THE USE OF $\delta^{15}N$ TO EXAMINE PAST MANGROVE STAND STRUCTURES

A Thesis

by

STEPHANIE M. GUDEMAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Oceanography

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Approved by:

Chair of Committee,	Luis Cifuentes
Committee Members,	Marilyn Fogel
	Dan Thornton
	Steve Davis
Head of Department,	John Morse

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ABSTRACT

The Use of $\delta^{15}N$ to Examine Past Mangrove Stand Structures. (August 2006)
Stephanie M. Gudeman, B.S., University of Rhode Island
Chair of Advisory Committee: Dr. Luis Cifuentes

Twin Cays, Belize, is dominated by *Rhizophora mangle L.* (red mangrove). Tall (>5m in height) *R. mangle* are located along the fringe of the island and dwarf *R. mangle* grow in the interior of the island. These stand structures can be differentiated using $\delta^{13}C$ and $\delta^{15}N$ analysis (mean tall $\delta^{13}C$ = -28 ‰, mean tall $\delta^{15}N$ = 0‰; mean dwarf $\delta^{13}C$ = -25‰, mean dwarf $\delta^{15}N$ = -10‰), which may also prove useful in examining past mangrove stand structures from sediment cores. ^{15}N label was traced in *R. mangle* leaves in a laboratory and field experiment over three months. The ^{15}N label was examined to determine distribution of nitrogen in various biochemical fractions of the leaf and to verify if nitrogen is fractionated in a predictable manner over time. This information could be beneficial in examining past mangrove stand structures. Experimental data indicate that nitrogen is mobile within each biochemical fraction of the *R. mangle* leaf over time and a measurable amount of nitrogen exists in each fraction after 3 months of incubation. Nitrogen immobilization was evident in each experiment, as the $\delta^{15}N$ values decreased ~200‰ in each of the labeled fractions of the laboratory experiment, which was mirrored by an increase in $\delta^{15}N$ in the control samples. The amount of nitrogen in the biochemical fractions of the field experiment varied over time either increasing or decreasing, which may be due to the various environmental conditions such as tidal fluctuation, temperature, oxygen concentrations and microbial activity. The $\delta^{15}N$ signature of the residual nitrogen fraction ($\delta^{15}N$ = 87‰) reflected that of the bulk fraction ($\delta^{15}N$ = 133‰) in the laboratory experiment as well as in the field experiment (residual nitrogen $\delta^{15}N$ = 759‰, bulk $\delta^{15}N$ = 770‰). To use isotope analysis to examine past mangrove stand structures it is essential that the original signature be maintained over time. The results of this study indicated that the simple interpretation of nitrogen isotopes may not be useful in examining past mangrove stand structures due to the variation over

time, although this type of analysis may be considered if coupled with additional proxies and diagenetic factors are taken into account.

ACKNOWLEDGEMENTS

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Special thanks to Brian Jones for all his help in the laboratory. Thanks to Carlton Rauschenburg, who made working the laboratory so much fun.

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1. INTRODUCTION: THE USE OF MANGROVES AS INDICATORS OF ENVIRONMENTAL CHANGE

The examination of nitrogen mangrove tissues is useful in paleoecological studies because of the high amount of plant production in these regions, which results in a high demand for nitrogen in plant tissues.[1,2,3,4,5] The term "mangrove" is used to describe a diverse group of trees and shrubs that are adapted to a wet, saline environment[6] that form the dominant plant communities in tidal, saline wetlands along tropical and subtropical coasts[7] and are highly productive ecosystems (2,500 mg C m^{-2} day^{-1}). They reduce erosion, act as a buffer during storm events (eg. hurricanes), provide a habitat for marine life, and maintain the quality of coastal waters,[8] making them an essential component to tropical and sub-tropical coastal environments. Mangrove ecosystems can be found as far north as Bermuda and as far south as Corner Inlet, Australia and are abundant in regions where there is a large input of fine-grained sediments, but can also grow in sand, peat, or coral substrates.[9] These ecosystems are particularly sensitive to variation in sea level and climate change,[10] making them good indicators of regional coastal dynamics.[7]

Rhizophora mangle L. (red mangrove) is particularly sensitive to variations in sea level as the physiology, stand structure (dwarf or tall), morphology, and reproduction of this species are governed by sea level.[11] In mangrove forests of Florida and the Caribbean there is a marked tree height gradient that runs parallel to the shoreline creating distinct zones.[12] The fringe zone, which has a slightly higher surface elevation, is dominated by tall *R. mangle*, and receives a larger nutrient load as a result of tidal flushing.[13] Dwarf *R. mangle*, located at the interior of mangrove islands, are fully mature trees that grow only to 1-2m in height. These forests are poorly flushed and severely nutrient limited.[12] They grow in continuously waterlogged conditions, which restrict root growth and metabolism, and often grow in higher salinities.[13,14] The sensitivity of mangrove ecosystems to minor alterations in coastal conditions, such as inundation, salinity, and nutrient status, make them good indicators of environmental changes.[7]

This thesis follows the style of *Geochemical Transactions.*

1.1 Isotope Analysis

Stable carbon and stable nitrogen isotope analysis may be used to examine environmental changes in the mangrove ecosystems of Twin Cays, Belize. The stand structure of *R. mangle* located in Twin Cays, Belize, whether dwarf or tall, is associated with unique stable isotopic compositions:[13,15,16,17] Tall *Rhizophora* trees are depleted in ^{13}C relative to dwarf trees, while tall mangroves, especially those on coastal fringe zones have enriched ^{15}N relative to more interior-living dwarfed trees. Specifically, on Twin Cays, Belize, Wooller et al., 2003 report that fresh dwarf *R. mangle* leaves have a lower $\delta^{15}N$ values (mean = -10 ‰) and higher $\delta^{13}C$ values (mean = -25.3 ‰), while fresh tall *R. mangle* leaves have higher $\delta^{15}N$ values (mean = 0 ‰) and lower $\delta^{13}C$ values (mean = -28.3 ‰).

Wooller et al., 2003, examined the taphonomic stages in the fossilization of *R. mangle* leaves to determine if the isotopic signature of the original leaf was reflected in preserved leaves. The results of the experiment indicate that preserved leaves have isotope values ($\delta^{13}C$ = -29 ‰ to –22 ‰; $\delta^{15}N$ = -11 ‰ to +2 ‰) that correspond with the values found in modern leaves. The isotopic composition of the surrounding peat matrix was analyzed and found to have little if any variation.[17] Isotopic analysis was also conducted at the molecular level.[15] The carbon and nitrogen isotopic composition of the bound amino acid fraction of the leaf was found to be unique between stand structures and reflect the isotopic composition of the parent leaf. In studies of other wetland plants, stable carbon isotope values are not significantly altered during decomposition,[18,19] but $\delta^{15}N$ changed in a period of months depending on the extent of microbial decomposition. In mangrove tissues, however, it is thought that nitrogen is preserved due to the anoxic and tannin rich composition of mangrove environments.[20,21]

This paper reports on the use of ^{15}N label in mangrove leaves in a controlled laboratory environment as well as in the field. The experiments were done to establish whether or not the ^{15}N label in the leaves remained constant over time. ^{15}N labeling experiments were conducted in a variety of environments to determine (1) if the ^{15}N label was present in all of the leaf's biochemical fractions including lipid, bound amino acid, free amino acid, and residual nitrogen fraction (2) if the label changed its distribution in

the various fractions in a predictable manner over time, and (3) if the results of this study could be used to strengthen the use of sub-fossil leaf fragments for paleoenvrionmental reconstruction of coastal mangrove islands.[15,16,17] The term “sub-fossil” fragments is used to describe leaf fragments that have been preserved with the sediment and are no longer mineralized.

2. METHODS: STUDY SITE

Fieldwork was conducted at Twin Cays, Belize, Central America in March 2004 and June 2004 (Fig 1). Twin Cays is a peat based mangrove island ~12km off the shore of Belize, within the Belizean Barrier Reef.[22] There is no direct terrigenous input to the island, the only input being aeolian dust. *R.mangle* dominates the island and forms a distinctive tree height gradient from the island fringe to interior. Dwarf *R.mangle* (canopy height <2.0m) are fully mature stunted trees located at the interior of mangrove islands, which is perennially flooded. Tall *R.mangle* dominate the fringe zone (canopy height ~2-6m), which is flooded and drained >700 times/yr.[13] *Avicennia germinans* (black mangrove) and *Laguncularia racemosa* (white mangrove) are also present in mixed stands in areas of slightly higher elevation.[13]

Twin Cays sits atop some of the thickest peat deposits in the world, measuring up to 10m deep.[23] The base of the peat deposits date back approximately 8,000 years before present.[23,17] These mangrove islands are unique in that they have been able to keep up with sea-level rise since the beginning of the Holocene, whereas islands to the south of this region have been totally submerged.[23]

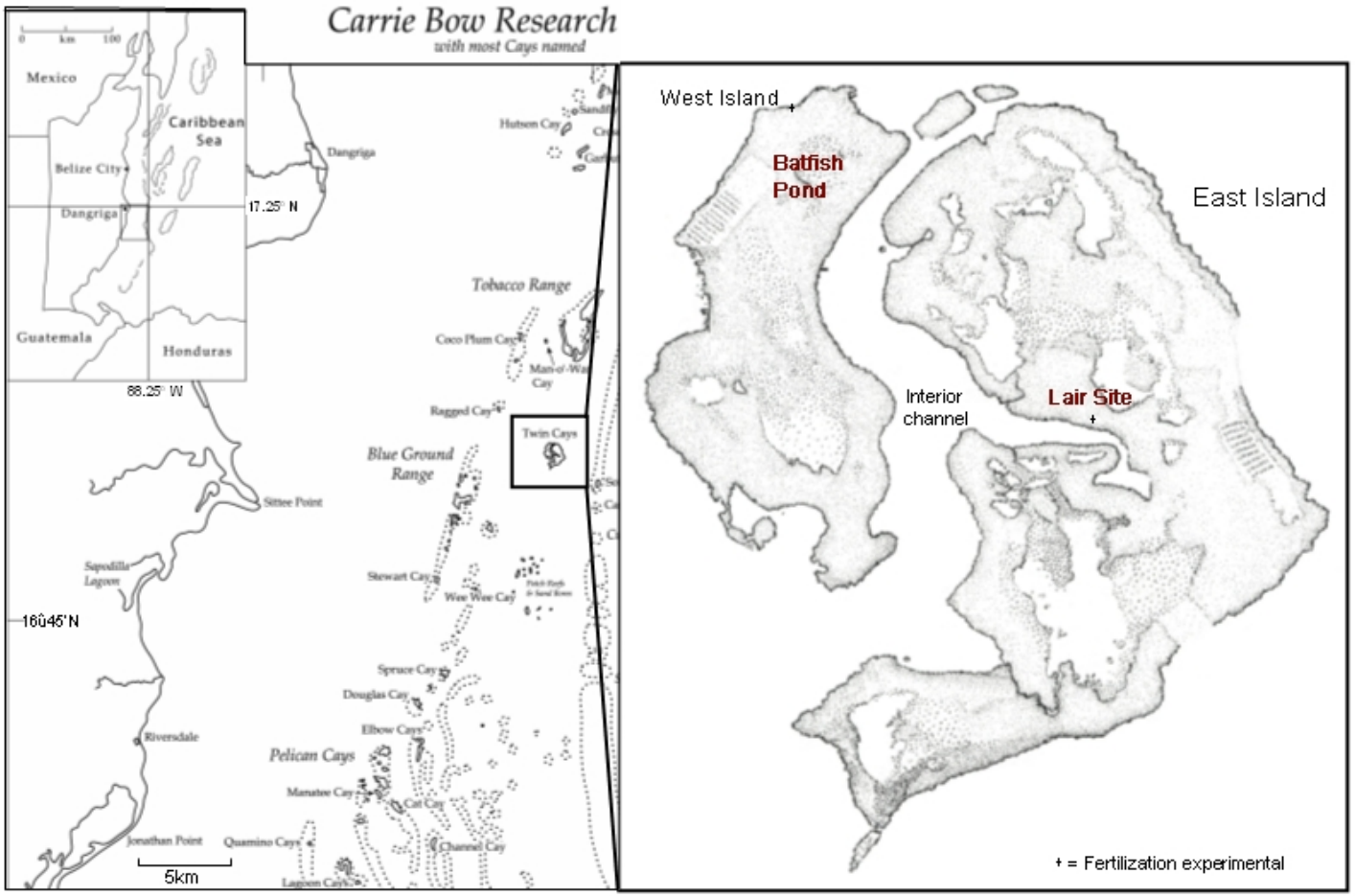

Fig. 1 Belizean Barrier Reef and Twin Cays, Belize. Large-scale map adapted from Smithsonian Caribbean coral Reef Ecosystems map and Twin Cays, Belize.

2.1 Laboratory Experiment

To examine ^{15}N over time in *R. mangle* leaves in the laboratory, a group of dwarf *R. mangle* trees located in Twin Cays, Belize (Lair Site), were labeled with ^{15}N for 24 hours. The trees chosen for the labeling experiment are part of a fertilization experiment (Fogel et al., in progress). Two control trees (identified as 401 and 402) and two phosphorus fertilized trees (identified as 408 and 409) were labeled by attaching a bag around a branch containing 8-10 leaves. The bag contained 100% ^{15}N urea and 1ml of 10M NaOH to convert the nitrogen to NH_3. The leaves did not come into contact with the liquid in the bag. After 24 hours the bag was removed from the branch and the leaves were rinsed thoroughly with tap water, followed by de-ionized water, then and dried (Fig 2).

Initial $\delta^{15}N$ values were obtained by sampling five fragments from each of the leaves (401, 402, 408, 409). Approximately 1mg of sample was weighed and placed into a tin boat for bulk EA analysis.

Approximately 100mg of leaf fragments were weighed and placed into 100μm mesh bags. The bags were tethered to a fishing line and placed in the polypropylene cups that contained a mixture of dried peat and 35‰ Instant Ocean (Fig 2).

The cups with sample mesh bags were placed into three different environments. Leaving the cups open to air created the “oxic” environment. The”oxic” samples were replenished with 35‰ Instant Ocean as needed. Purging the cups with N^2 for approximately 5 minutes and capping created the “suboxic” environment. Purging the cup with N_2, sealing with electrical tape, and placing the cup in an N_2-purged glove bag created the “anoxic” environment. As a control, an unlabeled *R.mangle* leaf was placed in each cup (Fig 2) For reproducibility, three cups (identified as A, B, and C) were created for each environment.

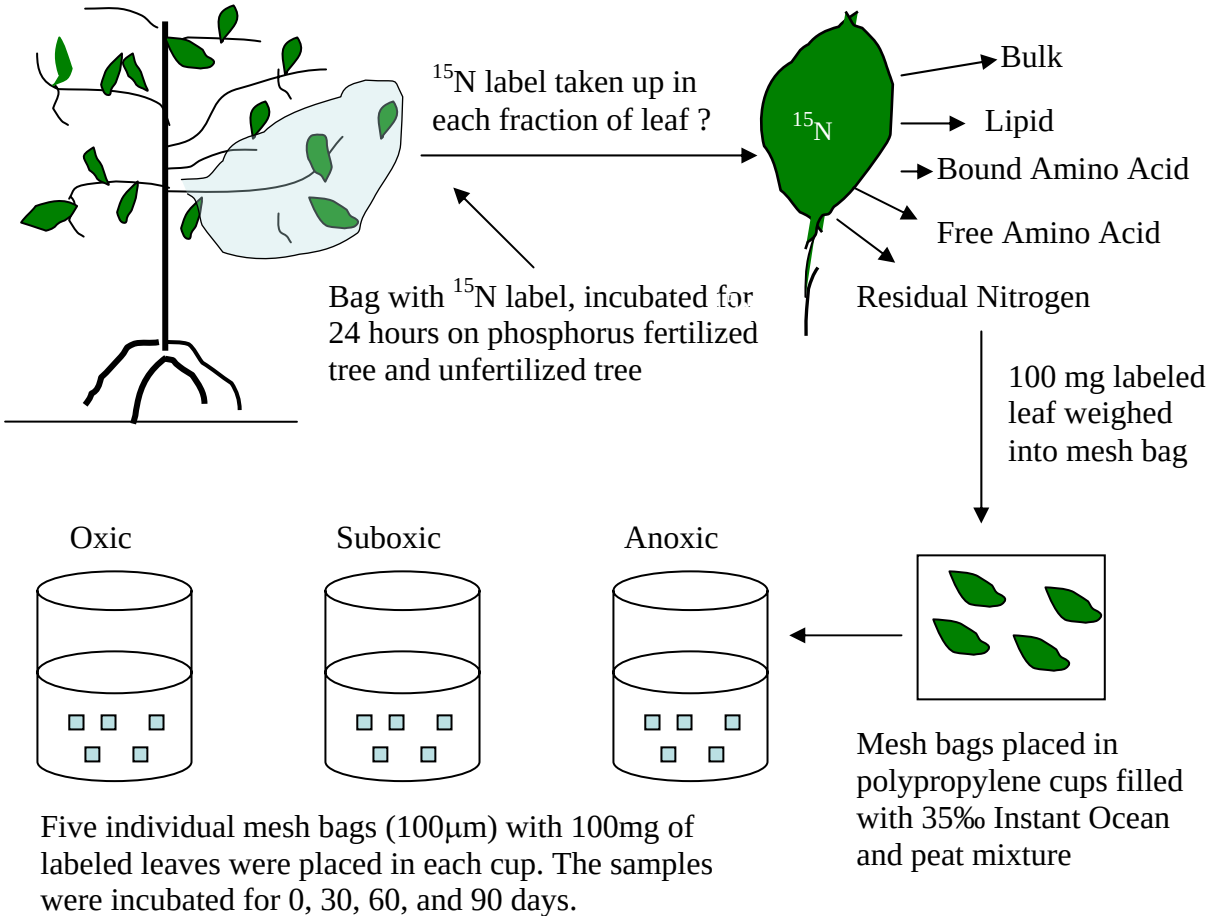

Fig. 2 Laboratory experiment materials and methods. The leaf samples were labeled in the field and incubated in the laboratory for 0, 30, 60, and 90 days.

At time 0, 30 days, 60 days and 90 days a mesh bag was removed from the "oxic", "suboxic", and "anoxic cups". The leaf samples were removed from each bag, rinsed with Mili-Q water and freeze dried. The samples were ground using a mortar and pestle.

Approximately 40mg of sample was weighed and placed into a culture tube for lipid extraction. Lipids were extracted with 10ml 9:1 CH_2Cl_2:CH_3OH and sonicated three times for ten minutes. The extract was separated via centrifuge and pipetted into a culture tube, and concentrated under N_2[14]. A small portion of the lipid extract was dissolved in 10µl of CH_2Cl_2 and pipetted onto pre-weighed muffled 3mm 45µm GC/F filter paper. The filter paper was dried, weighed, and placed in a tin boat and stored until analysis.

The bound amino acids were extracted from the lipid extracted leaf fragments. One milliliter of 6N HCl was added to the culture tube. The sample was purged with N_2 for 30 seconds and placed in an oven at 110ºC for 20 hours. After 20 hours, the samples were cooled and filtered through 3mm 45µm GC/F filter paper using MiliQ water as a rinse. The filtrate was freeze dried, weighed (~1mg) and placed in a tin boat and stored until analysis.[15]

The residual nitrogen fraction (which is comprised mainly of tannins and lignins[14]) was the solid that remained from the bound amino acid extraction. The solid was scraped from the filter paper, weighed (~1mg) and placed in a tin boat and stored until analysis.[15]

Approximately 40mg of leaf sample was weighed and placed in a culture tube to extract free amino acids. The free amino acids were extracted using 10ml of MiliQ water and sonicated for 10 minutes, three times. The extract was separated via centrifuge, pipetted into a culture tube, and freeze dried. The free amino acids were weighed (~1mg) and placed in a tin boat and stored until analysis.[15]

2.2 ^{15}N Field Experiment at Batfish Pond

To examine ^{15}N over time in *R. mangle* leaves in the field, a group of dwarf *R. mangle* trees located in Twin Cays, Belize (Batfish Site) were treated with fertilizer and labeled with 5% ^{15}N-urea. The trees were previously labeled and fertilized as part of a

separate experiment (Fogel et al., in progress). Two trees were fertilized with ^{15}N labeled nitrogen and will be referred to as the nitrogen tree, and two trees were fertilized with ^{15}N labeled nitrogen plus phosphate and will be referred to as the nitrogen plus phosphorus tree. Four trees did not receive any added ^{15}N: two were untreated control and two were given phosphate only, and will be referred to as the phosphorus tree. The fertilizer was added to the peat directly within the root zone of each tree.

Following 1 year of fertilization, approximately 3-4 leaves were removed from each tree, rinsed and dried. Approximately 100mg of dried sample was placed in 100μm mesh bag; five mesh bags were created for each of the treated trees (control, nitrogen, phosphorus, nitrogen/phosphorus). The mesh bags were tethered back onto the trees using approximately three feet of fishing line so they were free to float on the surface of the peat. As a control, a group of untreated dwarf *R.mangle* leaves were tethered to two of the treated trees (Fig 3).

For a control of the sediment location, mesh bags were tethered to a group of randomly selected trees approximately 100 yards from the study site similar to the first set of incubations at the Batfish site. A group of untreated dwarf *R.mangle* leaves were also tethered to two of the trees as an additional control. A bag was collected from each tree after 4 days and 87 days, rinsed with DI water and dried. The samples were stored at room temperature until the extractions could be performed. The samples from the field ^{15}N labeling experiment were subject to the exact same extraction procedure as the laboratory experiment.

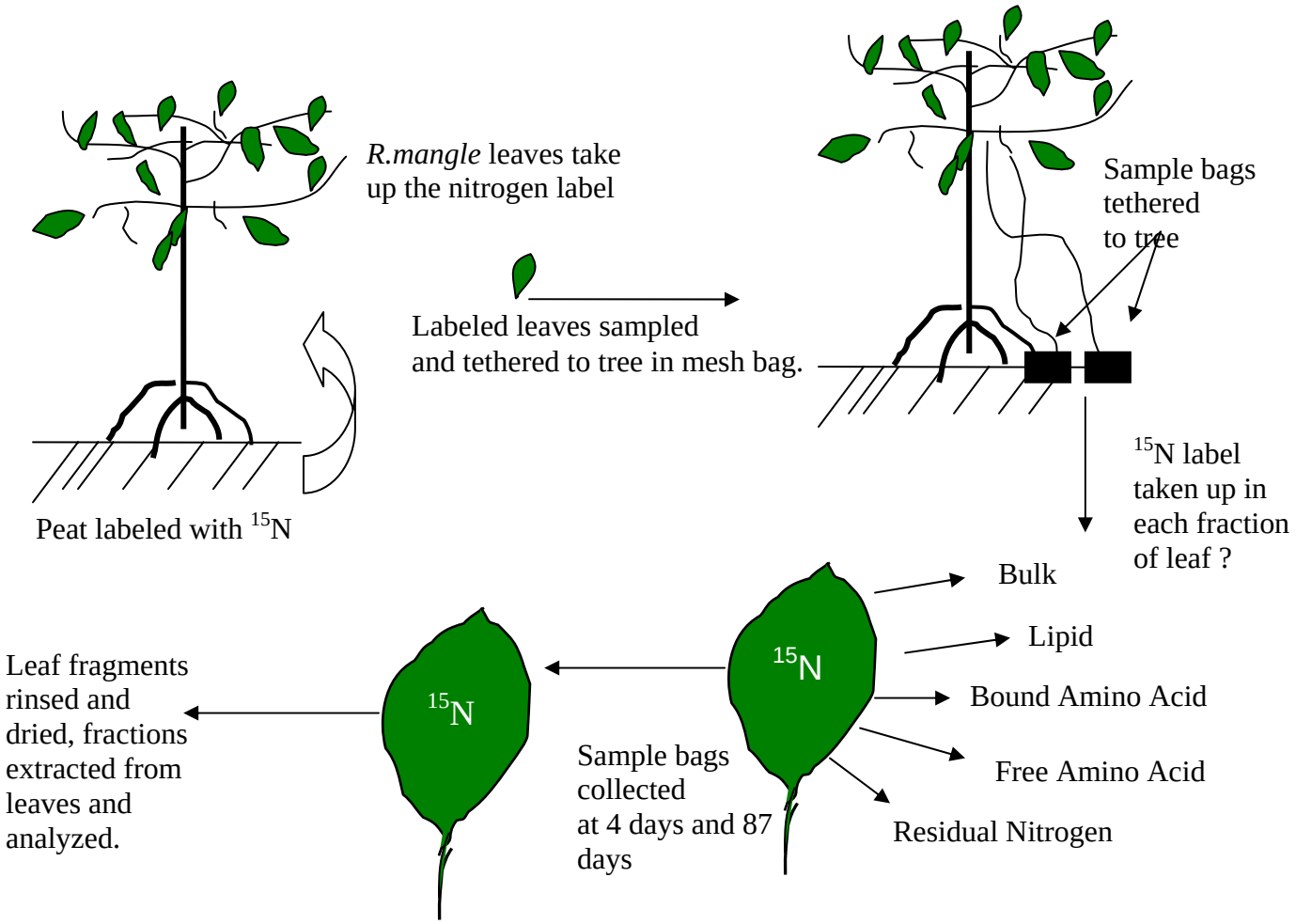

Fig. 3 ^{15}N field labeling experiment materials and methods. . The labeled leaves were removed from a control tree, phosphorus fertilized tree, nitrogen fertilized tree with ^{15}N urea, and a nitrogen plus phosphorus fertilized tree with ^{15}N urea. Labeled leaves were incubated for 4 days and 87 days in 100um mesh bags and tethered on the tree from which they originated.

2.3 Instrumental Analysis

All of the leaf fractions were dried and weighed into tin boats (0.3-1 mg) and crimped. The samples were introduced via the EA carousel[24] into the autosampler and analyzed using a CE instruments, NA 2500 series, elemental analyzer (EA). Isotope ratios of the combustion gases were analyzed using continuous-flow, stable isotope ratio mass spectrometry (Finnigan-Mat, Deltaplus XL). The results were determined using the following calculation:

$$\delta^{h}X = [((X^{h}/X^{i})SAM/(X^{h}/X^{i})STD) - 1] \times 1000$$

where X was either carbon or nitrogen, h was the heavier isotope, i was the lighter isotope, SAM, was the sample (μg/l), and STD was the standard (μg/l). Both N_2 and CO_2 samples were analyzed relative to internal, working gas standards. Stable nitrogen isotope ratios ($\delta^{15}N$) were expressed relative to air and reported in parts per mil (‰). Stable carbon isotope ratios ($\delta^{13}C$) were expressed relative to Pee Dee Belemnite and reported in parts per mil (‰). Acetanalide (C_8H_9NO) was analyzed as a check of accuracy and precision of isotopic ratios and elemental combustion by the EA. Precision for $\delta^{15}N$ was ±0.5% standard deviation (N% = ±0.8 S.D.) and for $\delta^{13}C$ was ±0.1% standard deviation (C% = ±2.9 S.D.).[16]

3. RESULTS: LABORATORY EXPERIMENT

After the 24-hour ^{15}N labeling incubation experiment in the field, the ^{15}N label was present in bulk and all of the molecular fractions of *R. mangle* leaves, however the label was not distributed evenly within each particular leaf fragment (Differences in isotopic labeling were probably the results of the variation in leaf physiology, exposure to the label, and other unknown causes.

Table 1 $\delta^{15}N$ values of bulk fraction of each leaf after the 24-hour incubation. The leaf sample is denoted by the tree number (401, control; 402, control; 408, phosphorus fertilized, 409, phosphorus fertilized). The number following the tree number denotes the sample fragment that was removed from the leaf.

Fraction	Parameter	Leaf Sample				
		401-1	401-2	401-3	401-4	401-5
Bulk	$\delta^{15}N$	193	474	567	352	354
		402-1	402-2	402-3	402-4	402-5
Bulk	$\delta^{15}N$	342	248	159	340	182
		408-1	408-2	408-3	408-4	408-5
Bulk	$\delta^{15}N$	754	215	331	633	694
		409-1	409-2	409-3	409-4	
Bulk	$\delta^{15}N$	408	388	561	492	

The results of the 90-day incubation indicate that there was little variation in $\delta^{13}C$ and %C for each leaf sample over time (ANOVA; $p>0.05$) (Appendix A). The $\delta^{13}C$ values ranged from –28‰ to –25‰ (mean = -26‰±2‰). The average %C of the bulk fraction measured 45% ± 2%, the lipid fraction measured 79%±8%, the bound amino acid fraction measured 17%±3%, the free amino acid measured 25%±8%, and the residual nitrogen fraction measured 61%±7%.

The %N of the total leaf composition was estimated for each fraction using the values obtained from the analytical results and the %N results reported by Smallwood et al., 2004 (Appendix A). The %N of the molecular fractions contributed 42%-73% of the total bulk nitrogen (100%). The %N of the bound amino acid fraction was the highest, measuring 0.28%±0.10% at time 0 and 0.40%±0.15% after 90 days. The %N of the lipid fraction was the smallest, measuring 0.03%±0.015% at time 0 and

0.04%±0.04% after 90 days. The change in %N of Leaf 401 over time is depicted in Figure 4.

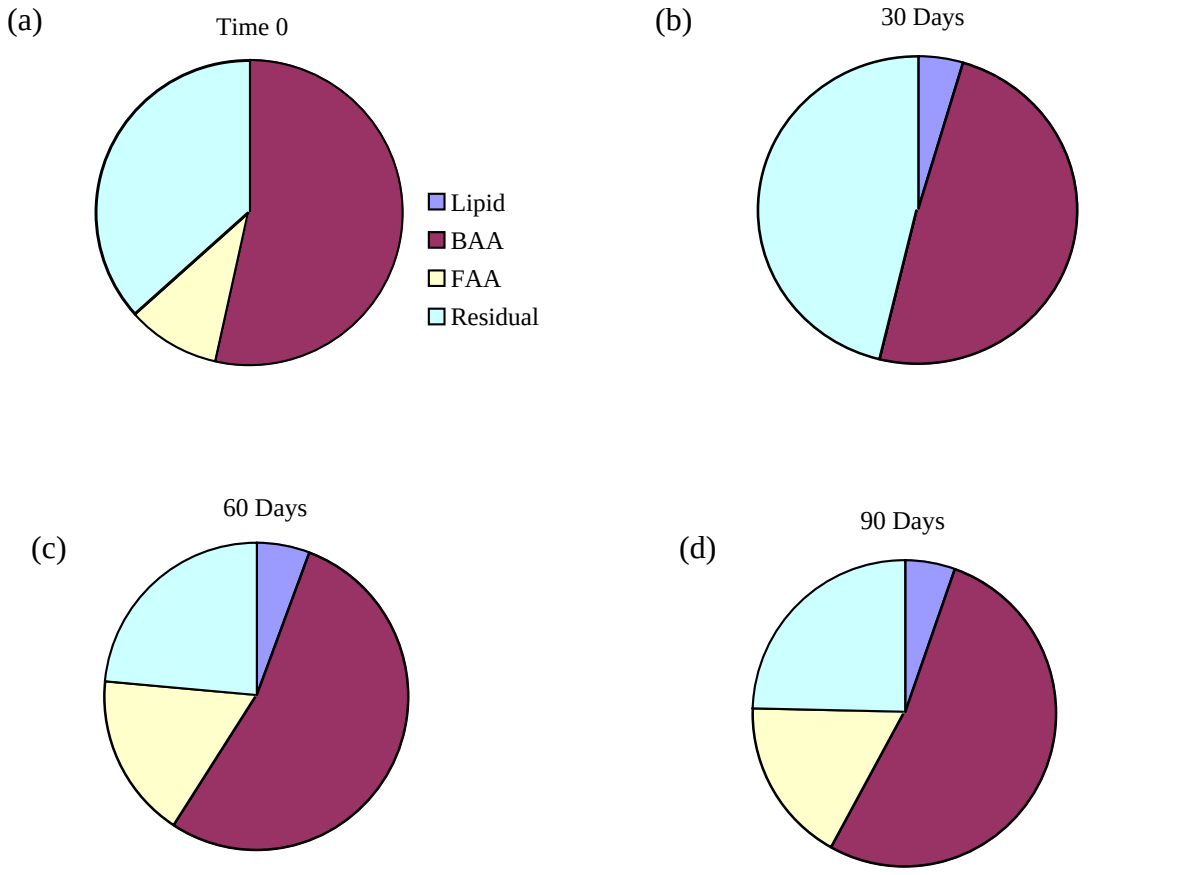

Fig. 4 Leaf 401 %N of leaf composition for the lipid, bound amino acid, free amino acid and residual nitrogen fraction. The results were estimated using the %N determined for each fraction as well as the results reported by Smallwood et al., 2004. 100mg. (a) Time 0, lipid fraction measured below detection. (b) 30 days, free amino acid fraction measured below detection. (c) 60 days. (d) 90 days.

The results of the experiment indicate that the ^{15}N label was present in each fraction during the 90-day incubation, although the label was not distributed evenly, nor was there a pattern of distribution (Appendix A, Fig 5). The $\delta^{15}N$ values were statistically different over time (t-test; $p<0.05$) but not between treatments (ANOVA; $p>0.05$). The bulk $\delta^{15}N$ values ranged from 90‰ to 710‰ (mean = 334‰±152‰). The lipid fraction measured 109‰ to 525‰ (mean = 254‰±137‰). The bound amino acid fraction measured 91‰ to 808‰ (mean = 336‰±188‰). The free amino acid fraction measured 181‰ to 861‰ (mean = 339‰±206‰) and the residual nitrogen fraction ranged from 95‰ to 654‰ (mean = 254‰±161‰).

There is a trend in the data of each particular fraction that shows a decrease in $\delta^{15}N$ values by 90 days. For example, within the leaves from Tree 401, there are negative shifts in $\delta^{15}N$ values of 190‰ (bulk), 230‰ (BAA), to 260‰ (FAA) after 90 days incubation with non-labeled mangrove peat. The $\delta^{15}N$ values did not decrease significantly within the lipid or the residual nitrogen fractions from this tree. Similar data was obtained for the leaves from Tree 408 and 409 as well, but all of the fractions including the lipid and the residual nitrogen material had more negative $\delta^{15}N$ values (Appendix A).

The control samples show a similar trend, even more conclusively, but from an opposite direction. Control leaves had $\delta^{15}N$ values from –0.2‰ to 2‰ for starting bulk tissue. After 90 days of incubation, the $\delta^{15}N$ values were elevated in all the fractions and $\delta^{15}N$ values of the residual nitrogen fraction were similar to the bulk fraction (Appendix B).

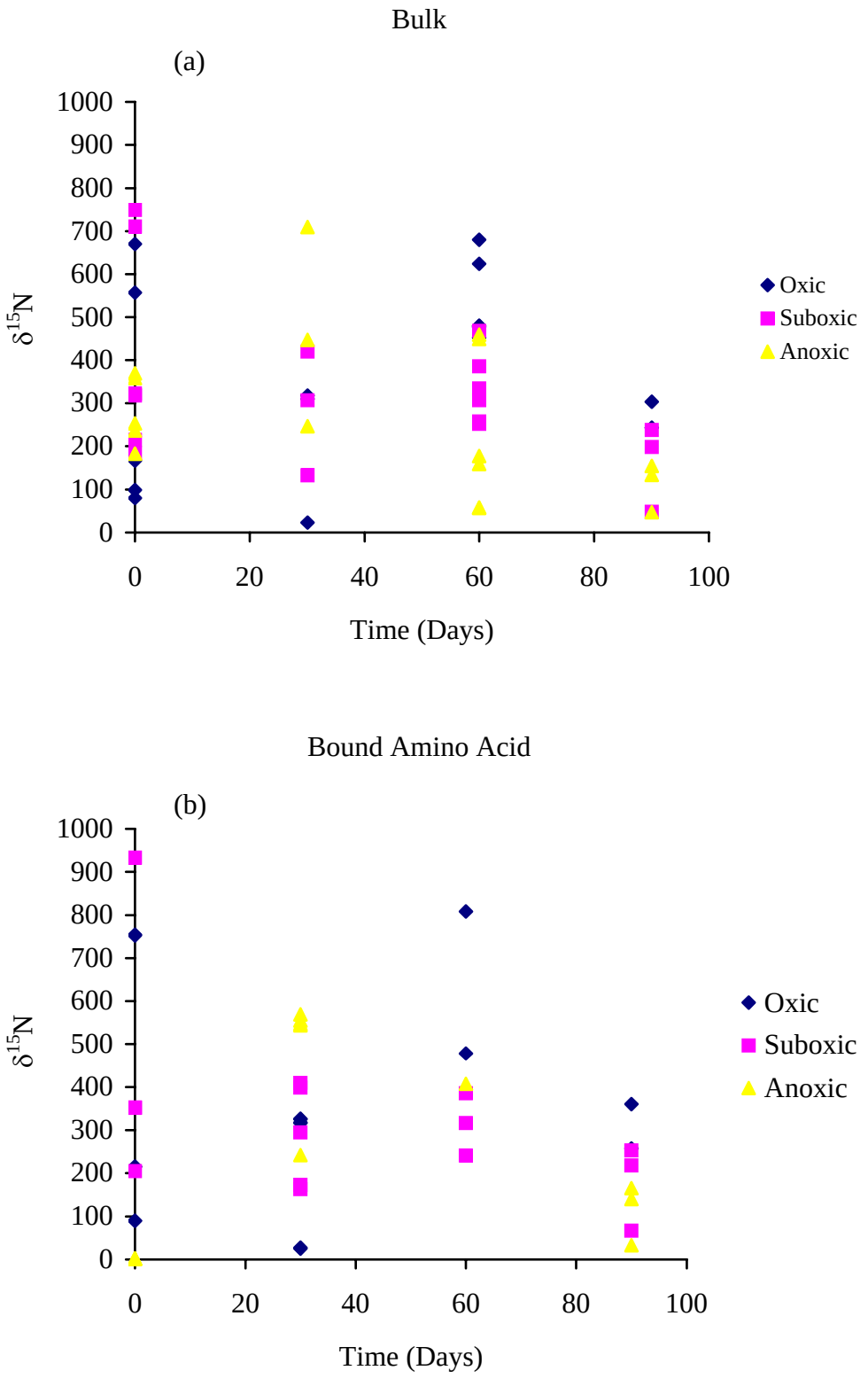

Fig. 5 The $\delta^{15}N$ values of leaves from the ^{15}N laboratory labeling experiment (a) Bulk fraction over time. (b) Bound amino acid fraction over time.

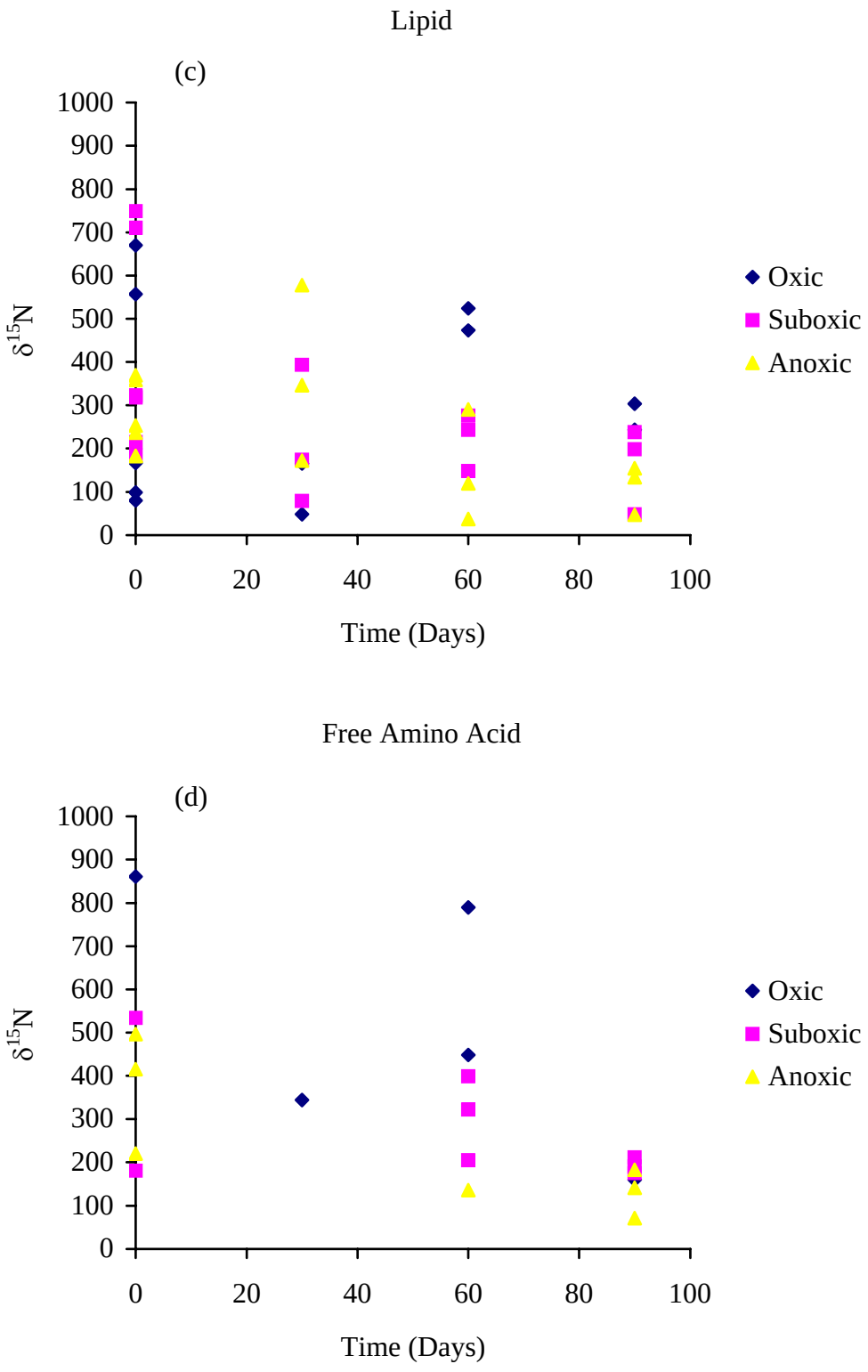

Fig. 5 Continued (c) Lipid fraction over time. (d) Free amino acid fraction over time.

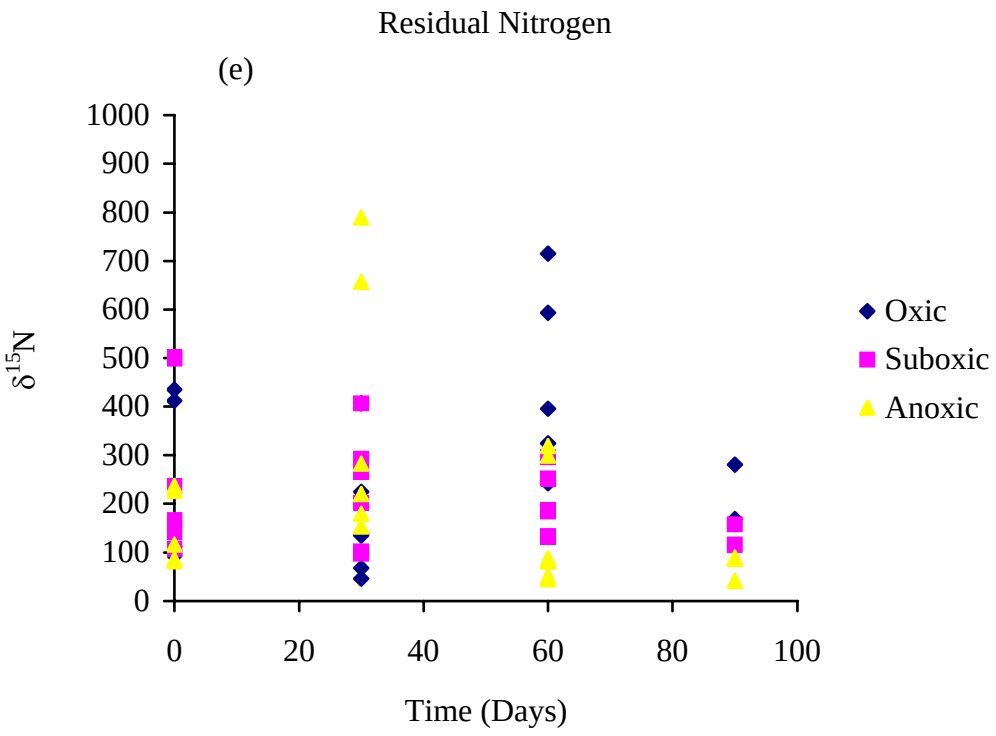

Fig. 5 Continued (e) Residual fraction over time.

3.1 Results: ^{15}N Field Labeling Experiment

The $\delta^{13}C$ results were similar for the nitrogen, phosphorus, nitrogen plus phosphorus, and control leaves, but differed between each fraction. Lipids were always 3-4‰ more negative in $\delta^{13}C$ than bulk tissue, as has been measured previously, and this difference did not change after almost three months incubation. The bound amino acid fraction was always 4‰ more positive in $\delta^{13}C$ than the bulk fraction and also did not change with time. The residual nitrogen $\delta^{13}C$ was nearly identical to the bulk $\delta^{13}C$ value, and almost remained constant with time. There was little variation in %C over time within the fractions (ANOVA; $p>0.05$); the highest %C was found in the lipid fraction (mean = 77% ± 7%) and the smallest amount was in the bound amino acid fraction (mean = 14%±6%) (Appendix C).

The %N of each fraction was measured and used to estimate the %N of the total for each fraction (Fig 6, Appendix C). The molecular fractions contributed 53% to 89% of the total bulk nitrogen (100%). The bound amino acid fraction had the highest %N, averaging 0.34%±0.09% at time 0 and 0.30%±0.1% after 87 days. The lipid fraction had the smallest %N, measuring 0.02% at 4 days and 0.05% after 87 days.

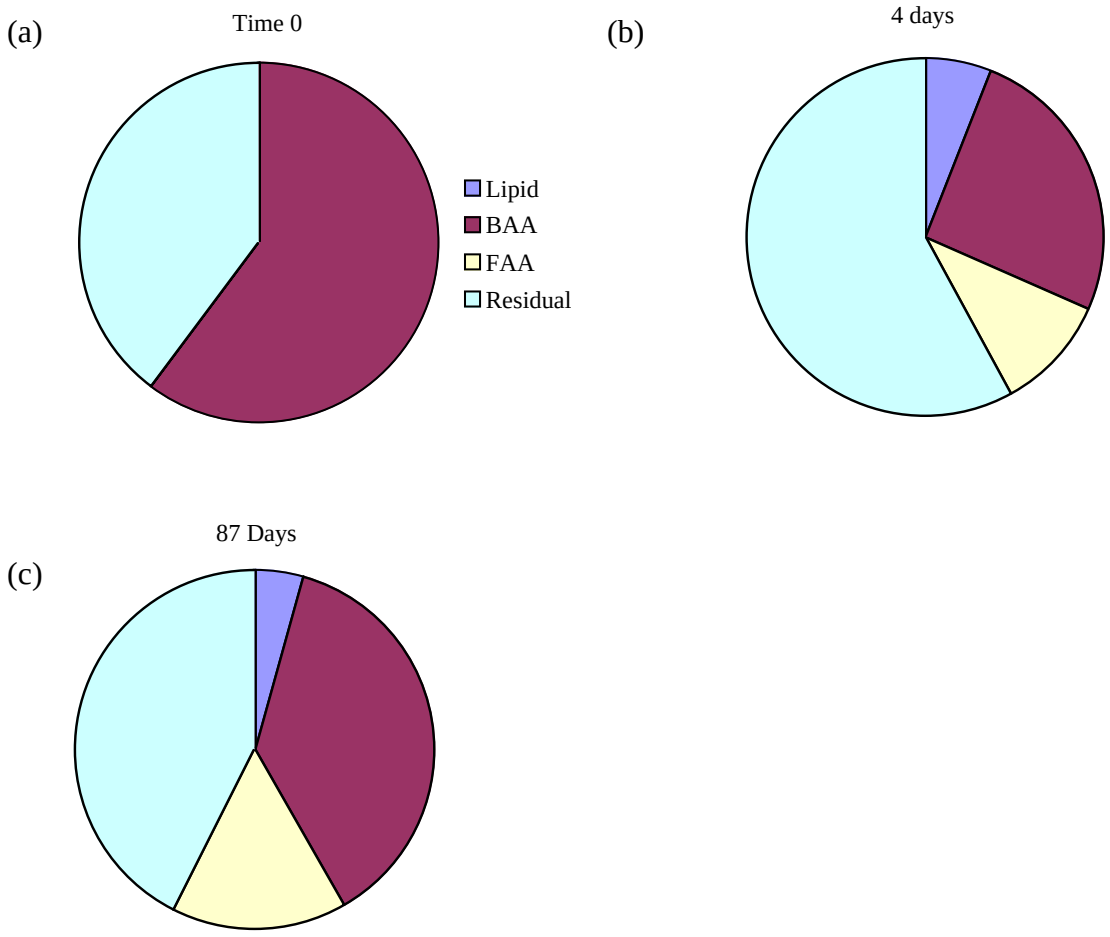

Fig. 6 %N of each fraction from the nitrogen tree (a) Time 0, the lipid fraction was below detection at time 0 and no value was measured for the free amino acid fraction. (b) 4 days. (c) 87 days.

The ^{15}N label was present in each fraction of the nitrogen and nitrogen/phosphorus fertilized trees, although the label was not distributed evenly. The nitrogen plus phosphorus fertilized trees had the highest $\delta^{15}N$ values in all fractions (502‰ to 1842‰). For the ^{15}N-only fertilized trees, the $\delta^{15}N$ values measured 661‰ to 940‰, with a similar decreasing pattern as the leaves fertilized with nitrogen plus phosphorus. The $\delta^{15}N$ values of the nitrogen tree did not vary much between fractions, with the exception of the free amino acid fraction, which was isotopically lighter: $\delta^{15}N$ = 338‰. The $\delta^{15}N$ results for the fractions from all ^{15}N-labeled leaves changed in every fraction over time by about 50% (Fig 7 a and c). The average $\delta^{15}N$ value of the ^{15}N fertilized trees measured 720‰ ± 94 ‰ after 87 days, with the exception of the free amino acid fraction which was isotopically lighter. The $\delta^{15}N$ values of the N only fertilized trees decreased an average of 208‰ after 87 days of incubation, which was somewhat less than that of the nitrogen plus phosphorus tree which decreased an average of 662‰ after 87 days.

The $\delta^{15}N$ results of the phosphorus leaves (-2‰ to 3‰) and the control leaves (-10‰ to -15‰) were at the natural abundance level. The residual nitrogen fraction of the control tree was the lightest fraction measuring -13‰± 2‰, which was reflective of the bulk (-12‰±2). In all of the components holding significant amounts of mangrove nitrogen, the $\delta^{15}N$ increased by several parts per mil after three months incubation. The residual nitrogen fraction, thought to be the most resistant to isotopic exchange[14], changed by only 4%, but the bound amino acid fraction, a biochemical pool of actively metabolized compounds, changed by 8‰.

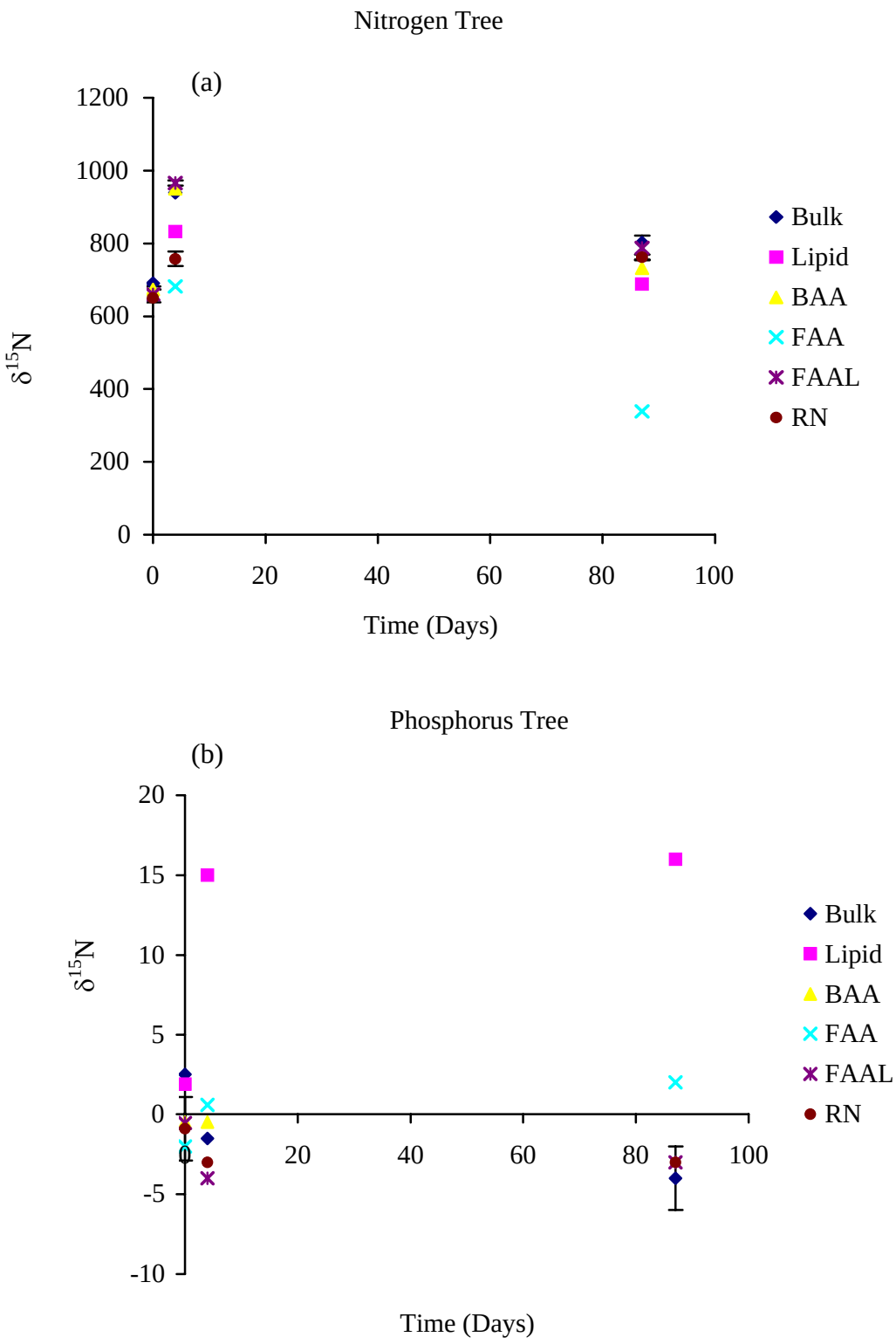

Fig. 7 $\delta^{15}N$ values of leaves from the ^{15}N field labeling experiment. (a) Nitrogen tree. (b) Phosphorus Tree.

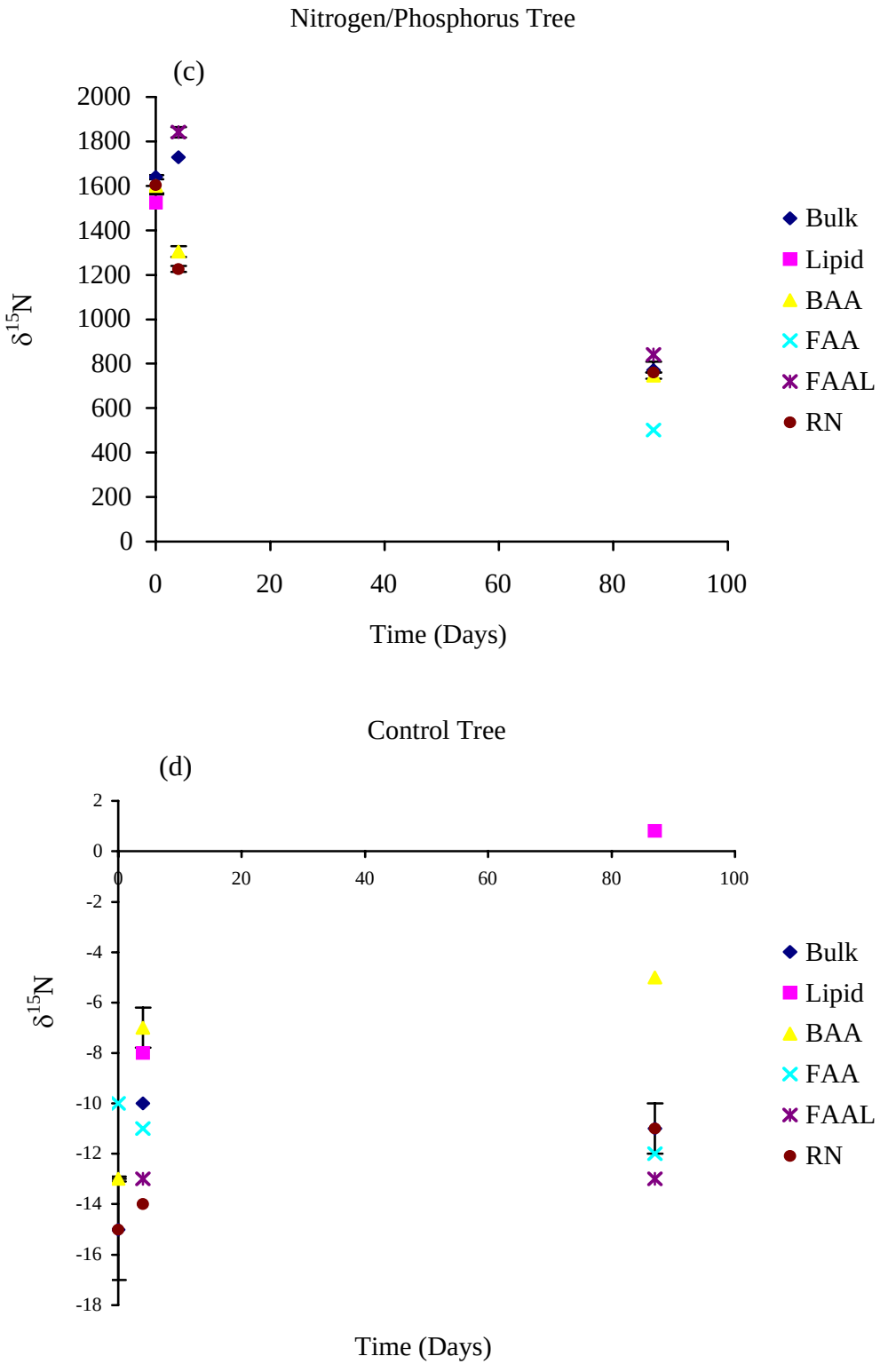

Fig. 7 Continued c) Nitrogen/Phosphorus Tree (d) Control Tree

As a secondary control, a group of unfertilized leaves were hung on the phosphorus fertilized tree and the nitrogen/phosphorus fertilized tree. The control leaves were picked from a group of dwarf *R. mangle* trees some distance away from the treated area. The $\delta^{15}N$ values did not vary significantly over time ($p>0.05$, t-test) and there is no indication that the label was picked up by the control samples. The bulk $\delta^{15}N$ value of the nitrogen/phosphorus fertilized tree measured -10‰ after 4 days. This was reflected in the residual nitrogen and bound amino acid fractions, which measured -10‰ and –11‰ (Appendix E).

As location control, a leaf from each of the treated trees was sampled and tethered to a group of unfertilized trees located approximately 100 yards from the treated Batfish Region. The bulk $\delta^{15}N$, $\delta^{13}C$, %C, %N, and C/N are listed in Appendix D. The results indicate that the label was present in each of the samples after 87 days of incubation. The highest $\delta^{15}N$ value was measured after 87 days in the residual nitrogen fraction of the nitrogen/phosphorus leaf (1540‰±13‰). The variation in $\delta^{15}N$ value within each sample indicates that the label was mobile through each fraction after isolation from the treatment area.

4. CONCLUSION

The results of the laboratory experiment indicate that the ^{15}N was mobile within each biochemical fraction (lipid, bound amino acid, free amino acid, and residual nitrogen) of the *R. mangle* leaves over time, although there was no temporal pattern to the distribution of the label. There was no significant difference between the various treatments ($p>0.05$), although there was an overall decrease in $\delta^{15}N$ over the 90-day incubation of approximately 200‰. The bound amino acid fraction carried the majority of the label (averaging 500‰ at time 0 and 277‰ at 90 days) and retained a similar signature as the bulk fraction (average 414‰ at time 0 and 246‰ at 90 days). The distribution of the ^{15}N label is consistent with the distribution of the %N of each fraction, as the bound amino acid fraction retained the majority of the nitrogen in the measured fractions over time. Smallwood et al., 2004 had similar results, reporting that the bound amino acid fraction contained the majority of the total nitrogen in both the dwarf and tall *R. mangle* samples and the $\delta^{15}N$ values (average = -8.78‰) were similar to the bulk $\delta^{15}N$ values (average = -9.99‰). The residual nitrogen fraction also retained a similar signature as the bulk fraction averaging 234‰ at time 0 and 185‰ after 90 days. Smallwood et al., 2003, report that the un-extractable fraction of the *R. mangle* leaf retains nitrogen and that the $\delta^{15}N$ composition of the un-extractable nitrogen is similar to that of the bulk fraction. Smallwood et al., 2003, also suggests that $\delta^{15}N$ analysis of the residual nitrogen fraction may be used to determine stand structures in mangrove sub-fossil leaves.

The results of the laboratory experiment did indicate the nitrogen was immobilized during the 90-day incubation. Nitrogen immobilization, the microbial conversion of inorganic N (NH_4^+ and NO_3^-) into organic forms during decomposition of organic matter,[25,26] is observed in the marine environment,[27,28,29,30,31] although the process is not well understood.[21] It was evident that even in a controlled laboratory environment nitrogen was immobilized as the label decreased approximately 200‰ after 90-days, which was mirrored by an increase in $\delta^{15}N$ in the control leaves (Appendix A and B).

The overall decrease in ^{15}N label in each of the molecular fractions of the laboratory experiment indicates that the original nitrogen signal was not retained over time.

The results of the field experiment indicate that nitrogen was mobile within each biochemical fraction of the *R. mangle* leaves. There was not an overall decrease in $\delta^{15}N$ of approximately 200‰, as seen in the laboratory experiment, although in general the $\delta^{15}N$ label in the samples of the treated region decreased over time in the leaves of the nitrogen tree and the nitrogen plus phosphorus leaves. The $\delta^{15}N$ label of the samples that were incubated in the untreated region increased in the nitrogen-fertilized leaves and decreased in the leaves from nitrogen plus phosphorus fertilized trees. In both sets of samples (treated and untreated regions) the residual nitrogen and bound amino acid fraction retained a similar signature as the bulk fraction. These results are similar to those of the laboratory experiment and the results reported by Smallwood et al., 2004, in which the bound amino acid fraction and the residual nitrogen fraction has a similar $\delta^{15}N$ signature as the bulk fraction. The majority of the total nitrogen was present in the bound amino acid fraction of the samples from the treated region (Appendix C), although the majority of the nitrogen measured in samples from the untreated region was found in the residual nitrogen fraction. Robertson, 1988, reports that the amount of nitrogen in decomposing mangrove litter increases during the first few months of degradation which may be due to the build of nitrogen in humic compounds that are unavailable to higher consumers.[32] In this experiment, the amount of nitrogen in each fraction varied over time and between each fraction, increasing, decreasing, or experiencing little change over time.

Unlike the laboratory experiment, the leaf fragments of the field experiment were subject to a variety of environmental conditions such as tidal fluctuation, temperature (increasing temperature increases the decay rate of leaf litter[33]), oxygen concentrations, nutrient source, and microbial activity.[34,35] Davis, 2003, reports after 361 days of incubation, that the mass of nitrogen in *R. mangle* leaves increased, which may be due to the long term accumulation of nitrogen into detritus.[36] Tidal inudation may also have an impact on mangrove leaf litter.[35] According to Twilley, 1986, mass loss of leaf litter was accelerated when the litter was constantly inundated, versus the little change that

occurred during the dry season. Nutrient content is also import when considering the decomposition of leaf litter. Fry, 2000, reports on the variation of $\delta^{15}N$ across riverine, fringe, and basin *R. mangle* forests and found that source of nitrogen had a greater impact on fractionation than microbial activity.[37] The various environmental conditions may have had an impact on the variation of %N over time, as well as the variation of $\delta^{15}N$ signature.

The information provided by the field experiment does not provide evidence that suggests that simple interpretation of the ^{15}N label in the molecular fractions of the *R. mangle* leaves could be used to interpret mangrove paleoenvironments, although the information may be useful if diagenetic changes are considered (eg. microbial activity, temperature regime, oxygen concentration). If past mangrove stand structures are going to be examined using $\delta^{15}N$ analysis, it is essential that the original nitrogen signal be maintained over time. Wooller et al., 2004, Smallwood et al., 2003, and Wooller et al., 2003, report that the $\delta^{13}C$ signatures and the $\delta^{15}N$ signature are unique between stand structures (dwarf and tall) and the signature of the sub-fossil leaves is similar to the signature of fresh *R. mangle* leaves. The laboratory and field experiment both provide information that suggests the $\delta^{15}N$ signature of the bound amino acid fraction and the residual nitrogen fraction retain a similar signature as the fresh leaf, but vary too much over time to be a sole proxy in examining mangrove sub-fossil leaves. The use of $\delta^{15}N$ analysis in sediment cores may be of use if microbial activity, tidal variations, temperature and oxygen concentrations are taken into account.

Both the lab and the field experiment indicate that the residual nitrogen fraction is similar to the bulk fraction, but over time the original signature is not retained. The findings of Smallwood et al., 2003, imply that further examination of the residual nitrogen fraction may show promise as an additional proxy in examining past mangrove stand structures. The results of the laboratory experiment and the field experiment do not provide conclusive evidence that supports the findings of Smallwood et al., 2003. The bound amino acid fraction and the residual nitrogen fraction did retain a similar signature as the bulk, but the results suggest that the nitrogen signature varied over time, making it difficult to rely solely on this information to examine mangrove subfossil leaves. Due to

the reflective nature of the residual ^{15}N signature, this may be considered useful in examining past mangrove stand structures if coupled with additional proxies.

Although the results of the laboratory experiment and the field experiment did not provide conclusive evidence that could be used to strengthen the interpretations of Smallwood et al., 2003; Wooller at al., 2003 and Wooller et al., 2004, some interesting results were obtained. Both experiments indicate that nitrogen is mobile within each molecular fraction of the *R. mangle* leaf during decay, but a measurable amount of nitrogen remains in each fraction after three months of incubation. Both the laboratory and field experiment suggest that nitrogen is immobilized after three months of incubation. In addition, insight was gained into the transformation of nitrogen compounds in mangrove leaves during decomposition. Of particular interest was the determination that the ^{15}N label was taken up in each biochemical fraction of the *R. mangle* leaf after 24 hours of incubation. This information could have implications for the translocation of nitrogen in *R. mangle* leaves and should be investigated further. Although simple interpretation if nitrogen isotopes in preserved leaves may not be possible, $\delta^{15}N$ analysis may be considered if diagenetic factors are taken into account.

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APPENDIX

Appendix A.1 The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of leaf 401 (control tree) from 0-90 days. The label letters and numbers following the tree label denote the incubation cup. The first letter indicates environment (O = oxic, S = suboxic). The second letter indicates the incubation cup (A, B, or C). The number represents the time period the sample was removed from the incubation cup (0= time 0, 1 = 30 days, 2 = 60 days, 3 = 90 days). Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf samples (Time 0)			Leaf Samples (30 Days)			Leaf Samples (60 Days)			Leaf Samples (90 Days)		
		401-OC0	401-SA0	401-SB0	401-OC1	401-SA1	401-SB1	401-OC2	401-SA2	401-SB2	401-OC3	401-SA3	401-SB3
Bulk	$\delta^{15}N$	193±38	730±26	320±3	318	420	307	466±21	320±19	427±59	303	198	238
	$\delta^{13}C$	-26±0.5	-27±0.1	-27±0.1	-27	-27	-26	-25±0.3	-27±0.1	-27±0.007	-26±0.2	-25	-27
	%N	1.4±0.03	.90±0.1	1.8±0.9	1.5	1.3	1.2	1.5±0.6	1.1±0.03	1.3±0.007	1.3±0.3	0.7	1
	%C	44±2	44±0.2	44	46	43	44	41±1.2	39±0.8	43±0.3	46±0.6	45	47
	%N of total	1.4	0.9	1.8	1.5	1.3	1.2	1.6	1.1	1.3	1.5	0.7	1.0
	C/N	37±.9	58±3	44±1.2	36	40	44	31±0.3	42±1	40±0.1	41±8	76	54
Lipid	$\delta^{15}N$	122	bd	bd	NA	393	174	474	243	277	272	196	194
	$\delta^{13}C$	-30	-31	-31	NA	-30	-31	-29	-30	-31	-31	-30	-31
	%N	0.34	bd	bd	NA	0.34	0.4	2	0.78	0.45	0.42	0.4	0.2
	%C	90	82	95	NA	79	82	120	81	86	78	67	75
	%N of total	0.03	bd	bd	NA	0.03	0.03	0.2	0.06	0.03	0.03	0.03	0.14
	C/N	307	NA	NA	NA	270	245	60	120	224	217	206	459
FAA	$\delta^{15}N$	bd	2010	535	344	bd	307	448	323	398	NA	176	211
	$\delta^{13}C$	-28	-28	-27	-27	bd	-26	-28	-27	-27	NA	-27	-28
	%N	bd	0.12	0.15	1.2	bd	bd	0.58	0.59	0.9	NA	0.3	0.4
	%C	26	16	22	44	bd	45	26	28	35	NA	24	31
	%N of total	bd	0.04	0.27	0.4	bd	0.18	0.27	0.18	0.27	NA	0.09	0.13
	C/N	NA	153	175	45	bd	44	44	48	39	NA	92	87
FAAL	$\delta^{15}N$	184±17	847	342±27	277	455	213	440±38	323	404	355	196	244±7
	$\delta^{13}C$	-27±0.06	-27	-26±.06	-26	-26	-26	-27±0.1	-26	-26	-26	-25	-27±0.1
	%N	1.9±.02	46	1.7±.04	1.8	1.4	1.7	8±0.2	1.4	1.4	1.8	0.8	1.2±0.14
	%C	47±0.7	1.3	48±.16	47	49	50	54±5	50	50	47	45	51±4
	C/N	29±0.1	43	33±0.06	30	42	35	36±0.5	42	42	30	70	49±2

Appendix A.1 Continued The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of leaf 401 (control tree) from 0-90 days. The label letters and numbers following the tree label denotes the incubation cup. The first letter indicates environment (O = oxic, S = suboxic). The second letter indicates the incubation cup (A, B, or C). The number represents the time period the sample was removed from the incubation cup (0= time 0, 1 = 30 days, 2 = 60 days, 3 = 90 days). Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (Time 0)			Leaf Samples (30 Days)			Leaf Samples (60 Days)			Leaf Samples (90 Days)		
		401-OC0	401-SA0	401-SB0	401-OC1	401-SA1	401-SB1	401-OC2	401-SA2	401-SB2	401-OC3	401-SA3	401-SB3
BAA	$\delta^{15}N$	216±0.8	933	353±0.8	325±0.5	404±8	295	NA	317	385	360	218	253
	$\delta^{13}C$	-23±0.1	-23.6	-22	-25±1	-23±0.09	-23	NA	-21	-22	-22	-22	-22
	%N	2±0.3	0.9	2±0.2	3.2±0.1	1.3±0.05	1.5	NA	2.3	2.5	3.4	1.9	1.9
	%C	15±3	13	15±2	20	10±0.3	12	NA	16	18	20	23	17
	%N of total	0.4	0.2	0.37	0.45	0.26	0.3	NA	0.54	0.35	0.7	0.27	0.27
	C/N	9±0.03	16	10±0.4	7.40	9±0.07	10	NA	7	7.5	7	14	10
RN	$\delta^{15}N$	107±7	501±2	202±49	244	278±19	304±144	360±50	186±3	274±31	280	116	158
	$\delta^{13}C$	-26±0.007	-27±0.04	-27±0.2	-27	-27±0.2	-27±0.6	-27±0.2	-27±0.1	-27±0.2	-26	-25	-27
	%N	0.3±0.02	0.2±0.007	0.18±0.05	0.32	0.4±0.04	0.03±0.08	0.32±0.03	0.35±0.007	0.32±0.04	0.44	0.18	0.3
	%C	66±5	62±0.4	58±6	44	56±10	61±5	71±0.3	70±0.6	70±0.4	59	64	57
	%N of total	0.2	0.14	0.12	0.2	0.25	0.2	0.05	0.23	0.2	0.3	0.13	0.3
	C/N	291±3	373±16	397±70	159	182±22	237±42	261±24	246±7	259±33	157	413	229

Appendix A.2 The molecular compositions, $\delta^{15}N$, and $\delta^{13}C$ values of leaf 402 (control tree) from 0-90 days. The label letters and numbers following the tree label denotes the incubation cup. The first letter indicates environment (O = oxic, A= anoxic). The second letter indicates the incubation cup (A). The number represents the time period the sample was removed from the incubation cup (0= time 0, 1 = 30 days, 2 = 60 days, 3 = 90 days). Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (Time 0)		Leaf Samples (30 Days)		Leaf Samples (60 Days)		Leaf Samples (90 Days)	
Fraction	Parameter	402-OA0	402-AA0	402-OA1	402-AA1	402-OA2	402-AA2	402-OA3	402-AA3
Bulk	$\delta^{15}N$	90±13	245±12	23	710	467±21	57±0.6	1886	47
	$\delta^{13}C$	-26±0	-25±0.2	-25	-25	-25±0.3	-25±0.1	-26	-25
	%N	1.2±0.03	1.2±0.02	1.6	1.3	1.5±0.06	1.6±0.09	1.2	1.5
	%C	45±0.9	46±1.3	48	46	41±1.2	41±0.2	46	47
	%N of total	1.2	1.2	1.6	1.3	1.5	1.6	1.2	1.5
	C/N	44±1.9	44±0.4	35	41	31±0.4	30±1.6	46	38
Lipid	$\delta^{15}N$	179	bd	48	578	NA	37	2089	52
	$\delta^{13}C$	-29	-29	-30	-29	NA	-29	-30	-29
	%N	0.6	bd	0.4	0.3	NA	0.6	0.2	0.5
	%C	75	79	85	79	NA	97	74	61
	%N of total	0.04	bd	0.03	0.02	NA	0.04	0.015	0.04
	C/N	147	NA	275	341	NA	192	511	143
FAA	$\delta^{15}N$	861	497	NA	bd	NA	bd	2328	71
	$\delta^{13}C$	-26	-26	NA	-28	NA	-25	-26	-25
	%N	0.15	0.2	NA	bd	NA	bd	0.4	0.4
	%C	18	15	NA	13	NA	25	21	29
	%N of total	0.07	0.05	NA	bd	NA	bd	0.12	0.13
	C/N	143	98	NA	NA	NA	NA	59	79
FAAL	$\delta^{15}N$	62±15	222±0.7	18	522	581	53±4	2787	34
	$\delta^{13}C$	-25±0.2	-25±0.1	-25	-25	-25	-25±0.1	-26	-25
	%N	1.7±0.08	1.9±0.2	1.8	1.8	1.9	2±0.2	1.3	2
	%C	49±0.6	51±0.6	51	52	57	54±6	51	47
	C/N	34±2	32±4	33	33	35	30±1	48	27

Appendix A.2 Continued The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of leaf 402 (control tree) from 0-90 days. The label letters and numbers following the tree label denotes the incubation cup. The first letter indicates environment (O = oxic, A = anoxic). The second letter indicates the incubation cup (A). The number represents the time period the sample was removed from the incubation cup (0= time 0, 1 = 30 days, 2 = 60 days, 3 = 90 days). Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Sample (Time 0)		Leaf Sample (30 Days)		Leaf Sample (60 Days)		Leaf Sample (90 Days)	
Fraction	Parameter	402-OA0	402-AA0	402-OA1	402-AA1	402-OA2	402-AA2	402-OA3	402-AA3
BAA	$\delta^{15}N$	91±0.6	257	26±2	562±10	479	NA	2820	33
	$\delta^{13}C$	-21±0.007	-22	-21±0.06	-21±0.1	-21	NA	-21	-21
	%N	1.8±0	16	3±0.2	1.8±0.3	2.5	NA	2	3
	%C	15±0.08	14	20±1	14±3	18	NA	16	19
	%N of total	0.37	0.31	0.42	0.13	0.36	NA	0.32	0.42
	C/N	9.7±0.06	13	8±0.2	9±0.2	7	NA	9	7
RN	$\delta^{15}N$	95±3	116	57±15	723±93	244±4	48±3	1725	41
	$\delta^{13}C$	-26±0.05	-26	-26±0.4	-26±0.7	-25±0.3	-26±0.09	-26	-25
	%N	0.2±0.007	0.2	0.2±0.02	0.2±0.02	0.3±0.007	0.5±0.2	0.3	0.4
	%C	64±1.5	63	44±12	44	71±1	70±2	61	62
	%N of total	0.16	0.15	0.14	0.18	0.23	0.39	0.11	0.29
	C/N	317±2	333	259±36	198	249±1	162±61	263	177

Appendix A.3 The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of leaf 408 (phosphorus tree) from 0-90 days. The label letters and numbers following the tree label denotes the incubation cup. The first letter indicates environment (A = Anoxic). The second letter indicates the incubation cup (C). The number represents the time period the sample was removed from the incubation cup (0= time 0, 1 = 30 days, 2 = 60 days, 3 = 90 days). Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Sample (Time 0)	Leaf Sample (30 Days)	Leaf Sample (60 Days)	Leaf Sample (90 Days)
Fraction	Parameter	408-AC0	408-AC1	408-AC2	408-AC3
Bulk	$\delta^{15}N$	363±8	246	454±7	155
	$\delta^{13}C$	-28±0.1	-27	-27±0	-27
	%N	1±0.06	1.6	1.5±0.06	1.5
	%C	42±0.2	47	45±2	47
	C/N	37±2	35	34±0.2	36
Lipid	$\delta^{15}N$	184	172	291	89
	$\delta^{13}C$	-30	-31	-30	-31
	%N	0.5	0.5	0.4	0.2
	%C	79	77	86	73
	%N of total	0.04	0.04	0.003	0.0015
	C/N	189	177	234	372
FAA	$\delta^{15}N$	415	bd	NA	142
	$\delta^{13}C$	-29	-26	NA	-28
	%N	0.2	bd	NA	0.4
	%C	17	23	NA	21
	%N of total	0.06	bd	NA	0.012
	C/N	106	NA	NA	56
FAAL	$\delta^{15}N$	327±32	237	459	154±6
	$\delta^{13}C$	-27±0.03	-26	-27	-27±0.03
	%N	2.3±0.08	2.3	1.8	1.8±0.007
	%C	48±0.3	52	52	51±2
	C/N	24±0.8	26	33	34±1

Appendix A.3 Continued The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of leaf 408 (phophorus tree) from 0-90 days. The label letters and numbers following the tree label denotes the incubation cup. The first letter indicates environment (O = oxic, A = anoxic). The second letter indicates the incubation cup (A). The number represents the time period the sample was removed from the incubation cup (0= time 0, 1 = 30 days, 2 = 60 days, 3 = 90 days). Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Sample (Time 0)	Leaf Sample (30 Days)	Leaf Sample (60 Days)	Leaf Sample (90 Days)
Fraction	Parameter	408-AC0	408-AC1	408-AC2	408-AC3
BAA	$\delta^{15}N$	356±0.007	242	408	165
	$\delta^{13}C$	-24±0.03	-24	-23	-23
	%N	2±0.3	3	2.6	2
	%C	15±2	19	18	15
	%N of total	0.42	0.40	0.06	0.03
	C/N	9±0.06	7	7	8
RN	$\delta^{15}N$	233±7	166±18	308±14	90
	$\delta^{13}C$	-28±0.02	-27±0.3	-26±0.2	-27
	%N	0.24±0.01	0.3±0.09	0.7±0.07	0.4
	%C	62±0.4	57±8	67±0.3	62
	%N of total	0.17	0.02	0.05	0.03
	C/N	302±20	248±49	109±10	202

Appendix A.4 The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of leaf 409 (phosphorus tree) from 0-90 days. The label letters and numbers following the tree label denotes the incubation cup. The first letter indicates environment (O = oxic, S = Suboxic, A = anoxic). The second letter indicates the incubation cup (B, C). The number represents the time period the sample was removed from the incubation cup (0= time 0, 1 = 30 days, 2 = 60 days, 3 = 90 days). Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicates samples that were done in replicate. The ± values indicate standard deviation.

		Leaf samples (Time 0)			Leaf Samples (30 Days)			Leaf Samples (60 Days)			Leaf Samples (90 Days)		
		409-OB0	409-SC0	409-AB0	409-OB1	409-SC1	409-AB1	409-OB2	409-SC2	409-AB2	409-OB3	409-SC3	409-AB3
Bulk	$\delta^{15}N$	614±80	199±22	183±0.4	309	133	447	652±40	255±4	168±13	243	48	133
	$\delta^{13}C$	-28±0.19	-28±0.2	-27±0.2	-28	-27	-27	-28±0.06	-27±0.03	-27±0.07	-27	-27	-27
	%N	1.4±0.05	1.1±0.1	1.4±0.01	1.6	1.6	1.6	1.6±0.02	1.6±0	1.6±0.03	1.7	0.7	1.3
	%C	45±1.6	47±1	44±0.1	48	49	47	46±0.9	46±0.8	46±2	47	44	48
	C/N	38±0.02	48±0.4	38±0.3	36	35	36	33±0.2	34±0.6	34±0.6	33	73	44
Lipid	$\delta^{15}N$	397	146	133	165	78	346	525	148	119	81	bd	109
	$\delta^{13}C$	-30	-31	-30	-31	-31	-30	-29	-31	-30	-30	-30	-31
	%N	0.32	0.54	0.7	1.6	0.6	0.6	1.2	0.6	1.4	0.4	bd	0.7
	%C	77	71	71	261	75	83	92	117	120	78	63	73
	%N of total	0.009	0.04	0.01	0.01	0.05	0.05	0.09	0.05	0.10	0.05	bd	0.05
	C/N	281	152	121	196	149	156	91	235	101	217	NA	122
FAA	$\delta^{15}N$	1514	181	217	bd	bd	bd	789	205	136	160	191	183
	$\delta^{13}C$	-28	-29	-28	-28	-28	-27	-28	-27	bd	-28	-28	-28
	%N	0.2	0.16	0.6	bd	bd	bd	0.5	0.4	0.3	0.3	1.3	0.3
	%C	25	19	37	27	27	22	25	25	bd	14	64	24
	%N of total	0.06	0.04	0.02	bd	bd	bd	0.15	0.12	0.09	0.09	0.4	0.08
	C/N	151	141	74	NA	NA	NA	54	64	NA	61	58	100
FAAL	$\delta^{15}N$	519±47	157±7	175±4	314	129	488	694±23	238	153±3	252	51±4	136
	$\delta^{13}C$	-27±0.1	-27±0.14	-27±0.1	-28	-27	-27	-28±0.3	-27	-27±0.04	-27	-26±0.2	-27
	%N	3±1.6	1.6±0.1	2±0.1	1.8	2	2	1.8±0.13	2	2±0.2	1.8	0.7±0.01	1.9
	%C	47±2	50±2	49±0.05	50	50	51	56±4	52	56±6	47	44±0.1	47
	C/N	21±11	36±0.7	26±1	33	29	28	37±0.09	32	31±0.1	30	69±1	30

Appendix A.4 Continued The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of leaf 409 (phosphorus tree) from 0-90 days. The label letters and numbers following the tree label denotes the incubation cup. The first letter indicates environment (O = oxic, S = Suboxic, A = anoxic). The second letter indicates the incubation cup (B, C). The number represents the time period the sample was removed from the incubation cup (0= time 0, 1 = 30 days, 2 = 60 days, 3 = 90 days). Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicates samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (Time 0)			Leaf Samples (30 Days)			Leaf Samples (60 Days)			Leaf Samples (90 Days)		
		409-OB0	409-SC0	409-AB0	409-OB1	409-SC1	409-AB1	409-OB2	409-SC2	409-AB2	409-OB3	409-SC3	409-AB3
BAA	$\delta^{15}N$	755±0.9	205	196±0.4	317±0.8	168±7	546±2	808	241	NA	257	66	140
	$\delta^{13}C$	-24±0.2	-24	-24±0.2	-24±0.04	-24±0.2	-23±0.05	-23	-22	NA	-23	-23	-23
	%N	2±0.2	2	1.9±0.4	3±0.5	4±0.2	3.5±0.05	3	3	NA	3	1.6	3
	%C	17±1	20	14±3	19±2.9	23±1	19±0.7	18	18	NA	17	18	19
	%N of total	0.40	0.30	0.30	0.60	0.50	0.50	0.50	0.40	NA	0.40	0.30	0.50
	C/N	8±0.03	10	9±0.06	6.7±0.02	7±0.05	6±0.1	6	6	NA	6	14	7
RN	$\delta^{15}N$	423±16	123±23	85±3	137±3	100±4	251±44	654±86	133±2	84±4	168	NA	87
	$\delta^{13}C$	-28±0.1	-27±0.1	-27±0.04	-27±0.3	-27±0.4	-27±0.3	-28±0.05	-27±0.04	-27±0.1	-28	NA	-27
	%N	0.23±0	0.2±0.007	0.2±0.007	0.3±0.1	0.2±0.03	0.25±0.13	0.7±0.3	0.8±0.05	0.3±0.007	0.3	NA	0.3
	%C	63±0.3	62±0.2	63±0.7	53±24	52±9	51±30	66±5	66±0.2	72±0.06	61	NA	62
	%N of total	0.16	0.14	0.14	0.20	0.14	0.18	0.50	0.60	0.50	0.20	NA	0.20
	C/N	318±2	322±9	325±7	231±3	286±9	233±23	130±68	91±6	250±6	228	NA	257

Appendix B The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of the oxic, suboxic, and anoxic control samples from 0-90 days. Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicates samples that were done in replicate. The ± values indicate standard deviation.

		Control Samples (Time 0)			Control Samples (30 Days)			Control Samples (60 Days)			Control Samples (90 Days)		
Fraction	Parameter	Oxic	Suboxic	Anoxic	Oxic	Suboxic	Anoxic	Oxic	Suboxic	Anoxic	Oxic	Suboxic	Anoxic
Bulk	$\delta^{15}N$	0.45±1.6	-0.21	2±4	5	0.8	-1.3	42±3	25±4	15±2	51	7	4
	$\delta^{13}C$	-29±0.2	-29±0.08	-28±0.2	-27	-28	-28	-29±0.1	-28±0.04	-28±0.08	-28	-29	-28
	%N	0.4±0.1	0.35	0.4	0.4	0.5	0.5	0.49±0	0.42±0	0.46±0.01	0.4	0.5	0.5
	%C	44±0.4	41±0.32	46±1	42	46	47	42±0.2	42±0.3	47±1	43	47	46
	% N of total	0.4	0.35	0.4	0.4	0.5	0.5	0.5	0.4	0.5	0.4	0.5	0.5
	C/N	135±4	46±1.4	138±4	117	109	118	100±0.5	116±0.8	119±0.8	118	112	120
Lipid	$\delta^{15}N$	7	bd	7	bd	17	bd	21	bd	10	bd	bd	21
	$\delta^{13}C$	-33	-33	-31	-31	-32	-31	-32	-32	-31	-33	-33	-32
	%N	0.09	bd	0.2	bd	0.12	bd	0.3	bd	0.4	bd	bd	0.04
	%C	76	83	70	76	79	89	85	92	81	83	80	79
	%N of total	0.007	NA	0.015	bd	0.009	bd	0.002	bd	0.003	NA	NA	NA
	C/N	984	NA	390	NA	768	NA	292	NA	248	NA	NA	1979
FAA	$\delta^{15}N$	-2	-0.4	5	bd	Bd	bd	23±2	6±2	5	79	NA	6
	$\delta^{13}C$	-29	-29	-27	-28	-28	-27	-28±0.07	-29±0.3	-29	-28	NA	-28
	%N	0.08	0.3	0.3	bd	Bd	bd	0.4±0.1	0.2±0.04	0.2	0.2	NA	0.3
	%C	18	21	57	9	45	22	34±10	19±4	29	19	NA	33
	%N of total	0.02	0.09	0.09	bd	Bd	bd	0.12	0.06	0.06	0.06	0.09	0.09
	C/N	263	66	208	NA	NA	bd	86±2	88±0.8	119	101	NA	113
FAAL	$\delta^{15}N$	0.2±0.08	3±0.02	0.5±0.2	4	0.6	-1.2	36±2	6±3	1±0.5	46	16	7
	$\delta^{13}C$	-28±0.1	-28±0.02	-28±0.06	-27	-28	-27	-29±0.8	-28±0.1	-28±0.2	-28	-28	-29±0.1
	%N	0.54	0.5±0.1	0.5±0.03	0.4	0.5	0.4	0.6±0.06	0.5±0.03	0.6±0.06	0.4	0.5	0.5±0.01
	%C	49±1	48±0.6	51±0.06	45	47	48	53±2	52±6	58±6	46	48	50±0.5
	C/N	105±2	110±2	111±6	147	117	130	107±0.1	120±7	118±0.8	124	105	127±5

Appendix B The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of the oxic, suboxic, and anoxic control samples from 0-90 days. Mean values indicates samples that were done in replicate. The ± values indicate standard deviation.

		Control Samples (Time 0)			Control Samples (30 Days)			Control Samples (60 Days)			Control Samples (90 Days)		
Fraction	Parameter	Oxic	Suboxic	Anoxic	Oxic	Suboxic	Anoxic	Oxic	Suboxic	Anoxic	Oxic	Suboxic	Anoxic
BAA	$\delta^{15}N$	2.6±0.4	3±1	2±0.2	34±3	34±3	8±3	86	57	9	70	15	16
	$\delta^{13}C$	-24±0.1	-24±0.4	-24±0.1	-23±0.4	-23±0.3	-22±0.1	-23	-22	-22	-22	-24	-23
	%N	0.7±0.06	0.6±0.007	0.6±0.02	0.8±0.01	0.9±0.2	0.6±0.05	0.6	0.9	0.9	0.8	1	1.5
	%C	14±1	11	17±0.7	16±3	16±3	15±1	12	15	18	14	17	16
	% N of total	0.15	0.13	0.09	0.17	0.17	0.19	0.13	0.19	0.19	0.17	0.2	0.3
	C/N	24±0.1	21±0.3	32±0.1	25±4	20±0.8	27±0.3	18	17	20	21	17	13
RN	$\delta^{15}N$	-3.2±6	2±0.7	-2±1	72	119	70	19±8	4±2	4	27	2	5
	$\delta^{13}C$	-29±0.1	-29±0.03	-28±0.2	-27±0.1	-28	-27±0.3	-28±0.2	-28±0.1	-27±0.3	-28	-29	-27
	%N	0.13	0.12	0.16	0.13±0.01	0.15	0.2±0.01	0.2±0.04	0.2±0.007	0.2±0	0.1	0.2	0.2
	%C	63±0.7	63±2	62±2	48±9	NA	57±2	70±3	71±1	72±1	58	60	57
	%N of total	0.09	0.08	0.08	0.09	0.1	0.14	0.14	0.14	0.14	0.07	0.14	0.14
	C/N	567±6	609±14	461	428±33	NA	335±11	344±76	403±8	399±5	618	438	418

Appendix C The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of the leaves from the ^{15}N labeling field experiment (treated region). C1, indicates the leaf sample from the control tree, N1 indicates the leaf sample from the nitrogen fertilized tree, P1 indicates the leaf sample from the phosphorus fertilized tree, and NP1 indicates the leaf sample from the nitrogen plus phosphorus fertilized tree. Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicates samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (Time 0)				Leaf Samples (4 Days)				Leaf Samples (87 Days)			
Fraction	Parameter	C1	N1	P1	NP1	C1	N1	P1	NP1	C1	N1	P1	NP1
Bulk	$\delta^{15}N$	-15	691	2.5	1641	-10	940	-1.5	1730	-11±1	802±25	-4±2	770±38
	$\delta^{13}C$	-25	-27	-26	-26	-25	-27	-26	-28	-26±0.1	-28±0.06	-25±0.08	-26±0.5
	%N	0.65	0.9	0.8	1.2	0.7	1.0	0.4	0.5	1±0.1	2±0.08	0.4±0.03	0.5±0.04
	%C	46	42	46	52	45	43	47	46	49±0.6	50±1	46±0.3	50±2
	C/N	83	53	64	49	73	50	148	110	51±5	36±1	130±8	121±16
Lipid	$\delta^{15}N$	bd	bd	1.9	1525	-8	833	15	bd	0.9	689	16	bd
	$\delta^{13}C$	-29	-31	-29	-30	-29	-31	-30	-31	-29	-31	-29	-30
	%N	bd	bd	0.2	0.2	0.18	0.3	0.08	bd	0.04	0.7	0.09	bd
	%C	83	84	38	84	76	62	77	68	80	71	80	82
	% N of total	bd	bd	0.015	0.015	0.014	0.02	0.006	bd	0.003	0.05	0.0067	bd
	C/N	NA	NA	234	493	492	267	1116	NA	2347	114	1037	NA
FAA	$\delta^{15}N$	-10	NA	-2	NA	-11	682	0.6	NA	-12	338	2	502
	$\delta^{13}C$	-26	NA	-27	NA	-25	-28	-27	NA	-26	-27	-25	-27
	%N	0.3	NA	0.2	NA	0.2	0.25	0.25	NA	0.9	0.6	0.25	0.3
	%C	39	NA	33	NA	30	25	37	NA	38	19	20	24
	% N of total	0.09	NA	0.06	NA	0.03	0.037	0.075	NA	0.027	0.002	0.08	0.09
	C/N	161	NA	211	NA	159	119	176	NA	51	34	93	98
FAAL	$\delta^{15}N$	NA	661±22	-0.54	NA	-13	966±7	-4	1842±23	-13	788±34	-3	840
	$\delta^{13}C$	NA	-27±0.3	-26	NA	-24	-27±0.09	-26	-28±0.2	-26	-27±0.4	-25	-26
	%N	NA	2.3±1.7	1.2	NA	0.8	1±0.08	0.4	0.7±0.02	1	1.5±0.08	0.4	0.4
	%C	NA	106±82	50	NA	48	47±0.2	53	49±0.08	48	46±0.4	48	49
	C/N	NA	51±2	47	NA	69	49±4	150	87±3	52	37±2	125	140

Appendix C Continued The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of the leaves from the ^{15}N labeling field experiment (treated region). C1, indicates the leaf sample from the control tree, N1 indicates the leaf sample from the nitrogen fertilized tree, P1 indicates the leaf sample from the phosphorus fertilized tree, and NP1 indicates the leaf sample from the nitrogen plus phosphorus fertilized tree. Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicates samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (Time 0)				Leaf Samples (4 Days)				Leaf Samples (87 Days)			
Fraction	Parameter	C1	N1	P1	NP1	C1	N1	P1	NP1	C1	N1	P1	NP1
BAA	$\delta^{15}N$	-13±0.1	674±0.2	-0.5±0.4	1599±31	-7±0.8	950	-0.5±0.03	1305±24	-5	732	NA	745
	$\delta^{13}C$	-21±0.03	-23±0.1	-23±0.5	-22±0.9	-21±0.3	-23	-22±0.5	-22±0.2	-20	-24	NA	-22
	%N	1.6±0.2	1±0.1	2±0.2	1.8±0.4	0.6±0.2	0.65	0.8±0.03	0.7±0.05	2	3	NA	1.4
	%C	21±2	18±1	25±1	19±6	8±2	8	16±0.7	10±0.5	9	13	NA	12
	% N of total	0.34	0.21	0.43	0.38	0.09	0.09	0.11	0.10	0.28	0.42	NA	0.20
	C/N	16±0.7	19±0.8	17±1	13±1	16±0.3	14	24±0.008	17±0.4	6	5	NA	14
RN	$\delta^{15}N$	-15±2	651	-0.9±3	1605±43	-14	758±20	-3	1227±13	-11	763±7	-3	759±0.01
	$\delta^{13}C$	-25±0.1	-27	-26±0.1	-26±0.01	-24	-27±0.006	-25	-28±0.08	-25	-28±0.09	-25	-26±0.1
	%N	0.2±0.007	0.2	0.2±0.007	0.19±0	0.2	0.3±0.04	0.16	0.16±0	1	0.7±0.07	0.8	0.14±0
	%C	60±0.5	61	61±0.06	60±0.5	61	61±0.9	64	62±0.08	48	53±0.7	111	54±0.7
	% N of total	0.14	0.14	0.14	0.13	0.21	0.30	0.11	0.11	0.70	0.49	0.56	0.10
	C/N	398±20	325	331±11	368±3	356	248±40	467	450±0.6	54	86±9	163	454±6

Appendix D The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of the leaves from the ^{15}N labeling field experiment (untreated region). C2, indicates the leaf sample from the control tree, N2 indicates the leaf sample from the nitrogen fertilized tree, P2 indicates the leaf sample from the phosphorus fertilized tree, and NP2 indicates the leaf sample from the nitrogen plus phosphorus fertilized tree. Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (Time 0)				Leaf Samples (4 Days)				Leaf Samples (87 Days)			
Fraction	Parameter	C2	N2	P2	NP2	C2	N2	P2	NP2	C2	N2	P2	NP2
Bulk	$\delta^{15}N$	-15	691	2.5	1641	-8.88	802	3	1569	-9±0.1	1024±9	2.5±2	1445±60
	$\delta^{13}C$	-25	-27	-26	-26	-26	-27	-27	-27	-26±0.1	-27±0.08	-27±0.3	-27±0.2
	%N	0.65	0.9	0.8	1.2	0.6	0.5	0.5	0.5	0.7±0.03	0.8±0.04	0.7±0.01	0.5±0.007
	%C	46	42	46	52	46	47	48	49	43±0.1	49±0.5	48±2	46±1
	C/N	83	53	64	49	92	110	124	124	74±3	73±3	84±5	102±1
Lipid	$\delta^{15}N$	bd	bd	1.9	1525	8	bd	14	1212	-12	909	bd	1004
	$\delta^{13}C$	-29	-31	-29	-30	-30	-31	-30	-30	-29	-31	-31	-30
	%N	bd	bd	0.2	0.2	0.14	bd	0.13	0.1	0.09	0.2	bd	0.2
	%C	83	84	38	84	76	72	72	73	80	77	75	153
	% N of total	bd	bd	0.15	0.15	0.10	NA	0.09	0.08	0.07	0.20	NA	0.1
	C/N	NA	NA	234	493	634	NA	648	854	2347	409	NA	941
FAA	$\delta^{15}N$	-10	NA	-2	NA	NA	NA	2.6	NA	-10	911	bd	1108
	$\delta^{13}C$	-26	NA	-27	NA	NA	NA	-28	NA	-26	-27	-28	-27
	%N	0.3	NA	0.2	NA	NA	NA	0.2	NA	0.2	0.2	bd	0.2
	%C	39	NA	33	NA	NA	NA	38	NA	14	13	18	18
	% N of total	0.90	NA	0.60	NA	NA	NA	0.80	NA	0.30	0.30	NA	0.30
	C/N	161	NA	211	NA	NA	NA	211	NA	69	70	NA	100
FAAL	$\delta^{15}N$	NA	661±22	-0.54	NA	NA	1062±53	2.5	1313±155	-10	1057	0.6	1540±13
	$\delta^{13}C$	NA	-27±0.3	-26	NA	NA	-27±0.07	-27	109	-25	-27	-27	-27±0.1
	%N	NA	2.3±1.7	1.2	NA	NA	0.6±0.2	0.5	0.5±0.03	0.7	0.3	0.6	0.6±0.007
	%C	NA	106±82	50	NA	NA	50±14	50	50±0.08	47	16	50	51±0.5
	C/N	NA	51±2	47	NA	NA	95±11	109	111±2	80	67	102	101±0.2

Appendix D Continued The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of the leaves from the ^{15}N labeling field experiment (untreated region). C2, indicates the leaf sample from the control tree, N2 indicates the leaf sample from the nitrogen fertilized tree, P2 indicates the leaf sample from the phosphorus fertilized tree, and NP2 indicates the leaf sample from the nitrogen plus phosphorus fertilized tree. Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (Time 0)				Leaf Samples (4 Days)				Leaf Samples (87 Days)			
Fraction	Parameter	C2	N2	P2	NP2	C2	N2	P2	NP2	C2	N2	P2	NP2
BAA	$\delta^{15}N$	-13±0.1	674±0.2	-0.5±0.4	1599±31	-9±2	NA	208	1305±24	-8	1057	11	1505
	$\delta^{13}C$	-21±0.03	-23±0.1	-23±0.5	-22±0.9	-21±0.8	NA	-23	-22±0.2	-19	-23	-23	-21
	%N	1.6±0.2	1±0.1	2±0.2	1.8±0.4	1.0±0.1	NA	0.5	0.5±0.04	1.4	1.5	1.4	NA
	%C	21±2	18±1	25±1	19±6	14-±2	NA	8	11±1	16	14	12	NA
	% N of total	0.30	0.20	0.40	0.40	0.70	NA	0.10	0.40	0.10	0.10	0.10	NA
	C/N	16±0.7	19±0.8	17±1	13±1	17±0.2	NA	18	24±1	14	11	10	NA
RN	$\delta^{15}N$	-15±2	651	-0.9±3	1605±43	NA	NA	-0.6±1.0	1118±56	-9±1.5	967±14	4	1560±3
	$\delta^{13}C$	-25±0.1	-27	-26±0.1	-26±0.01	NA	NA	-27±0.1	-26±0.04	-25±0.2	-27±0.05	-27	-26±0.04
	%N	0.2±0.007	0.2	0.2±0.007	0.19±0	NA	NA	0.2±0.02	0.18±0.03	0.2±0.06	0.2±0.01	0.3	0.18±0
	%C	60±0.5	61	61±0.06	60±0.5	NA	NA	61±3	74±15	58±6	57±0.2	55	57±0.2
	% N of total	1.4	1.4	1.4	1.0	NA	NA	1.0	1.0	1.0	1.0	2.0	0.10
	C/N	398±20	325	331±11	368±3	NA	NA	408±31	476±21	295±99	353±25	246	370±1

Appendix E The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of the control leaves from the ^{15}N labeling field experiment. P1 indicates unlabeled leaf that was hung on the phosphorus fertilized tree. NP1 indicates the unlabeled leaf that was hung on the nitrogen plus phosphorus fertilized tree. Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (4 Days)		Leaf Samples (87 Days)	
Fraction	Parameter	P1-Control	NP1-Control	P1-Control	NP1-Control
Bulk	$\delta^{15}N$	NA	-10	-10±0.2	-7±4
	$\delta^{13}C$	NA	-27	-25±0.1	-25±0.01
	%N	NA	0.5	0.7±0.01	0.5±0.05
	%C	NA	48	48±0.06	46±2
	C/N	NA	112	77±1	98±5
Lipid	$\delta^{15}N$	NA	-8	bd	bd
	$\delta^{13}C$	NA	-29	-29	-29
	%N	NA	0.1	bd	bd
	%C	NA	78	82	82
	C/N	NA	831	NA	NA
FAA	$\delta^{15}N$	-12	-6	10	-4
	$\delta^{13}C$	-25	-27	-25	-25
	%N	0.3	0.2	0.4	0.3
	%C	33	35	26	14
	C/N	142	177	70	60
FAAL	$\delta^{15}N$	-13	-13	-11	-9
	$\delta^{13}C$	-23	-27	-25	-26
	%N	0.4	0.5	0.7	0.6
	%C	46	44	50	49
	C/N	135	104	84	99

Appendix E Continued The molecular compositions, $\delta^{15}N$ and $\delta^{13}C$ values of the control leaves from the ^{15}N labeling field experiment. P1 indicates unlabeled leaf that was hung on the phosphorus fertilized tree. NP1 indicates the unlabeled leaf that was hung on the nitrogen plus phosphorus fertilized tree. Bd indicates values that measured below detection, NA indicates values that were not measured. Mean values indicate samples that were done in replicate. The ± values indicate standard deviation.

		Leaf Samples (4 Days)		Leaf Samples (30 Days)	
Fraction	Parameter	P1-Control	NP1-Control	P1-Control	NP1-Control
BAA	$\delta^{15}N$	6	-11	1	NA
	$\delta^{13}C$	-19	-20	-19	NA
	%N	0.85	0.6	1	NA
	%C	16	13	11	NA
	C/N	22	24	11	NA
RN	$\delta^{15}N$	-6	-10	-13	-9
	$\delta^{13}C$	-24	-25	-25	-24
	%N	0.2	0.2	0.2	0.4
	%C	63	63	56	53
	C/N	432	352	270	172

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