Annual Report for Period: 06/2002 - 06/2003
Submitted on: 04/15/2003
Principal Investigator: Feller, Ilka C.
Award ID: 9981535
Organization: Smithsonian Institution
Title:
BIOCOMPLEXITY: Collaborative Research: Microbial and Nutrient Controls in Mangrove Ecosystems

Project Participants

Senior Personnel

Name: Feller, Ilka
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Fogel, Marilyn
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Lovelock, Catherine
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Urish, Daniel
Worked for more than 160 Hours: Yes
Contribution to Project:
Dan Urish surveyed the island and measured the bathymetry of Twin Cays

Name: Wright, Raymond
Worked for more than 160 Hours: Yes
Contribution to Project:
Ray Wright measured flow rates and tidal exchange across Twin Cays

Post-doc

Name: Wooller, Matthew
Worked for more than 160 Hours: Yes
Contribution to Project:
Wooller is working with Marilyn Fogel doing stable isotope analysis for the biocomplexity project. He helped Chamberlain build the walkway through our mangrove field site.

Name: Scharler, Ursula
Worked for more than 160 Hours: Yes
Contribution to Project:
Scharler is serving as data manager for the mangrove biocomplexity project. She is working with all the PI's to produce metadata for the data they generate during the project. She is also working on characterizing the macrozoobenthos associated with the mangrove study site at Twin Cays

Graduate Student

Name: Pittek, Mary
Worked for more than 160 Hours: Yes
Contribution to Project:
Pittek has revised her research plans. She is working on her M.s. within the mangrove biocomplexity project. She is comparing nutrient dynamics in mangrove forests subjected to different management practices at our satellite site in the Indian River Lagoon.

analyses.

Name: Rodriguez, Wilfrid

Worked for more than 160 Hours: Yes

Contribution to Project:

Wilfrid Rodriguez built a geospatial database and mapped the hydrology and bathymetry of Twin Cays, Belize

Undergraduate Student

Name: Akob, Denise

Worked for more than 160 Hours: Yes

Contribution to Project:

Akob did her senior research project within the mangrove biocomplexity project. She investigated the presence and expression of Nif genes in cyanobacteria associated with sponge epibionts on mangrove prop roots. She received a grant to attend the 2002 ASLO/AGU meeting in Hawaii where she presented her results in a poster. Here presentation took the first place award in the undergraduate poster session. During Summer 2002, she will work in Fogel's laboratory to learn stable isotope techniques. She will use stable isotopes to assess fiddler crab trophic connections.

Name: Clancy, Amanda

Worked for more than 160 Hours: No

Contribution to Project:

Mandy Clancy is a summer 2002 undergraduate intern at SERC from Baldwin Wallace College. She will help Feller prepare for the mangrove biocomplexity field course at Calabash Cay for June and July. She will visit a mangrove ecosystem in Florida in June. In August, she will travel to Belize to participate in the field course.

Technician, Programmer

Name: Chamberlain, Anne

Worked for more than 160 Hours: Yes

Contribution to Project:

Chamberlain works as a research assistant for Feller and Lovelock in the laboratory and field. She works 32 hr/wk. Her greatest acheivement was building a walkway through the mangrove to each of the trees in our fertilization experiment on Twin Cays. Chamberlain has been on a leave of absence since Aug. 2001. She will return to the Mangrove Biocomplexity project in Aug. 2002.

Name: Borgatti, Aimee

Worked for more than 160 Hours: Yes

Contribution to Project:

Borgatti is working full time as a research technician on the mangrove biocomplexity project. She does nutrient analysis of plant and animal tissue; image analysis of leaves for herbivory; maintains experiments in Belize, Panama, and Florida; updates website information;

Name: Roberts, Quinn

Worked for more than 160 Hours: Yes

Contribution to Project:

Roberts went to Belize with Fogel and Wooller in January for 2 weeks. Prior to this she helped pack supplies for the trip. Following, she weighed and dried most of the samples for isotopic analysis.

Other Participant

Name: Levine, Mia

Worked for more than 160 Hours: No

Contribution to Project:

Levine developed curriculum material for planned mangrove courses and boardwalks. She wrote and submitted grant proposals to support these projects. Levine left the project to attend graduate school

Name: Venable, George

Worked for more than 160 Hours: Yes

Contribution to Project:

Venable is designing a virtual mangrove nature trail and an on-line mangrove ecology course. Support is provided by a subcontract specified in the proposal budget.

Research Experience for Undergraduates

Organizational Partners

Carnegie Institution of Washington

University of Maryland College Park

Graduate Student Mary Pittek is a full time Ph.D. student at the University of Maryland working on the mangrove biocomplexity project. Dr. Andrew Baldwin, Pittek's senior advisor, is providing in-kind support, facilities, etc. available for this student. Baldwin will also collaborate with the mangrove biocomplexity project in fy2002. The Univ.MD is also providing additional financial support for Pittek and Baldwin to compare the mangrove system at Twin Cays with Belizean mangroves. Pittek will compare mangroves in pristine environments (Twin Cays) with mangroves associated with shrimp farms. She will focus specifically on how the fish fauna and food webs are affected by shrimp farming.

Nature Conservancy

We are collaborating with Drs. Will Heyman and Dan Campbell of The Nature Conservancy to design, fund, and build an educational boardwalk and trail system in the Paynes Creek National Park in southern Belize.

Smithsonian Tropical Research Institute

The Director of STRI and affiliated staff have provided funding, laboratory space, technical support, boats, and accommodations for Feller and Lovelock to establish a satellite experimental site associated with their marine lab at Bocas del Toro.

Smithsonian Marine Station at Fort Pierc

Smithsonian Marine Station at Fort Pierce has provided funding, accommodations, equipment, field and laboratory facilities, and technical support for Feller, Lovelock, McKee, Fogel, Jacobson, Cheeseman, and Ball to work at their research facility. We have set up a satellite experimental site where all participants in the mangrove biocomplexity have access.

Smithsonian Institution Museum of Natural History

Financial support, in-kind support, technical support, and facilities have been provided by Dr. Klaus Ruetzler, Director of the Smithsonian's Marine Station in Belize, a research field station for the Smithsonian National Museum of Natural History.

Australian Institute of Marine Science

Dr. Dan Alongi has provide facilities and technical support for Feller and Lovelock to set up satellite sites in Queensland Australia.

University of Belize

Eden Garcia, Director of the Institute of Marine Studies for the University of Belize, is collaborating with us to teach a joint marine ecology course at their field station on Calabash Cay in Turneffe Islands, Belize. We are also working together to build an

Australian National University

Dr. Marilyn Ball, Ecosystem Dynamics Group of the Research School of Biological Science, has conducted collaborative experimental studies at Carrie Bow Cay and the Florida Marine Station satellite sites which contribute to the Biocomplexity Project. She provides facilities and support to establish a satellite site in Southern Australia. Additionally, a Mellon Award to her contributes post-doctoral support to the project.

University of Vienna

Drs. Marianne Popp and Wolfgang Wanek of the Institute of Plant Physiology at the University of Vienna are collaborating on the mangrove biocomplexity project. They measured stable isotopes in leaves from fertilized mangrove trees from Twin Cays. They are also sending a graduate student, Julia Hofman, to work at the field site in Belize to study the effects of herbivory on nutrient cycling in the mangrove. They are providing the finanical support for Hofman's travel and per diem; the mangrove biocomplexity project and CCRE are contributing financial support for her stay at the field station.

New Zealand NIWA

Feller and Lovelock are collaborating with Dr. Joann Ellis at the New Zealand National Institute of Water and Atmosphere to establish a satellite site in a black mangrove forest on North Island. The first trip to this field site is scheduled for July 29-Aug. 3 for the purpose of setting up a long-term fertilization experiment and to compare the responses to similar experiments in Belize, Florida, Panama, Queensland, and New South Wales. Other members of the mangrove Biocomplexity team will also visit this site pending acquisition of funding. Funding to support the collaboration with NIWA is provided by a competitive grant to Feller and Lovelock from the Smithsonian.

University of Rhode Island

Professors Urish and Wright are hydrological engineers in the URI, Department of Civil and Environmental Engineering. They are collaborating with Feller to map Twin Cays and to measure the hyrology and bathymetry. Our goal is to understandy 1) how hydrological processes affect patterns of nutrient availability and forest structure, 2) how anthropogenically altered flow patterns affect habitat heterogeneity.

Wilrid Rodriguez is a Ph.D. candidate in GIS and Remote Sensing at URI, specializing in geographical information systems and remote sensing technologies to the study of land and coastal ecosystem processes at the landscape, regional, and global scale. He has created a geospatial database to accommodate the hydrological data generated by Urish and Wright.

Other Collaborators or Contacts

1. Klaus Ruetzler, Director, Caribbean Coral Reef Ecosystems Program, Smithsonian Institution
2. Dennis Whigham, Plant Ecologist, SERC
3. Jos Verhoeven, University of Utrecht, the Netherlands
4. Marianne Popp, Plant Physiologist, University of Vienna, Vienna, Austria
5. Wolfgang Wanek, Stable Isotope Ecologist, University of Vienna, Vienna, Austria
6. Daniel Urish, Hydrologist, University of Rhode Island
7. Florence Thomas, Oceanographer, University of South Florida
8. Marilyn Ball, Ecophysiologist, Australian National University
9. Dan Alongi, Mangrove Ecologist, Australian Institute of Marine Sciences
10. Eric Wolanski, Australian Institute of Marine Sciences
11. Joanne Ellis, Benthic Ecologist, NIWA, New Zealand
12. M. L. Ewe, Plant Physiologist, Smithsonian Marine Station, Ft Pierce Florida
13. William Davis, Ichthyologist, Environmental Protection Agency
14. Craig Cary, Microbial Ecologist, University of Delaware
15. Penelope Barnes, Seagrass Ecologist, Smithsonian Tropical Research Institute
16. Hector Guzman, Coral Reef Ecologist, Smithsonian Tropical Research Institute
17. Joe Holtum, Ecophysiologist, James Cook University, Australia
18. Christina Diaz, Sponge Ecologist, UC Davis
19. Robert Twilley, Mangrove Ecologist, University of Louisiana
20. Dan Childers, FIU, PI/PD Florida Everglades Coastal LTER
21. Will Heyman, The Nature Conservancy, Punta Gorda, Belize
22. Eden Garcia, Director, Institute of Marine Studies, University of Belize
23. Andrew Baldwin, Biogeochemist, University of Maryland
24. Joel Trexler, Ecologist, FIU
25. William Loftus, Ecologist, National Park Service
26. Steve Davis, Texas A & M Uni

Activities and Findings

Research and Education Activities:

Our goals and objectives were to:
(1) Characterize the bacterial and fungal community structure and diversity of key functional genes associated with N and carbon (C) cycling rates and nutrient availability [Frischer, Joye, Shearer]
(2) Measure enzymatic activity associated with nutrient acquisition and decomposer pathways for bacteria and fungi [Jacobson, Shearer]
(3) Measure rates of intermediary and terminal physiological processes and bacterial biomass in the sediment, relative to nutrient availability and stable C and N isotopes for sediment processes [Joye, Jacobson, Fogel]
(4) Measure arthropod, fungal, and bacterial species associated with litter decomposition [Feller, Frischer, Shearer]
(5) Determine stable C and N isotopes in mangroves associated with nutrient availability and fertilization experiment [Fogel]
(6) Quantify soil physico?chemical variables and their interactions with hydrology and nutrient enrichment [McKee]
(7) Determine rates & patterns of elevation change, vertical accretion and subsidence as well as peat composition, and stratigraphy [McKee]
(8) Assess rates of nutrient delivery across the intertidal gradient and determine the effects on growth, resource allocation, and gas exchange [McKee, Cheeseman, Lovelock, Feller]
(9) Determine the effects of nutrient availability and sediment processes on leaf chemistry, the photoprotective mechanisms associated with survival under sub?optimal conditions, and the feedback of tissue quality and quantity on sediment nutrient dynamics [Lovelock, Cheeseman]
(10) Characterize communities of primary and secondary consumers of mangrove tissue, measure the effects of tissue quality on herbivory, nutrient loss from trees, and detritivory, and determine how these processes impinge on nutrient cycling [Feller, Fogel]
(11) Integrate field observations using Network Analysis to study and model systematically and quantitatively the trophic interrelationships among the major components of the mangrove community [Ulanowicz].

SUMMARY OF SPECIFIC INDIVIDUAL AND INSTITUTIONAL RESPONSIBILITIES
I. Management: Ilka C. Feller (SERC) is the project director.
Web Development: Website Designer, George Venable, has developed a Tangled Roots public website, which currently contains general project information, an web-based mangrove ecology course, outline and description of mangrove field course, publication and meeting abstracts, and annual reports (http://www.mangroves.si.edu). A virtual mangrove nature trail is under construction for this website; activation is planned for August 2003. Venable also maintains a project ftp site where participants in the project share and exchange data, manuscripts, maps, digital images, and pdf copies of publications. Marilyn Fogel hosts a listserve at Carnegie Institute of Washington (CIW) to facilitate intraproject communication.
All-Hands Meeting: Our third all-hands meeting was held in October 2002 USGS National Wetland Research Center in Lafayette, LA and was hosted by Karen McKee. Planning is underway for our next all hands meeting in Year 4.
Mangrove Ecology Field Course: 1) In August 2002, we inaugurated a mangrove ecology field course for the Mangrove Biocomplexity project. This course was taught at the Institute of Marine Sciences (IMS), University of Belize, on Calabash Cay, Turneffe Islands Atoll, for both U.S. and Belizean undergraduate students, in collaboration with Eden Garcia (Director, IMS) and Professor Steve Davis (Texas A & M University). In preparation for this course, we revised and published on line a new course manual (www.mangroves.si.edu). 2) In 2003, we are collaborating with Dr. Kelton Clark (Coordinator, Smithsonian's NSF's LSAMP Program) and LSAMP Puerto Rico to offer this field course to undergraduate students at the University of Puerto Rico at their La Parguera Marine Laboratory, May 26-June 6. Five members of the mangrove biocomplexity project will participate in teaching this course, along with instructors from the Univ. PR and the US Fish & Wildlife Service in PR. 3) In Nov. 2002 and May 2003, Feller and her graduate students (Faustino Chi and Cyril Piou, University of Bremen) participated in a course conducted by World Bank's MesoAmerica Barrier Reef System (MBRS) project to teach mangrove ecology, research and monitoring methods to resource managers from Mexico, Belize, Honduras, and Guatemala. 4) June 16-July 18, 2003, Feller and Dr. Penelope Barnes (STRI) are co-coordinating an OTS graduate course in Tropical Marine Ecology (www.stri.org/tropical) course at the Smithsonian Tropical Research Institute's Marine Laboratory at Bocas del Toro, Panama. Seven members of the mangrove biocomplexity team will participate as instructors and guest lecturers for this course. 5) Feller initiated a new collaboration with the North Carolina Natural History Museum to teach an annual tropical marine ecology course for primary and secondary teachers from North Carolina and Belize, beginning summer 2004 at the IMS in Belize. 6) Feller initiated a new collaboration with the Toledo Institute for Development and the Environment (TIDE) to design and build a network of mangrove boardwalks in Punta Gorda, Belize. These boardwalks will be for teaching mangrove field courses, public education, research, and monitoring.
To extend our education efforts to primary and secondary school teachers and students, Feller is working with Mark Kormann, Director of the Project View, a distance-learning program at Ball State University (funded by Best Buy Co., Inc.), the Apple Learning Interchange, and the SERC's Education Department to conduct a mangrove biocomplexity electronic field trip (EFT) for May 13, 2003, at the IMS in Belize. As of April 4, 13 million students in 45 states had been registered to participate online in this EFT. To prepare for the EFT, we have conducted training programs and provided resource materials for the primary school teachers who, in turn, developed grade-appropriate curriculum materials for the EFT. The web-based mangrove ecology course (described above) has provided additional in-depth training, curriculum development material for participating schools, and website materials for the EFT website developed by Ball State (www.bsu.edu/eft/belize).

During 2001-2003, Feller collaborated with the Florida Coastal Everglades LTER program (Caribbean Initiatives) to link facilities and share data at multiple sites around the Caribbean. We have drafted a multi-authored paper on Caribbean mangrove ecosystems, which has been submitted to BioScience. Currently, we are working together to develop a grant proposal for an international, multi-country LTER project focused on mangrove biocomplexity of the Caribbean Region.
Satellite Field Sites: Feller and Lovelock have received additional grant funding to support extension of our research on mangrove biocomplexity at satellite sites in Florida, Panama, Australia, and New Zealand the Indo-Pacific Region. We received a grant from the Smithsonian's Johnson fund to set up sites in Australia and New Zealand. Lovelock and Feller received another grant from the AAAS/NSF Women's International Scientific Collaboration (WISC) Program to fund collaborations with Drs. Marilyn Ball (Australian National University, Canberra, Australia) and Joanne Ellis (National Institute for Water and Atmospheric Science, New Zealand). This grant is supporting the development of a research proposal to examine mangrove biocomplexity on a global scale with our Australian and New Zealand collaborators for the International Program at NSF. In collaboration with Marilyn Ball, Lovelock and Feller received a grant from the Mellon Foundation to support our research efforts in Florida and Belize. Feller and Lovelock each received grants (2000-2003) from the competitive grants program at the Smithsonian Marine Station in Fort Pierce to support the satellite experiments in Florida. Feller and Lovelock also received 2 yr of funding (2003-2005) through the competitive grants program of the Smithsonian Marine Science Network to support our research activities at the STRI Marine Field Station in Bocas del Toro, Panama.
During Year 3, we initiated three new collaborative projects which contribute additional expertise and experience: 1) Daniel Urish and Ray Wright (University of Rhode Island) to measure bathymetry and hydrology of Twin Cays; 2) Steve Davis (Texas A&M University) and Linda Franklin (SERC) to determine the role of macroalgae and periphyton on the formation of floc and it effect on the heterogeneity of the mangrove ecosystems at Twin Cays; 3) Scott Taylor (Brevard County Mosquito Control Project), Will Davis (EPA), and Carol McIvor (USGS) to characterize the fish communities at Twin Cays. These collaborators will allow us to pursue these projects, and were chosen because of their expertise and prior experience at Twin Cays or similar mangrove ecosystems. Because of high demand for limited space and facilities at the Smithsonian's Marine Station on Carrie Bow Cay, the number of visits by mangrove biocomplexity participants cannot exceed limitation established at the onset of this project. Thus, adding new collaborators to work at that site is carefully coordinated with the Director of the Caribbean Coral Reef Ecosystems (CCRE) Program, Dr. Klaus Ruetzler, at the National Museum of Natural History. Funding to support these additional projects is provided by a competitive grant from CCRE. With the support and approval of the Smithsonian, we have added these studies, which will expand the process-level studies that we are currently doing at Twin Cays to a landscape level and provide important data for the network analysis.
Data management: The project Data Manager Ursula Scharler (postdoctoral fellow, UMD and SERC) is responsible for data management for trophic flow networks. During Year 3, the management of data for the network analysis continued throughout the year, and the Metadata management lasted from July 2002 until September 2003. The data gathering for the 18 networks describing the Twin Cays ecosystem continued from old and new literature sources to update the previously established trophic flow networks. The 18 networks describe three vegetation zones (fringe, transition, dwarf), 2 seasons (wet, dry) and three flow currencies of material transfer (carbon, nitrogen, phosphorus). Data generated by the participants of this Biocomplexity project are exchanged via a project specific ftp site and are incorporated, as they are made available by the researchers. The networks are continuously updated.
Metadata records according to the NBII Profile are generated on a continuous basis as the researchers in this group generate new data and data files. The researchers enter descriptive information about their data files in a Metadata Table of Contents, which the data manager uses to create Metadata records. The communication of data takes place via a project specific ftp site. Metadata records have been established for all datasets available to the Data Manager

II Fertilization experiments, field station facilities, and host-country interactions: In Year 3, Feller made four trip to Belize to maintain the long-term fertilization experiments in the mangrove forest at our primary site at Twin Cays, to orientate new collaborators from the University of Rhode Island, to do a photographic flyover, to conduct the mangrove ecology field courses, (http://www.mangroves.si.edu/Tropical/abstract.html), to work with graduate student Chi and Piou at Turneffe Atoll, to meet with the staffs of the Belize Fisheries Department and the MBRS Project, and to collaborate with TIDE staff in Punta Gorda on the mangrove education boardwalks in Punta Gorda. Feller worked with the Director and Facilities Manager of CCRE to arrange accommodations, support, logistics, facilities, and sponsorship for each of the co-PI's from collaborating institutions to work at their Marine Field Station on Carrie Bow Cays. Each of the collaborating scientists and/or their associates visited the field site during Year 3 (FY2003).

III Nutrient cycling rates and processes: In Year 3, Samantha Joye (University of Georgia); Marilyn Fogel (Carnegie Institute of Washington); and Myrna Jacobson (University of Southern California) conducted and coordinated experiments and measurements related to the effects of nutrient availability and tidal position on microbial processes and the transfer, recycling, and storage of nutrients.

Joye: Sediment nutrient cycling rates and processes: We conducted experiments and measurements to examine the effects of nutrient availability and tidal position on microbial processes and the transfer, recycling, and storage of nutrients.
Joye and graduate student, Rosalynn Lee, have conducted six field expeditions to Twin Cays, Belize in August and November 2000, June and October 2001, and March and September 2002. In August 2000, study sites were selected, and in subsequent trips, diel experiments have been conducted to quantify rates of carbon, nitrogen and oxygen transformations. The goals during the first two years of the project were to

characterize the spatial (from micrometers to hundreds of meters) and temporal (from daily to seasonal) patterns of nitrogen fixation, denitrification, oxygenic photosynthesis and respiration in different mangrove habitats and to perform a baseline evaluation of the nutrient status of sediment microbial communities. These goals were achieved by quantifying rates of processes during diel experiments and by performing short-term (2-day) nutrient addition bioassays. Additional samples for stable nitrogen and carbon isotopic analyses, pore water chemical analyses and chlorophyll analyses were also collected. Diel experiments consisted of rate measurements for nitrogen fixation, denitrification and photosynthesis, and microelectrode profiling of sediment oxygen concentrations. We focused our work this year on completing our picture of seasonal variation in Twin Cays microbial mat composition and carbon, oxygen and nitrogen cycling processes. We also began to integrate our research into the Twin Cays network model as we near the end of this project.
The following list describes the samples collected in 2002: 56 samples of pore water and overlying water nutrients, 3456 measurements of photosynthetically active radiation, 412 samples of benthic chlorophyll, 196 stable isotope samples for natural abundance and carbon fixation rate assays, 621 gas samples from nitrogen fixation and denitrification rate assays, and 223 profiles of sediment pore water oxygen concentration and gross oxygenic photosynthesis rate (each profile consisting of 20-30 samples over a depth of 2-7 mm). Pore water profiles were collected using equilibration meters ('peepers') to profile nutrient chemistry with sediment depth (30 chambers over 50 cm). Five peepers were collected in January and September 2002 and three will be collected in May 2003. Nutrient samples from spatial surveys and each peeper chamber were analyzed for pH, salinity, dissolved organic matter (DOC, DON, DOP), dissolved inorganic nutrients (NH4, NO3/NO2, PO4, Si), redox metabolites (H2S, DIC, Fe2+, Mn2+), and dissolved gases (O2, N2, CH4, N2O in peepers only). Data from this project were presented at the February 2003 American Society of Limnology and Oceanography meeting in Salt Lake City, Utah, in an oral presentation entitled 'The importance of microbial mats in the C and N cycle of mangrove ecosystems.' Selected data and images are available on Joye's web page at: http://www.marsci.uga.edu/FacultyPages/Joye/belize.html. Selected images of the microbial mat collected this year are available on Lee's web page at: http://www.arches.uga.edu/~rosalynn/photos/twin1.html and http://www.arches.uga.edu/~rosalynn/photos/twin2.html.

Fogel: During Year 3, Marilyn Fogel and postdoctoral fellow, Matthew Wooller, worked in close collaboration with the Jacobson and Cheeseman groups. We confirmed and extended the isotopic measurements published in prior work by McKee, Feller, and their Austrian colleagues. Fertilized red mangrove trees at Twin Cays have different N and C isotopic ratios depending on whether or not they have been fertilized with urea or phosphate or are controls. The body of our work, however, rests on over 1,000 measurements on red and black mangrove leaves, roots, stems, and wood from grid stations, vector experiments (radiating from one point), transects, new P fertilization experiments, and samples of opportunity, in addition to the fertilized trees, at Twin Cays. Although our measurements are similar to those of McKee et al., we have been pursuing alternative hypotheses for explaining the very unusual range in N isotopic compositions, particularly the very negative d15N values (range û8 to û18 ë) that are found in certain places on the islands.
Together with the Cheeseman and Jacobson groups, we have worked together to assemble an integrative picture of the N cycling at Twin Cays. We have tested numerous hypotheses including the supply and demand hypothesis argued in McKee et al. (2002). Our measurements in addition to isotopic compositions include porewater ammonia, total solid phase phosphate (see Jacobson section), atmospheric ammonia concentrations, rainwater N concentrations, the isotopic compositions of porewater, rainwater, and atmospheric ammonia, photosynthetic measurements (see Cheeseman section), lichen N isotopic compositions, N2 fixation measurements (see Jacobson and Joye's sections), compound specific isotopic fractionations (Smallwood et al., to be submitted 2003), senescent leaf N isotopic compositions (Wooller et al., in press), and C and N isotopic compositions of leaf fragments in a 10 m core dated to be as old as 8,500 years old (Wooller).

Jacobson: Our objectives for Year 3 were to: 1) describe the types and relative activity of the microbical physiological processes associated with mangrove trees across the tree-height gradient in fertilized and unfertilized areas at different seasons. This exercise will enable us to look at the differences in microbial process emphasis and its associated interaction with tree height and fertilization; 2; to develop and calibrate with traditional biogeochemical measures, additional enzyme activity markers associated with nitrogen and carbon metabolism in the sediments and describe the availability of nutrients using enzyme analogs; 3) continue collaboration with Dr. M Fogel, Dr. J. Cheesman on objectives associated with determining the reasons for anomalous negative d15N in mangrove leaves; 4) collaborate with Dr. M. Frischer and C. Shearer (in coordination with Dr. B. Smallwood's lab for the latter) to describe bacterial and fungal activity and types found across the tree height gradient and with depth in fertilized and unfertilized areas; 5) associate with the effort the NIF gene diversity as well as physiological processes associated with nitrogen metabolism and acquisition was measured by post doctoral associate James Burns; 6) begin to address the effect of root zone physiology on acquisition of nutrients for bacterial and plants. 7) Establish a method to compare visually, and for educational purposes, the data array generated by contributing associates using the EASY program, an interactive GIS program established by D. Kiefer a USC associate.
Methods: 1) Nutrients found seasonally in pore water was measured using peepers and sippers. Microbial physiological processes were measured by incubation experiments using a series of selective metabolic inhibitors. Incubation of gas, pore water and solid phase materials associated both with a discrete tree height gradient, and fertilization histories were accomplished. In addition, seasonal data on nutrient status/depth across the tree height gradient was measured at one station (dock site) using in situ peepers. In addition to the peeper measurements, selective sipper samples were taken at discrete depths to compare with peeper information. Measurements were taken to describe the relative contribution of processes including, sulfate reduction, methanogenesis, nitrate reduction, nitrogen fixation, and total sediment respiration. In addition, sipper samples were taken to describe the nature of the organic C and N found in pore waters associated with tree height gradient in selected areas. 2) Fluorescent enzyme substrates associated with N,C and P metabolism were introduced into sediments

from both fertilization areas and tree height areas with out added fertilizer. In addition, experiments with an emphasis on enzyme comparative kinetics were initiated in an attempt to model the amount of bacterial 'perceived' available substrate. 3) Supportive data associated with objectives of M. Fogel and J Cheesman include: measurement of enzymes, solid phase, and pore water nutrients associated with many areas described in their part of the report, and discrete laboratory experiments to test the hypothesis posed for the negative del nitrogen levels. 4) Bacterial types (NIF gene DNA analysis), respiration, and activity measured by gas chromatograph and by ATP extractions are being compared to flow through measurements. This information is also being correlated to biogeochemical measures and bacterial type and number at selective areas including both natural and fertilized trees. Samples were collected for Smallwood to analyze at Texas A&M for amino acid and free lipid distribution from fringe through transition to dwarf and floc zones to determine changes in microbial vs. fungal vs. mangrove biomass. Stephanie Macdonald is a new grad student that will be working with Smallwood, Schmidt and Shearer to determine fungal biomass at Twin Cays. 5) Field measurements of root exudates associated with mangrove trees across the tree height gradient and under different nutrient regimes was initiated to begin to understand the interaction of tree and sediment in processes associated with nutrient acquisition by desorption as well as by creation of unique microenvironments for bacterial growth.

IV Microbial activity (bacterial/fungal): Marc Frischer (Skidaway Institute of Oceanography) and Carol Shearer (University of Illinois) conducted and coordinated characterization of bacterial and fungal communities at the study site.

Frischer: The primary role of the Skidaway Institute of Oceanography group directed by Frischer is to elucidate the indirect interactions between bacterial communities and other mangrove ecosystem components that influence the overall biogeochemical behavior of mangrove bacterial communities. Efforts during the second project year continued studies begun during the first project year aimed at conducting a census of the composition and diversity of microbial assemblages associated within each ecological zone in the mangrove system and in association with the ongoing long-term fertilization experiment. Studies continued to be conducted closely with the mycology team (led by C. Shearer at the University of Illinois) to determine the linkages between bacterial and fungal communities. These studies focused primarily on differences related to nutrient availability, seasonality (wet vs. dry season), and the turnover of senesced leaf material.
In addition to Frischer, the project at the Skidaway Institute of Oceanography has benefited from the assistance of two technicians (J. Danforth and T. Williams), a postdoctoral associate (M. Booth), and two undergraduate interns (T. Foy û Armstrong Atlantic University and C. Archer û Savannah State University). Savannah State University is a historically black university located in Savannah Georgia. C. Archer and T. Williams are both African Americans. With the exception of one part time technician and Frischer, alternative funds were used to support personnel. Notably, undergraduate research assistantships were provided through NSF Collaboration to Integrate Research and Education (CIRE) activity at Savannah State University and the Skidaway Institute of Oceanography. Frischer is a co-PI associated with the CIRE program. Support of undergraduate research experiences has been the primary education outlet provided by the Skidaway group.

During the past year, one 1-week research expedition to Belize was made during the wet season. This trip was made in conjunction with the mycology group from the University of Illinois. Studies continued to focus on microbial and fungal communities during the wet seasons. Sediment cores along the natural nutrient gradient and associated wither fertilized trees were collected and the abundance, biomass, and diversity of microbial and fungal communities were determined. Bacterial abundance and biomass was determined by direct microscopy while community diversity/structure was determined using molecular techniques (Denaturing Gradient Gel Electrophoresis [DGGE] using 16S rRNA targeted primers). In addition, freshly collected sediments and purified DNA was provided to several other researchers to facilitate enzyme activity studies and molecular studies focused on nitrogen fixing bacterial populations.

Shearer & Schmit): 1) Collections were made in Belize in December 2002 and February 2003. We collected wood, leaves, and sediment samples from across the nutrient and tidal gradient. Collections were also made on the Glovers Reef atoll east of Twin Cays. We have continued to identify and isolate fungi from these collections. So far we have collected 380 sediment samples, 1200 leaf samples and 1200 wood samples. 2) We have begun to determine the enzymatic capabilities of the fungi we isolated. Enzymes include amylase, lipase, pectinolytic enzymes, cellulolytic enzymes and lignin degrading enzymes.3) We are continuing to collaborate with Marc Frischer, looking at the response of fungal and bacterial communities to the nutrient treatment. 4). In collaboration with Marc Frischer we are carrying out a leaf decay experiment. Leaves from each of the three nutrient treatments from each of the three vegetation zones were collected. These were placed in the field and left to decay for various times 1, 2 or 4 weeks. A fourth treatment that will decay for approximately 6 months is still in the field. Leaves are being analyzed for fungi and bacteria and will be used to determine the effects of nutrient addition on the decomposer community. We are also measuring the changes in carbon, nitrogen and phosphorus concentrations as the leaves decay and will be comparing this data with changes in the decomposer community. 5) In conjunction with Candy Feller, we are measuring the amount of standing and fallen deadwood in the mangrove forest. We are taking these measurements in a variety of vegetation types on Twin Cays. 6) In conjunction with Barbara Smallwood, we plan to measure the biomass of fungi that occur in peat samples. This work was pushed back due to delays in the set up of critical equipment in the Smallwood lab. 7) We have continued to update our database of all reported occurrences of fungi in mangrove swamps. The database records the geographic distribution, host and substrate preference for 860 fungi, and covers nearly all of the literature on mangrove fungi. This database is available online at http://fm5web.life.uiuc.edu:23523/mangrove/. This database is also the basis for two manuscripts.

V Mangrove growth and internal nutrient dynamics: Feller, Lovelock, and McKee worked closely at the Twin Cays study site to coordinate measurements of above- and belowground growth responses by mangroves as well as to assess external nutrient sources, internal nutrient dynamics, and interactions with hydro-edaphic stressors using stable N and C isotope analyses (in collaboration with Drs. Marianna Popp and Wolfgang Wanek, University of Vienna, Austria). We published two papers describe the effects of nutrient availability of growth and internal nutrient dynamics (McKee et al. 2002, Feller et al. 2003). Another experiment installed in a dwarf stand of black mangrove (Avicennia germinans) at Twin Cays is being used to examine interspecific differences in the effects of nutrient enrichment on growth and nutrient dynamics. Growth responses indicate that trees at this site are severely P-deficient. A manuscript is in preparation to compare growth and nutrient dynamic with this P-limited black mangrove forests with a parallel experiment in a N-limited black mangrove forest in the Indian River Lagoon, Florida (Feller et al. in preparation).

In Year 3, Feller and Lovelock, along with Marilyn Ball (ANU) and Joanne Ellis (NIWA), maintained our fertilization experiments in mangrove forests at satellite sites in: Queensland, Australia (Townsville, Hinchinbrook Channel, Port Douglas), New South Wales (Batemans Bay) and New Zealand (North Island) in June-July 2002; Panama (Bocas del Toro) in February-March 2003; and Florida (Indian River Lagoon) in November 2002 and April 2003. In February 2003, we set up a second fertilization experiment on North Island, NZ, in a coastal, sandy mangrove forest to compare with the experiment we installed the previous year in a muddy estuary. In July-Aug. 2001, Feller and Lovelock set up additional experimental sites in New South Wales (with Marilyn Ball, ANU), Western Australia, and North Island, New Zealand (with Joanne Ellis, NIWA). In April 2002, we set up another experiment at the southern end of the Indian River Lagoon near Hobe Sound. At all satellite sites, similar experiments were set up to compare ecological processes with our core site at Twin Cays. Trees were fertilized, and growth, photosynthesis and rates of herbivory were measured. Harvested leaves are currently being analyzed for nutrient concentrations. Data from Bocas del Toro, Panama have been analyzed and two manuscripts describing the characteristics of the mangroves of the region, and the response of dwarf mangroves to fertilization are close to completion. At the satellite site in Florida, postdoctoral fellow Sharon Ewe (funded by a Mellon foundation grant to Lovelock, Ball, and Feller) is assessing the effects of fertilization on tree phenology and establishing a greenhouse experiment assessing the effects of salinity on growth and physiological responses to nutrient additions in three mangrove species. In November 2002, we will set up another experiment on Merritt Island at the northern end of the IRL.

For the Florida satellite site, we completed the first phase of this experiment and published two papers describing the study site, physical-chemical properties and plant growth and physiological responses, and comparing these parameters to the Belize fertilization experiment (Feller et al. 2003, Nitrogen limitation of growth and nutrient dynamics in a mangrove forest, Indian River Lagoon, Florida, Oecologia; Lovelock & Feller. 2003, Oecologia, Photosynthetic performance and resource utilization of two mangrove species coexisting in a hypersaline scrub forest).

During our two research trips to Twin Cays, the Feller Group measured forest structure across and within all zones. We began quantifying the coarse woody debris (CWD) across the island. We also measured the influence of algal floc on growth experiment. We collected and processed the litter bags from the three ongoing decomposition experiments associated with the ecotone fertilization experiment. We measure growth, surviva,l and herbivory of the 1620 R. mangle seedling (20 at each of the 81 trees in the ecotone experiment) planted in Sept 2001 at each of the fertilized trees. For each seedling, we quantified damage by crabs, miners, borers, bud moths, and folivores.

VI. Plant physiological responses: John Cheeseman (University of Illinois) and Catherine Lovelock (SERC) focused on gas exchange and chlorophyll fluorescence to provide fundamental data on mangrove physiological responses to differences in nutrient availability across environmental gradients.

Lovelock: Attendance at the field site in Belize was from November 27, 2002 to January 8, 2003. Dr. Marilyn Ball collaborated with me during this trip. During this time we tested whether hydraulic conductance and architecture varied over different vegetation zones and species, and whether hydraulic properties were influenced by fertilization.

Measures of leaf area index (LAI) were made over the fertilization experiment using hemispherical photography. Respiration fine wood was also measured over the fertilization experiment using segments of 1-2 cm diameter twigs. Biomass of algal communities adhering to mangrove tree roots was measured in each tree height zone and each fertilization treatment in a 0.5 m square. These data are being used to parameterize production in the tree compartments of the Network analysis models (Ulanowicz and Shcarler).

Using a chlorophyll fluorescence imaging system (Walz Imaging PAM), we also investigated the effects of herbivory on photosynthetic carbon gain in fertilized and control trees.

Field trips to satellite sites in Australia and New Zealand were undertaken in June and July 2002. All sites (Western Australia, Queensland, New South Wales, with collaborator Marilyn Ball (ANU) and New Zealand (with Joanne Ellis, NIWA) were visited. Trees were fertilized, and growth, photosynthesis and rates of herbivory were measured. Harvested leaves are currently being analyzed for nutrient concentrations. Data from satellite sites in Bocas del Toro, Panama have been analyzed and one manuscript describing the response of dwarf mangroves to fertilization is in review. A second manuscript describing the characteristics of the mangroves of the region is close to completion. The satellite site in Florida was visited in November 2002. Postdoctoral fellow Sharon Ewe based at the Smithsonian Marine Station at Fort Pierce is assessing the effects of fertilization on tree phenology and has completed a greenhouse experiment assessing the interacting effects of nutrient enrichment and salinity on growth and physiological responses in three mangrove species.

Cheeseman: During Year 3, we have extended our work on four projects. Manuscripts detailing the results are in the final stages of preparation for two of them, with the expectation that they be submitted by the end of May, following review by project collaborators. 1) Analysis of flavonoid and tannin composition of mangrove leaves. Additional participants, Drs. Fayez Kandil and Mary Grace (post-doctoral research associates), Professor David Seigler (University of Illinois); 2) Analysis of leaf peroxidase activity, including its possible role in photoprotection; 3)Analysis of mangrove fertilization, including the impact of zonation and fertilization (collaboration with Cath Lovelock, SERC); 4) Examination of potential in planta mechanisms which would account for the extreme d15N found in dwarf trees and their response to P-fertilization (in collaboration with Marilyn Fogel, Mat Wooller, Myrna Jacobson and Barbara Smallwood).

VII. Herbivory and detritivory of mangrove tissue: Feller is responsible for measurements of herbivory and detritivory. In Year 3, we measured the effects of nutrient enricment on growth, survival and herbivory of the R. mangle seedlings (20 at each of the 81 trees in the ecotone experiment) planted in Sept 2001 at each of the fertilized trees at Twin Cays. For each seedling, we characterized and quantified damage by crabs, miners, borers, bud moths, and folivores. We also continued our measurements to determine the effects of nutrient enrichment and zone on damage caused by foliage-feeding herbivores in the canopy of fringe, transition, and dwarf trees for trees in the fertilization experiments at Twin Cays and at all the satellite experiments. Damage was characterized according to specific herbivore. We also measured the density of the major herbivores (number/m2) within the forest.

VIII. Hydro?edaphic and peat analyses and elevation change: McKee is responsible for measurement of hydro-edaphic conditions at the experimental site, peat accumulation rates and patterns, soil surface elevation change and ability of the mangrove ecosystem to accommodate sea-level rise. McKee made several trips to the main field site in Belize (Twin Cays) during 2001-2003 to determine surface and sub-surface root growth responses by mangroves to natural hydro-edaphic conditions and to nutrient enrichment. Surface and sub-surface root production was measured at the experimental sites using 1 mm mesh screens (placed on the soil surface) and in-growth cores (30 cm depth), respectively. Final samples were processed in June 2002, and seasonal and annual belowground production rates were determined. In all, 103 surface and 80 sub-surface plots were analyzed to measure root production by mangroves. In addition, similar measurements were initiated at five satellite sites in Australia (3 locations), New Zealand, and Panama during July 2002 and February 2003 to provide a global perspective on nutrient effects on belowground production by mangroves.

Surface elevation tables (SET's) and marker horizons established at the experimental site in Belize were used to document effects of nutrient enrichment on soil surface elevation change, vertical accretion and subsidence relative to sea-level rise. Measurements have been made at ~ 6 month intervals for two years at 27 SET's and 27 marker horizons. In addition, measurements of peat decomposition were initiated in Belize and Panama to provide information about nutrient effects on organic matter accumulation rates.

IX. Network Analysis: Robert Ulanowicz (University of Maryland). During the first year of network analysis, the initial configuration of the Twin Cays ecosystem network was established and a preliminary quantification of the biomasses and material transfers among the compartments of the network was initiated. Throughout the course of the second year, data for the 18 networks that are to describe the ecosystems of Twin Cays were assembled from various literature sources by U. Scharler in conjunction with her duties as data manager for the project. The 18 networks describe 3 vegetational zones (fringe, transition, dwarf), 2 seasons (wet and dry) and 3 flow currencies (carbon, nitrogen and phosphorus) of material transfers. During the past year, first estimates were made of the balanced networks of ecological exchanges, based partially on data already collected by project investigators and partly on data from mangrove ecosystems elsewhere. Data on biomass and the material transfers of carbon, nitrogen and phosphorus within the ecosystems are constantly being upgraded to make the networks gradually more representative conditions on Twin Cays.

To inform the public of the results of the network analysis of the Twin Cays ecosystems as they become available, a website (http://cbl.umces.edu/~scharler/mangnets/TwinCays.htm) has been established. The site describes network analysis in general and the development of the Twin Cays networks in particular. The biomasses, inputs to and outputs from each compartment of the network will be listed according to species. A comprehensive listing of the species inhabiting Twin Cays, including photographs, is gradually being added. The arrays of biomasses and exchanges in the 18 networks are being updated on a continuing basis.

Findings:

Mangrove growth and internal nutrient dynamics (Feller, McKee, Lovelock): At Twin Cays, we discovered that although there was spatial variability in response, growth by Rhizophora mangle was generally nitrogen (N) -limited fringe zone; phosphorus (P) -limited in the dwarf zone; and, N- and/or P-limited in the transition zone. Phosphorus-resorption efficiency decreased in all three zones, and N-resorption efficiency increased in the dwarf zone in response to P enrichment. The addition of N had no effect on either P or N resorption efficiencies. Belowground decomposition was increased by P enrichment in all zones, whereas N enrichment had no effect (Feller et al. 2003a). To determine if this N-to-P gradient was present in other mangrove ecosystems, we used fertilization experiments at satellite sites (n = 8) to compare the responses at Twin Cays with global patterns of nutrient limitation and to determine the effects of nutrient enrichment on ecological processes, including aboveground growth, nutrient dynamics, and herbivory. Like Twin Cays, we found that primary production in mangrove forests in Florida, Panama, Australia, and New Zealand were nutrient limited. However, patterns of nutrient limitation and plant growth responses to nutrient enrichment varied by position within the forest, latitude, region, or disturbance history. Nutrient enrichment also had dramatic effects on herbivory that varied by treatment, species of herbivore and mangrove, as well as forest position, latitude, region, and disturbance. Results from these experiments demonstrate: 1) patterns of nutrient availability within and among mangrove ecosystems are complex; 2) similar habitats are not limited by the same nutrient when different mangrove forests are compared (Feller et al. 2003b; 3) not all species respond similarly to increased nutrient availability (Lovelock and Feller 2003); and 4) not all ecological processes within a mangrove ecosystem respond similarly to, or are limited by, the same nutrient.

McKee: Surface and sub-surface root production by red mangroves varied across the environmental gradient from fringe to dwarf zones and was significantly stimulated by P enrichment in the dwarf zone. Under control treatment, root production varied from 82 (dwarf) to 394 (transition) to 525 (fringe) g m-2 yr-1, a pattern highly correlated with flooding duration (r2 = 0.80), but not nutrient availability. Addition of N, which stimulated aboveground growth in fringe and transition trees, had no significant effect on root production. Enrichment with P, however, caused an increase in root production by dwarf trees to 742 g m-2 yr-1 and eliminated the correlation with flooding. The results show that effects of nutrients on root production cannot be inferred from aboveground responses and are consistent with other findings that not all processes in this ecosystem are limited by the same nutrient. Elimination of the correlation between flooding and root production by P-addition further indicates that P-limitation to mangrove growth in this system is secondary to flooding stress.

Nutrient cycling rates and processes (Joye, Fogel, Jacobson)

Joye: Every dwarf mangrove sediment examined has continued to consistently maintain a diverse array of laminated microbial mat organisms including diatoms, coccoidal and filamentous cyanobacteria, and purple and green sulfur bacteria over 3 mm to cms in depth. We conducted benthic chlorophyll surveys to gauge the distribution of microbial mat across each study site. The variability within individual sites is high, e.g. from 94 to 468 mg chl a m-2 at the West Pond in March. The variability across different sites is also high (e.g. average of 80 mg chl a m-2, standard deviation of 59 mg chl a m-2 in September). Despite seasonal variations in the degree of microbial mat dessication, the average photosynthetic biomass at any site during any season was similarly high at about 80 mg chl a m-2 of dwarf mangrove sediment.

Sediment nutrient availability was measured in pore water and overlying water across each site and in peepers inserted at selected sites. Throughout the seasons, pore water analysis indicates reduced sediment conditions with concentrations of up to 0.5 mM NH4, 40 mM H2S, and 50 mM CH4, and less than 5 mM NO3/NO2. Trace metal concentrations are consistently low down to 10 cm below the sediment surface, but increase up to 10 uM Fe2+ and 100 mM Mn2+ with depth. Peeper profiles also illustrate sulfate reduction occurring at depth in mirrored SO4 and H2S profiles. A significant fraction of nutrients are bound in the organic pool (~ 20 mM DOC, 0.2 mM DON, 0.001 mM DOP) relative to the inorganic pool. The stoichiometry of C:N:P in the organic (20:0.2:0.001 mM) and inorganic (15:0.4:0.0005 mM) nutrient fractions at depth clearly show strong P-limitation (relative to the Redfield ratio) in the dwarf mangrove sediments.

Primary production rates are high (up to 45 mmol C m-2 d-1 and 45 mmol O2 m-2 h-1 gross under full daylight illumination) and variable across different mat types. Inorganic carbon in Twin Cays microbial mats is fixed primarily by phototrophs, with a small, but significant percentage by chemolithotrophs. Detailed studies of gross oxygenic photosynthesis demonstrate high average maximum rates of about 18 mmol O2 m-2 h-1 independent of season. Half-saturation of activity occurs at relatively low light levels (200 mE m-2 s-1) illustrating the ability of the microbial mat organisms to fix carbon efficiently over diel variations in light.

Diel patterns of nitrogen fixation and denitrification were observed at all sites, with some sites exhibiting daytime maxima and other sites exhibiting night-time maxima. Rates of nitrogen fixation are related to the seasonal state of microbial mat dessication. Extreme low tides and warmer temperatures during the dry season increase water stress and cause decreased rates of nitrogen fixation (e.g. 3.88 mmol N d-1 m-2 in June 2000) compared to higher rates during the cooler wet season (e.g. 12.48 mmol N d-1 m-2 in September 2002, 15.43 umol N d-1 m-2 in October 2001). Potential (nitrate and glucose amended) denitrification rates exceed rates of nitrogen fixation by orders of magnitude, but at in situ nitrate and glucose concentrations, nitrogen fixation dominates the diel N cycle. Results from bioassay experiments showed that nitrogen fixation rates were primarily controlled by the availability of reductant (organic carbon), trace metals, and oxygen, while denitrification rates were controlled by the availability of nitrate and glucose.

Comparisons of C, O and N processing in surface sediments collected from fringe, transition and dwarf environments show that dwarf mangrove zones support the highest rates carbon and nitrogen fixation. Primary production rates and photosynthetic biomass (i.e. chlorophyll concentration) are variable across Twin Cays, but relatively constant throughout the year. Rates of nitrogen fixation and denitrification are also variable between sites, but in contrast, rates vary seasonally according to relief from the water stress of low tides during the dry season. The patterns of N input in the dwarf zones via activity in microbial mats may contribute to the larger, ecosystem scale patterns of nutrient limitation observed in trees (i.e. P limited trees in the dwarf zone and N limited trees in the fringe).

Fogel: During Year 3, we confirmed and extended the isotopic measurements published in prior work by McKee, Feller, and their Austrian colleagues. Red mangrove trees in the fertilized plots at Twin Cays have different N and C isotopic ratios depending on whether or not they have been fertilized with urea or phosphate or are controls. The body of our work, however, rests on over 1,000 measurements on red and black mangrove leaves, roots, stems, and wood from grid stations, vector experiments (radiating from one point), transects, new P fertilization

experiments, and samples of opportunity, in addition to the fertilized trees, at Twin Cays. Although our measurements are similar to those of McKee et al, we have been pursuing alternative hypotheses for explaining the very unusual range in N isotopic compositions, particularly the very negative d15N values (range û8 to û18 ë) that are found in certain places on the islands.
Together with John Cheeseman and Myrna Jacobson and her group at USC, we have worked together to assemble an integrative picture of the N cycling at Twin Cays. We have tested numerous hypotheses including the supply and demand hypothesis argued in McKee et al. (2002). Our measurements in addition to isotopic compositions include porewater ammonia, total solid phase phosphate (see Jacobson, Romero, and Smallwood's report), atmospheric ammonia concentrations, rainwater N concentrations, the isotopic compositions of porewater, rainwater, and atmospheric ammonia, photosynthetic measurements (see Cheeseman's section), lichen N isotopic compositions, N2 fixation measurements (see Jacobson and Burns' and Joye's sections), compound specific isotopic fractionations (Smallwood et al, to be submitted 2003), senescent leaf N isotopic compositions (Wooller et al., in press), and C and N isotopic compositions of leaf fragments in a 10 m core dated to be as old as 8,500 years old (Wooller).

Hypothesis 1: The N isotopic compositions of mangrove tissues are related to the inorganic nitrogen concentrations in sediments. At higher N concentrations, there is increased N isotopic fractionation during uptake and biosynthesis in the roots. Based on the ammonium concentrations in porewater and the coexisting N isotopic compositions in mangrove leaves, we find no evidence to support the hypothesis. Trees growing in floc zones or at slightly higher elevations have the highest levels of porewater ammonium, but d15N near zero. Conversely, trees fertilized with P grow in sediments with porewater ammonium levels <2 mM or even below detection, and these trees also have d15N closer to zero. Dwarf trees fertilized with urea do have higher porewater ammonium concentrations (e.g., 200 mM) and d15N values down to û10ë (2003 survey at the Dock site). Unfertilized dwarf trees growing in sediment with 20-30 mM ammonium, however, have d15N as low as û18ë. The relationship between substrate concentration and product in this ecosystem is not straightforward.

Hypothesis 2: The supply and demand for N determines the d15N of mangrove trees. High demands from rapidly growing trees result in lower isotopic fractionation, whereas in slowly growing trees isotopic fractionation is expressed (McKee et al, 2002). In general, this hypothesis supports many of the measurements we have made outside of the fertilization plots. In an extensive survey of unfertilized dwarfed mangrove trees, both the red and black mangrove species, however, we find a continuum of d15N from +2 to û18 ë. Red mangrove trees growing in the fringe zones around the islands may also have lower growth rates in certain areas, as evidenced by short internodes, thicker leaves, and 'stunted' appearance. All fringing red mangrove leaves from trees with long or short internodes had d15N values from +3 to û2 ë in sites distributed over the islands. Although Feller et al. (2003) have proven that fringing mangrove trees at Twin Cays are N limited for growth, the d15N values of N fertilized trees (-4ë at the Dock site, 2003) do not support the above hypothesis. Growth rate and supply of N are important controls on the d15N, but cannot be the only explanation for the range in d15N we have measured.

Hypothesis 3: Phosphorus limitation limits the active uptake of N through roots. Severe P limitation results in roots that have significantly diminished function. Mangroves growing under P sufficient conditions will have more positive d15N owing to the active transport of ammonium into roots rather than passive uptake. A relationship between the d15N and the N/P in the leaves and underlying sediments has been measured. Trees fertilized with P and some trees growing nearby with equivalent sedimentary and leaf total P concentrations have the most positive d15N (+2 to û1 ë), whereas those trees with lower available P have more negative d15N (-5 to û17 ë). The relationship between total P in leaves or sediments with d15N of leaves is not a linear or exponential function, however. In 2002, we started P fertilization experiments that followed the time course of d15N in dwarf mangrove leaves to see if and when the d15N became more positive and how that was expressed in the growth of the trees. After 5 months, the d15N increased from û14ë to û9ë in leaves that had P applied directly at the roots, as opposed to 1 m away or sprayed on the leaves. After 8 months, all P fertilized trees, regardless of the application method, showed evidence of responding by having longer internodes, thinner leaves, more leaves, new prop roots, and reproductive tissues. Control trees were unchanged. At thirteen months, the P fertilized trees had doubled in size. d15N in leaves increased in all P treatments: direct root application, d15N = -2 ë; 1 m distant root application d15N = -4 ë; foliar application, d15N = -8 ë. In conclusion, phosphorus can explain the continuum of d15N values from negative to positive values, but not the negative d15N itself.

Hypothesis 4: A pool of nitrogen in the ecosystem has a very negative d15N. This pool is important for the growth and maintenance of dwarf mangrove trees. In dwarf trees, the coarse and fine roots had more positive d15N by 4-13 ë than aboveground tissues, leaves, prop roots, stems, wood, and bark. The differences between roots/leaves are especially accentuated in trees with very negative d15N. To explain these intraplant differences in d15N, which are larger than any literature values, would require a previously unrecognized mechanism for the transport of isotopically light material from roots to leaves. Second, d15N of lichens collected from trees in the dwarf, transition, floc, and fringe ecotones of the island also have very negative values (as low as d15N of -22 ë) on trees that do not necessarily have such negative isotopic values. Lichens use atmospheric sources of N for their nutrient requirements; therefore the possibility that an isotopically depleted N source is available to them for growth is strong. The N isotopic compositions of the available pools were measured in 2003. Sedimentary, microbial and detrital N over the islands have d15N of 0.0?1 ë (n=150). Porewater ammonium values from mats, floc zones, dwarf regions, and underneath some N fertilized trees averaged d15N of 4?4ë (n=12). Ammonia in the atmosphere was always depleted in 15N, with isotopic compositions averaging û18?4ë (n=20), as was the ammonium in rainwater (d15N = -9?3ë; n=4). Uptake of N from the atmospheric pool then would explain the d15N of the most negative dwarf trees and lichens, and in addition explain depletion of 15N in leaves relative to roots.

Hypothesis 5: Foliar uptake of ammonia is an important, critical source of nitrogen for the Twin Cays mangrove ecosystem. Because phosphorus limitation is extreme here, dwarf trees have adapted to resolving nitrogen limitation and root dysfunction through their leaves. Isotopic compositions of dwarf mangrove leaves should be related to concentrations and isotopic compositions of ammonia in the air. Ammonia concentrations in the air above microbial mats, amongst the fertilization plots, and in interior ponds were measured with colorimetric badges (K&M Environmental). Ammonia concentrations in the air were highest just above the water surface in the floc zones, over mats found at the edges of large, interior ponds, and over N fertilized trees. Ammonia emissions from zones fertilized with P were some of the lowest values measured on Twin Cays. In general, atmospheric ammonia concentrations were extremely high (e.g., 1000 mmoles/m-3) in comparison to literature values. In almost all locations at Twin Cays, the concentrations exceeded the compensation point for ammonia in leaves. Short term, contained experiments with 15N-labeled urea confirmed that leaves of dwarf mangrove trees took up N within 24 h. Therefore, the conditions for leaf uptake of atmospheric ammonia are favorable, and mangrove leaves will take it up when available. Phosphorus-limited dwarf trees, growing adjacent to ammonia sources, in addition to lichens on trees in floc and dwarf zones, obtain a considerable portion of their N from the plentiful atmospheric sources with isotopically distinct isotopic compositions. Although it has been shown for crop plants that foliar uptake is an important pathway for nutrient uptake, the possibility that the N cycle at Twin Cays has a large atmospheric component supporting mangroves has not been demonstrated previously.

Nitrogen fixation by the microbial population is the most likely source of the ammonium found in sediments and the ammonia in the atmosphere (see Joye's and Jacobson's sections). For each molecule of N2 fixed by a microbe, however, 16 ATP molecules are required. Phosphorus addition to sediments increased N2 fixation at fertilization experimental sites (see Burns and Jacobson section), therefore phosphorus availability to microbes also appears to be limiting for microbial nitrogen fixation. Phosphorus cycling on Twin Cays, then, is closely linked to the nitrogen cycle through the microbial community. Without available phosphorus, not only do the mangrove trees grow slowly, but the microbial sedimentary community will fix nitrogen at rates comparable, but not in excess, to other ecosystems (Rosalynn Lee and Joye). Phosphorus additions to the microbial community include influx from the ocean during tidal or hydrological exchange or from anthropogenic additions via experiments. If solid-phase phosphorus was available to the microbial community but unavailable to P-limited mangrove trees, excess fixed nitrogen might support the quantity of ammonia found in the air at Twin Cays. Further work will concentrate on measuring and linking d15N of mangrove tissues with sedimentary P cycles, including GIS mapping of different P pools as they relate to N concentrations, reactivity, and isotopic distributions in the geosphere (Jacobson, Romero, Thornton, Burns, Fogel, Wooller, & Smallwood), biosphere (Cheeseman, Jacobson, Romero, Burns, and Fogel), and atmosphere (Fogel and Wooller).

Jacobson: 1-Similarity indices run using nutrient comparisons for all control zones (dwarf, transition and fringe trees) indicate that the fringe and transition zones show greatest similarity to each other than the dwarf zone. Relative presence of sulfide and ammonium at these stations is thought to be controlling the similarity indices. Nutrient content of sediments indicates that total P decreases in sediments going from fringe to dwarf zones in transects across the environment. Organic and inorganic P followed the same trend of decrease. The OP/IP ratio was not significantly different within zones for solid phase measures. Porewater phosphate measures are very low across all zones except where fertilization has been historically added. Phosphate measured across the entire island in a GIS generated grid system using the EASY program (Dale Kiefer) illustrate unequal distribution of P in sediments across the grid and no significant difference in organic/inorganic P ratio. This data is currently being compared with leaf P and N data. Most P is bound in the various organic fractions with the residual pool as the single most important. It seems that sediment P is hard to get except in the experimentally enriched sites.

Seasonal variation in porewater nutrient chemistry is evident. Ammonium - seasonality is evident across the sampling grid with Flocc >Fringe> Transition> Dwarf . Sulfide is seasonally evident in each zone but the pattern in more complex. Stoichemetric modeling of this data in conjunction with nitrate, phosphate, alkalinity, fatty acid, and respiration data is ongoing with the goal of understanding the relative importance of the various processes (sulfate reduction,methanogenesis, nitrate reduction, nitrogen fixation, etc).

Processes associated with nitrogen metabolism and acquisition were measured in the tree zones plus in the microbial flocc zone associated with the dock site. These processes varied across the tree height gradient. Incubation experiments done with process inhibitors indicate that sulfate reduction (and/or sulfide metabolism) seems to be more important in fringe and flocc sites while other processes associated with nitrogen acquisition (possibility associated with photosynthetic metabolism and N acquisition), seem to dominate in dwarf and transition areas in locations measured. Seasonal shifts are also observed. In experimental plots, the highest Nitrogenase activities were measured in associated with P additions across all zones, relative to N additions and controls. The highest response to P fertilization was measured in the dwarf zone. Nitrogenase activity in control transects measured was found to be highest in fringe and microbial flocc zones. At experimental plots where nitrogen was added, complex responses depending on location and tree height and N fertilization were observed.

2- Florescent substrate analysis: Enzyme substrates of carbon, nitrogen and phosphorous acquisition and metabolism were tested across a gradient of tree height at two seasons. In addition, phosphatase was measured in incubation and experimental samples. These enzymes are thought to indicate the 'perceived available substrate' as seen by organisms secreting these enzymes and are therefore correlates to nutrient limitation as well as eutrophication. These numbers are currently reported in terms of sediment mass but correlates of activity of bacterial are in progress. We currently feel that these enzymes have two components, on constitutive, and one induced by nutrient changes in the environment. Phosphatase activity is the highest in all zones followed by enzymes using leucine substrate. Carbon substrates (Glucoside and Galactoside) have the lowest activity. A comparison of regions and enzyme activity leads one to suspect that there are different nutrient

constraints on different zones both in nutrient type and the degree of limitation. This is not necessarily parallel to the finding relative to tree nutrient limitation. This complex mosaic is still under analysis Phosphatase activity is highest in the Flocc zones followed by dwarf, fringe, and then transition. We believe that this is an indication of bacterial activity, nutrient limitation and the availability of organic P in these zones. Proteases show a different pattern with flocc having highest activity followed by dwarf, transition and then fringe. Carbon substrates tested show a variable pattern where glucosidase activity highest in both the Dwarf and Fringe, and galactosidase activity is similar in fringe, flocc and transition zones and low in the dwarf zones. We believe that the latter are correlates of available carbon substrates however more substrate testing is planned. Enzyme kinetic models are in progress, which we will use to indicate the 'organism perceived nutrient load' in the selected environments.
3- Nitrogen isotopic values for Twin Cayes mangrove leaf tissue are found to be extremely negative relative not only to root tissues but also to all types of tissues world wide. This isotopic fractionation was also observed in lichens collected by M. Fogel and M. Wooller. Isotopic measures have been published on previously by McKee and Feller and Dr M. Fogel has continued this work. In support of this initiative we have measured phosphatase, total P, organic P, nitrate, nitrogen fixation (Burns, Jacobson, and Joye) phosphate, ammonium, sulfide, and compound specific isotopic fractionation (Smallwood) at selected sample stations associated with tree sampling for isotopes. We are currently working on coordination of the nutrient pool information with the stable isotope measures to determine the degree of ammonium/ammonia partitioning in the mangrove ecosystem. The integrative picture of ammonification, denitrification, nitrogen fixation, N cycling to the ocean and atmosphere will need to be a coordinated effort with Dr. Joye, Dr. McKee, and Dr. Fogel, and associates, all who have measured different components of N cycling in this ecosystem. This information may be placed on the EAsy system that we have developed for Twin Cayes (see information below).
4- Bacterial activity at transects across the gradient of tree height measured using ATP were found to be very low and highly variable. Experiments (joint with Dr. M. Frischer) are currently being analyzed using respiration technologies to measure bacterial activity. Bacterial types have been measured at experimental sites across the tree hight gradient. Terminal restriction fragment length polymorphisms (TRFLP) were utilized to assess differences in diazotrophic community structure between nutrient addition transects and tree height gradients. TRFLP analyses shows that certain members of the diazotroph community are present in all transects and samples, regardless of treatment. Conversely, certain fragments were unique to either tree height zone or treatment. The fragment pattern changed for all sites in nutrient addition transect. A few fragments remained important in both control and nutrient addition transects.
5- Root flux studies were initiated this February in selected zones in non-experimental sites with the objective of determining tree/microbial interactions in nutrient cycling, availability and acquisition by both microbes and trees. In addition, laboratory studies on small trees has just begun with the same objectives. This work is being done by Dr. D. Thornton and I Romero, both associates in the laboratory of M. Jacobson.
6- The easy program (a spatial, temporal, interactive GIS based program developed for Twin Cayes by D. Kiefer and associates) is now in a web based, password protected environment for those individuals who have expressed interest in participating with the initiative. Data associated with historical as well as recent experiments is being entered into a data base where it will be available for viewing and manipulation in time and space but the participants including Ulanowitz, Fogel, Wooller, Cheeseman, Smallwood, Jacobson. Details of programs and results may be found at Jacobson's
web site: http://wrigley.usc.edu/faculty/jacobson/home/.

Microbial activity (Frischer): Total bacterial abundance in peat cores ranged from 0.9 to 4 x 109 cells/g sediment corresponding to 90 to 300 ÁgC/g sediment. In general, fertilization treatments depressed the abundance of bacteria in the sediments by two to three-fold with the largest effects observed in the mangrove Fringe and Dwarf zones. Interestingly, this affect appears to be inversely related to nutrient limitation pattern of the trees suggesting a direct link between tree and bacterial population growth. For example, in the Dwarf zones where the trees are P-limited bacterial abundance is depressed relative to unfertilized sediments while in the Fringe zone where the trees are N-limited bacterial abundance is depressed by P-fertilization. Preliminary comparison of the species richness of mangrove peat bacterial communities suggests that relative to unfertilized sediments P-fertilization increases the diversity of bacterial communities while N-fertilization decreases bacterial species richness.
A manuscript describing these results is currently in preparation and the results were presented in August 2001 at the International Society of Microbial Ecology meeting in Amsterdam. In addition, this work served as the basis for Tara Foy's undergraduate senior thesis at Armstrong Atlantic State University. Ms. Foy plans to continue her involvement with this project as a graduate student beginning in the Fall 2002.

Microbial community structure (Shearer, Schmit): Fungi in Sediment:
In general, we found that the diversity and abundance of fungi in sediments is influenced by their location along the dwarf to fringe gradient. Fungi in the fringe zone are significantly more diverse and abundant than fungi in the transition or dwarf zones. There is no difference in fungal diversity or abundance between the transition and dwarf zones. There is no significant difference in fungal diversity or abundance in sediments related to nutrient treatment or depth, although there is a non-significant trend for higher fungal species diversity and abundance in nitrogen fertilized transects.

In leaves, fungi are most diverse and abundant early in the decay process. As decay continues fungal diversity declines, but bacterial abundance increases, which indicates a shift in the decomposer community. Early in the decay process oomycetes are ubiquitous, but they are not found later in the decay process.

Fungi that live in wood are strongly influenced by the nutrient addition. Wood in the phosphorus transect has a significantly higher fungal diversity and abundance than wood from the other two transects. Location of the wood did not play a role in determining fungal diversity or abundance, although there was a non-significant trend for wood in the fringe zone to have a higher fungal diversity.

Plant physiological responses (Cheeseman, Lovelock):
Cheeseman: Regardless of their location within the Twin Cays system, Rhizophora mangle leaves contain approximately 20% of their dry weight as proanthocyanidins (condensed tannins) and another 20% as flavonoids. The major flavonoids were quercetin-3-O-arabinopyranoside (F4), quercetin-3,7-O-diglucoside (F5) and rutin (quercetin-3-O-rutinoside, F7). Quercetin-3-O-rhamnoside (F2), quercetin-3-O-glucoside (F3), myricetin-3-3'-O-diglucoside (F6), and kaempferol-3-O-rutinoside (F7) were identified as minor flavonoids. The aglycone, quercetin, was itself present at low to undetectable levels except in senescent leaves. The proanthocyanidins are based mainly on (+)-catechin and (-)-epicatechin units in polymers up to octamers at least. The mangrove tannins are also characterized by an unusually high composition of catechin and epicatechin glycosides, and by the occurrence of (+)-epicatechin.During senescence, flavanol glycosides decreased in abundance, as did low ûmer tannins. Quercetin and caffeic acid became significant components of the polyphenolic pool, as did a previously unidentified compound, 5, 4'-dimethoxy-7, 3', 5'-triyhydroxyflavone. Because flavonoids are constructed through the phenylpropanoid pathway using phenylalanine as the major precursor, the molecules are metabolically expensive, and in mangroves, represent a major sink for carbon and energy that is not immediately productive. The diversity of components in the pool indicates evolutionary 'attention' to the value of individual components rather than simple use of flavonoids as sinks for excess carbon.
Peroxidase activities in leaf extracts reflect leaf development (being higher in mature leaves than young leaves) and overall growth rate of the trees (being higher in dwarf trees and sun leaves than in fringe trees and shade leaves of P-fertilized trees). There is probably one major peroxidase isozyme in mangrove leaves, not associated with cell walls. It is highly stable at high temperatures, losing little to no activity when incubated at 60˚C for 24 h, whether that be in crude extracts or purified form. Peroxidase also maintains its activity through leaf senescence and abscission. The enzyme is much more active with aglycones (e.g. quercetin) than their glycosides (e.g. rutin) or other polyphenolics. This activity may reflect the lifetime of free radicals generated during flavonoid oxidation. This feature also affects the coupling of peroxidase activity to non-enzymatic but peroxidase-dependent ascorbate oxidation. The enzyme will not oxidize ascorbate directly. Because the preferred substrates are not, apparently, present in non-senescent leaves, peroxidase detoxification of H2O2 may require the presence of glycosidases to maintain a small but efficient pool of aglycones.
At irradiances below 300 to 500 Ámol m?2s?1, mangrove leaf photosynthesis is linearly dependent on light intensity. Above that level, it is apparently light saturated, despite the much higher irradiances that it may experience in situ. Neither the low-light quantum efficiency nor the saturated rates of CO2 assimilation or electron transport are affected by growth zone (fringe or dwarf) or fertilizer regime. Because fringe zone trees and P-fertilized dwarf zone trees grow much faster than dwarf zone, unfertilized trees, this result is counter intuitive. At irradiances above 300 Ámol m?2s?1, photosynthesis is linearly related to stomatal conductance, at least up to 0.15 mol m?2s?1. This is true both at atmospheric and at elevated CO2. Again, there is no effect of growth zone or fertilization. For much of the year, there are no differences in stomatal conductance dependent on zonation, but analysis of d13C indicates a small but persistent reduction of stomatal conductance in dwarf zone trees relative to those in the fringe zone. At low stomatal conductance, photosynthetic electron transport and CO2 assimilation are substantially uncoupled. P-fertilization, and to a lesser extent, N-fertilization of dwarf trees increases stomatal conductance, but without changing the relationship between conductance and CO2 assimilation. We have made the first measurements of Rubisco activity in Rhizophora mangroves. Rubisco is active in the dark in mangrove leaves, indicating absence of the nighttime inhibitor found in some other plants. The mean activation state is similar in the dark and in the light and across fertilization treatments (ca. 80%). At irradiances below 300 Ámol m?2s?1, Rubisco activity is linearly related to irradiance at the time of leaf sampling. Above that level, it is linearly related to stomatal conductance at the time of leaf sampling. These characteristics are similar to those found for gas exchange, and represent the first such report in any species. An abstract has been submitted for the Ecological Society of America annual meeting.
Objectives for Year 4: Analysis of biochemical pathways of N assimilation and metabolism; analysis of the biochemical relationship of N metabolism to phenolic metabolism through phenylalanine ammonia-lyase; meta-analysis of leaf-to-air NH3 exchange in plants, the ammonia compensation point, and direct atmospheric ammonia fertilization of plants.

Lovelock: We clearly established that hydraulic conductance and architecture differed across the tree height gradient at Twin Cays, and that fertilization with N and P affected the hydraulic conductance of plants, with P limited dwarf trees showing a doubling of conductance when fertilized compared to control trees. Hydraulic architecture was also sensitive to fertilization. Mean xylem sizes were enhanced under fertilization and the frequency distribution of xylem sizes was shifted to larger size classes with the addition of P and to a lesser degree with the addition of N. Hydraulic properties of Avicennia germinans when fertilized were altered in a similar way to those of Rhizophora mangle. Responses of hydraulic conductance and architecture closely correlate with growth enhancements measured in these trees and lead us to the hypothesis that nutrient limitations are in some way limiting water transport in these mangrove forests.
Measures of leaf area index (LAI) also closely correlated with previously published growth responses, with dwarf P fertilized trees having LAI similar to trees in the taller fringing forest. Therefore, after only 5 years of fertilizer inputs, dwarf trees have similar structural characteristics as

those of taller fringing forests.
Respiration fine wood was enhanced in P fertilized dwarfs. Biomass of algal communities adhering to mangrove tree roots was greater in fringing forest than in interior dwarf stands and this was not affected by the fertilizer treatments. These data are being used to parameterize production in the tree compartments and macroalgal compartments of the Network analysis models (Ulanowicz and Shcarler).
Using a chlorophyll fluorescence imaging system (Walz Imaging PAM) we found that the leaf area influenced by the dominant herbivore, the mangrove crab Arratus pisonnii, was approximately double the size of the area of leaf tissue actually removed. Photosynthetic rates of leaf area directly adjacent to the crab wound was depressed by approximately 20% compared to leaf area not affected by herbivory. Fertilization with P in the dwarf ameliorated the effects of herbivory. This work is on going, but our preliminary conclusions are that affects of herbivory are greater than the amount of canopy removed, and that fertilization reduces the impact of crab herbivory.

Herbivory of mangrove tissue (Feller): Nutrient enrichment and zone had significant effects on herbivory that varied by functional feeding group or specific herbivore. Based on mass consumption that occurred over the lifetime of a leaf, zone had a significant effect on leaf area consumed or damaged by folivores. Mass consumption of leaves from fringe zone trees (9.1% û10.3%) was significantly greater than in the transition zone (6.6%-8.7%), which was significantly greater than in the dwarf zone (3.5%-4.6%). However, when damage is scaled up to the whole canopy, nutrient enrichment also had a significant effect on damage patterns such that the P-fertilized dwarf trees lost 8x as much leaf area to herbivores as control dwarf trees. Although N enrichment had a significant effect on growth of fringe trees, it had no effect of the amount of leaf area damaged in the fringe. The mangrove tree crab (Aratus pisonii) was responsible for ~83% of the leaf damage across zones, whereas the red mangrove leaf miner (Marmara undescribed sp.) was responsible for <3% of leaf damage. The remaining damage is caused by a puss moth (Megalopyge dyerii), a bagworm (Oikeiticus kirbii), and crickets. The densities of the tree crab and leaf miner (~0.2 m-2; 4.3 m-2, respectively) did not vary significantly by zone or by nutrient treatment. The most dramatic and significant source of damage to R. mangle leaves was caused by the mangrove bud moth (Ecdytolopha undescribed sp.), whose damage is the result of loss of yield rather than direct leaf mass consumption. Leaves produced from apical buds attacked by Ecdytolopha sp. are misshapen and reduced in size (~41% smaller). The percent of leaves exhibiting Ecdytolopha damage varied significantly by zone (dwarf: 12% ? 2; transition zone: 23% ? 3; fringe: 11% ? 2). An additional 3 to 6% of the leaves in each zone were aborted following Ecdylolopha damage. In addition, 2 to 9% of the apical buds were completely killed by Ecdytolopha. Because of apical dominance in R. mangle, this type of damage results in the death of the twig. These results demonstrate nutrient enrichment sometimes affects herbivory patterns, but it depends on the species of herbivore and location within the mangrove environment. Coupled with earlier studies on mangrove woodborers (e.g., Feller 2002), these findings show that primary consumption is a extensive and important in mangrove ecosystems.

Hydro?edaphic and peat analyses and elevation change (McKee): The rates of soil elevation change and vertical accretion varied across the study site at Twin Cays and in response to nutrient treatment. Control rates of elevation change varied from û0.52 cm/yr (dwarf) to 0.23 cm/yr (fringe). Addition of N doubled elevation loss rates (û1.03 cm/yr) in the dwarf zone, but P caused elevation gain (0.36 cm/yr). Vertical accretion (0.14-0.19 cm/yr), due to surface deposition of detrital/algal/ microbial/root mats, did not vary by zone or treatment, but mat type affected soil strength/compaction. Together, root production and vertical accretion explained 90% of variation in elevation change. The results indicate that habitat stability of these mangrove islands is controlled by a delicate balance between surface and subsurface biotic processes that are limited by different nutrients.

Network Analysis (Ulanowicz, Scharler):
Although the existing networks have been subjected to the network analysis routines, it would be premature to report such results, as they are still significantly influenced by the requisite data borrowed from remote mangrove ecosystems. Already the majority of data necessary for the carbon networks comes from Twin Cays, and a recent flurry of measurements on the processing of nitrogen will soon be used to amend N networks, so that some preliminary results worth reporting should soon be forthcoming.
R.E. Ulanowicz has related the demands of consumers to the supplies of prey items through a program that estimates the dietary proportions according to information on biomass, trophic topology, and physiological demands. The program partitions the flow magnitudes in joint proportion to both supplies and demands. The collection of stable isotope measurements among Twin Cays biota, currently being collected by M. Fogel and colleagues as part of this project, was vital in establishing the trophic links between species. Ulanowicz is now embarking on a program that will automatically update the magnitudes of the previously estimated exchanges according to the observed isotopic ratios of respective predators and prey.

Training and Development:

The project has provided opportunities for young scientists to conduct research in a unique ecosystem and to learn new techniques. :
(1) Ursula Scharler, post-doc
(2) Matthew Wooller, post-doc
(3) Mary Pittek, Masters student
(4) Julia Hofman, Masters student
(5) Mia Levine, Masters student
(6) Feller provided a 3-day training session for the staff of the

Institute of Marine Science, University of Belize
(7) Quinn Roberts, technician, went on her first trip abroad and
learned field work in difficult conditions.
(8) Denise Akob, undergraduate intern, learned sponge systematics and physiology and isotopic analysis
(9) Samantha Clancy, undergraduate intern, studied animal-plant interactions in mangroves
(10) Wilfrid Rodriguez, Ph.D. student

Outreach Activities:

Mangrove Biocomplexity outreach program:

(1) Graduate course in tropical marine ecology: Feller is co-coordinator of a new OTS tropical marine ecology course that will be offered for the first time June 15-July 18, 2003. This course will be taught at the Smithsonian's marine laboratory at Bocas del Toro, Panama. Seven co-PI's from the Mangrove Biocomplexity program will participate as guest lecturers and research advisors in this course.

(2) An undergraduate field course on the Biocomplexity of Mangrove Ecosystems: Feller collaborated with Stephen Davis (Texas A & M Univ.) and Eden Garcia (Director of the Institute of Marine Studies at the University of Belize) to organize a mangrove ecology course for undergraduates from TAMU and the Univ. BZ. This course took place August 1-13, 2003 at the Institute of Marine Studies on Calabash Cay, Turneffe Atoll, Belize. Undergraduates from both institutions earned course credit and participated in group research projects and conducted their own independent research.

(3) Mangrove Boardwalks: Feller is working with Toledo Institute for Development and the Environment, The Nature Conservancy, agencies within the Government of Belize, the University of Belize, and the Belize Audubon Society to plan and design educational boardwalks at four mangrove sites in Belize, including our field site at Twin Cays (which is now a World Heritage Site). We are currently seeking funding to proceed with construction.
(4) Mangrove Biocomplexity Electronic Field Trip in Belize: On May 13-15, 2003, we will conduct a mangrove biocomplexity electronic field trip (EFT) on Calabash Cay, Belize. Feller is collaborating with SERC's Education Department, with Ball State University, which has a grant from Best Buys to fund the EFT, and with Project View, a distance learning program based in the NY State school system, which has another grant from the US Dept. of Education to participate in EFT's and to develop the age- and grade-appropriate classroom curriculum. During Year 3, worked with participating teachers and held a mangrove ecology workshop for them. We will provided the teachers with training, field experience, and reference materials, including an on-line mangrove ecology course. The teachers spent the first part of next year working up the appropriate classroom material. In May, junior high school students, along with a crew of technicians, will accompany Feller to Calabash Cay at Turneffe for the actual EFT. As of midApril 2003, 13 million students had registered to participate live in the EFT from their classrooms. Students in the classrooms will be able to call with questions or send them by email, and have those questions answered in real time on line.

(5) Web-based mangrove ecology course: A teaching manual developed for our mangrove field course (Feller 1996) is being updated and converted into an online mangrove ecology course. We anticipate that this web-based course was completed in September 2002.

(6) Feller is collaborating with Dr. Kelton Clark, Smithsonian's NSF LSAMP program, and the University of Puerto Rico's LSAMP program to organize an undergraduate mangrove ecology course at the La Parguera Marine Laboratory. This course will take place May 26-June 4, 2003.

Outreach Activities in-house at SERC include:
a. Lectures to undergraduate interns on the mangrove biocomplexity project;
b. Poster presentations and discussion with the public during SERC's annual Open House;
c. Discussion and explanation of the project to numerous VIPs during visits to SERC (VIP's have included local and national political figures and business leaders).
(6) Feature article in the Spring 2002 issue of the Smithsonian
Environmental Research Center's Newsletter

SEMINARS presented on the Mangrove Biocomplexity project

SEMINARS present by Feller:
1. Feller, IC. (Symposium organizer) Microbial and Nutrient Controls on the Biocomplexity and Emergent Properties of Mangrove Ecosystems. INTECOL 7th International Wetlands Conference. Utrecht, The Netherlands, July 2004.

2. Feller IC and Duke N. (Symposium organizers) Symposium on Biocomplexity in Mangrove Forests: an Indo-West Pacific vs. Atlantic-East Pacific Comparison. Symposium Organizer. ERF2003, Sept. 2003. Seattle, WA.
3. Feller, I. C. and Sitnik, M. Science and Education in Belize: An international partnership for biodiversity and conservation. Conservation International Annual Conference. Washington DC, April 28, 2003.
4. Feller, IC, (Symposium Organizer) Symposium on Emergent Properties and Biocomplexity of Mangrove Ecosystems û How Will They Respond to Nutrient Over-Enrichment? Symposium Organizer, ASLO 2003, Salt Lake City, UT, February 6-10, 2003.
5. Feller, I. C. Lovelock, C.E., McKee, K. L. Mangrove Ecosystems: Nutrient controls on growth and herbivory. ASLO 2003, February 6-10, 2003
6. Feller, I. C. Nutrient controls on growth and herbivory in mangrove ecosystems. Department of Entomology Seminar Colloquium, University of Maryland, October 18, 2002.

7. Feller, IC. Effects of nutrient availability on mangrove growth and herbivory across a tree-height gradient. 3rd International Canopy Conference. Cairns, Australia. 23-28 June 2002.
8. Feller IC. Nutrient Controls on Ecological Processes in Mangrove Ecosystems. Workshop on LTER-Based Tropical Coastal Research across the Caribbean. LTER Network Support for Coordinating Collaborative Research. Florida Coastal Everglades LTER Program. University of Louisiana, Lafayette, LA. March 21-24, 2002.
9. Feller, IC. Mangroves in the Indian River Lagoon: Patterns of Nutrient Availability. Estuarine Research Federation 2001: An Estuarine Odyssey, St. Pete Beach, FL. November 4-8, 2001.
10. Feller, IC. Effects of nutrient enrichment on ecological processes in mangrove ecosystems. NIWA, Hamilton, New Zealand, July 30, 2001
11. Feller, IC. Ecological drivers in mangrove ecosystems. The Nature Conservancy Conservation Training Week. Miami, FL June 18-22, 2001.
12. Feller, IC. Mangrove research within the Smithsonian Institution's Marine Science Network. Workshop on LTER-Based Tropical Coastal Research across the Caribbean. LTER Network Support for Coordinating Collaborative Research. Florida Coastal Everglades LTER Program. Florida International University, Miami, FL. March 20-25, 2001;
13. Feller IC. Species richness and abundance of insects in mangroves. Symposium on Caribbean Clades and their Distributions. The Smithsonian Institution, Conservation International, The Center for Marine Conservation, March 27-28, 2001.
14. Feller IC. Ecology of mangrove ecosystems. Indian River Community College, March 1, 2001, Fort Pierce, FL.
15. Feller IC. Wood-feeding insects as ecosystem engineers in mangrove ecosystems. Biology Department, South Florida University, Tampa, FL, Nov. 15, 2000.
16. Feller IC. The role of wood-feeding insects in mangrove ecosystems. Plant Biology Department, James Cook University University, Townsville, Australia. Sept. 2000.
17. Feller IC, McKee KL, Whigham DE, Lovelock, CE. Decomposition and Nutrient Limitations in Tropical Mangroves. INTECOL 6th International Wetland Symposium. Quebec City, Quebec. August 6-12, 2000

SEMINARS presented by Lovelock:
(1) Lovelock CE, Heath K, Kandil F, Ball MC, Cheeseman JM. 2001. Do flavonoids and tannins have a role in photoprotection in mangroves? Abstract S20-032. 12th International Congress on Photosynthesis. August 12, Brisbane, Austalia.
(2) Ewe ML, Lovelock CE, Feller IC. 2002. Vegetative and reproductive phenology of fertilized mangroves in a nitrogen-limited ecosystem. Submitted Abstract, Ecological Society of America Annual Meeting, Tuscon, Arizona.
(3) Lovelock CE, Ball MC, Feller IC. 2002. Utilization of water and nutrients for photosynthesis in mangrove canopies across gradients in nutrient availability. Accepted Abstract, International Canopy Conference, July 2002, Cairns, Australia.
(4) Lovelock CE, Feller IC. 2003. Productivity in mangrove canopies across environmental gradients. Association for Limnology and Oceonography Annual Meeting, March 2003, Salt Lake City, Utah.
(5) Lovelock CE. 2003. Mangrove ecosystems: Responses to nutrient enrichment. University of Queensland, March 2003, Brisbane, Queensland, Australia.
(6) Lovelock CE, Ball MC, Feller IC. 2003. Petterns in photosynthetic carbon gain and resource allocation under nutrient enrichment in two biogeographic regions. Annual meeting of the Estuarine Federation, Seattle, WA.

Journal Publications

(1) Feller, I. C., K. L. McKee, D. F. Whigham, & J. P. O'Neill, "Nitrogen vs. phosphorus limitation across and ecotonal gradient in a mangrove forest.", Biogeochemistry, p. 145, vol. 62, (2003). Published

(2) McKee, K. L. I. C. Feller, M. Popp, & W. Wanek., "Mangrove isotopic fractionation (d15N and d13C) across a nitrogen versus phosphorus limitation gradient.", Ecology, p. 1065, vol. 83, (2002). Published

Lovelock CE and Feller IC, "Photosynthetic performance and resource utilization of two mangrove species coexisting in a hypersaline scrub forest", Oecologia, p. 455, vol. 134, (2003). Published

Lovelock CE, Feller IC, McKee K, Engelbrecht BMJ, Ball MC, "Nutrient limitation on growth in extensive scrub mangroves in Bocas del Toro, Panama.", Functional Ecology, p. , vol. , ( ). In preparation

Lovelock CE, Feller IC, McKee K, Thompson R, "Forest structure of the extensive Caribbean mangrove forests of Bocas del Toro, Panama.", Journal of Tropical Ecology, p. , vol. , ( ). In preparation

Feller IC, "The role of herbivory by wood-boring insects in mangrove ecosystems in Belize.", Oikos, p. 1657, vol. 97, (2002). Published

Feller IC, Whigham DF, McKee KL, Lovelock CE, "Nutrient limitation on growth and nutrient dynamics in a mangrove forest, Indian River Lagoon, Florida", Oecologia, p. 405, vol. 134, (2003). Published

Wooller, M. J., B. J. Smallwood, U. Sharler, M. Jacobson, and M. L.
Fogel
, "A taphonomic study of d13C and d15N values in Rhizophora
mangle leaves for a multi-proxy approach to mangrove paleoecology
", Organic Geochemistry, p. , vol. , ( ). Accepted

Wooller, M. J., B. J. Smallwood, M. Jacobson, and M. L. Fogel, "Carbon and nitrogen stable-isotopic variation in Laguncularia racemosa from Florida and Belize
", Biotropica, p. , vol. , ( ). Accepted

Books or Other One-time Publications

Lovelock C. E. & M. C Ball., "Influence of salinity on photosynthesis of halophytes.", (2002). Book, Published
Editor(s): A Lauchli and U Luttge (eds.)
Collection: Salinity: Environment - Plants - Molecules
Bibliography: Kluwer Academic Publishers

Web/Internet Site

URL(s):
http://www.serc1.si.edu:8003/biocomplexity/biocomplexity_index.htm

www.mangroves.si.edu

Description:

Other Specific Products

Product Type: Data or databases
Product Description:
Metadata for all data collected by each of the investigators involved with the mangrove biocomplexity project are being prepared.

Sharing Information:

Metadata files are being made available on the the website http://entomology.si.edu/tangled/roots.html

Contributions

Contributions within Discipline:

Our findings show that: (1) mangrove communities are far more complex than previously recognized; (2) nutrients are not uniformly distributed within or among mangrove ecosystems; (3) soil fertility can switch from nitrogen to phosphorus limitation along narrow gradients; (4) not all ecological processes respond similarly to, or are limited by, the same nutrient; (5) not all species respond the same to nutrient over-enrichment. We hypothesize that nutrient over-enrichment will alter ecological processes and generate changes in ecosystem properties, but that the influence exerted on mangroves by nutrient loading will vary depending on the nutrient, the location, and the ecological processes being measured.

Stable isotopes at the natural abundance level can be effectively used to provide linkages for developing the food web model for Twin Cays. Spatial distributions of isotopic composition can determine transfers of N and P from one part of the island to another and at the level of a specific tree can be used to rapidly and effectively tease out photosynthesis, nitrogen loading, and tree

Contributions to Other Disciplines:

On the stable isotope side, this is a test project to use the laws of biocomplexity and the power of stable isotopes to study non-linear processes. It turns out that nitrogen isotopic compositions of organisms from Twin Cays are an 'emergent property' that we are attempting to understand. Nothing is simple, even on this small island. In addition, we are working with the USC portion of the team to develop a GIS-based system that will be used to see patterns in the landscape that might be useful in tracking environmental change. Last, we are using stable isotopes for food web modeling, and there is a lot of interest in the community of ecologists, astrobiologists, and others to use isotopic data for modeling

Contributions to Human Resource Development:

Our project has engaged undergraduate, graduate, and postgraduate students in research. We have advised governmental officials in Belize on potential impact of nutrient over-enrichment on coastal ecosystems.

We have contributed to training of field station staff at STRI's and the Univ. Belize's field station. We have given public lectures and tours of our research activities and facilities. We have presented lectures and posters at scientific meetings. We have educatedprimary and secondary students in mangrove ecology through an electronic field trip.

Contributions to Resources for Research and Education:

We have developed a teaching manual for mangrove ecology. This manual is also presented on the web. We have developed a field course for students and teachers in mangrove ecology. We are hosting an electronic field trip to Belize mangrove, in collaboration with SERC's Education Department, Ball State University's Teachers College, and Project View, supported by grants from Best Buys and the US Department of Education.

At CIW, students have been trained in stable isotope technology and analysis. We have a 'hands on' lab, and students learn to prepare samples, run the mass spectrometer, and figure out what their data means. During this past year, 3 students and 1 postdoc, who are all working on some aspect of the project.

Contributions Beyond Science and Engineering:

We have provided advice to the Department of Foresty in Belize and our results have contributed to reforming regulatory policy for mangrove ecosystems in Belize. We have participated in training courses for resource managers from countries participating in the World Bank Mesoamerican Barrier Reef System Program (Mexico, Belize, Guatemala, and Honduras); this training is being used by members of the MBRS to monitor the status of their coastal zone and to improve management practices. We have provided training and education to personnel from environmental NGO's in Belize that have enhanced their abilities to manage and protect the coastal zone.

Special Requirements

Special reporting requirements: None
Change in Objectives or Scope: None
Unobligated funds: less than 20 percent of current funds
Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported: