Mangroves as Indicators of Nutrient Pollution in Guam

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Abstract

On the U.S. territory Guam, a small island in the Pacific, there are major concerns about local wastewater treatment. The island's population is expected to grow rapidly by more than 10% by 2014 due to an increase in military presence, which raises concerns about the infrastructure's ability to keep up with new demands. Most of the island runs on poorly mainly sewage plants or septic systems, which leads to nutrient pollution in the coastal sea. Mangroves, flowering trees that live along ocean coasts, absorb nutrients from run-off before it enters the sea. Nitrogen table isotope analysis is a useful technique for determining where nutrient pollution comes from. This study attempts to determine if mangroves effectively record differences in nitrogen pollution across different sites, and to use that data to identify sewage hotspots around Guam's coast. Nitrogen stable isotope analysis of *Rhizophora stylosa* and *Bruguiera gymnorrhiza* mangrove leaves indicate that *R.stylosa* is an ideal species in pollution studies. Data also show significant differences in nitrogen pollution levels around Guam, with the highest level of pollution in the waters around Merizo, a town on the southern end of the island with a stream that likely drains nearby septic systems.

Introduction

Guam

Guam is a U.S. territory located between Australia and Japan, at the southern end of the Mariana Islands (Figure 1). It is home to U.S. naval and air force bases. It has approximately three times the area of Washington, DC., and with more than 183,000 people, its population is slightly more dense than that of D.C. More than 60% of the population consists of indigenous Chamorros or Filipinos (CIA 2011). The U.S. military bases, followed by tourism, constitute most of the economy on Guam. In the next few years, the military intends to relocate almost 20,000 troops from Okinawa to Guam by 2014 in an effort to strengthen relations between the U.S. And Japan (Kan 2011).

Figure 1: Map of Guam

Wastewater Treatment Concerns

A sudden population increase of over 10% during this relocation raises concerns about Guam's ability to keep up with infrastructure demands. Already, 2 of the 7 water treatment plants in Guam, which handle approximately 60% of the wastewater, do not operate in concordance with the Clean

Water Act. The other 40% of the island operate on septic systems, individual primary sewage treatment structures which allow solid wastes to accumulate within an underground tank before liquid waste is leached into surrounding drainage fields. These systems are often poorly maintained and prone to leaking when subjected to frequent heavy rainfall. As of July 2011, multiple beaches in 8 regions on Guam (including a collection site in this study) were closed to public use due to unsafe levels of infectious bacteria in the water (Guam EPA 2011).

The addition of military troops on Guam provides an opportunity to study the changes in the coastal ecosystem as pressure on wastewater treatment increases. This contributes to the long-term monitoring of wastewater on Guam. Despite governmental concern about the coastal water quality on Guam, funding and staffing limitations prevent effective monitoring from being completed. In addition, this study also provides an opportunity to attempt to use mangrove trees to document changes in nutrient pollution. This use of mangroves has been little, if at all, documented.

Mangroves

Mangroves are terrestrial flowering plants (Angiosperms) that thrive in salinities unlike most other terrestrial plants. Mangroves constitute a diverse group of plants comprised of over 70 species of small shrubs to palms, and can be found along protected tropical shores (Alongi 2002).

In general, mangrove forests serve many ecologically and economically important functions. They serve as nursery habitats for commercially harvested species such as a the spiny lobster, blue crabs, mullet, and other reef fish species. Many endangered species also find shelter amount mangrove forests, such as the Ridley sea turtle and the American crocodile (Nybakken 2005). Mangroves serve as barriers against erosion, flooding, storm surges, and tsunamis, because of their ability to reduce wave energy and increase sedimentation rates. Mangrove forests are large carbon sinks, and their destruction might exacerbate global warming by increasing atmospheric carbon dioxide. Historically, the greatest threats to mangroves were filling of the forests for development and conversion into aquaculture habitats. These stressors may not be as impacting as climate change effects, such as sea-level rise, an increase in extreme high water events, increased intensity and frequency of tropical storms, changes in global precipitation patterns, changes in temperatures, and increased atmospheric CO_2 (Gilman 2008).

In this study, two species of mangrove trees were studied, *Rhizophora stylosa* and *Bruguiera gymnorrhiza* (Lovelock 1993). These trees have characteristic aerial prop root systems that form above-ground entanglements that are inundated or exposed as the tides move in and out. In addition, they have pneumatophores, which are root extensions that re-emerge from the ground. These adaptations al-

Figures 2a and 2b: Mangroves in Guam with prop roots (left) and pneumatophores (right) visible low the trees to thrive in an anoxic sediment. In addition, these extensive above-ground root systems decrease water movement, leading to accumulation of sediment around the roots (Nybakken 2005). The biomass of mangrove roots is roughly equal to the above-ground tree biomass (Alongi 2002). Mangroves can also survive in saline waters because of various adaptations to reducing their internal salinity. Among these adaptations are salt excretion glands, fresh water storage, and the ability to extract fresh water from salt water without absorbing salt. In addition to absorbing water, mangroves tend to act as biochemical filters of runoff, making them ideal organisms for studying terrestrial pollution inputs (Nybakken 2005).

Mangrove forests, or mangals, often exhibit zonation, or bands of specific species from the sea moving inland. Typically, *B. gymnorrhiza* grows more inland than does *R. stylosa*. In locations with extensive mangrove forests, there is ample space for mangroves to expand and exhibit zonation (Nybakken 2005). In contrast to the normal, distinct zonation patterns in mangrove forests, mangroves on Guam can only be found in a few locations on the island, restricting their ability to exhibit strong zonation patterns. Mangrove forests are found on the southern tip and western coast of the island, two locations likely to have different sewage inputs. Because runoff and sewage on Guam have little treatment before entering the coastal ocean, mangroves effectively absorb this pollution, leading to records of differing levels of pollution around the island. Although they are sparsely distributed, they are the first marine ecosystem exposed to runoff as it enters the ocean. Combined with pollution data recorded in sea grass beds and corals (work that has also been conducted by students at AU), these data may construct a comprehensive analysis of nutrient pollution on Guam.

Nitrogen Stable Isotope Analysis & Nutrient Pollution

Nutrient pollution in the context of this study refers to an input of excess nitrogen into a system. In general nitrogen pollution can come from two primary sources, artificial fertilizer in agriculture or from human waste in sewage. Stable isotope analysis is a useful technique for determining where nitrogen pollution comes from. Nitrogen exists in two stable isotopic forms, ^{14}N and ^{15}N. These means that these two types of nitrogen have a different number of neutrons, the non-reactive elements of an atom. The ratio of the heavier nitrogen to the lighter nitrogen, ^{15}N:^{14}N, can be used as a fingerprint for the source of pollution. The notation for these measurements is $\delta^{15}N$, and the units for this measurement is per mil, or ‰. This measurement is similar to a percent, but measures the amount of the ^{15}N isotope per thousand nitrogen atoms because the heavier isotope is more rare than the lighter isotope. The $\delta^{15}N$ value of atmospheric nitrogen, or N_2 gas, is about 0‰. This does not mean that there is no ^{15}N in nitrogen gas because $\delta^{15}N$ values are measures of the amount of heavy nitrogen in a sample relative to some standard. Since atmospheric N_2 is used as the standard, its $\delta^{15}N$ is set to zero. As nitrogen moves through a food chain, consumers tend to be more enriched, or have a higher $\delta^{15}N$ ratio, than their prey. For example, the $\delta^{15}N$ values of human fingernails can range from 8.5‰ to 10.5‰, depending on the amount of meat in individuals' diets. Human waste tends to be more enriched in ^{15}N relative to the individual. These processes of producing different isotopic signatures in organisms is called fractionation. Although light and heavy isotopes of nitrogen can both be used in metabolic systems, lighter nitrogen reacts faster and easier than does the heavier nitrogen. For this reason, biochemical systems tend to favor the use of lighter nitrogen, leaving behind a pool of nitrogen with more ^{15}N than before the reactions. However, when organisms are nitrogen limited, they do not discriminate as well against ^{15}N, which causes species at higher trophic levels to become more enriched. In addition, fractionation drives the differences in $\delta^{15}N$ values between fertilizer and sewage. Sewage is more enriched in ^{15}N than fertilizer because sewage is mostly human waste, which has a similar $\delta^{15}N$ value to the human body.

Plants tend to mimic the δ15N values of their source pools of nitrogen. This makes them a useful tool for studying differences in pollution. Essentially, a higher δ15N value in one plant compared to another of the same species indicates a higher δ15N value in the source of nitrogen and ultimately, larger inputs of sewage pollution in the area. Similar studies have been conducted using corals as proxies (Baker 2010), but not with mangroves. Nitrogen stable isotope analysis is difficult to use to trace the source of nitrogen when both fertilizer and sewage are present, because the lighter isotope signature of fertilizer may mask the effects of sewage on the isotope values (Fry 2006). However, Guam is an ideal place for using nitrogen stable isotope analysis to study pollution because there is essentially no agriculture on the island, so all nutrient pollution comes from sewage inputs (CIA 2011). Therefore, it is possible to infer differing levels of sewage pollution around the island of Guam by looking at the δ15N values of mangroves around the island.

Project Goals

The fundamental goals of this project are:

1) To determine if mangroves effectively record nutrient pollution in coastal ecosystems.

2) To identify sewage hotspots on Guam by quantifying how nitrogen isotope values vary around the island.

Materials & Methods

Study Sites and Collections

Mangrove leaves were collected in 3 sites around Guam - Sasa Bay, Merizo, and Achang Bay (Table 1). These sites were the only beaches that contained mangroves and had public-access by land. In each site, 2 leaves from 2 species, *Bruguiera gymnorrhiza* and *Rhizophora stylosa*, were collected. Leaves were dried in Guam using a kitchen oven set to warm at the University of Guam. The map highlights the 3 collections sites given in Table 1 (Figure 2). Note that in the Guam EPA's July 2011 advisory report (during collection time), Merizo was closed to public access due to high levels of bacteria associated with sewage pollution. Merizo had few mangrove trees on rockier outcroppings, and was located near a tiny stream that likely drained nearby septic systems. Achang Bay was a narrow, muddy beach about 5-10 meters wide with little zonation. The two species were interspersed between each other. The Sasa Bay site was a muddy shore along a very large bay that was open to the ocean. It was approximately 15-20 meters wide, allowing for more distinct zonation between *B. gymnorrhiza* (further inland) and *R. stylosa*.

Table 1. Summary of the number of individual trees sampled. Merizo had the least number of samples due to limited number of mangroves growing in that site.

SITE	SPECIES	n
Achang Bay	*B. gymnorrhiza*	13
	R. stylosa	8
Sasa Bay	*B. gymnorrhiza*	7
	R. stylosa	12
Merizo	*B. gymnorrhiza*	3
	R. stylosa	3

Figure 3: Map of collection sites on Guam

Nitrogen Stable Isotope Analysis

For leaf bulk N isotope analysis, 3-6 leaves from each tree were collected. Veins and any fungal damage or discoloration were removed from the analyzed sample. Approximately 1mg of dried leaf sample from each tree was analyzed using continuous-flow, stable isotope mass spectrometry (Thermo Delta V) at the Carnegie Institution of Washington Geophysical Laboratory (Fogel 2008). Analysis of variance was used to test for differences in $\delta^{15}N$ values between the 3 sites, the 2 species, and an interaction between the two with a 95% significance level.

RESULTS

Analysis of variance indicated that species and sites are significant factors in d15N values of mangroves leaves; moreover, the analyses revealed a significant interaction (Table 2). Mangrove leaves exhibited $\delta^{15}N$ values ranging from about 0.6‰ to 3.5‰ across all sites and species. There was no significant difference between the isotopic signatures of for species within the same site, except for trees within Sasa Bay. *B. gymnorrhiza* had significantly higher $\delta^{15}N$ values than did *R. stylosa* within Sasa Bay. Both species within Merizo had significantly higher $\delta^{15}N$ values than did mangroves from the other sites (Figure 4). *B. gymnorrhiza* exhibited significantly higher $\delta^{15}N$ values in Merizo than in Sasa Bay and Achang Bay, with no significant difference within the species' $\delta^{15}N$ values between Achang Bay and Sasa Bay. *R. stylosa* exhibited the highest $\delta^{15}N$ values in Merizo, followed by Achang Bay, then by Sasa Bay. All differences were significant (Figure 5). Significance tests showed that there were species, site, and an interaction effects on $\delta^{15}N$ values of the leaves (Table 2; figures 4, 5).

DISCUSSION & CONCLUSIONS

Mangroves as effective recorders of nitrogen pollution

Mangrove leaves show significant variation in $\delta^{15}N$ values across sites and between species (Table 2). However, *R. stylosa* shows significant differences in its $\delta^{15}N$ values across all three sites, while *B. gymnorrhiza*'s $\delta^{15}N$ values in Sasa Bay and Achang Bay do not differ significantly from each other (Figure 5). In addition, there may be some threshold below which *B. gymnorrhiza* $\delta^{15}N$ values do not exist, perhaps due to differential fractionation between the two species. This hypothesis needs more rigorous testing, but indicates that *R. stylosa* may record lower enrichment values in pollution input better than *B. gymnorrhiza*. This indicates that *Rhizophora stylosa* is the more useful species for studying differential nitrogen pollution on Guam.

Identifying sewage hotspots on Guam

The data indicate that Merizo is the most polluted of the 3 study sites. The $\delta^{15}N$ values of both Merizo's mangroves (both species) are significantly higher than the $\delta^{15}N$ values in mangroves in Sasa Bay and Achang Bay (Figure 4). This conclusion is supported by the Guam EPA's water quality testing, which published a restriction again public coastal water use in Merizo the same week that the samples in this study were collected due to high levels of bacteria. Because enriched $\delta^{15}N$ values indicate the presence of sewage pollution, it is reasonable to expect that bacteria counts would increase in areas with highly enriched nitrogen inputs. However, these bacteria counts are a small snapshot of pollution levels. Nitrogen stable isotope analysis assesses pollution levels across the amount of time it takes the leaves to grow. Therefore, while bacteria counts are useful for closing beaches for small periods of time due to short-term increases pollution, stable isotope analysis is more useful for assessing pollution levels across a greater period of time.

The sampled mangroves in Merizo all grew along a small freshwater input. The data indicate that this stream is draining sewage, most likely from septic systems in the upstream small towns into the coastal oceans. In Belize, *Rhizophora mangle* mangroves (related to *R. stylosa*) located on the other edges of the islands have average $\delta^{15}N$ values of -0.6±1.7‰ (Fogel 2008; see also McKee 2002). The highest $\delta^{15}N$ values for Guam's *R. stylosa* mangroves are higher at 2.7±0.3‰, indicating that the nitrogen source in Guam is enriched relative to that in a location with little direct sewage input such as Belize (see Table 3). In the Philippines, at least one study shows mangrove leaves with higher $\delta^{15}N$ values, similar to Guam, around 3.8‰. This location probably has an enriched nitrogen source for similar reasons to those on Guam - poor sewage treatment (Primavera 1996). The $\delta^{15}N$ values for *Rhizophora* leaves in Guam were also similar to those found in a study in the Bahamas, suggesting that both places suffer from similar poor wastewater treatment (Kieckbusch 2004).

Future Work

As stated above, mangrove leaves provide a longer term record of nutrient pollution in coastal waters than do bacteria counts. However, leaves are constantly falling off of trees and therefore, can only record average levels of nitrogen pollution across months. Mangrove tree rings many provide a record of temporal changes in $\delta^{15}N$ values across many years, even decades, spanning the entire lifetime of a tree. As this work continues, tree cores collected from the same mangroves in Guam will be divided by annual or seasonal growth rings and analyzed using the same technique to study how nitrogen pollution has changed on Guam over a much longer timescale.

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Figure 4: Nitrogen stable isotope signatures across Sasa Bay, Achang Bay, and Merizo on Guam. $\delta^{15}N$ values are grouped within site by species. There was no significant intra-site differences between species in Achang Bay, and Merizo. The $\delta^{15}N$ value for *B. gymnorrhiza (B.g.)* was significantly higher than the $\delta^{15}N$ value for *R. stylosa (R.s.)* in Sasa Bay ($p<0.05$).

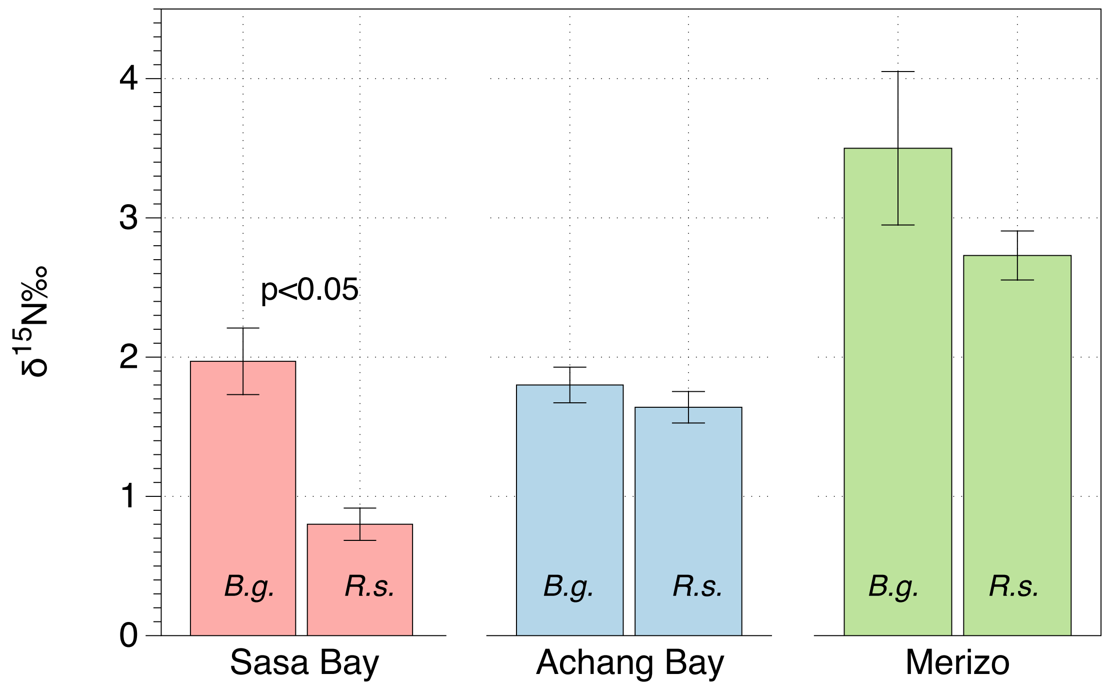

Figure 5: Nitrogen stable isotope signatures of 2 species in Guam. There was no significant difference within *B. gymnorrhiza* between Sasa Bay and Achang Bay (A). *B. gymnorrhiza* in Merizo (B) had significantly higher $\delta^{15}N$ values. *R. stylosa* trees in Merizo had the highest enrichment in $\delta^{15}N$, followed by those in Achang, then by those in Sasa. The differences between *R. stylosa* across all sites was significant. ($p<0.05$)

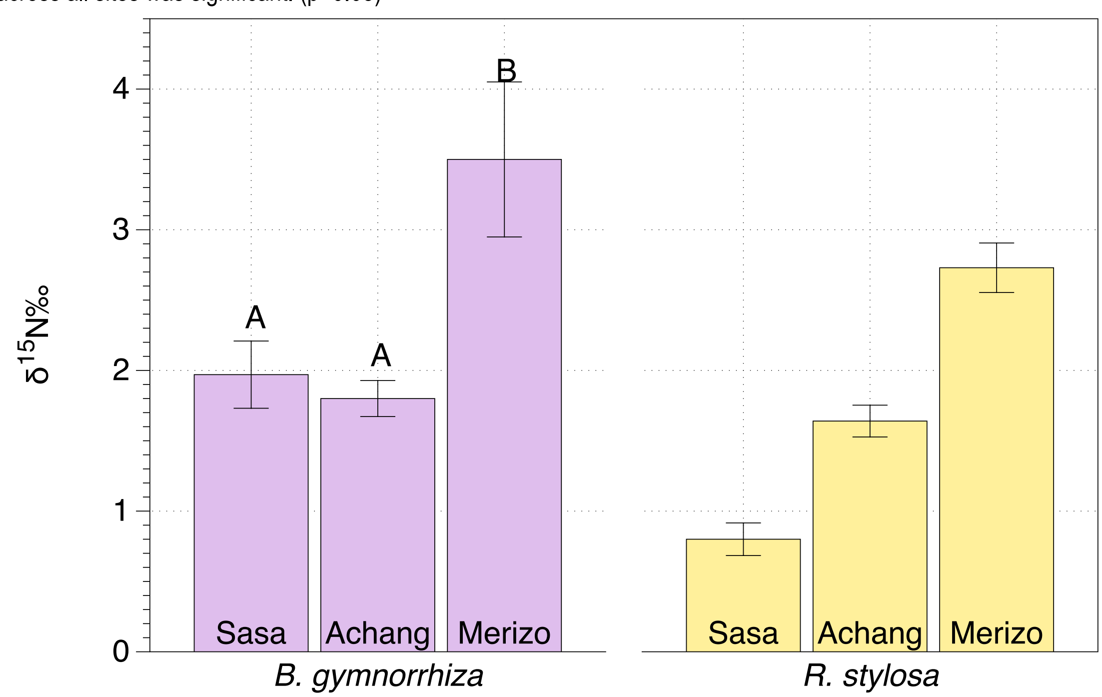

Table 2. The data were analyzed using ANOVA with 2 factors (species and site, with interaction) and 1 variable, $\delta^{15}N$. Post-hoc tests were done using Tukey's HSD tests.				
Source	**DF**	**SS**	**F Ratio**	**P**
Species	1	4.030	17.079	0.0002
Site	2	13.542	28.694	<0.0001
Site*Species	2	2.399	5.082	0.0108

Table 3. Summary of the $\delta^{15}N$ values of leaves of similar mangrove species in previous studies around the world.

SITE	SPECIES	$\delta^{15}N$ values	STUDY/SOURCE
Belize, fringe mangroves	*Rhizophora mangle*	-0.6±1.7‰	Fogel 2008
Guimaras, Philippines	*Rhizophora mucronata*	3.8‰	Primavera 1996
Belize, fringe mangroves	*Rhizophora mangle*	0.10‰	McKee 2002
Biscayne Bay, Miami, Florida	*Rhizophora mangle*	0.1±1.00‰	Kieckbusch 2004
Bahamas	*Rhizophora mangle*	2.3±2.97‰	Kieckbusch 2004