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Degradation of mangrove tissues by arboreal termites (*Nasutitermes acajutlae*) and their role in the mangrove C cycle (Puerto Rico): Chemical characterization and organic matter provenance using bulk $\delta^{13}C$, C/N, alkaline CuO oxidation-GC/MS, and solid-state ^{13}C NMR

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[1] Arboreal termites are wood decaying organisms that play an important role in the first stages of C cycling in mangrove systems. The chemical composition of *Rhizophora mangle*, *Avicennia germinans*, and *Laguncularia racemosa* leaf, stem, and pneumatophore tissues as well as associated sediments was compared to that of nests of the termite *Nasutitermes acajutlae*. Nests gave $\delta^{13}C$ values of −26.1 to −27.2‰ (±0.1) and C/N of 43.3 (±2.0) to 98.6 (±16.2) which were similar to all stem and pneumatophores but distinct from mangrove leaves or sediments. Organic matter processed by termites yielded lignin phenol concentrations (Λ, lambda) that were 2–4 times higher than stem or pneumatophores and 10–20 times higher than that of leaves or sediments, suggesting that the nests were more resistant to biodegradation than the mangrove vegetation source. ^{13}C NMR revealed that polysaccharide content of mangrove tissues (50–69% C) was higher than that of the nests (46–51% C). Conversely, lignin accounted for 16.2–19.6% C of nest material, a threefold increase relative to living mangrove tissues; a similar increase in aromatic methoxyl content was also observed in the nests. Lipids (aliphatic and paraffinic moieties) were also important but rather variable chemical components of all three mangrove species, representing between 13.5 and 28.3% of the C content. Termite nests contained 3.14 Mg C ha^{-1} which represents approximately 2% of above ground C storage in mangroves, a value that is likely to increase upon burial due to their refractory chemical composition.

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1. Introduction

[2] Mangrove forests, the intertidal wetlands of the tropics, comprise a unique succession of halophytic plants belonging predominantly to the *Avicenniaceaea* and *Rhizophoracea* families. In the Caribbean, the lone *Rhizophora mangle-Avicennia germinans-Laguncularia racemosa* association produces appreciable quantities of forest litter and root biomass which may accumulate as peat under favorable geochemical, sedimentary, and tectonic conditions [*Gleason and Cook*, 1926; *Golley et al*., 1962]. Therefore, mangrove forests play a key role in the cycling and storage of organic carbon in tropical coastal ecosystems and have been postulated to contribute approximately 15% of the C in contiguous marine sediments [*Donato et al*., 2011; *Jennerjahn and Ittekkott*, 2002]. The chemical composition of mangrove sediments, including vertically accreted mangrove peats, depends in part on the initial proportions of leaf, wood, and root matter, their resistance to physical maceration by crustaceans such as fiddler crabs (*Uca* spp.) and susceptibility to decay by fungi aerobic, and anaerobic, bacteria as well as tidal movement and redeposition [*Benner et al*., 1990; *Huxham et al*., 2010; *Marchand et al*., 2005; *Middleton and McKee*, 2001; *Skov and Hartnoll*, 2002]. However, the chemical transformation of mangrove forest leaves, wood, and root matter by insects such as termites has not been widely reported. Consequently, there is only a partial understanding of the organic matter decay processes caused by insects and the linkages between canopy litter fall, forest floor decay, and belowground diagenetic reactions, all of which lead to the chemical stabilization of mangrove peats.

[3] Termites are a successful group of insects that mainly feed on soil (humivorous), dead-decaying wood (xylophagus), leaf litter, or a combination of these lignocellulose-rich substrates. Lower termites are distinguished from higher termites on the basis that the former contain cellulose fermenting protists in their hindgut, whereas the latter do not [*Hongoh*, 2010; *Hyodo et al*., 1999; *Watanabe et al*., 2003]. Both higher and lower termites host a diverse range of bacteria and to a lesser extent archea in their gut. Metagenomic analysis of the anaerobic third proctodeal segment of the hindgut of the higher termite (*Nasutitermes coringer*) identified a diverse range of genes for cellulose and xylan hydrolysis; in contrast, no genes were expressed for lignin degrading enzymes [*Warnecke et al*., 2007]. Additionally, *Warnecke et al*. [2007] also confirmed that Gram-negative bacteria (spirochete) and fibrobacter (a cellulitic bacterium present in the rumen of cattle) played an important role in the decay of lignocellulose by the termite *N. coringer*. On balance, although the decomposition of cellulose by termites is widely accepted, no lignolytic enzymes such as those observed in the lignin degrading fungi (*Basidomycota)* have been reported. However, a recent review emphasized the fact that 99% of symbiotic microflora in the gut of termites are difficult to culture using traditional techniques, substantiating the view that more molecular level microbiological studies are required [*Kudo*, 2009].

[4] Understanding of the chemical alteration of lignocellulose during digestion by termites was first studied using ^{14}C labeled wood, which provided either tentative or no clear evidence of lignin degradation. For example, when the ability of *N. exitious* to decompose lignin was investigated by

incubating ^{14}C labeled aspen wood with termite mound material and measuring the amount of $^{14}CO_2$ released upon combustion, no significant lignin decay was observed over the entire 5 week time series [*Cookson*, 1992]. Conversely, an earlier study reported *N. exitious* degraded approximately 8.5% of ^{14}C labeled aspen after 14 days cultivation, whereas the feces of *Coptotermes acinaciformis* and *Mastotermes darwiniensi* were little changed compared to the original wood [*Cookson*, 1987].

[5] Chemical degradation of structural polysaccharides and lignin by both higher and lower termites has also been studied at the molecular level. Comparison of solid-state ^{13}C NMR spectra of Japanese red pine with *Coptotemes formosanus* feces after 2 weeks cultivation showed that the lower termite preferentially degraded cellulose and xylans with no change to the lignin structure [*Hyodo et al*., 1999]. Both ^{1}H NMR and nitrobenzene oxidation have been used to identify molecular changes in the subtropical hardwoods by examining the Bjorkman lignin extracts in feces of the lower termite *Cryptotermes brevis* [*Katsumata et al*., 2007]. The study reported that the concentration of lignin phenols of the feces was lower than the original woods and that the proportion of syringyl to vanillyl (S:V) increased in Apitong wood from an S/V 1.09 to 1.40 and in Ilang-ilang wood from an S/V of 0.88 to 1.32 [*Katsumata et al*., 2007]. Lignin biodegradation of Ponderosa pine by the Pacific dampwood termite *Zootplophora glabripennis* was studied by comparing tetra-methyl ammonium hydroxide thermochemolysis (^{13}C TMAH) depolymerization products from original and fecal matter (frass) using GC-MS analysis [*Geib et al*., 2008]. The lower termite caused three main alterations to the conifer lignin structure: (1) oxidative decay of alkyl side chains; (2) hydroxylation of aromatic rings and; (3) demethylation of intact lignin. In addition, these modifications to the lignin structure occurred within hours of digestion rather than the many weeks commonly taken to bring about a similar type/magnitude of change by white-rot and soft-rot fungi [*Geib et al*., 2008; *Vane et al*., 2001, 2005]. Field studies of termite feeding, digestion, and nest building are scarce; however, alkaline CuO treatment of lignin in six species of Amazonian termite nests has shown that different species of termites occupy different ecological niches (wood/soil/interface) and utilized different food sources. For wood decaying *Nasutitermes* spp., the increased yield in lignin phenols and lower proportion of syringyl acid to syringyl aldehydes was interpreted to indicate a lignin preservation as compared to degradation process [*Amelung et al*., 2002]. The combined molecular characterization studies of *Katsumata et al*. [2007], *Geib et al*. [2008], and *Amelung et al*. [2002] indicate, but do not necessarily prove, that some species of termites host a consortium of microbes that can depolymerize lignin.

[6] Puerto Rico has 21 termite species, of which 17 are endemic; however, the majority are soil dwelling, which largely precludes colonization of tidally flushed mangroves [*Scherffrahn et al*., 2003]. This ecological niche is dominated by the arboreal *Nasutitermes* species, which feed on decaying wood [*Scherffrahn et al*., 2003]. Of these, *N. acajutlaea* distribution is mainly restricted to littoral forests where it builds distinctive large ellipsoid carton nests (1–2 m height, 0.3–1 m diameter) that are dark-brown to tan-brown colored and are constructed from fecal matter cemented by saliva [*Scherffrahn et al*., 2003]. Given the paucity of research regarding the cycling of organic carbon by insects in mangrove forests and the clear succession of red (*Rhizophora mangle*), black (*Avicennia germinans*), and white (*Laguncularia racemosa*) mangroves hosting *N. acajutlaea* nests, we aim to elucidate (1) the chemical transformations caused by termite digestion and (2) provenance the sources of mangrove litter utilized by *N. acajutlaea* and (3) understand the sources and processes affecting organic matter (OM) accumulation in mangrove sediments using the bulk geochemical methods of C/N ratios and stable C isotopes and molecular methods such as alkaline CuO oxidation as well as solid-state ^{13}C NMR.

2. Method

2.1. Sample Collection

[7] The mangrove degradation study site located on the north coast of Puerto Rico comprises *R. mangle* (N18°11.587, W65°41.497), *L. racemosa* (N18°11.587, W65°41.508), and *A. germinans* floral zones (N18°11.586, W65°41.499) (Figure 1). The area has a tropical climate and receives average annual rainfall of 2724 mm with an average temperature of 80°C. On 10–12 May 2010, leaf and wood stems were from healthy, mature specimens of each mangrove tree were collected. In addition, the aerial roots (pneumatophores) were collected from *L. racemosa* and *A. germinans*

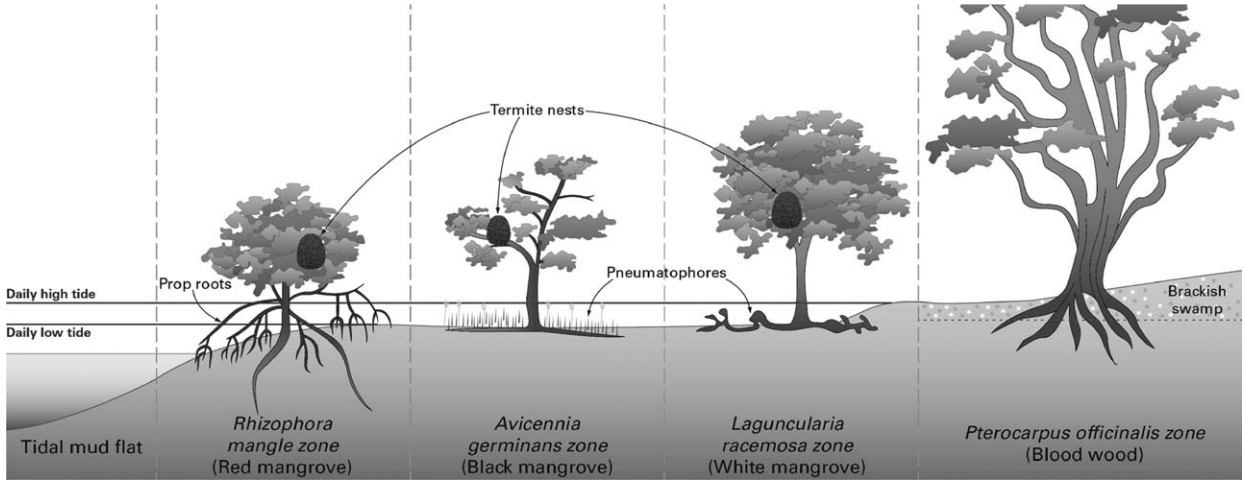

Figure 1. Schematic illustrating the major mangrove vegetation zones Sabana Seca, Puerto Rico. Site video is available at http://www.bgs.ac.uk/research/climatechange/environment/coastal/home.html.

(Figure 1). All leaf samples were green in color indicating that they had not undergone senescence. Combined stems and leaves of the understory colonizing nonwoody halophytes, *Sesuvium portulacastrum* and *Batis maritime* were also collected along with the seagrass *Thalassia testudinium*. Carton nests of *Nasutiteremes acajutlae* constructed around the branches of *R. mangle, A. germinans*, and *L. racemosa* were harvested. Each nest was immediately tapped free of termites and the outer galleries (10 cm wall thickness) stored in a separate polyethylene bags; the inner royal cells housing the queen termites were not included in this study. Surface sediments (top 1 cm) at each nest site were collected using a stainless steel trowel. Plant tissues and sediment were sealed in polyethylene plastic bags and transported to the Long Term Ecological Research Station (LTER) El Verde field station (within the Luquillo Critical Zone Observatory) in a cool box (~4°C) where they were dried in an oven at 60°C for 72 h.

2.2. The %TOC, C/N, and Carbon Isotope Ratios

[8] For measurement of $\delta^{13}C$ and C:N, plant samples were treated with 5% HCl for 2–3 h, washed with deionized water, dried in an oven at 40°C overnight and milled to a fine powder using a freezer mill. Mangrove sediment samples were treated using an identical method to that published for moorland peats [*Vane et al.*, 2013]. $^{13}C/^{12}C$ analyses were performed on plant and sediment samples by combustion in a Costech Elemental Analyser coupled online to an Optima dual-inlet mass spectrometer. The $\delta^{13}C$ values were calculated to the Vienna Peedee belemnite scale using a within-run laboratory standard (cellulose, Sigma Chemical prod. no. C-6413) calibrated against National Bureau of Standards (NBS–19 and NBS–22). C:N ratios were analyzed on the same instrument and the ratios were calibrated using an acetanilide standard. The $\delta^{13}C$ and C/N values presented herein are the mean of three separate treatments and analyses for each sample (Table 1). C:N values are expressed on a weight ratio basis (Table 1).

2.3. Microwave-Assisted Alkaline CuO Oxidation

[9] A recovery standard stock solution was prepared in pyridine, containing 5-bromovanillin and tetradecanoic-14,14,14-d3 acid at 1 μg/µL and a internal standard containing 4-fluoro-3-hydroxybenzoic acid and hexadecanoic-16,16,16-d3 acid at 10 ng/µL. Each reaction vessel was loaded with vegetation (3.035–3.929 mg) or soil (65.39–128.2 mg) to which CuO powder (500 mg), ferrous ammonium sulfate (50 mg), and NaOH solution (2 *M*, 15 mL) are added. Cupric oxidation was performed using a cloud ensemble model (CEM) MARS microwave fitted with 6 Teflon vessels (130 mL) (CEM) installed in series [*Goni and Montgomery*, 2000]. The microwave was operated for 60 min plus a 5 min warm at 600 W at 75% power at 150°C and a pressure of 65 psi (±5 psi). The vessels were then cooled for 30 min and vented to atmospheric pressure. The contents were transferred to a 50 mL glass centrifuge tube, and the recovery standard (5 µL) added to each and centrifuged (3000 rpm, 10 min). Quantitative

Table 1. Elemental and Stable C Isotopic Composition of Puerto Rican Mangrove Tissues

Mangrove Species	$\delta^{13}C$ (‰)	TOC (% wt)	N (Total) (% wt)	C/N
Rhizophora mangle				
Leaf	−29.8 (±1.9)	41.3 (±2.8)	0.9 (±0.3)	52.4 (±8.8)
Stem	−28.5 (±0.1)	40.2 (±0.3)	0.5 (±0.1)	82.3 (±6.9)
Surface sediment	−17.8 (±0.5)	1.4 (±0.2)	0.2 (±0.1)	8.1 (±0.2)
Termite Nest	−27.2 (±0.1)	49.3 (±1.5)	0.5 (±0.4)	98.6 (±16.2)
Avicennia germinans				
Leaf	−28.5 (±0.1)	40.4 (±0.1)	1.8 (±0.2)	23.1 (±2.7)
Pneumatophores	−26.6 (±0.1)	38.8 (±0.6)	0.7 (±0.2)	53.6 (±9.3)
Stem	−26.7 (±0.2)	46.0 (±1.3)	0.4 (±0.1)	114.5 (±11.2)
Surface sediment	−25.5 (±0.1)	4.0 (±0.1)	0.3 (±0.1)	11.7 (±0.3)
Termite nest	−26.3 (±0.1)	50.9 (±0.6)	1.2 (±0.1)	43.3 (±2.0)
Laguncularia racemosa				
Leaf	−27.9 (±0.1)	36.5 (±0.9)	1.2 (±0.1)	30.0 (±2.0)
Pneumatophores	−24.2 (±0.1)	46.7 (±1.0)	0.5 (±0.1)	90.7 (±17.2)
Stem	−25.6 (±0.1)	43.7 (±0.4)	0.5 (±0.1)	77.6 (±15.7)
Surface sediment	−22.8 (±0.1)	7.7 (±1.1)	0.5(±0.1)	16.7 (±2.4)
Termite nest	−26.1 (±0.1)	52.4 (±0.5)	0.8 (±0.1)	63.3 (±5.4)
Sesuvium portulacastrum	−26.1 (±0.1)	33.2 (±0.4)	1.4 (±0.1)	23.6 (±0.8)
Batis maritima	−29.1 (±0.1)	36.5 (±0.9)	1.2 (±0.1)	30.0 (±2.0)

transfer of the supernatant was achieved by rinsing the centrifuge tube with NaOH (2 *M*, 5 mL), recentrifuging as previously described and combining the two supernatants. The resulting supernatant was acidified to pH 1 using concentrated HCl. Liquid-liquid extraction was performed by adding ethyl acetate (6–7 mL). The supernatant was transferred to a 60 mL glass vial through a Pasteur pipette packed with anhydrous sodium sulfate (1 g) to remove any moisture. The solvent was removed using a stream of N_2 gas. The residue was transferred to a 2 mL septum-sealed glass vial using pyridine and reconstituted to 1 mL with pyridine. This was stored at <0°C in darkness prior to derivatization. A 200 μL glass insert was placed in a 2 mL glass septum-sealed vial, into which the reconstituted sample (10–100 μL) was spiked with the internal standard (10 μL) and made up to 160 μL with pyridine. The sample was derivatized with 40 μL of BSTFA + TMCS (1%). The vial was sealed, mixed by gentle agitation, and placed in an oven (60°C, 15 min). The sample was allowed to cool and analyzed by gas chromatography/mass-spectrometry (GC/MS) within 36 h.

2.4. Gas Chromatography/Mass Spectrometry

[10] GC/MS was performed using a Varian 3800 gas chromatograph (GC) directly coupled to a Varian 1200L triple quadropole mass spectrometer operating in full scan mode (ionization energy 70 eV, mass range 47–500 amu). Sample application (1 μL) was by splitless injection from 0 to 0.7 min and thereafter a 1:20 split was applied (injector temperature 250°C). The GC was fitted with a fused silica Agilent DB-1MS UI column (60 m length × 0.25 mm i.d. × 0.25 μm film thickness). The GC was temperature programmed from 100°C (1 min isothermal) to 320°C (at 4°C/min) and held isothermally at 320°C for 10 min. Helium was used as carrier gas at a flow rate of 1 mL/min.

[11] The following products were tentatively identified using mass spectra and chromatographic retention order: vanillin (Vl); acetovanillone (Vn); vanillic acid (Vd); syringealdehyde (Sl); acetosyringone (Sn); syringic acid (Sd); *p*-coumaric acid (pCd); ferulic acid (Fd); *p*-hydroxybenzaldehyde (P); *p*-hydroxyacetophenone (Pn); *p*-hydroxybenzoic acid (Pd); benzoic acid (Bd); *m*-hydroxybenzoic acid (m-Bd); 3,5-dihydroxybenzoic acid (3,5-Bd); 5-bromovanillin (5BrVl); 4-fluoro-3-hydroxybenzoic acid (4F,m-Bd). Quality control used consisted of an oak wood sample introduced in every batch of six vessels and a procedural blank in every fourth batch. The possibility of crossover contamination between vessels was investigated and found not to occur.

2.5. Solid-State ^{13}C Nuclear Magnetic Resonance

[12] Solid-state ^{13}C NMR spectra were obtained for plant and termite nest samples using a Bruker DSX200 instrument equipped with double-bearing probes for cross polarization (CP) and magic angle spinning (MAS). The resonance frequency for ^{13}C was 50 MHz, the sample was spun at the magic angle with a speed of 6.0 kHz. Typically 10,000 scans were accumulated with high power ^{1}H decoupling for the CP experiments. For CP, the

contact time was 3.0 ms and the relaxation delay was 1.5 s. All spectra were obtained at ambient temperature and processed with a line broadening factor of 50 Hz. Chemical shifts were calibrated using an external sample of tetrakistrimethylsilane (TKS). Peak integrations were determined using Fityk v.0.9.8. software. Sediment samples were not analyzed by ^{13}C NMR due to the potentially high concentration of paramagnetic metals in sediments that can diminish the sensitivity of carbon species [*Gelinas et al*., 2001]. Errors are typically ±1 mol % C for the major types measured (more than 10% of the total C).

2.6. Estimate of Termite Nest Carbon Store

[13] The carbon store survey was conducted on 6–8 January 2012, at Sabana Seca (18°27 N and 66°12) situated on the north coast of Puerto Rico, about 11 km west of San Juan. Four plots (30 × 30 m) were established along a 500 m transect running perpendicular to the broadly E-W orientated coastline (Figure 1). Each plot was situated within continuous mature stands of mixed *Avicenna germinans* and *L. racemosa*. For each plot, the number, size, and shape of aboveground (arboreal) and fallen termite nests (on the sediment surface) nests were recorded. These were categorized into large, medium, and small classes with a height of 1.6, 0.9, and 0.3 m, respectively. The shape of the nests were approximated to that of an ellipsoid where the volume was calculated by $V\ (m^3) = 4/3\pi\ (a,b,c)$, where a, b, and c are the radii (m) along each axis. The mass of an object can be written as $m = vp$, where p is the nest density, and the mass of carbon for each nest class was calculated by multiplying the % carbon of average *N. acajutlae* nest by its mass. The loss of nest volume due to the presence of mangrove branches running through the nests was not accounted for; conversely the considerable number of termite galleries emanating from the nests was not measured.

3. Results and Discussion

3.1. Storage of C in Arboreal Termite Nests

[14] A total of 10 large, 31 medium, and 41 small termites nests were observed in mangrove trees within four plots within the Sabana Seca site (total of 3600 m^2), and 1 large, 9 medium, and 11 small category nests were recorded fallen on the forest floor. The large termites' nests (1.6 m ht) contained 70 kg C, whereas medium nests (0.9 m) gave 6 kg C and small nests (1.5 kg C). Arboreal termites nests contained 270 t C/km^2 (2.75 Mg C ha^{-1}) as compared to 39 t C/km^2 (0.39 Mg C ha^{-1}) for fallen nests on the forest floor. These results are entirely plausible given that average aboveground C storage in living and dead wood in mangroves is reported to be about 159 Mg C ha^{-1} [*Donato et al*., 2011], thus termite nests represent about 2% of above ground C storage, this finding is to the best of our knowledge the first estimate of its type.

3.2. The $\delta^{13}C$, TOC, C/N of Mangrove Plant Tissues

[15] Total organic carbon (TOC) values for all mangrove leaf, stem, and pneumatophores ranged from 36.5 (±0.9) to 46.7% (±1.0) (Table 1). The $\delta^{13}C$ composition of *R. mangle*, *A. germinans*, and *L. racemosa* varied between species, as well as between leaf, stem, and pneumatophore tissues (Table 1). With the exception of the seagrass (*T. testudinium*) which is a C_4 grass with $\delta^{13}C$ value of −6.1 to −12.3‰ [*Anderson and Fourqurean*, 2003], all of the $\delta^{13}C$ values were typical of terrestrial plants that utilize the C_3 photosynthetic pathway with $\delta^{13}C$ values between −24 (±0.1) and −30‰ (±1.9).

[16] Fresh mangrove leaves $\delta^{13}C$ ranged from −29.8‰ (±1.9) to −27.9‰ (±0.1‰) and were depleted in ^{13}C (more negative values) compared to stem −28.5‰ (±0.1) to −25.6‰ (±0.1) and pneumatophore tissues −26.6‰ to −24.2‰ (±0.1), whereas the $\delta^{13}C$ of pneumatophores were enriched in $\delta^{13}C$ relative to the stems and leaves from corresponding mangrove tree species (Table 1).

3.3. The $\delta^{13}C$ and C/N of Mangrove Sediments

[17] The greatest magnitude of difference between $\delta^{13}C$ values of the same sample type was from the mangrove sediments (Figure 2). The $\delta^{13}C$ of *R. mangle* sediment −17.8‰ (±0.1) was considerably higher (more positive stable C isotope value) than the *L. racemosa* sediment −22.8‰ (±0.1) and *A. germinans* sediment −25.5‰ (±0.1) (*t* test, $p \leq 0.05$). In Puerto Rico, mangrove forests form elevation/nutrient dependent floral communities with *R. mangle* generally dominating from mean tide level to mean higher high water. As a result, sediments in the lower mangrove zone receive a greater amount of marine OM compared to

L. racemosa or *A. germinans* stands which grow at slightly greater elevations and are consequently subject to less tidal flushing and associated deposition of imported OM [*Gleason and Cook*, 1926; *Golley et al*., 1962; *Lugo and Snedaker*, 1974]. The seagrass *T. testudinium*, growing near the *R. mangle* shoreline gave a δ^{13}C value of −11.1‰ (±0.1) and the C/N ratio 19.0 (±2.3). These are similar to the *R. mangle* sediment values, supporting the possible import of local seagrass from the tidal mud flat into the *R. mangle* zone (Figure 1).

[18] Fiddler crabs are an abundant mangrove fauna (50–70 animals/m^2) that mainly consume mangrove leaves at the sediment floor or store leaves in their burrows for later consumption [*Hogarth*, 2007]. Studies in the mangroves of the Indo-West Pacific reported that although leaf storage in burrows does not significantly alter C/N ratio [*Skov and Hartnoll*, 2002], it was postulated that the physical fragmentation/ingestion/excretion of leaf litter by crabs accelerates microbial decay and decreases mangrove sediment C/N ratios. Therefore, the low C/N ratios observed in all three sediments could be due in part to the processing of leaf litter and sediment by crabs (Table 1). Nevertheless, on balance, the unusual combination of ^{13}C enrichment and low C/N ratios in the *R. mangle* sediment suggest input from seagrasses (*T. testudinium*), as well as other sources including bacteria, brown algae, green algae, and phytoplankton [*Tyson*, 1995, and references therein; *Goni and Hedges*, 1995].

3.4. The δ^{13}C and C/N of Termite Nests

[19] One common approach to source ascription of organic matter in carbon-rich coastal systems such as salt marshes and mangroves is to utilize the differences in δ^{13}C and C/N ratios of terrestrial plant tissues, marine, and freshwater organisms as well as dissolved and particulate organic matter [*Cloern et al*., 2002; *Kemp et al*., 2010; *Lamb et al*., 2006, 2007; *Middelburg et al*., 1997]. We adopted this same approach to investigate whether the source of organic matter in arboreal termites' nests could be tracked on the basis of δ^{13}C and C/N ratios to their corresponding leaves, stems, pneumatophores, and underlying surface sediments (Figure 2). Inspection of the δ^{13}C and C/N biplot shows that the termite nests in *R. mangle*, *A. germinans*, and *L. racemosa* stands did not correspond to a particular leaf, stem, or pneumatophore counterpart. However, in a general sense, the termite nests combined δ^{13}C values −26.1 to −27.2‰ (±0.1) and C/N ratio (43.3–98.6) were typical of stem and pneumatophore tissues and distinct from leaf and sediments (*t* test, $p \leq 0.05$) (Figure 2). Termites such as *Nasutiteremes* spp. construct their nests from fecal matter that has passed through a hindgut that contains a diverse consortium of microbes with the capacity to degrade wood polysaccharides, so it is not surprising that the mangrove plant tissue δ^{13}C values and C/N ratios are not precisely conserved.

[20] *R. mangle*, *A. germinans*, and *L. racemosa* leaf tissues gave low C/N ratios (<52) and δ^{13}C depleted (<−27‰) values which were distinct from that of the nests, substantiating the inference that leaf material was not a major source of organic matter for the wood decaying insects.

[21] Previous studies reported that the different distributions of biochemical structural groups (lipids versus unextractable lignins/cellulose/tannins) in *R. mangle* leaves showed that the lipid component was depleted in δ^{13}C (−29.8‰) relative to the bulk leaf values (−28.7‰) [*Smallwood et al*., 2003]. Therefore, in this current study, the more δ^{13}C depleted values observed in the mangrove leaves compared to other plant tissues (stems and pneumatophores) is explained by the high lipid content of leaf tissues. Although the δ^{13}C values of the halophytes *S. portulacastrum* and *B. maritima* were similar to that of the mangrove forest tissues and termites nests, the C/N values of 23.6% (±0.8) and 30.0% (±2.0) were far lower, suggesting that the halophytes were not a direct source of OM digested by termites (*t* test, $p \leq 0.05$).

3.5. ^{13}C NMR of Mangrove Plant Tissues and Termites Nests

[22] The solid-state CP/MAS ^{13}C NMR spectra of *R. mangle*, *A. germinans*, and *L. racemosa* and their counterpart termites nests are presented in Figure S1 (supporting information)[1] together with corresponding carbon distributions, given in Table 2. *R. mangle* leaves showed a broad resonance in the 20–40 ppm region centered at 33 ppm from aliphatic and paraffinic structures (CH_2) present in waxes, cutins, and proteins; the shoulder at 21 ppm is most likely from methyl carbons of acetyl groups in xylans [*Gamble et al*., 1994; *Vane et al*., 2001, 2005]. The high lipid (aliphatic and paraffinic moieties) content of *R. mangle* leaf material has been previously reported to account for approximately 20% carbon in *R. mangle* leaves collected from Bahamas mangrove swamp [*Benner et al*., 1990]. In this current work, the % C

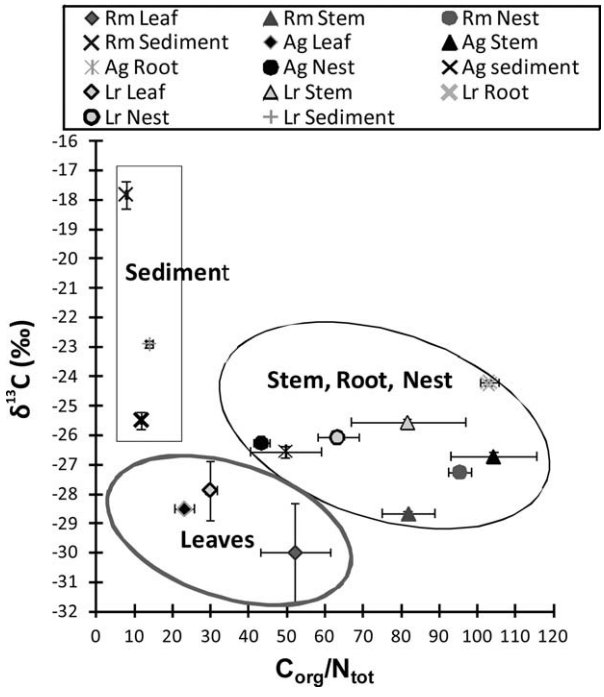

Figure 2. The δ^{13}C and C/N values measured from mature specimens of common mangrove forest plants and bulk-organic sediment in Puerto Rico. Specimen abbreviations: Rm, *Rhizophora mangle* (Red mangrove); Ag, *Avicennia germinans* (Black mangrove); and Lm, *Laguncularia racemosa* (White mangrove).

lipid content of *R. mangle* leaves was 24.5%, 27.2% for *A. germinans*, and 25.9% for *L. racemosa* leaves, which shows that all three mangrove wood species leaves have a high lipid content compared to woods and grasses [*Vane et al.*, 2003, 2001, 2005]. The geochemical implication of this finding being that mangrove peats sourced mainly by *R. mangle*, *A. germinans*, and *L. racemosa* leaves may yield elevated lipid contents as compared to mangrove peats, which are primarily sourced from root and stem tissues [*Middleton and McKee*, 2001].

[23] The resonance at 56 ppm from methoxyl carbon confirmed the presence of lignin in leaf tissue (see Figure S1, supporting information). In contrast, previous ^{13}C NMR characterization of *R. mangle* leaves at various stages of decomposition including attached senescent and detached detritus reported the absence of a prominent methoxyl C signal due to a low concentration of lignin [*Benner et al.*, 1990]. This difference could be due to either (1) the age of the leaf tissues giving rise to different lignin contents; (2) natural variation in lignin contents from leaf to leaf; (3) differences in sampling and post collection treatment; and (4) variation in leaf lignin content due to different nutrient stresses between sites caused by hydroedaphic conditions.

[24] The resonances from cellulose and xylans dominated the *R. mangle* leaf spectra from 60 to 110 ppm, the prominent peak centered at 72 ppm is from C-2, C-3, and C-5 carbons in both cellulose and xylans. Additional cellulose resonances are at 66 ppm (C-6), 106 ppm (C-1) as well as 84 and 90 ppm C-4 (supporting information Figures S1 and S2). The resonance at 84 ppm is attributed to cellulose in three-dimensionally disordered regions (amorphous), whereas the resonance at 90 ppm is from highly ordered crystalline cellulose biosynthesized away from lignin at the center of the fiber matrix [*Atalla and VanderHart*, 1999;

Table 2. Distribution of Carbon in Mangrove Tissues and Associated Arboreal Termites Nests as a Percentage of the Total Carbon From ^{13}C NMR Spectra

Sample	Acetyl, Lipid, Protein (5–40 ppm)	Methoxyl (50–60 ppm)	Carbohydrate (C-2,C-3,C-4,C-5 and C-6 of Cellulose and Xylans) (60–90 ppm)	Carbohydrate (C1 of Cellulose and Xylans and Aliphatic Lignin) (95–110 ppm)	Aromatic Lignin (110–160 ppm)	Carboxyl/Carbonyl (160–210 ppm)
Rhizophora mangle						
Leaf	24.5	6.5	44.7	13.4	9.7	1.2
Stem	13.5	7.6	55.6	12.8	8.2	2.4
Termite nest	23.5	11.5	32.5	13.9	16.2	3.0
Avicinnea germinans						
Leaf	27.2	7.1	40.4	9.7	10.0	5.7
Stem	11.7	6.4	56.5	12.7	8.4	4.3
Pneumatophore	28.3	7.1	44.9	8.4	8.9	2.4
Termite nest	16.6	10.4	39.0	12.7	18.6	2.6
Laguncularia racemosa						
Leaf						
Stem	16.2	7.5	50.3	13.2	11.6	1.3
Pneumatophore	16.4	6.6	48.7	13.0	12.4	2.9
Termite nest	18.4	8.0	41.2	9.36	19.6	3.6

Haw et al., 1984]. Comparison of the resonance at 90 ppm (crystalline cellulose) for fresh mangrove tissues and termites nests showed a decrease in peak intensity, which is entirely consistent with the preferential decay of crystalline cellulose regions and simultaneous decay of amorphous and crystalline regions. A similar loss of signal at 89 ppm has been reported during the decay of Japanese red pine wood by the lower termite *Coptotermes formosanus*; thus, it appears that both higher and lower termites are able to elicit the decomposition of crystalline cellulose structures during digestion/excretion [*Hyodo et al*., 1999]. Combined the areas of resonances at 65, 72, 84, 89, and 106 ppm provide an estimate of the relative percentage amount polysaccharide (Table 2). However, it should be borne in mind that the resonance at 106 ppm may also be contributed to by C-2 and C-6 carbons in syringyl units in lignin. Nevertheless, taken together the ^{13}C NMR spectra show that the *N. acajutlae* nests have a lower cellulose content than mangrove OM (stems, leaves of pneumatophores) found near the nests, confirming the notion that the termite caused a decrease in polysaccharide (cellulose and xylans) concentration. If we assume that the chemical shifts at 60–90 and 95–110 ppm are from polysaccharides, whereas those between 110 and 160 ppm arise from lignin, then the ratio of polysaccharides to lignin can be estimated. Leaf, stem, and pneumatophore tissues from all three mangrove woods yield polysaccharide to lignin ratios of between 5.0 and 8.2. Whereas, all three termite nest materials gave low values ranging from 2.6 to 2.8, confirming that the nests were enriched in lignin relative to undigested mangrove tissues (Table 2).

[25] Tannins are minor components of most plant tissues but comprise as much as 5% (wt) of tropical leaves suggesting that they and their decay products are important biomolecules within mangrove leaf litter, sediments, and waters [*Benner et al*., 1990; *Hernes and Hedges*, 2004]. The structure of condensed tannins in *R. mangle* leaves has been previously characterized using ^{13}C NMR with prominent resonances at 145 ppm from C-3 and C-4 procyanadins and 154 ppm from C-5, C-7 carbons, as well as at 104 and 108 from C6′ and C2′ carbons from prodelphinidins (see supporting information Figures S1 and S2) [*Zhang et al*., 2010]. The NMR spectra of the termite nests were markedly different from leaf, stem, and pneumatophore mangrove tissues. Termite nests gave distinct peaks at 56 ppm from aryl methoxyl carbons in lignin, and the percentage contribution of methoxyl C in fresh *R. mangle* and *A. germinans* and *L. racemosa* tissues ranged from 6.4% to 7.6%, whereas the methoxyl content of termites nests ranged from 8 to 11.5 (Table 2). This rise in methoxyl C observed in termites nests is most probably due to a relative increase in lignin caused by the decomposition of cellulose and xylans (Table 2). For the *R. mangle* nest, there is also some evidence that the increase in methoxyl C may also be due to an increase in syringyl content since the resonance at 154 ppm from C-3 and C-5 in syringyl units that are O-alkylated clearly increase relative to other lignin and polysaccharide resonances. Other workers have observed that the syringyl to guaiacyl ratio increased in the nitrobenzene products from feces of the lower termite *Cryptotermes brevis* following 6 months incubation with Ilang-ilang wood [*Katsumata et al*., 2007].

[26] The NMR spectra of termite nests in the 144–145 ppm region assigned to C-3 and C-4 tannins (proanthocyanidins) (Figures S1 and S2, supporting information) shows moderate signals confirming that tannins are not major components of the mangrove nests when compared to polysaccharides and lignin structures. The low intensity of tannin resonances indicates that plant tissues such as *R. mangle* bark which contain appreciable amounts of tannins are either not digested by termites or that the tannin structure is decomposed in the higher termite gut to such an extent that clear resonances from tannin carbons are not observed [*Benner et al*., 1990; *Wilson and Hatcher*, 1988; *Zhang et al*., 2010]. The former explanation has some credence given that tannins are reported to be important compounds in the defense against herbivory due their astringency and specifically by the observation that tannin concentration in mangrove leaves is strongly negatively correlated with leaf consumption by the tropical sesarminid crab *Neosamatium smithi* [*Neilson et al*., 1986]. Previous studies of apricot wood decayed by the soft-rot fungi *Hypocrea sulphurea* and oak bark decayed by the white-rot fungus *Lentinula edodes* over 8 years showed a decrease in resolution at 144–145 ppm when compared to fresh counterparts, confirming that tannins are susceptible to fungal decomposition [*Vane et al*., 2006, 2005]. However, given the manifold sources of organic matter available to termites in a mangrove system, our ^{13}C NMR do not confirm or refute extensive tannin decay during digestion by the higher termite but do show that tannins are not concentrated in the residual nest material as a result of

polysaccharide decay (supporting information Figure S1). This is entirely consistent with the view that tannins are of intermediate resistance to biotic and abiotic processes that occur during the early stages of diagenesis [*Hernes and Hedges*, 2004; *Benner et al*., 1990]. One important biogeochemical process observed in mangrove swamps is the leaching of tannins from leaf litter as evidenced by a 30% loss of tannin and an increase in reactive prodelphinidin after 7 weeks immersion in water [*Benner et al*., 1990; *Hernes et al*., 2001]. It is therefore plausible that the moderate tannin signals observed in the outer walls of the nests is caused by either (1) leaching by rain water or (2) partial decomposition/leaching by tidal action of the organic tissues collected and subsequently digested by the termites or both. The carbonyl and carboxyl (160–210 ppm) content of *R. mangle* leaf and stem (1.2–2.4%) were low relative to the corresponding termites nest (3.0%); however, neither of the other nests showed a particularly clear increase in this region when compared to the fresh tissues (see Figures S1 and S2, supporting information).

3.6. CuO Oxidation Products From Nests, Litter, and Sediments

[27] The CuO reaction products were analyzed using GC/MS. Inspection of the chromatograms (Figure S3, supporting information) shows that the main lignin-derived compounds from *R. mangle*, *A. germinans*, and *L. racemosa* tissues, sediments, and arboreal termites nests were vanillin (Vl), acetovanillone (Vn), *p*-coumaric acid (Pd), syringylaldehyde (Sl), acetosyringone (Sn), and syringic acid (Sd). Other aromatic moieties observed included *p*-hydroxbenzaldehyde (Pl), *p*-hydroxyacetophenone (Pd) as well as 3,5-dihydroxybenzoic acid (3,5-Bd). The presence of a variety of fatty acid and dicarboxylic acid compounds was also observed, of which the products 2-carboxypyrole, octane-18-dioic acid, and tetradecanoic acid were most abundant. Termite nests had the lowest distribution and concentration of fatty acid and dicarboxylic acid products as compared to their counterpart leaf, root, stem, and sediments, suggesting that these products are used and/or destroyed during digestion-excretion by termites.

3.6.1. Lignin Concentrations

[28] The sum of vanillyl, syringyl, and cinnamyl reaction products (Λ, lambda) provides a quantitative measure of total lignin content in a variety of matrices including fresh and decayed plant tissues and sediments [*Goni and Thomas*, 2000; *Goni et al*., 1993; *Hedges and Mann*, 1979a, 1979b; *Hedges et al*., 1982; *Loh et al*., 2008]. Although *p*-hydroxybenzenes and benzoic acids are also produced during CuO oxidation of plant tissues, these compounds are generally excluded from the Λ parameter because they have multiple structural biochemical precursors, such as tannins and aromatic amino acids [*Hedges et al*., 1982; *Tesi et al*., 2007; *Vane et al*., 2001]. In this current study, the highest Λ values of 32.5 (*R. mangle*), 43.1 (*A. germinans*), and 24.2 (*L. racemosa*) were observed in the arboreal termite nests (Figure 3). If it is assumed that wood is as an example of one likely source for termite food/excreta/nest, the arboreal termites nests from *R. mangle*, *A. germinans*, and *L. racemosa* increased the lignin content by 3.5, 2.6, and 2.2 times, respectively. Similarly, if pneumatophores were then the source of nest material, the termites would have to concentrate the lignin by 3–6 times. If leaf tissue were the sole nest OM source, the lignin would have to be concentrated by approximately 10–20 times, which seems improbable (Figure 3). The Λ value obtained from the termites nests are higher those previously reported in lignin-rich tropical hardwoods (*Zanthoxylum compactum*, Λ 24.11) and exceed those of softwoods (pine, Λ 14.2), confirming the field observation that the nests comprise a hard protective casing [*Goni and Montgomery*, 2000; *Goni and Thomas*, 2000]. Metagenomic analysis of the hindgut bacteria populations of wood feeding higher termites (*Nasutitermes* species) demonstrates a wide range of bacterial genes capable of cellulose and xylan (hemicellulose) hydrolysis [*Warnecke et al*., 2007]. Therefore, the elevated lignin content of termites nests observed in this current study is due to the preferential decay of cellulose and xylans in mangrove plant tissues mediated by symbiotic bacteria within the termite digestional tract.

[29] The Λ values of *R. mangle*, *A. germinans*, and *L. racemosa* wood tissues ranged from 9.2 to 16.5 and the pneumatophores varied from 6.6 to 10.1 whereas the leaf tissues had far lower lignin contents with Λ values of 1.2 (*R. mangle*), 3.1 (*A. germinans*), and 1.2 (*L. racemosa*) (Table 3). The lower Λ values of mangrove leaves are entirely consistent with a molecular level study of *R. mangle* leaves, which reported that lignin only comprised 2–5% [*Hernes et al*., 2001]. Overall, the lignin content of leaves from three mangrove tree species is similar to those previously reported for *R. mangle* (Λ 2.01) and *A. germinans* (Λ 3.54) [*Benner et al*., 1990; *Opsahl and Benner*, 1995].

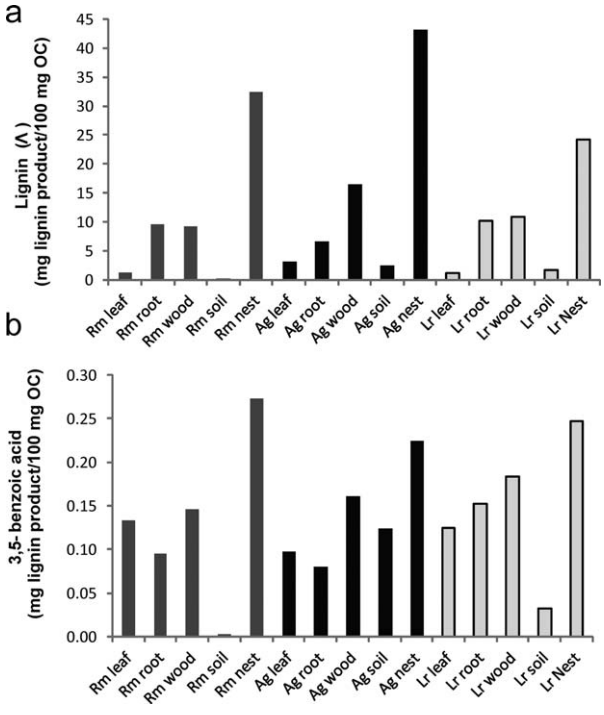

Figure 3. (a) Comparison of lignin concentrations (Λ) in mangrove tissues, surface sediments, and arboreal termites nests. High amounts of lignin are observed in the termites nests due primarily to the loss of cellulose during digestion by *N. ajaculatae*. (b) Concentration of 3,5-benzoic acid (3,5-Bd) in mangrove tissues, surface sediments, and termites nests.

All three mangrove sediments have low Λ values compared to the plant tissues (0.01–1.7), which substantiates the inference that mangrove sediments were an unlikely source of OM for termite excreta/nests. The low Λ values of the sediments surrounding the nest sites were not unexpected given the high inorganic mineral content and comparatively low TOC (Table 1).

3.6.2. Lignin Phenol Compositional Parameters

[30] *R. mangle* leaf tissue gave S/V of 1.2 and C/V 0.73, which is typical of nonwoody angiosperm tissues [*Hedges and Mann*, 1979b] (Table 3). Inspection of the *R. mangle* leaf tissue total ion chromatogram shows that that elevated C/V value is due to the high abundance of the *p*-coumaric acid rather than a paucity of vanillyl moieties (Figure 4). Previous studies of CuO oxidation products from fresh *R. mangle* leaf tissues collected from mangrove forests of the Caribbean and South America reported S/V values ranging from 0.92 to 1.50 and C/V values ranging from 0.67 to 4.14 [*Benner et al*., 1990; *Dittmar and Lara*, 2001; *Lallier-Vergès et al*., 2008; *Marchand et al*., 2005]. Therefore, the relative proportions of syringyl to vanillyl phenols from Puerto Rican *R. mangle* leaves are broadly similar to those reported from other mangrove forests. In contrast, the S/V and C/V values for *R. mangle* wood (S/V 4.99, C/V 0.16) are higher than that reported for *R. mangle* wood collected from the mangroves of Guadoloupe (S/V 1.52, C/V 0.02) and French Guiana (S/V 1.82, C/V 0.10). The most plausible explanation for these slight differences in S/V and C/V values is that the mangrove wood collected from each of the studies was of different ages and therefore contained syringyl and vanillyl and cinnamyl phenols in slightly different proportions. The small differences in S/V and C/V cannot be attributed to the use of a microwave as compared to a parr-bomb apparatus for the high-temperature oxidation in basic solution because *Goni and Montgomery* [2000] showed no appreciable difference in the compositional parameters (S/V, C/V, and [Ad/Al]) obtained upon alkaline CuO oxidation treatment of *Zanthoxylum compactum* wood and red oak leaves. However, it should also be borne in mind that molecular level methods involving the depolymerization of the lignin structure such as CuO oxidation and thermochemolysis do not necessarily yield the same S/V values as methods such as ^{13}C NMR spectrometry, although the general trends observed during plant tissue decay by white-rot fungi are the same [*Vane et al*., 2003].

[31] All three mangrove leaf tissues show low S/V values (0.79–1.23) and high C/V values (0.73–1.41) making them compositionally distinct from the woods and pneumatophores as well as understory halophytes illustrated in the S/V and C/V plots (Figure 4). Previous CuO oxidation of *L. racemosa* and *A. germinans* leaf tissues reported S/V values in the range of 1.79–1.05 and C/V values of 0.87–2.15 [*Dittmar and Lara*, 2001; *Lallier-Vergès et al*., 2008; *Marchand et al*., 2005]. Thus, the S/V values presented here are slightly lower than those previously reported where as the C/V values are closer. The acid to aldehyde ratios for *R. mangle* leaves (Ad/Al)v 0.93 and (Ad/Al)s 0.59, *L. racemosa* leaves (Ad/Al)v 0.76 and (Ad/Al)s 0.66 and *A. germinans* (Ad/Al)v and (Ad/Al)s 0.56 values were higher than either the counterpart wood, pneumatophores, or nests (Table 3). Graphical representation of the data in the S/V to C/V plot and (Ad/Al)v to (Ad/Al)s plot also indicates that the leaf tissues of *R. mangle, A. germinans*, and *L. racemosa* are compositionally distinct from wood, pneumatophore, and termite nests, which suggests that leaf litter was not a major source of

Table 3. Concentrations and Ratios of CuO Oxidation Products From Plant Tissues

Sample	V	S	C	P	B	Λ*	S:V	C:V	[Ad:Al]v	[Ad:Al]s
Rhizophora mangle										
Leaf	0.41	0.51	0.30	0.39	0.13	1.2	1.23	0.73	0.93	0.59
Stem	1.50	7.51	0.23	0.19	0.15	9.6	4.99	0.16	0.32	0.20
Surface sediment	0.01	0.02	0.01	0.04	0.00	0.1	1.40	0.42	0.69	0.39
Termite nest	8.17	23.99	0.32	0.56	0.27	32.5	1.23	0.73	0.93	0.59
Avicennia germinans										
Leaf	1.12	0.88	1.11	0.53	0.10	3.1	0.79	0.99	2.45	0.56
Pneumatophores	1.35	4.76	0.45	0.21	0.08	6.6	3.52	0.33	0.28	0.20
Stem	3.92	12.20	0.38	0.12	0.16	16.5	3.11	0.10	0.24	0.18
Surface sediment	0.93	1.30	0.20	0.61	0.12	2.4	1.40	0.21	0.54	0.52
Termite nest	5.25	37.36	0.52	0.13	0.22	43.1	7.12	0.10	0.27	0.22
Laguncularia racemosa										
Leaf	0.33	0.44	0.46	0.18	0.13	1.2	1.35	1.41	0.76	0.66
Pneumatophores	5.06	4.74	0.30	0.94	0.15	10.1	0.94	0.06	0.42	0.40
Stem	2.22	8.45	0.25	0.38	0.18	10.9	3.81	0.11	0.37	0.27
Surface sediment	0.79	0.77	0.13	0.21	0.03	1.7	0.97	0.16	0.43	0.24
Termite nest	7.09	16.59	0.47	0.69	0.25	24.2	2.34	0.07	0.40	0.37
Sesuvium portulacastrum	0.48	0.50	0.20	0.21	0.10	1.2	1.04	0.42	1.17	0.52
Batis maritime	1.00	5.11	0.13	0.69	0.08	6.2	5.13	0.13	0.66	0.36

Λ* (lambda) carbon normalized yields of 8 lignin phenols (mg 100 mg organic carbon).
V, vanillyl phenols; S, syringyl phenols; C cinnamyl phenols; P *p*-hydroxybenzene products; B, Benzoic acids; [Ad:Al]v, vanillic acid to aldehyde; and [Ad:Al]s, syringic acid/syringic aldehyde.

termite nest building material (Figure 4). This notion is further supported by the high relative amounts of 3,5-dihydroxybenzoic acid (3,5-Bd) in all leaf and sediment samples and its low abundance relative to lignin phenols in the termites nests (Figure 3). Conversely, the general distribution of lignin phenols and particularly the abundance of syringylaldehyde (Sl), acetosyringone (Sn), and vanillic acid (Vn) (supporting information Figure S3) is more consistent with a wood or pneumatophore source or both. Inspection of the C/V to S/V and (Ad/Al)v to (Ad/Al)s plots reveal that *R. mangle* and *L. racemosa* woods plot just below their respective nests and all the woods have slightly elevated (Ad/Al)s compared to the termites nests (Figure 4). Decreasing S/V and increasing (Ad/Al)s are common chemical alterations caused by white-rot fungal decay and result

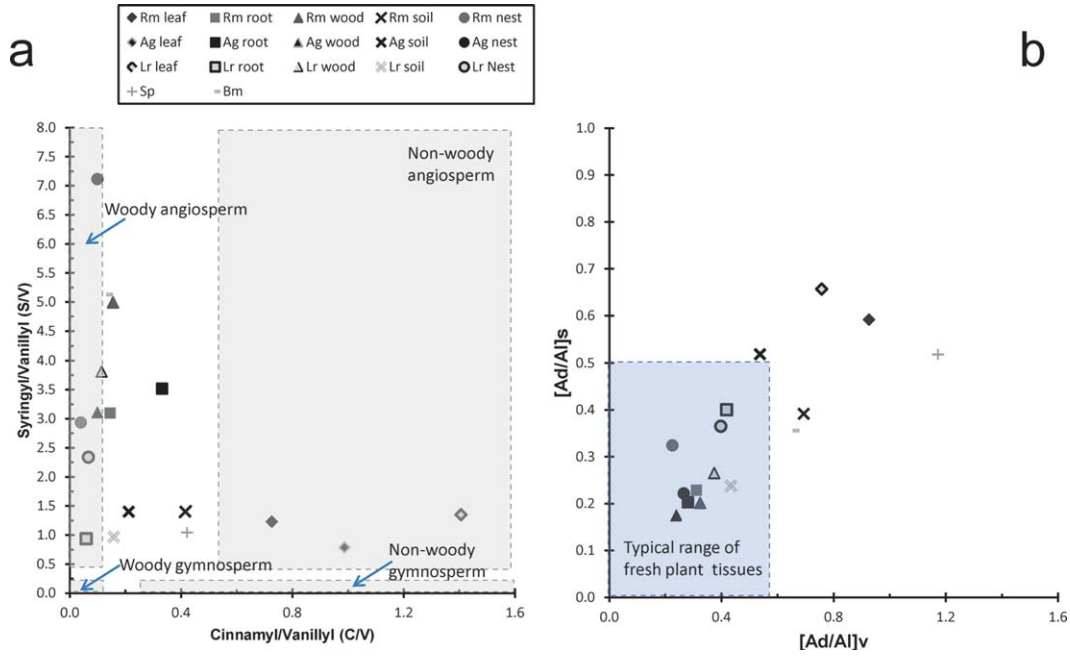

Figure 4. Source plot of (a) cinnamyl to vanillyl ratios and syringyl to vanillyl ratios and (b) vanillic acid to aldehyde and syringyl acid to aldehyde ratio (Ad/Al_{vs}).

from the preferential decay of syringyl as compared to guaiacyl units due to either demethylation or demethoxylation and the oxidative side chain cleavage between the Cα-Cβ at the Cα atom [*Vane et al*., 2003, 2001, 2005]. However, we do not assert that the transformation of mangrove wood to termite nest is caused by a particular group of fungi but do note that no bacteria have been shown to cause these transformations to lignin.

[32] The compound 3,5-dihydroxybenzoic acid (3,5-Bd) is a product of soil humification and decay processes that has been used to track terrestrial soil organic matter input into lake, coastal, and marine sediments and provide insights into hydrodynamic sorting effects at shelf sea and prodelta sites [*Goni and Hedges*, 1995; *Houel et al*., 2006; *Kuzyk et al*., 2008; *Prahl et al*., 1994; *Tesi et al*., 2007]. However, it should also be borne in mind that 3,5-Bd is not an entirely unambiguous indicator of terrestrial input since it has been measured in high concentrations in a variety of common marine organisms including, brown, green, and red algae as well as two species of phytoplankton [*Goni and Hedges*, 1995]. In this current study, the high concentration of 3,5-Bd relative to other lignin compounds in all three mangrove sediments (Figure 3) could be due to mangrove humification and decay processes (soil/sediment process origin) or mixing of marine organic matter or both. Furthermore, any attempt at source apportionment is confounded by the presence of 3,5-Bd in fresh *R. mangle*, *A. germinans*, and *L. racemosa* leaf, stem, and pneumatophore tissues as well as arboreal termite nests of *N. acajulatae*. Overall, the presence of 3,5-Bd in fresh plant tissues and soil/sediment as well as in previous studies of marine organisms suggests that there are at least three sources of 3,5-Bd in sediments accumulating offshore of mangroves.

4. Conclusions

4.1. Organic Matter Decay by (*N. acajutlae*) Termites

[33] Comparison of fresh mangrove tissues and nest material provided valuable information on the role played by termites in mangrove systems. ^{13}C NMR revealed that polysaccharides (cellulose and xylans) were the most abundant components of living mangrove tissues accounting for between 50 and 69% C, whereas the associated arboreal termite nests had lower polysaccharide contents with values ranging from 46 to 51% C. This showed that ingestion and excretion of mangrove tissues by *N. acajutlae* causes a significant decrease in polysaccharides, the main structural polymer of wood. Conversely, lignin accounted for between 16.2 and 19.6% C of nest material, a twofold to threefold increase relative to living mangrove forest tissues. Lipids (aliphatic and paraffinic moieties) were important but rather variable chemical components of all three mangrove forest species, representing between 13.5 and 28.3% of the C pool. Irrespective of mangrove species, the leaf and pneumatophore tissues had the highest lipid contents and stems the lowest, whereas the termites nests gave intermediate lipid contents ranging from 16.6 to 23.5% C. Alkaline cupric oxidation showed that the lignin phenol concentration of termites nests was 2–4 times higher than mangrove stem and pneumatophores and 10–20 times higher than that of leaves or sediments indicating that arboreal termites nests are highly lignified. Chemical decomposition of wood in mangrove systems is mainly associated with (1) aerobic fungal decay and (2) aerobic and anaerobic bacterial decay [*Hyde and Lee*, 1995]. Aerobic decay in mangrove systems is reported to elicit a decrease in lignin content, increase in Ad/Al values, and relatively stable C/V and S/V, whereas bacterial-mediated anaerobic degradation causes a decrease in total lignin content, a decrease in C/V, and stable Ad/Al ratios [*Dittmar and Lara*, 2001]. The pattern of lignin decay caused by termites in this current work is distinct from direct microbial decay in that we observed (1) an increase in lignin content; (2) an increase in S/V; (3) an increase in benzoic acids; and (4) an increase in (Ad/Al)s.

4.2. Provenance of Organic Matter in Carton Nests of *N. acajutlae*

[34] Nests gave δ^{13}C values that were similar to mangrove stem and pneumatophore tissues but distinct from leaves or local surface sediments. Overlapping δ^{13}C and C/N values *of R. mangle*, *A. germinans*, and *L. racemosa* stems and pneumatophores preclude any further δ^{13}C and C/N-based source apportionment. Taken together, the various lignin phenol descriptors namely, Λ, S/V, C/V, (Ad/Al)v, (Ad/Al)s, and high relative abundance of compounds such as *p*-coumaric acid (pCd), 3,5-dihydroxybenzoic acid (3,5-Bd), and aminoacid-

derived carboxypyrrole (Cp) suggest that soil and leaf tissues were not utilized for termite nest building. Therefore, the molecular level analysis supports the bulk chemical data and confirms that the nests are composed of partially decayed wood material sourced from the stems and branches of mangrove trees. However, none of the bulk or molecular analyses utilized in this study were able to unequivocally distinguish whether the termites were utilizing *R. mangle*, *A. germinans*, or *L. racemosa* stems or were indiscriminate in their choice of wood used in constructing carton nests. The absence of soil-derived organic matter in the nests is not entirely unexpected since it has been postulated that *Nasutitermes* (spp.) nests are built away from the soil not only to avoid environmental pressures (flooding, overheating, and competition) but also to decrease infection risks from soil inhabiting pathogens such as fungi [*Postava-Davignon*, 2010].

4.3. The Role of Arboreal Termites in Nutrient Cycling in Mangroves

[35] Woody tissues comprise up to 50% of the total net primary productivity of mangrove forests, characterizing the first stages of decay initiated by wood decaying termites must be considered a key step in understanding early diagenesis within mangrove systems. We have shown using bulk $\delta^{13}C$, broad scale (^{13}C NMR), and specific molecular (lignin) methods that decay mediated by termites is chemically distinct from that reported for wood decomposed by white-rot fungi. Furthermore, the polysaccharide deficient, lignin-rich composition of the carton nests indicate a high preservation potential in the sedimentary column, a notion supported by visual observation of fallen nests being incorporated into the sediment within the *A. germinans-L.racemosa* floral zone. There is little knowledge of the amounts of C stored in arboreal termite nests—we estimate that this comprises about 2% of the total above ground mangrove C store. Notwithstanding physical maceration by macrofauna, we suggest that the nests may be preferentially preserved in the sedimentary column relative to less aromatic and more labile mangrove tissues such as leaves.

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