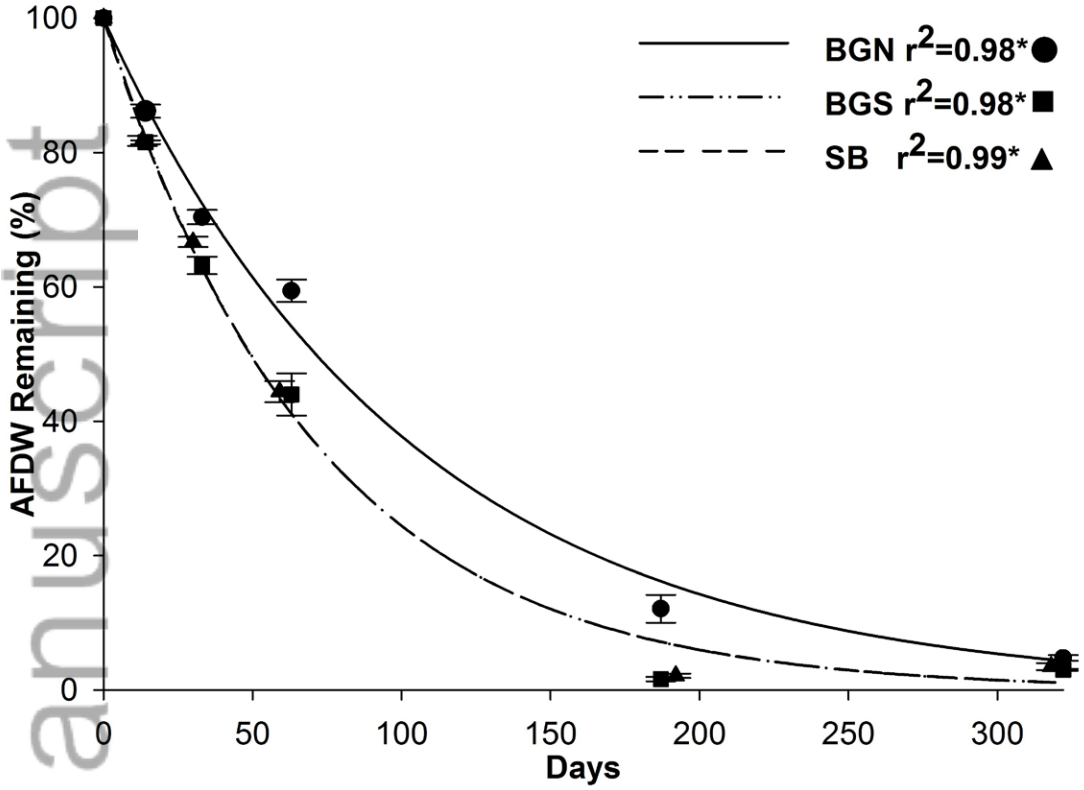

Figure 3: Mass remaining expressed as percent ash-free dry weight (AFDW) of *Avicennia germinans* leaf litter in three study sites in the Lower Laguna Madre, Texas over a 320 d period. Lines are the best fit for data from eight sampling stations at every site. Symbols indicate mean values (± SE). Abbreviations and sample size as in Fig. 2. Only two lines are visible because SB and BGS overlap. * = $p < 0.001$.

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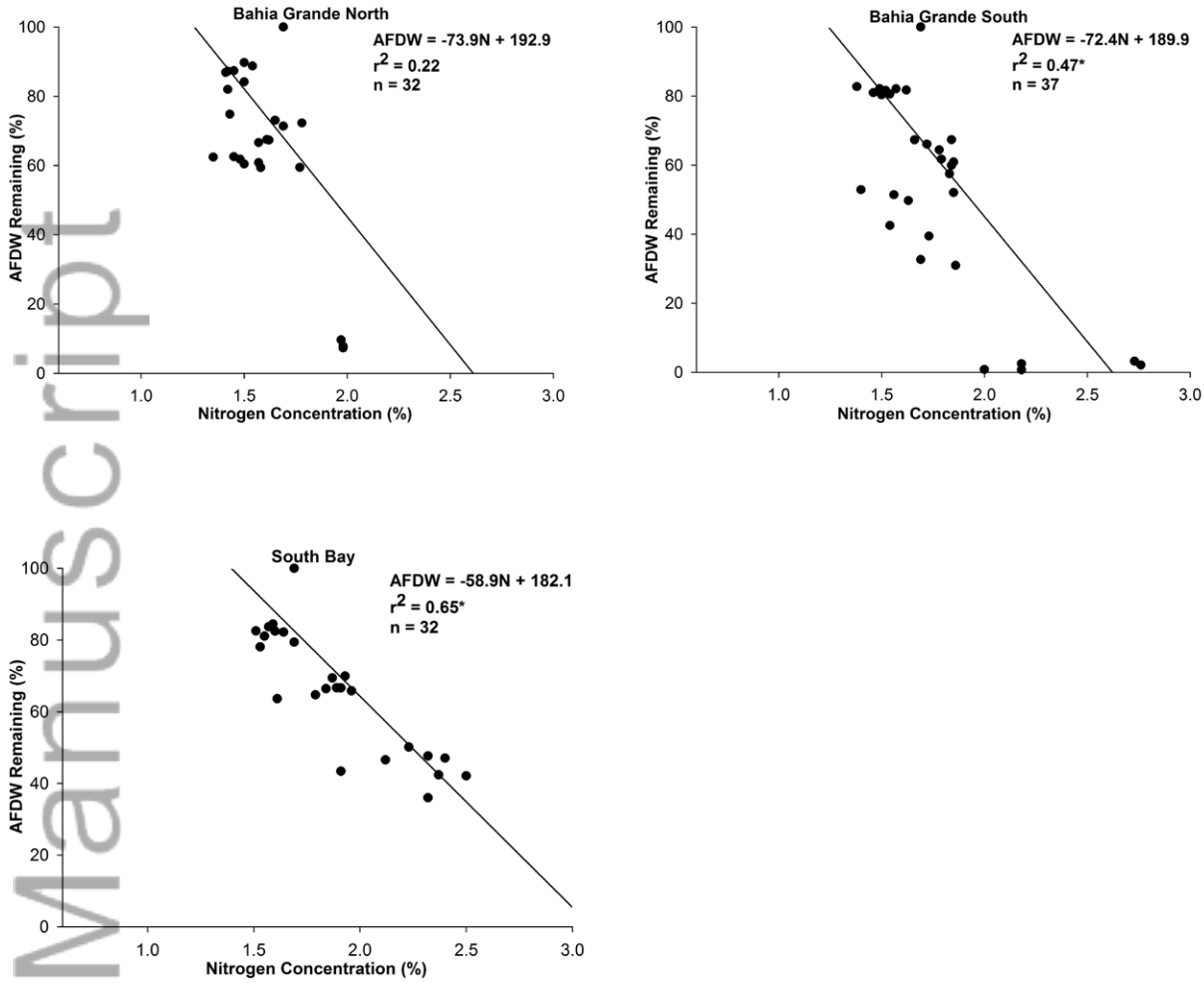

Figure 4: Original mass remaining of Black mangrove (*Avicennia germinans*) leaf litter expressed as percent ash-free dry weight (AFDW), as a function of N concentration (%) in remaining material, in three estuaries linked to the Lower Laguna Madre, Texas. Some values overlap thus not all points are visible; * = $p < 0.001$.

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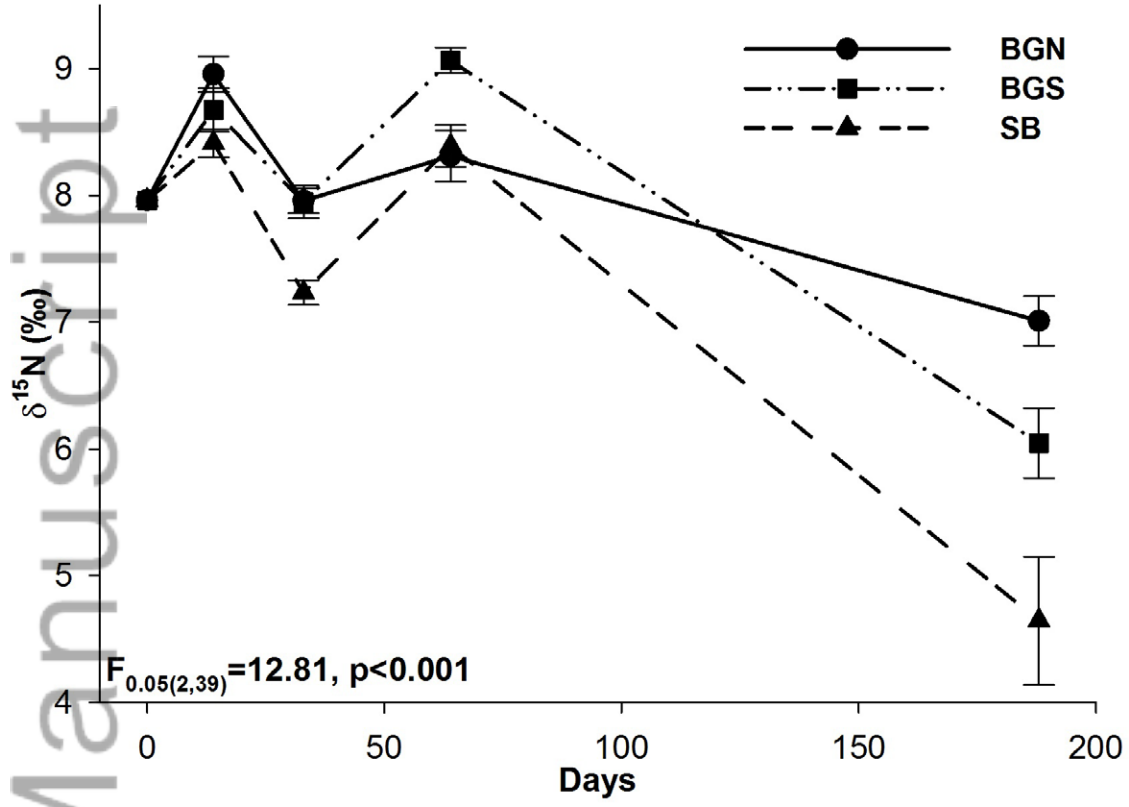

Figure 5: Changes in ´ ^{15}N (mean values ± SE) over about 190 d in decomposing leaf litter of *Avicennia germinans* in three study sites in the Lower Laguna Madre, Texas. Bahia Grande South = BGS, Bahia Grande North = BGN, South Bay = SB, n = 8 samples/site/retrieval date.

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