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Belowground productivity of mangrove forests in southwest Florida

Beatriz Eugenia Giraldo Sánchez
Louisiana State University and Agricultural and Mechanical College

BELOWGROUND PRODUCTIVITY OF MANGROVE FORESTS IN SOUTHWEST FLORIDA

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DEDICATION

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Thanks God for blessing me during this journey….

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ABSTRACT

Studies in belowground dynamics are limited mainly due to the difficulty of studying roots despite wide recognition of its importance. This dissertation focused on methods for analyzing mangrove roots, root responses to phosphorus and flooding, and variation in root production between forest types.

Techniques to separate live and dead roots such as colorimetric, fluorescence, buoyancy, and visual assessment were compared. The traditional method of visual assessment combined with root buoyancy was accurate, fast, and applicable to larger samples. Additionally, techniques such as rhizotrons, root ingrowth cores, and root image analysis were useful to study mangrove roots.

Root and litter production, and hydro-edaphic conditions were determined seasonally for one year at eight sites (fringe, basin, and scrub forest types) in Southwest Florida. Root production was equal or greater than litter production showing spatial variation, especially in biomass allocation. Correlations with soil variables indicated that above and belowground processes respond differently to environmental conditions. The combined root+litter production was a good predictor of flooding and nutrient stress. Mangrove root production and morphology responded to nutrient enrichment (additions of nitrogen or phosphorus) depending upon forest type and stress factors interacting with resource acquisition. Root production increased with low nutrient availability and high flooding, and decreased with high salinity and nutrient availability. Nutrient enrichment increased the specific root length and surface area at the basin-monospecific site, and decreased the specific surface area at the scrub forest.

Greenhouse experiments were conducted to study root dynamics of *Rhizophora mangle* and *Avicennia germinans* seedlings in response to phosphorus availability and flooding regimes.

Mangrove species differed in their tolerance of flooding, and their plasticity to nutrients availability. The more flood tolerant species, *R. mangle*, was slower growing. The faster-growing species, *A. germinans*, exhibited limitations to flooding and changes in root morphology that altered the surface area for absorption of nutrients. These results indicate a trade-off between root strategies to tolerate flooding and to acquire nutrients.

This study contributes to a better understanding of how mangrove ecosystems function. Additional work in other geographic areas and sedimentary settings is needed to provide a broader perspective on belowground processes in mangrove systems.

CHAPTER 1
LITERATURE REVIEW

1.1. INTRODUCTION

1.1.1. Mangrove Ecosystems and Productivity

Studies have shown that mangrove ecosystems play an important ecological role along tropical and subtropical coastlines, contributing to primary productivity of the coastal zone and indirectly increasing secondary productivity of nearby coastal waters. In Terminos Lagoon, Mexico, for example, positive net fluxes of dissolved and particulate nitrogen from the mangrove to the coastal lagoon have been measured (Rivera-Monroy et al. 1995). This input of nutrients and organic matter enhanced the net primary productivity in the water column, especially during the rainy season due to increased run-off (Rivera-Monroy et al. 1998). Besides the importance of mangroves as sources of organic matter and nutrients, researchers found evidence recently in the Caribbean supporting their role as feeding and protection areas for juveniles of commercial and endangered fish species (Mumby et al. 2004). The presence of mangroves enhanced the structure of coral fish communities. Also, the biomass of some commercial species was doubled or increased up to 25 times near mangroves, even under fishing pressure (Mumby et al. 2004). Modeling analyses also support this functional role of mangroves (Barbier and Strand 1998). Using mangrove area and shrimp fishery data of Campeche, Mexico, modeling studies suggested a negative impact on shrimp production due to mangrove deforestation in combination with over-fishing (Barbier and Strand 1998). Mangrove deforestation may also have a negative impact on related coastal ecosystems by reducing the primary productivity of seagrasses and biodiversity of coral reefs due to increased turbidity of coastal waters (Rivera-Monroy et al. 2004). However, different types of mangroves (riverine, fringe, and basin) vary in functioning according to differences in hydrology, nutrient dynamics, and productivity (Ewel et al. 1998).

Studies of mangrove biomass and productivity have primarily focused on the aboveground component. Litterfall, which provides an idea of the amount organic matter and carbon available for turnover and export, is the most common estimate of aboveground production in mangrove forests. Based on this information, global and local patterns of aboveground dynamics have been described. Aboveground biomass and litterfall correlate positively with forest development, but decrease with increasing latitudes (Saenger and Snedaker 1993). In the New World, mangrove forests are classified by types: fringe, riverine, overwash, basin, and dwarf, based on local differences in hydrology, such as tides, waves, and runoff (Lugo and Snedaker 1974). Many studies have suggested a gradient in both mangrove productivity among mangrove types, dominant species, or stage of development, e.g., high values in riverine forests to low values in scrub forests (Odum et al. 1982, Twilley et al. 1986, Day et al. 1996, Twilley et al. 1997, Chen and Twilley 1999b) (Table 1.1). This variability has been attributed to the differences in hydrology between mangrove forest types, which controls salinity level, nutrient inputs, and sulfide concentration (Twilley et al. 1986, Day et al. 1989, Chen and Twilley 1999b). Ecological processes such as herbivory by crabs may also control litter turnover rates even under high energy-effects by tides (Twilley et al. 1997). Nevertheless, litterfall, degradation rates, and physical, chemical, and biological processes have been recognized as important factors for organic matter accumulation in mangrove soils, the importance of belowground processes (production and biomass accumulation) may be underestimated, considering the lack of information for this component.

Fine root production represents a large proportion of total annual net primary production in most ecosystems. Fine root production in terrestrial forests may account for up to 75% of total net primary production (Nadelhoffer and Raich 1992), but this process remains the least explored

Table 1.1. Biomass production (g m^{-2} yr^{-1}) of mangrove forests. ANPP: Aboveground net primary productivity

	Source	Location	Latitude	Longitude	Dominant spp.	Forest Type	Biomass Production
Root production	McKee and Faulkner 2000a	Naples, Florida	30º23.'N	91º8.6'W	*A. germinans* *R. mangle*	Basin	18-1,146
	Cahoon et al. 2003	Bay Islands, Honduras			*R. mangle* *A. germinans*	Fringe Mixed basin	311 333
Photosynthesis	Bunt et al. 1979	Hinchinbrook Island Queensland			*Rhizophora sps.* *C. tagal, B. gymnorrhiza*	Fringe and basin	584-949
Litterfall	Twilley et al. 1986	Estero Bay and Rookery Bay, Florida	26º02'N 25º02'N	81º45'W 81º34'W	*A. germinans* *R. mangle* *L. racemosa*	Basin mixed Basin mono	810 444
	Amarasinghe and Balasubramaniam 1992	Dutch Bay, Sri Lanka	8º15'N	79º50'E	*A. marina* *R. mucronata*	Fringe	407-588
	Day et al. 1996	Terminos Lagoon, Mexico	18º40'N	91º80'W	*R. mangle* *A. germinans*	Fringe Basin	793 307-496
	Twilley et al. 1997	Guayas River Estuary, Ecuador	2º25'S	79º40.5'W	*Rhizophora*	Riverine	647-1064
	Dawes et al. 1999	Cockroach Bay Tampa Bay, Florida	27º41'N	82º31'W	*R. mangle*	Overwash	1,132
	Sherman et al. 2003	Dominican Republic	19º10'N	69º40'E	*R. mangle* *L. racemosa*		1,140
Wood production	Golley et al. 1962	Puerto Rico					307
	Chen and Twilley 1999b	SW Everglades, Florida					320-1,200
ANPP	Amarasinghe and Balasubramaniam 1992	Dutch Bay, Sri Lanka	8º15'N	79º50'E	*A. marina* *R. mucronata*	Fringe	694-1,208
	Ong et al. 1995				*R. apiculata*		1,224* 1,135**
	Day et al. 1996				*A. germinans*	Basin	399-695
	Ross et al. 2001	Florida	25º27'N	80º 20'W	*R. mangle*	Fringe Dwarf	2,610 810
	Sherman et al. 2003	Dominican Republic			*R. mangle* *L. racemosa* *A. germinans*		1,970

* data obtained by allometric calculations, that may be 1700 g m^{-2} yr^{-1} assuming that root turnover is similar to aboveground

** data obtained by the gas exchage methodthat did not include respiration of branches, trunk, and roots.

component of terrestrial and wetland ecosystems (Symbula and Day 1988). In mangrove forests, simulation models of nutrient biogeochemistry suggest that the contribution of root production to soil organic matter is more important than litterfall (Chen and Twilley 1999a). However, there are almost no estimates of belowground dynamics, such as biomass accumulation (Clough 1992) or root turnover in mangrove forests (Clough 1992, Ong et al. 1995), and limited information is available about root growth and physiological responses to flooding and/or nutrients (McKee and Mendelssohn 1987, McKee 1996, 2001). Studies of belowground biomass and productivity of mangroves forest are needed to estimate root contribution to net primary productivity, and also help identify possible global or local patterns between forest types and species and their response to environmental factors. This information may also contribute to a better understanding of the carbon global cycle and climate change effects on coastal ecosystems.

Biomass production and accumulation, particularly belowground, provides an important function. These processes contribute to vertical accretion and ability of mangrove forests to keep pace with sea-level rise (McKee and Faulkner 2000b, Middleton and McKee 2001a). Studies have concluded that accretion rates in mangrove forests in Florida (Lynch et al. 1989, Parkinson et al. 1994, Cahoon and Lynch 1997), and Mexico (Lynch et al. 1989) are in equilibrium with reported sea-level rise rates. However, the stability or vulnerability of mangrove wetlands to sea-level rise is better explained by changes in soil elevation, which reflect not only the contribution of sedimentation or accretion, but also the effect of “shallow subsidence” by subsurface process such as compaction, decomposition, and dewatering as well as positive processes such as peat production (Cahoon and Lynch 1997). The contribution of mangroves to soil building is especially important in carbonate settings such as Florida and the Caribbean, where sedimentation depends mostly on autochtonous processes (Parkinson et al. 1994). For

example, mangrove leaf litter accumulation appears to be the main mechanism of soil accretion for basin forests in Southwest Florida (Cahoon and Lynch 1997). In Micronesia, the carbon accumulation rate was 93 g C m^{-2} yr^{-1} in conjunction with a sedimentation rate of 2 mm yr^{-1} during sea-level rise phases, but may be higher in other sites where higher accretion rates have been observed (Fujimoto et al. 1999). Mangrove peat has been reported to vary from 2 m depth (Golley et al. 1962, Fujimoto et al. 1999) to over 9 m depth (Macintyre 2004), indicating large stores of plant biomass beneath some mangrove forests. In Puerto Rico, Golley et al 1962 studied partitioning of the total biomass (above-ground and below-ground) contained in a mangrove tree and found that peat and fine roots (smaller than 0.5 cm diameter) exceed all other biomass components combined by 5:1. This accumulation of organic material as peat in mangrove soils serves as a sink for carbon, and also nutrients, especially nitrogen.

Some studies have estimated the total carbon and nutrient accumulation in mangrove forests. In arid and oligotrophic areas in Northwest Australia, most internal phosphorus and nitrogen of *R. stylosa* and *A. marina* resided in the leaves. However, roots, both live and dead, were next in storage of these nutrients (except P in *A. marina* roots). The estimation of carbon accumulation belowground (in soil, live and dead roots) ranged between 140-330 t C ha^{-1} for *R. stylosa* forests, and between 120-360 t C ha^{-1} for *A. marina* forests (Alongi et al. 2003). Also, the average total carbon accumulation in Australian mangroves was 471 t C ha^{-1}, with 64, 25, and 11% contribution from sediment, aboveground roots, and belowground roots, respectively (Matsui 1998). In coral-reef or estuarine mangrove habitats of tropical Pacific islands, total carbon accumulation of about 1300 t C ha^{-1} and nitrogen accumulation of 23 to 51 t N ha^{-1} have been estimated (Fujimoto et al. 1999). The location and high capacity of carbon storage in mangroves contrasts with that of terrestrial forests in which most nutrients are stored in the litter

(Alongi et al. 2003). This information supports the importance of mangroves in the cycling of nutrients, and also their role as C sinks.

The function of wetlands as carbon storage ecosystems has been further analyzed based on published information in mangroves and salt marshes (Chmura et al. 2003). Estimations of average soil carbon density are higher for mangroves than salt marshes (0.055 and 0.039 g cm^{-3}, respectively), and carbon accumulation rate for both wetlands is about 210 g CO_2 m^{-2} yr^{-1}, (which is an order of magnitude higher than the estimates in northern peatlands. Considering global estimates of mangrove coverage, Chmura et al. (2003) calculated that C sequestration in mangroves is about 38 Tg C yr^{-1}, which suggests that mangroves sequester carbon faster than terrestrial forests. Also, they suggested that the role of mangroves as carbon sinks may be ecologically more important since decomposition in mangrove soils occurs mainly through sulfate reduction, which would contribute less to greenhouse gases.

The accumulation of organic matter in mangrove soils reflects the interaction of soil processes under anaerobic conditions, which slows decomposition. Mangrove root decay rates are generally slower than leaves. For example, 90% of leaves degraded within a 5 mo period (Albright 1976), but 50 to 88 % of root tissues still remained after a year (Albright 1976, McKee and Faulkner 2000a). When roots remain buried, degradation rates are even slower (Albright 1976). In Belize, twigs and roots degraded slower than leaves, but roots were thought to contribute most to peat formation due to high root biomass production (Middleton and McKee 2001b). In Australia, decomposition rates of organic matter were only about 3-7% of the net primary production, which was consistent with the large accumulation of dead roots (Alongi et al. 2003). Additionally, rates of root decomposition may be higher in natural sites compared to restored forests (McKee and Faulkner 2000a). Interspecific differences in the rate of root

decomposition have been reported *L. racemosa* < *R. mangle* < *A. germinans* (McKee and Faulkner 2000a).

1.1.2. Belowground Biomass and Biomass Partitioning

The few studies that estimated belowground biomass of mangrove forests suggest that it is high in comparison to other tropical, temperate (Golley et al. 1975), and freshwater forested wetlands (Lugo et al. 1988) and marshes (Connor and Chmura 2000) (Table 1.2). High values of belowground biomass suggest an important allocation of carbon underground, and a fine root contribution of 69% (Tabuchi et al. 1983). Also, the reported data of belowground biomass suggest a high spatial variability, that may be controlled by global and local factors (Clough 1992), as described for the aboveground component (Saenger and Snedaker 1993). However, non-uniformity in the methodologies applied may also be reflected in these data.

Plants have different adaptive patterns of acquisition, storage, and allocation of resources to maximize benefits to support growth or other plant functions (Chapin et al. 1990). Studies of allocation patterns to above or belowground components in 22 herbaceous species supported the hypothesis of balanced growth, i.e., under nutrient limiting conditions, more biomass was allocated to roots, and under limited irradiance, more biomass was allocated to leaves (Shipley and Meziane 2002). In forest ecosystems, C allocation belowground was positively correlated to aboveground production at a global scale. Comparison of above and belowground production in forest systems at a global scale suggests that belowground production increases as aboveground production increases. These processes seem to be controlled by the same factors, but contrasting results were obtained depending on the methodology applied to estimate fine root production (Nadelhoffer and Raich 1992). Studies of *Phragmites australis* found a higher productivity

Table 1.2. Comparison of belowground biomass measured in mangrove forests.

Source	Location	Sampling/Analysis Method	Species	Belowground Biomass (t ha^{-1})	Fine root proportion (%)
Golley et al. 1962	Puerto Rico	Harvesting plots	*R. mangle*	50	-
Golley et al.1975[1]	Panama	Harvesting plots	*R. mangle*	73	-
Briggs 1977[2]	Australia	Soil cores	*A. marina*	147-160	-
Tabuchi et al.1983	Thailand	Trench	*R. apiculata*	338	**69**
Komiyama et al. 1987	Thailand	Trench and root density model	*R. apiculata Sonneratia*	438	50
			Sonneratia &Bruguiera	172	60
			Bruguiera	85	66
			Rhizophora	243	56
				510	46
Komiyama et al. 1988	Indonesia	Trench	*Sonneratia*	39	16
			Bruguiera	111-181	<
			Rhizophora	177-196	<
Komiyama et al. 1989[3]	Japan	Trench and root density distribution model	*B.gymnorrhiza*	**3,048**	11
			R.stylosa	890	16
Mackey 1993[2]	Queensland	Soil cores	*A. marina*	109-126	-
Saintilan 1997a[4]	Australia	Stratified coring	*A. marina*	70-166	
			A. corniculatum	35-106	
Saintilan 1997b[5]	Queensland	Soil cores	*A. marina*	15-60	
			A. corniculatum	25-80	
			E. agallocha	10-60	
			R. stylosa	100-110	
			C. tagal	35-45	
Matsui 1998	Australia	Allometric equations	*Rhizophora & Ceriops*	52	
Komiyama et al. 2000	Thailand	Trench, root density model	*C. tagal*	87.51	
Alongi et al. 2003	Australia	Soil cores	*R. stylosa*	40-60	15-19
			A. marina	10-20	8-27
Sherman et al. 2003[6]	Dominican Republic		*R. mangle*	67.8	14
			L. racemosa, A. germinans		

[1] Data calculated for depths up to 0.3 m. [2] Includes biomass of pneumatophores. [3] Calculated using the same procedure as Tabuchi et al. 1983, [4] Calculated based on regression equations of belowground biomass vs salinity. [5] Calculated based on figures provided, [6] Data includes live and dead roots.

belowground (8.5-9.7 g $m^{-2}day^{-1}$), compared to above (4.8 g $m^{-2}day^{-1}$) (Scartron et al. 1999). Furthermore, considering that this species was regularly flooded up to 20 cm during high tides and low river discharges, the high below/aboveground ratios for biomass (7.14) and production (2.58) suggest different responses of each of these components to controlling factors such as salinity. However, controls on biomass allocation patterns are not clearly understood (Raich and Nadelhoffer 1989).

Few studies have analyzed biomass allocation patterns in mangrove forests. Some of these studies suggest an increasing biomass allocation to root biomass with decreasing tidal inundation inland (Komiyama et al. 1987). This response may reflect more stressful conditions such as higher salinity, which increases the root/shoot ratio (Saintilan 1997b, a, Sherman et al. 2003). Mangrove studies under controlled conditions have found that when resources such as light and nutrients are limited, plants allocate more biomass to leaf area and to roots to maximize uptake. However, species differ in their responses according to specific abilities to cope with resource limiting environments (McKee 1995b). More studies are needed to understand controls on biomass partitioning in mangrove forest in response, especially to flooding and nutrient regimes.

McKee and Faulkner 2000a provided the first direct estimates of root production rates in basin mangrove forests (natural and restored) of Florida. They found that root production varied from 0.05 to 3.14 g m^{-2} d^{-1}, suggesting that C input by roots was 60-70% of litter fall. These estimates of belowground production in mangroves are similar to values for marshes in the Louisiana Gulf Coast in saline and less saline habitats dominated by *S. alterniflora* and *S. patens*, respectively (1.60 and 3.84 g m^{-2} d^{-1}; Nyman et al. 1995), but higher than values reported for temperate forests (0.44-1.62 g m^{-2} $d^{-}1$; Nadelhoffer et al. 1985) and lower than estimates in

Phragmites australis marshes in the Po delta, Italy (8.5-9.7 g m^{-2} d^{-1}; Scartron et al. 1999). These data further indicate the need to quantify belowground processes in mangroves to better understand the contribution of mangroves to global net primary production and carbon cycle.

Hydrology controls the biotic and abiotic characteristics of wetlands. Hydroperiod in most forested wetlands is extremely variable, and biomass allocation patterns appear to change in response to a flooding gradient (Day and Megonigal 1993). Productivity is hypothesized to decrease under extremes of continually and irregularly flooded conditions. Studies in belowground dynamics of Atlantic white-cedar, *Chamaecyparis thyoides* (L.), a coniferous forested wetland species, suggest that in overall belowground production (estimated from total root length) did not vary between permanent and intermittently flooded forests. However, production decreased in response to altered hydrology (lower water table) in the intermittently flooded forests (Rodgers et al. 2003). In bald cypress, *Taxodium distichum*, responses to different water regimes (continuous and periodically flooded) changed with age since total production of 1 yr old seedlings was reduced under constant flooding, and after three years, there were no differences in total production (shoots and roots) due to morphological and physiological adaptations by roots. However, differences in allocation patterns were evident (low root/shoot ratio under constant flooding with an optimum water and nutrient supply) (Megonigal and Day 1992). Mangroves experience predictable hydroperiods due to tidal flooding, but tidal regime may vary substantially among mangrove forest types (Lugo and Snedaker 1974). Such differences in hydrology influence salinity level, nutrient availability, and sulfide concentrations that will strongly control mangrove productivity (Twilley et al. 1986, Day et al. 1989, Chen and Twilley 1999b). However, there is a lack of information of the effect of hydrology on the belowground dynamics of mangroves.

In wetlands, flooding reduces the oxygen supply to the soil and promotes reducing conditions (Pezeshki et al. 1993). Reducing conditions may limit root growth if the supply of oxygen to the root system is less than that required for respiration (Sorrell and Armstrong 1994). Even in plants well adapted to waterlogged conditions, root development and growth may be affected by flooding (Gregory 1987). However, wetland plants, including mangroves, have different adaptations to cope with soil flooding and anaerobic conditions. Scholander and Scholander (1955) demonstrated gas exchange through the lenticels, allowing aeration of underground roots in *A. nitida* (= *germinans*) and *R. mangle*. Other studies also indicate this functional role of aerial roots (Curran 1985, McKee et al. 1988), pneumatophores (Curran 1985, Thibodeau and Nickerson 1986). Some work specifically showed transport of oxygen to the roots belowground under anaerobic conditions and the formation of the rhizosphere in mangrove species such as *A. germinans* and *R. mangle* (McKee et al. 1988). The shallow root system of *Avicennia* and the short distance between pneumatophores and the active roots is favorable for supplying oxygen (Wada and Takagi 1988). Under flooded treatments, pneumatophores of *A. marina* increased in height and density in response to anaerobic conditions (Toma et al. 1991).

In saltmarshes, nitrogen is the limiting factor to the growth of short *Spartina,* however this is a secondary effect to factors such as excessive soil waterlogging (Mendelssohn 1979, Mendelssohn et al. 1982). Available data indicate that porewater concentrations of P in mangrove forests are generally <40 μM for dissolved inorganic phosphorus and <4 μM for dissolved organic phosphorus. In mineral sediments, most of the inorganic P in mangrove sediments is either bound in the form of Ca, Fe, and Al phosphates or as soluble reactive phosphorus adsorbed onto, or incorporated into, hydrated Fe and Al sesquioxides. Total organic P concentrations are proportionally greater in the surface sediments (0-25 cm) due to the

influence of roots, whereas inorganic fractions, mainly Fe-P, increase with depth and reducing conditions below the root layer (Alongi et al. 1992). In mangrove soils at North Queensland, Australia, Boto and Wellington (1984) found that phosphorus decreased with depth and increasing elevation (total phosphorus ranged from 100-500 µg P g^{-1}, and (extractable phosphate ranged from 5 to 20.1 µg P g^{-1}). However most soil P was bound in organic forms and in combination with high salinity and low redox potential, was the major factor affecting the aboveground biomass of that area

In tropical montane forests of Jamaica, nutrient availability was an important factor controlling root production, which increased significantly in response to N and P additions (Stewart 2000). In mangroves, seedlings may alter their growth to maximize carbon acquisition in the absence of the most common stressors such as flooding, salinity, and herbivores (McKee 1995b). However, species responded differently according to their tolerance to limiting resources including nutrients (McKee 1995b). In mangroves, different responses in growth and productivity to nutrient limitation may be attributed to a more complex interaction between stress factors, such as salinity, flooding, and sulfide accumulation with nutrient soil dynamics and availability, and the plant internal demand and ability to acquire nutrients (McKee et al. 2002).

In mangroves, several studies have found that mangrove growth and productivity may be controlled by nutrient limitations of either nitrogen (N) or phosphorus (P) or both nutrients depending on local or regional characteristics. Researchers analyzed the effects of nitrogen by natural (Onuf et al. 1977) or experimental enrichment (Naidoo 1990, McKee 1995b, Feller et al. 1999, Feller et al. 2002, Feller et al. 2003, Lovelock et al. 2004) on mangrove growth. In the Indian River at Fort Pierce, Florida, (Onuf et al. 1977), found that trees fertilized by bird guano had higher growth rates (increased number of leaves, reproductive parts, branches, and biomass)

and higher N concentrations in tissues. In solution culture experiments, Naidoo (1990) analyzed the effects of nitrate, ammonium, and salinity on the growth of *Bruguiera gymnorriza* (L.) Lamk. He found that at low salinity, N additions increased biomass accumulation especially aboveground (total, leaves, and stems dry mass), yielding a greater shoot/root ratio. However, at high salinities biomass was reduced even at high levels of added nutrients. In Florida, a study was conducted in a mangrove area disturbed by mosquito impoundment to determine the effect of nutrient enrichment on plant growth, nutrient conservation mechanisms, and photosynthesis (Feller et al. 2003). The area was characterized by perpendicular gradients in tree-height and mangrove species (from *R. mangle* to *A. germinans*), from fringe, transition, to dwarf mangrove zones. Results showed that mangrove production was limited by N; increased photosynthetic electron transport, shoot growth, and leaf production were observed along all forest types enriched with N. In *A. germinans* growing under saline stress in the dwarf zone, N and P conservation mechanisms were enhanced, and carbon fixation increased in response to N fertilization, suggesting that N limitation was in part due to hypersaline stress conditions in the interior dwarf zone (40-50% higher salinity), which may have increased the physiological demand for N (Feller et al. 2003). In Florida and the Caribbean region, several other studies have found P to be a limiting factor for primary productivity of mangroves. Feller (1995) conducted fertilization experiments with P in an intertidal mangrove island at Twin Cays, Belize, area characterized by oligotrophic conditions. She found that P was a limiting factor of primary productivity of dwarf *R. mangle*. At the same area, Feller et al. (1999) determined that P enrichment decreased nutrient-use efficiency mechanisms, and controlled litter and belowground decomposition rates of *R. mangle*. They found that after fertilization, P resorption from senescing leaves and biomass per unit of P decreased, while nitrogen resorption efficiency

increased. These responses altered litter quality, but not decomposition rates. Thus, P enrichment in the Belize area might increase productivity, but the litter would have a higher P and lower N concentration, which may affect nutrient dynamics in the forest. At Shark River in the Florida Everglades, soil P explained changes in community composition and productivity of mangroves along an estuarine gradient of soil nutrients (Chen and Twilley 1999b).

At Twin Cays, (Feller et al. 2002) conducted a N and P fertilization experiment to study the patterns of nutrient limitation across a mangrove tree height/productivity gradient of *R. mangle*. They found that tall fringe trees were N-limited, dwarf trees were P-limited, and transitional trees were co-limited by both N and P. This patterns was related to spatial variation in nutrient availability and flooding effects on root acquisition of nutrients, particularly P. Studies conducted in a dwarf *R. mangle* zone at Bocas del Toro, Panama found that mangrove growth was limited to some extent by N but strongly by P. In that area, fertilization with N and P increased the number of leaves, but addition of P also increased the shoot elongation rate, stomatal conductance, specific leaf area, and decreased P resorption efficiency. Also, the photosynthetic nutrient used efficiency increased with nutrient additions showing a co-limitation pattern between N and P (Lovelock et al. 2004).

1.1.3. Mangrove Root Growth and Morphology

Root production is important to understand the role of plants in carbon cycling. However, root architecture influences plant productivity due to the uneven distribution of soil resources, i.e., the spatial deployment of the root system will influence the ability of a plant to exploit nutrient resources (Lynch 1995). The study of root architecture and morphology provides insights into function. (Lynch 1995). Architectural variables such as topology (branching pattern), link length, root diameter, and branching angle can be modified to increase root length

density and to enhance the exploitation of limiting resources (Fitter 1994). Root distribution refers to the orientation of roots in a spatial gradient (such as root biomass or root length as a function of factors such as depth in the soil, distance from the stem, and position between neighboring plants). Root morphology refers to the surface factors of single roots (Lynch 1995). Root morphology can be described by attributes such as root weight, root length, root surface area, root volume, and also ratios among these features. However, root length and weight are the most commonly used (Boot 1989). Root acquisition of water and nutrients is based more upon root length or root surface area than mass (Eissenstat 1992). The ratio of length to weight or specific root length (SRL) is an indicator of the gross morphology of the roots (Boot 1989). Root morphology is generally an inherent characteristic, but the final configuration of a root system is strongly influenced by environmental factors (Schiefelbein and Benfey 1991).

Root size and morphology influence rates and patterns of nutrient uptake (Boot 1989). Changes in root allocation and/or root morphology will depend on particular growth strategies of the plant species, and the availability of the limiting resource (Gregory 1987, Fitter 1994). When resources such as N, P, or water are limiting, a higher proportion of plant biomass may be allocated to root growth (Stitt and Scheible 1998), thereby incresing acquisition of the limiting resource and increasing the overall rate of growth (Eissenstat 1992). However, root proliferation in response to spatial variability of resources is an adaptive response that requires the investment of plant resources and is directly related to the diameter of the new roots (Fitter 1994). Fine roots have a high SRL ratio, which makes them more efficient at resource acquisition since they invest less biomass (Eissenstat 1992) and their cost for maintenance is lower (Fitter 1994). Fine roots tend to have greater plasticity in root growth, greater physiological capacity for water and nutrient uptake, and lower root longevity (Eissenstat 1992). Such plasticity may favor root

production where limiting resources are patchy. In contrast, coarse roots may be important for soil exploration. Their cost to the plant may be higher, but their production and maintenance may be worthwhile if resources are high (Fitter 1994). In nitrogen-deficient conditions, roots are much finer and more highly branched (Grime et al. 1991). In phosphorus limited conditions, P-efficient plants of the common bean *Phaseolus vulgaris* have a vigorous, highly branched root system and an increased number of apices with a high plasticity to respond to localized changes in P availability (Lynch 1995).

In mangroves, nutrient acquisition may also be influenced by root growth strategies involving changes in root morphology and biomass allocation. In the oligotrophic forests of Belize, mangrove roots of *R. mangle* and *A. germinans* proliferated in decaying roots and old root channels upon contact with decomposing organic matter and nutrients, which may enhance the uptake of relatively immobile P (McKee 2001). Field experiments involving nutrient enrichment have also found that mangrove species may enhance their growth and change patterns of biomass allocation as a response to increased availability of limiting resources (Feller 1995, Feller et al. 1999, Feller et al. 2002, McKee et al. 2002, Feller et al. 2003, Lovelock et al. 2004). However, these responses were strongly controlled by stress factors such as salinity and flooding regimes.

Mangroves also have developed metabolic responses to anaerobic soil conditions that may alter morphological or allocation patterns. Roots of *Avicennia germinans* respond metabolically to hypoxic conditions by increasing the capacity for alcoholic fermentation to maintain ATP production (McKee and Mendelssohn 1987). Physiological differences in the response of mangrove species to salinity and flooding have been evident in changes in biomass partitioning, a higher total plant and root biomass in *R. mangle* and *A. germinans* in comparison

to *L. racemosa* (Pezeshki et al. 1990). *Rhizophora mangle* and *A. germinans* responded differently to flooding under strongly reducing conditions and the presence of sulfide in growth and biomass partitioning showing opposite changes in total biomass; 20-40% decrease in *A. germinans* and 9-24% increase in of *R. mangle* seedlings (McKee 1993). Under low oxygen conditions, *A. germinans* and *L. racemosa* have lower respiration and root extension rates compared with *R. mangle*, which makes them more sensitive to soil oxygen stress and suggesting that *R. mangle* has a higher degree of plasticity to adapt to changes in the environment (McKee 1996). General changes in morphology (color and diameter), and anatomy of mangrove species roots in relation to the soil aeration status have also been described. *Rhizophora* species growing under well-drained conditions develop first and second laterals and a limited number of fine roots, while under waterlogged conditions, they produce a normal root system with several lateral roots near the soil surface (Gill and Tomlinson 1977).

Mangrove root systems are concentrated mainly in shallow horizons (top 50 cm) of the soil (Komiyama et al. 1989). They are composed of aerating, absorbing, or anchoring and cable components (Tomlinson 1986). The aerating component projects aboveground and controls gas exchange to the belowground root system, e.g., columns of prop roots of *Rhizophora* spp., the pneumatophores of *Avicennia* spp. and *Laguncularia racemosa*. The roots belowground include mainly soft, non-woody (that have little or non secondary thickening) roots with less than 10 mm in diameter that produce laterals up to three orders including fine roots, fibrous roots of less than 1 mm in diameter (Gill and Tomlinson 1977, Clough 1992). The distal part of the growing root is always branch- free (Gill and Tomlinson 1977). The anchoring roots grow belowground in a vertical direction (Chapman 1975), and their main function is to support the plant. The cable component extends horizontally from the tree base and unifies the aerating, absorbing, and

anchoring parts. For *R. mangle* this function is carried out by the arches, which are part of the aboveground component. The absorbing component is composed of fine roots that function in nutrient absorption and grow directly from thick, supporting roots (Wada and Takagi 1988).

1.1.4. Root Methodology

1.1.4.1. Standing Biomass and Production of Roots

Measurement of root dynamics is problematic. Different methods have been used for estimating root production such as sequential core, maximum-minimum biomass, in-growth core, and N budget methods, which have given inconsistent results (Nadelhoffer and Raich 1992). However, comparison of root production of freshwater swamps obtained by sequential coring and in-growth core methods yielded similar results for fine roots, but was less successful in measuring the production of coarse roots (>5mm) (Symbula and Day 1988). The in-growth core technique, which involves the implantation of root-free soil packed in a nylon bag into the sediment, was tested for use in mangrove forests. One of the advantages of this technique is that any roots growing inside the in-growth core are considered new production (Symbula and Day 1988). Other advantages include use of a standardized substrate that facilitates comparisons, rapid separation of roots, and smaller sample sizes for processing. Although this approach may not provide absolute measure of root production, it does provide a relative measure of production, which is appropriate for comparisons among sites and treatments. Consequently, this method was suitable for comparison of belowground production of mangroves among forest types and in response to nutrient treatments in the present study.

Since mangrove roots may proliferate in microsites with higher nutrient concentrations (McKee 2001), in-growth cores with added nutrients were used to examine N and P enrichment effects on root production, biomass allocation, and morphology. Fertilizer in the form of Triple

Super PO_4 or Urea (as solid granules to prevent significant losses by nutrient leaching) were added to in-growth bags. This modification of the in-growth core technique was successfully applied to test nutrient limitation in montane forest of the tropics (Stewart 2000).

The fine root system is the principal pathway for water and nutrient absorption (Eissenstat 1992). Consequently, quantifying the processes influencing fine root dynamics (production, longevity, and mortality) is important for an understanding of forest functioning (Hendrick and Pregitzer 1992). Also, since the possible interpretations of aboveground plant responses can be considerably different with the addition of belowground data (Day and Megonigal 1993), data on root production is essential for a complete picture of forest production and controls on forest functioning. However, there are inherent difficulties in studying roots due to their relative inaccessibility (Symbula and Day 1988). Also, it is difficult to distinguish between living and dead roots (Clough 1992). Thus, the development of practical methodologies is vital to continued progress in root research (Symbula and Day 1988). In addition, a more complete knowledge of root demography could contribute to on understanding of ecosystem processes (Hendrick and Pregitzer 1992).

In studies of root dynamics, researchers need to find ways to separate roots by their viability to obtain information about root production, mortality, and decomposition rates. In this study, laboratory techniques that have been used in root research to separate live and dead roots were assessed and tested for use in mangrove research. Triphenyltetrazolium Chloride (TTC) and colorimetric analysis have been used to determine percentages of living tissue in woody fine root samples (Joslin and Herderson 1984), fine-root vitality and its seasonal variation in coniferous species (Clemensson-Lindell 1994), and cold injury in woody plants (Steponkus and Lanphear 1967). In other studies, authors have verified the efficiency of colloidal silica for

separation of live and dead fine roots (Robertson and Dixon 1993) and by staining techniques (Stadelmann and Kinzel 1972, Bartholomew et al. 1981). These are potentially valuable tools to get more accurate estimates of mangrove root productivity, since turnover rates may be calculated and biomass losses by decomposition can be taken into account.

Separation of live and dead roots has been used previously in marshes. For example, live roots of *Spartina* spp. were characterized by turgid tissues, light color, and positive flotation, and dead ones were darker, with flaccid tissues, and negative flotation (Connor and Chmura, 2000). However, separation of *Plantago maritima* roots was not possible because they were darker, woodier, and more friable than other species (Connor and Chmura 2000). In freshwater swamp forests, live and dead roots have been also separated by inspection of physical features. In these forests, live roots were described as resilient, and dead roots were non-flexible, easily broken, with a damaged cortex, lack of lateral roots, and a loose stele (Symbula and Day 1988). Separation of mangrove roots by condition has also been attempted. Komiyama et al 1987 separated living and dead mangrove roots by inspection and described mangrove live roots as fresh, soft, and whitish in color, and dead roots as dark-brown hard tissues. McKee (2001) described mangrove live roots as light in color, turgid and structurally intact with positive buoyancy in fresh water. A method for separating fine roots of mangroves *Ceriops tagal* and *Rhizophora stylosa* using solutions of colloidal silica has been described (Robertson and Dixon 1993). Although these researchers achieved 94% efficiency, the technique requires optimization with roots of different plant species and root sizes. In the present study, several techniques applied in root research such as microscopic analysis, flotation, and staining methods, and colorimetric analysis were assessed to define their efficiency in separating live from dead mangrove roots.

1.1.4.2. Root Growth Rates and Patterns

Minirhizotrons, which require the insertion of clear tubes in the soil to obtain pictures of roots by the used of mini cameras, is a common and accurate technique used to monitor root dynamics directly in the field (Vogt et al. 1998). A similar technique, rhizotrons, provides an alternative option that can be applied in greenhouse facilities. In the present study, rhizotrons (clear acrylic boxes) were used to study root dynamics, especially patterns of root growth and changes in root density of mangrove seedlings of two mangrove species under controlled conditions. Rhizotrons were held in two wooden frames at an angle of about 30 ° from the vertical to promote root growth on the viewing face and to facilitate root tracing and monitoring of root dynamics. Rhizotrons were filled with moistened commercial peat soil over a bottom layer of 4 cm of pea gravel and covered with black plastic sleeves up to soil level to prevent light penetration into the soil. This technique has not been used previously to study root dynamics in mangroves.

1.1.4.3. Root Morphology

Several image analysis methods exist to quantify root morphological attributes. In this study, MacRHIZO, a root analysis software (Regent Instruments, Inc.) was selected because it provides rapid quantification of root traits such as total length, diameter, surface area, and branching using an scanned image of roots. This tool was used to study root morphology of fine and medium class size mangrove roots following the scanning protocol suggested by Bouma et al. (2000). Initial validation of measures obtained with MacRHIZO was accomplished by comparison with direct measurements of root length and diameter distribution on separate root samples and sketches.

Dimensional ratios also provide insights about morphological responses of root systems to controlling or limiting factors. In this study, biomass allocation to roots was analyzed using ratios such as root biomass ratio (RBR), root:shoot ratio, relative root growth rate (RRGR) and specific root length (SRL), calculated as follows:

$$\text{RBR} = \text{Root biomass} / \text{Total plant biomass} \quad (1)$$

$$\text{Root:shoot ratio} = \text{Root biomass} / \text{Shoot biomass} \quad (2)$$

$$\text{RRGR} = \ln(\text{final root dry weight}) - \ln(\text{initial root dry weight}) / \text{time} \quad (3)$$

$$\text{SRL} = \text{root length} / \text{root biomass} \quad (4)$$

SRL was calculated for all scanned roots whose dry weight was recorded after root length was obtained through image analysis.

1.2. RESEARCH OBJECTIVES

The major goal of this research was to investigate belowground processes in mangrove forests by describing how two major forcing functions, hydrology and nutrient concentrations, affect root dynamics. The specific objectives of this research were: (1) to assess laboratory and field techniques that have been used in root research and test them for use in mangrove systems (2) determine the effect of biotic (species composition and forest types) and abiotic (hydroperiod and nutrient level) factors on mangrove root dynamics under experimental and field conditions, (3) to describe architectural aspects of mangrove roots (distribution and morphology) from field and laboratory experiments. The research questions addressed in this study will contribute to a

better understanding of controlling factors on mangrove primary productivity and nutrient cycling.

The research questions addressed were:

1. Which are appropriate techniques to study mangrove root traits such as root production (growth, density, biomass), root morphology, and to separate live and dead mangrove roots?
2. How does root standing biomass and production vary between mangrove forest types and species composition?
3. How does root standing biomass and root production vary relative to abiotic factors?
4. What is the effect of nutrient amendments on root growth patterns and production?
5. Do patterns of belowground production across different forest types and species composition correlate with aboveground production (litterfall)?
6. How do dynamics and distribution of mangrove roots vary in relation to different flooding and nutrient regimes under controlled conditions?
7. What are the morphology and root turnover rates of mangrove roots and how are they altered by different flooding and nutrient regimes?

Evaluation of different techniques for mangrove root analysis is covered in Chapter 2 and addresses research question 1, listed above. However, these techniques were further assessed in field and greenhouse experiments described in Chapters 3-4. The root ingrowth technique was used to obtain data of biomass and production of roots in mangrove forests of southwest Florida and these results are presented in Chapter 3. Root separation techniques were used to distinguish between live and dead mangrove roots in both field and greenhouse studies. Rhizotons were used to assess root growth and morphology in greenhouse experiments reported in Chapter 4.

Finally, root image analyses (MacRHIZO) were used to study the morphology of mangrove roots in the field and greenhouse experiments.

Chapter 3 focuses on belowground dynamics such as root production, root standing biomass, and effect of nutrient amendments on root production and morphology in the field. Above-ground productivity (estimated from litterfall rates) was also quantified and compared between forest types and species composition in relation to below-ground productivity. This study was conducted at three locations in the Naples area, southwest Florida, and it included three mangrove types, fringe, basin, and shrub, with different species composition allowing the testing of research questions 2, 3, 4, 5, and 7.

The results of two greenhouse experiments are described in Chapter 4. These experiments assessed growth, density (by root size class), distribution, biomass and biomass partitioning, and morphology of mangrove roots of *R. mangle* and *A. germinans* under different hydrology and nutrient levels. The experimental design included a factorial treatment with three flooding treatments and two phosphorus treatments. This chapter addressed research questions 6 and 7.

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CHAPTER 2

ASSESING METHODS TO STUDY MANGROVE ROOTS

2.1. INTRODUCTION

Terrestrial primary production and climate appear to be correlated, but these interactions may differ depending on local dynamics of nutrients (Melillo et al. 1993). The ecological role of terrestrial plants in the carbon cycle has motivated research to improve root study techniques in forests around the world. The function of wetlands, especially mangroves, in carbon storage has been suggested by different studies and reviews (Matsui 1998, Fujimoto et al. 1999, Alongi et al. 2003, Chmura et al. 2003). Belowground carbon storage in mangrove systems has been estimated to be around 38 Tg C yr^{-1}, higher than other terrestrial forests (Chmura et al. 2003). This high C accumulation has been facilitated by the slow decomposition rate of organic matter in mangrove soils, especially roots (Albright 1976, Alongi et al. 1992, McKee and Faulkner 2000a, McKee 2001), lowering potential release of greenhouse gases (Chmura et al. 2003). Despite the importance of evaluating root dynamics in terrestrial forests, the estimation of the contribution of roots to plant productivity has proven to be difficult and time consuming.

Researchers have used several direct and indirect techniques to estimate belowground production, especially the contribution of fine roots. Some of these approaches include the sequential core, maximum-minimum biomass, ingrowth core, and N budget methods, which have shown contrasting results (Nadelhoffer and Raich 1992). Some researchers have evaluated limitations of these techniques when measuring root production (Symbula and Day 1988, Nadelhoffer and Raich 1992, Vogt et al. 1998, Makkonen and Helja-Sisko 1999).

The objective of this study was to assess laboratory and field techniques that have been used in root research and test them for use in mangrove systems. Different techniques for root

analysis were used to examine mangrove response to spatial variation in environmental conditions as well as manipulation of nutrient availability. Some of these methods were applied in the field or greenhouse studies. In this study, several techniques were tested to study mangrove root growth and production, some of which had not been used previously in mangrove research.

The ingrowth core technique was used to study root production in mangrove forest, which allows estimates of new production (Symbula and Day 1988). This technique was combined with nutrient additions to examine the effect of nutrient enrichment on belowground production, biomass allocation, and root morphology of mangroves. This modification of the basic ingrowth core technique has been applied previously in a few instances, e.g., to test nutrient limitation in montane forest of the tropics (Stewart 2000).

In the greenhouse, rhizotrons were employed to study root elongation and distribution (anchor root growth rates, and root density per class size and depth) in response to nutrient (phosphorus) and flooding regimes. Additionally, I used an image analysis software to study root morphology, and I calculated some ecological ratios to evaluate morphological responses of root systems to controlling or limiting factors such as hydrology and nutrients.

Laboratory techniques to separate live and dead roots were assessed, including separation of roots by physical features, flotation (Robertson and Dixon 1993), colorimetric (Steponkus and Lanphear 1967, Towill and Mazur 1975, Joslin and Henderson 1984, Clemensson-Lindell 1994), and fluorescence (Rotman and Papermaster 1966, Heslop-Harrison and Heslop-Harrison 1970, Stadelmann and Kinzel 1972, Bartholomew et al. 1981).

2.2. METHODS

2.2.1. Root Vitality

Microscopic and colorimetric techniques were used to determine percentages of living tissue in mangrove roots. Mangrove seedlings of *A. germinans, R. mangle*, and *L. racemosa* (25 individuals per species) were collected from Terra Cia Isle in the Tampa Bay area and established in the greenhouse. Each seedling was assigned to one of four decomposition groups (duration after root mortality). Shoots of the decomposition groups were severed at the base to kill the seedling, and root systems were harvested at 1, 4, 8, and 12 wks after mortality (n = 5 per time interval). A set of five pots with roots of live seedlings was used as the control group, which were collected at the end of the experiment. Data collection included percent of live and dead roots, which were determined using the following laboratory techniques.

2.2.1.1. TTC

The colorimetric technique was based on Triphenyl Tetrazolium Chloride (TTC) This technique is based on the reduction of TTC by enzymes, mainly dehydrogenases (DH), and thereby provides a rough estimate of DH activity (Clemensson-Lindell 1994).

If red formazan is produced, the tissue is viable, whereas in dead tissues these dehydrogenases will be inactive and low or no formazan produced. The analysis followed the protocol described by Clemensson-Lindell (1994), Joslin and Herderson (1984), and Steponkus and Lanphear (1967). Mangrove roots were weighed (200 mg), cut into small segments (1-2 mm), and put in a test tube with 6 ml TTC (Triphenyl Tetrazolium Chloride) 0.6% w/v in 0.06 M Na_2HPO_4 – KH_2PO_4 buffer (pH 7.4) + 0.05 % (v/v) wetting agent. Next, samples were infiltrated under vacuum (680 mm Hg) for 15 min and incubated at 32°C for 24 hrs. The TTC solution was drained, and root samples were rinsed once with DI water, and 7 ml ethanol (95%) was added for

extraction of the water–insoluble Red Formazan in a boiling water bath at 80°C for 15 minutes. When extracts were cold, the volume was adjusted to 10 ml with ethanol (95%), and absorbance was determined in a spectrophotometer (using ethanol as the blank). Absorbances were read at 480 nm wavelength, which had the highest absorbance of Formazan in mangrove roots (Fig. 2.1). Dry weight of root samples was calculated based on the relationship between dry weight of live tissue and the absorbance of the Formazan (Joslin and Henderson 1984). Standard curves per species were obtained by mixing known masses of live and dead roots of each species at different proportions.

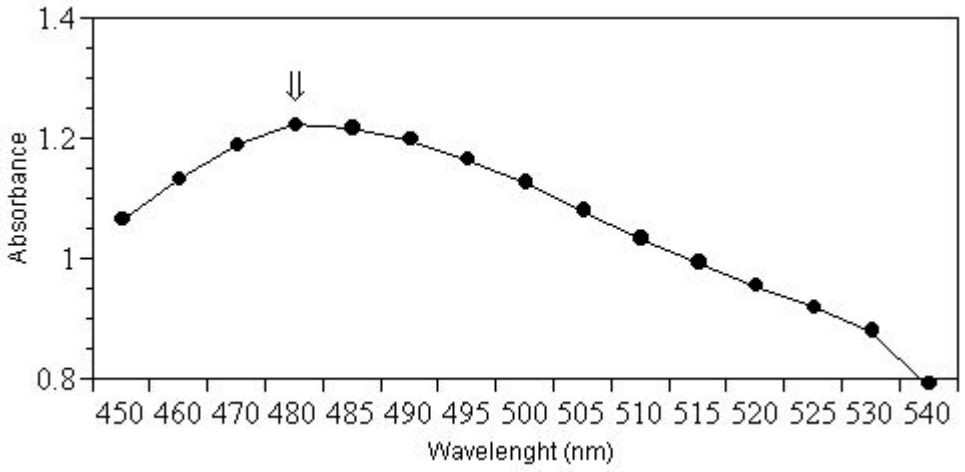

Figure 2.1. Absorbance at different wavelengths of a formazan solution extracted from mangrove root samples.

2.2.1.2. FDA

A technique to separate live and dead animal tissues proposed by (Rotman and Papermaster 1966) that uses fluorescein diacetate (FDA) and modified by (Heslop-Harrison and Heslop-Harrison 1970) for plant cells (pollen) was applied to identify viable cells of mangrove roots. The applicability of this method is based on the integrity of the cell membranes which can be assessed by the fluorochromatic reaction (FRC). This reaction involves the rapid entry of non-fluorescent FDA molecule into the cell, where it is hydrolyzed by esterase forming a polar

product, fluorescein, a fluorescent substance that accumulates in the cell depending on the integrity of the plasmalemma. In cells with damaged cell membranes, FDA is lost easily and does not produce a fluorescent molecule (Bartholomew et al. 1981). The procedure involves the preparation of two solutions, a stock solution made by dissolving Fluorescein diacetate (Sigma #7378) with acetone (at 1mg ml^{-1}) and a 0.5 M sucrose solution. The working FDA solution was made by adding 10µL FDA solution to10 ml 0.5 M sucrose solution. A small sample of roots was immersed in 100 µL of working FDA stock solution and infiltrated under vacuum to facilitate the entrance of FDA into the cells. Then roots were mounted on a slide for analysis and sorted using a Hertz Ortholux II epifluorescent microscope with a H2 filter cube.

2.2.1.3. Colloidal Silica

A method proposed by Robertson and Dixon (1993) using solutions of colloidal silica was tested to separate roots of *A. germinans*, *R. mangle,* and *L. racemosa*. The method is based on separation of live and dead roots by flotation in colloidal silica (Ludox) dissolved in water at different proportions (6 and 11%). Mangrove roots contain large amounts of air-space tissue, which is filled with air when alive and water when dead. Consequently, live roots float and dead roots sink. Each sample was placed into the successive solutions for 15 min, and floating roots were separated from those that sank. The root fractions from the two separations were combined, dried, and weighed.

2.2.1.4. Visual Assessment

Live and dead roots were examined according to visual assessment of physical features such as color, turgidity, structural integrity, and presence of water or air bubbles in the cortical air-spaces.

2.2.2. Root Standing Biomass and Production

The in-growth core technique was applied to estimate root production. This technique requires the insertion of a nylon bag packed with root-free soil into the sediment; consequently, any roots growing into the bag are considered new production (Symbula and Day 1988).

Root in-growth cores were made of nylon bags with a flexible mesh of 3 mm^2. The cores were about 30 cm long and 4.5 cm diameter, which corresponds to a volume of 377 cm^3. Core bags were filled with approximately 85 g of root free peat soil (Hyponex, Canadian Spagnum Peat Moss, Hyponex Corporation 14111 Scottslacon Rd., Marysville OH 43041) and labeled. In April 2001, in-growth cores were taken to the field where they were plugged into vertical holes made with a corer, making sure they were not either forced inside the soil and that the top of the core was even with the soil surface. The site was flagged to facilitate later collection, which was made seasonally. Bags were extracted with a sharpened corer (5 cm diameter) to cut roots and sediment that were surrounding the in-growth cores. After bag retrieval, external material and protruding roots were severed with scissors.

Bags were divided into depth increments and opened lengthwise. Roots were rinsed and placed in a tray with tap water. Roots were sorted by condition as live/recently dead, and dead based on physical features, and then by size (fine, medium and coarse). In the separation process of living and dead mangrove roots by gross visual inspection or microscopic analysis, features such as buoyancy, color, and integrity of stele and tissues, and air bubble presence were considered. This analysis allowed the description of the roots according to their physical attributes. A lamp with a magnifying lens was used to facilitate separation of roots from soil particles and wood. Subsamples of roots were preserved in a 1:10 methanol solution for later

digital analysis. Finally, sorted roots were put in labeled paper bags and dried at 70ºC until constant weight to obtain dry biomass (g).

Nutrient enriched in-growth cores were used to test the effect of nutrient enrichment on root production, root biomass allocation, and root morphology. This study involved P and N fertilization by mixing 14 g of fertilizer granules (Triple Super PO4 or Urea) with approximately 85 g of peat soil that was packed into mesh bags. The insertion, collection, and analysis of roots for this experiment followed the same methodology as described previously for in-growth cores samples.

To estimate standing root biomass, soil cores were extracted with a 30 cm long corer in Spring 2002. Once collected, cores were divided by depth, top and bottom layers of approximately 15 cm each, and stored in a tagged plastic bag. In the laboratory, roots were washed on 1 and 2 mm screens, separated from soil particles and wood, placed in labeled paper bag,s and dried at 70ºC until constant weight to obtain root biomass (g).

2.2.3. Root Elongation and Distribution

Clear acrylic boxes called rhizotrons (0.6 L capacity, 56 cm x 38 cm x 5 cm) were used to study mangrove root dynamics of two species, *A. germinans*, and *Rhizophora mangle*, at the greenhouse facility of the Wetland Biogeochemistry Institute at Louisiana State University (Fig. 2.2). Rhizotrons were held in two wooden frames at an angle of about 30 ° from the vertical to promote root growth on one side of the rhizotron and facilitate root tracing and monitoring of root dynamics. A layer of pea gravel (4 cm) was placed at the bottom of the rhizotron to facilitate drainage and overlain with commercial peat soil. Rhizotrons were permanently covered by black plastic sleeves up to soil level to prevent light penetration into the soil. The

rhizotrons had an outlet at the base made of aquarium tubing, which allowed control of water level, and salinity.

Root growth patterns were traced onto plastic films using a different color marker at each sampling date. This root tracing allowed estimation of root growth rates and root density (per size class fine <2mm, medium 2-5 mm, and coarse roots >5mm) at different depths. Root tracing of all emerging roots began between two and six weeks after the experiment started. Initially they were traced weekly, then at bi-weekly or monthly intervals for about six months, After root density became high, quadrants 10x10 cm (top and bottom) were traced until the end of the experiment. Linear equations of root growth were obtained for each species fitting the curve for the root growth observed before roots reached the bottom of the rhizotron (9 and 32 weeks for *A. germinans*, and for *R. mangle*, respectively). Root density rates for each species were calculated based on the slopes of root density variation over time obtained for each rhizhotron, treatment combination and depth.

Figure 2.2. View of the greenhouse and the rhizotrons.

2.2.4. Root Morphology

MacRHIZO, a root image analysis software (Regent Instruments, Inc.), which allows the quantification of morphological traits, was used to analyze roots in greenhouse and field experiments. The analysis followed the scanning protocol suggested by (Bouma et al. 2000). Prior validation of the method was based on measures of root length and diameter that were directly made on root samples and root sketches.

The analysis required the scanning of root samples, and the importing of its image to MacRhizo. The digital analysis superimposed a colored root skeleton over the image, with each color representing a different root diameter class. Data generated such as total root length, diameter, surface area, and branching were saved into an Excel file. The analysis was performed following the scanning protocol suggested by Bouma *et al* (2000). Finally, these root samples were dried to calculate the specific root length (SRL, meters of root per gram of root tissue) as root length/ root biomass ratio (Eissenstat 1992).

Ratios also provide insights about morphological responses of root systems to controlling or limiting factors. In this study biomass allocation to roots was analyzed using ratios such as root biomass ratio (RBR), root:shoot ratio, relative root growth rate (RRGR) and specific root length (SRL, meters or centimeters of root per gram or milligram of root tissue, respectively), that were calculated as follow:

RBR = Root biomass / Total plant biomass (1)

Root:shoot ratio = Root biomass / Shoot biomass (2)

RRGR= ln root dry weight / lenght of the experiment (3)

Assuming that initial root weight was zero

SRL= root length/ root biomass (4)

SRL was calculated for all scanned roots whose dry weight was recorded after root length was obtained through image analysis.

2.3. RESULTS

2.3.1. Root Vitality

2.3.1.1. TTC

TTC standard curves for *A. germinans*, *R. mangle*, and *L. racemosa* are illustrated in Figs. 2.3, 2.4, and 2.5, respectively. Formation of the formazan product was evident by the red colored solution obtained after extraction. These equations were used to calculate the proportion of live roots in the mixed samples from the greenhouse experiment. The average time needed to analyze a sample with the FDA technique was 111 min.

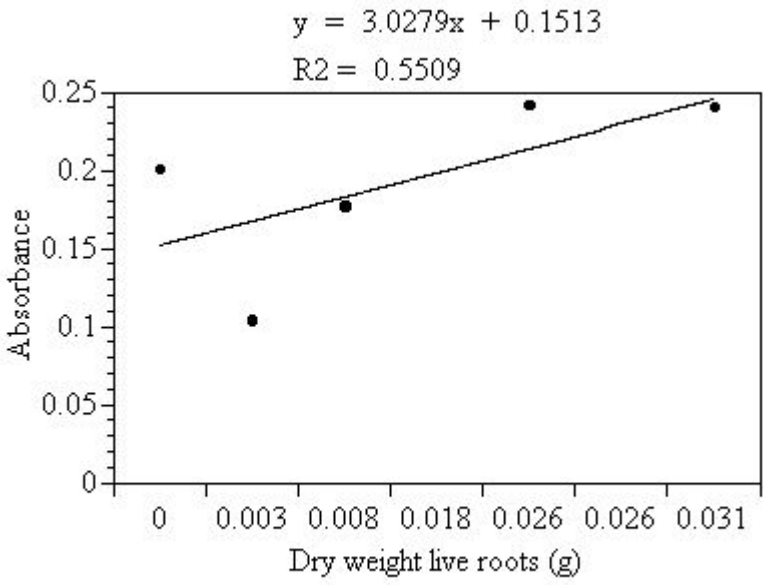

Figure 2.3. Standard curve that shows the relationship between proportions of live roots of *A. germinans* and dehydrogenase activity measured by the production of formazan (absorbance recorded at 480 nm). Regression equation and correlation coefficient are on top.

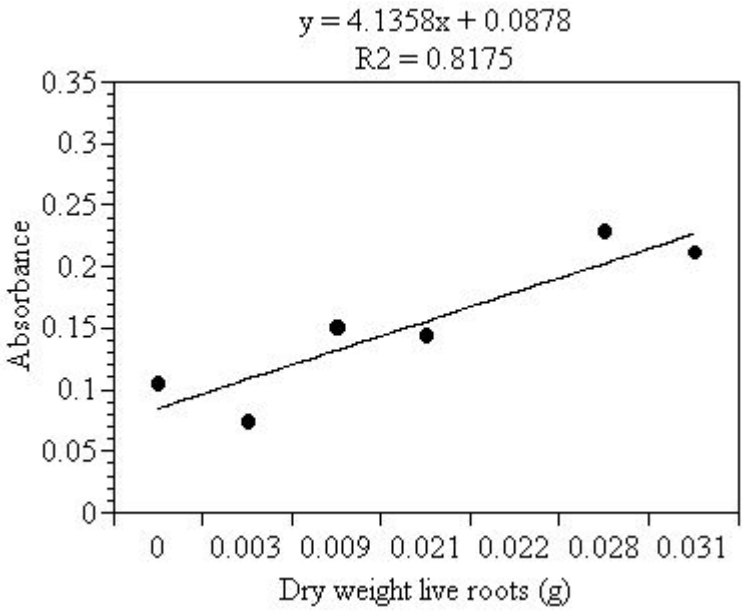

Figure 2.4. Standard curve that shows the relationship between proportions of live roots of *R. mangle* and production of formazan (absorbance recorded at 480 nm). Regression equation and correlation coefficient are on top.

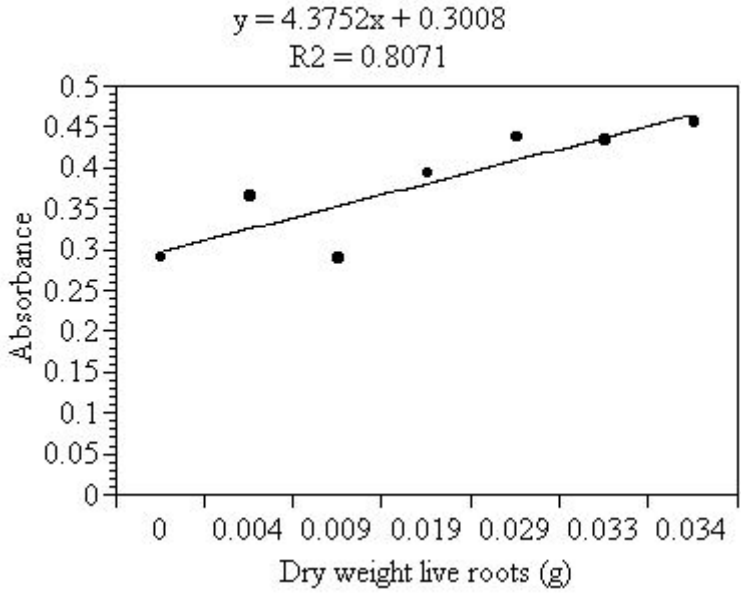

Figure 2.5. Standard curve that shows the relationship between proportions of live roots of *L. racemosa* and production of formazan (absorbance recorded at 480 nm). Regression equation and correlation coefficient are on top.

2.3.1.2. FDA

The FDA technique was optimized before application to the experimental plants. A description of these analyses is in Table 2.1.

Table 2.1. Testing the FDA technique under different experimental conditions (mounting and time) using mangrove roots.

Mounting	Time in solution	Cytosol	Cell wall	Root	Condition
Dry, control No FDA added	-	-	-	White, light brown	Live
				Dark brown to black	Dead
Immersed * plate	30 min			Light yellow	Live
	3 hrs	Brown	Bright yellow	Dark brown	Dead
Dry plate	40 min	-	-	**Green**	Live
	3 hrs			Light yellow	
				Dark brown	Dead
Wet * slide	-	Transparent	Green/yellow	Light yellow	Live
		Dark brown	Yellow	Dark brown	Dead
Dry slide	-		-	**Light green**	Live
				Yellow	
		Brown		Brown	Dead

*If the roots were immersed/wetted for observation under the microscope, the area surrounding them looked green.

A set of sorted (live and dead) root samples of each of the three mangrove species *A. germinans*, *R. mangle*, and *L. racemosa* in FDA solution was used as controls (Figs. 2.6 to 2.11) to facilitate the process of sorting mixed samples of roots. In general, live or recently dead roots fluoresce green to yellow, and dead roots looked brown to black. The surface of coarser roots had a uniform color (yellow to brown) and dark fluorescence. In dead roots some cell structures were still able to fluoresce. This can be observed in the cross section of a *L. racemosa* root where the vessels and the epidermis fluoresce green and the parenchyma does not, having a brown color (Fig. 2.10). Observation on the same root on a longitudinal view presents a mixed coloration (Fig. 2.11).

The average time needed to analyze a sample with the FDA technique was 40 min.

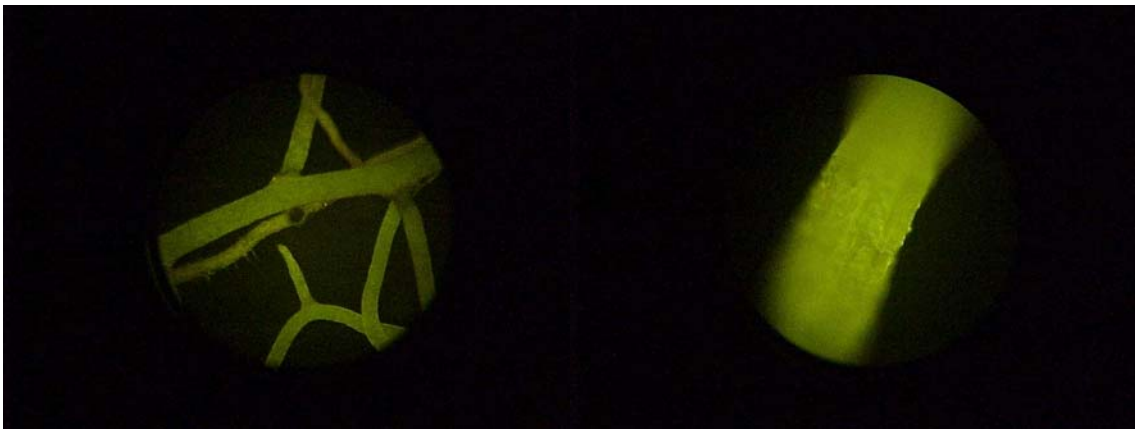

Figure 2.6. FDA analysis for live roots of *Avicennia germinans*. Magnification 4X (left) and 25X (right).

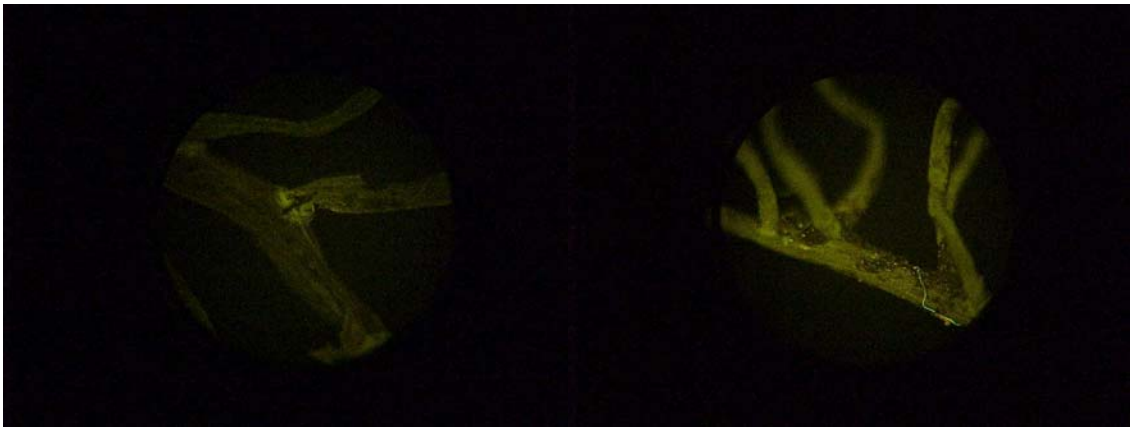

Figure 2.7. FDA analysis for dead and recently dead roots of *Avicennia germinans*. Magnification 4X (left) and 25X (right).

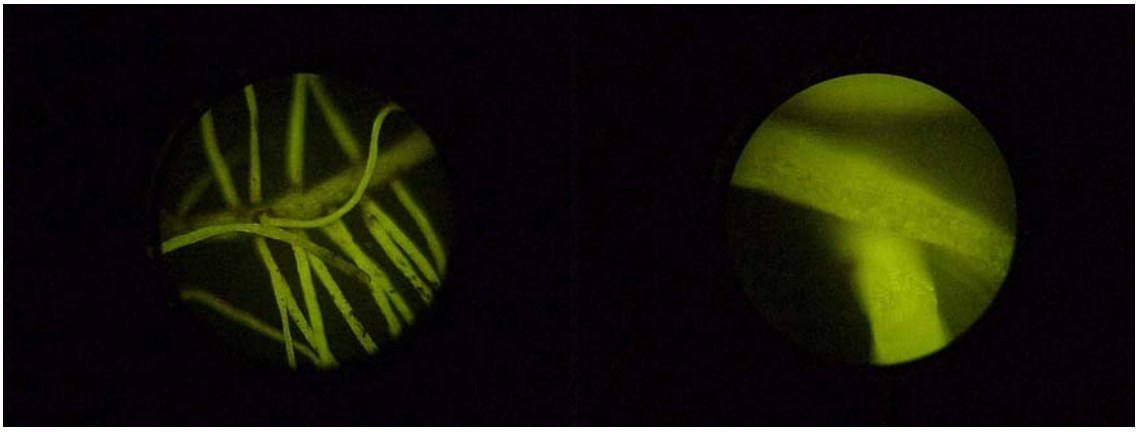

Figure 2.8. FDA analysis for live roots of *Rhizophora mangle.* Magnification 4X (left) and 25X (right).

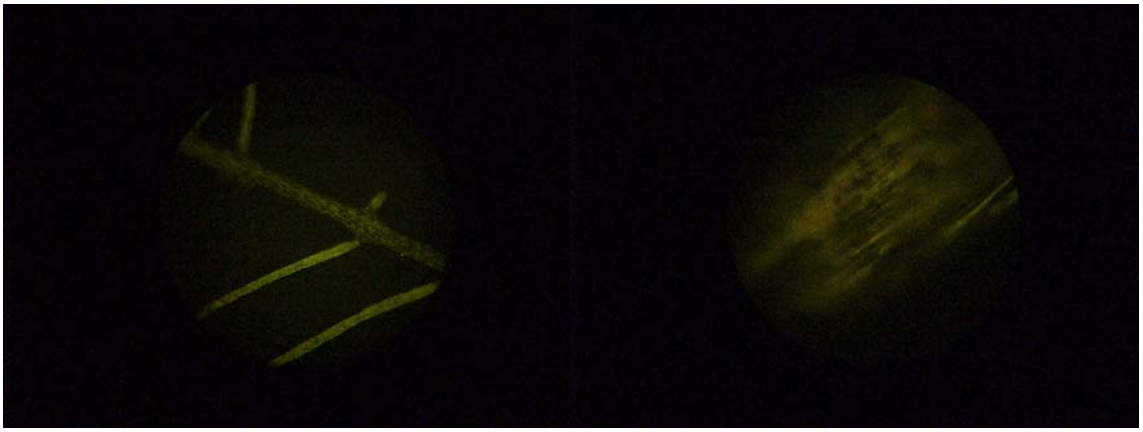

Figure 2.9. FDA analysis for dead and recently dead roots of *Rhizophora mangle*. Magnification 4X (left) and 25X (right).

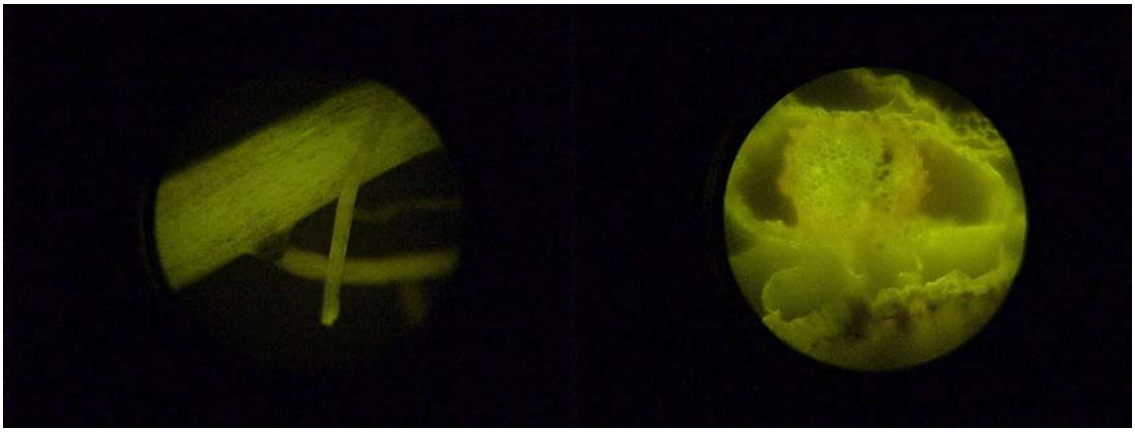

Figure 2.10. FDA analysis for live roots of *Laguncularia racemosa.* Magnification 4X (left) and 25X (right).

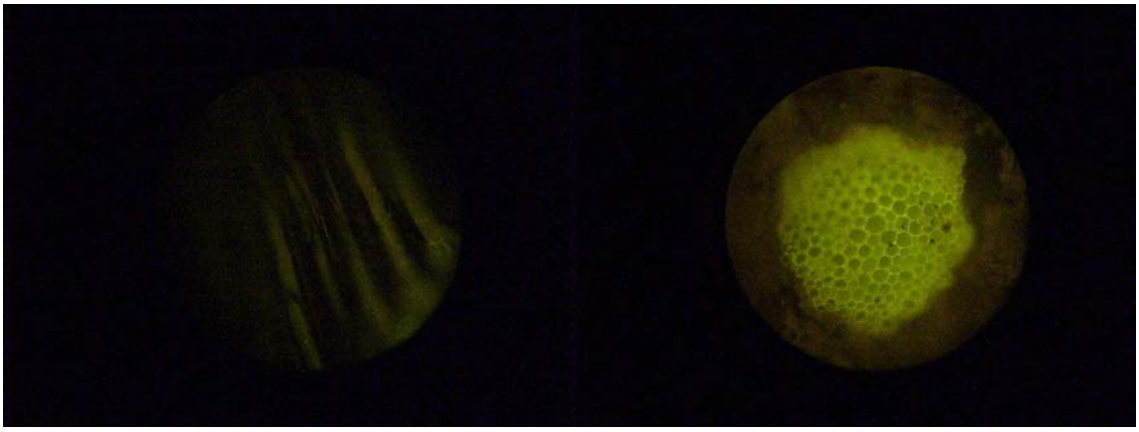

Figure 2.11. FDA analysis for dead and recently dead roots of *Laguncularia racemosa.* Magnification 25X.

2.3.1.3. Colloidal Silica

Separation of live and dead mangrove roots using the colloidal silica technique averaged 37 min. Most of the dead roots collected from the killed seedlings floated in colloidal silica. This result indicate that this technique may not readily distinguish recently dead from live roots. A comparison with flotation in water indicated that the colloidal silica was not really essential to distinguish live and dead roots by their buoyancy. Live roots readily floated in water due to air-

filled cortical lacunae, whereas dead roots filled with water sank. Thus, use of water as a medium to assess root buoyancy was more straightforward and require no chemicals.

2.3.1.4. Visual Assessment

A general description of the features used to separate live and dead roots by inspection are in Table 2.2. Live roots were mostly white and sometimes light brown, when they are beginning to die. Dead roots were mostly dark brown. Roots that floated in water were considered alive, while dead roots sank in a few minutes. When detailed observation was made under the stereoscope or magnified lenses, if the root tissue was mostly intact, it was not possible to see through the root. In contrast, dead roots were flattened and transparent with a loose stele. Air bubbles were observed inside dead roots, but not inside live roots. These air bubbles could lead to flotation of a dead root, but if squeezed to remove bubbles, the root would quickly sink. Roots that contained both live and dead tissues, they often floated in the middle of the water column. Buoyancy in freshwater and physical features of mangrove roots successfully classified roots by vitality. Therefore, this method was applied to separate samples from the field (cores).

2.3.2. Root In-growth Bags

The root in-growth bag technique was successful in providing an estimate of relative root production. Other workers have detailed the drawbacks of this technique (Symbula and Day 1988, Nadelhoffer and Raich 1992, Vogt et al. 1998, Makkonen and Helja-Sisko 1999). Because the bag contains unoccupied soil, the technique may overestimate root production due to greater root growth without competition. On the other hand, cutting of existing roots during insertion of the bag may delay root in-growth, thus leading to underestimation of root production. However, the method allows comparison of root production using a standardized substrate, thus giving a measure of relative production. Because the mass of in-grown roots is generally less than the

standing biomass, separation is faster and less subject to problems of separation of roots by viability. Detailed results obtained using the in-growth core technique are explained in chapter 3.

Table 2.2. Summarized description physical features of mangrove roots used to visually separate them as dead and live roots.

Physical Features	Living roots	Dead roots
Vitality	soft	hard
Buoyancy	positive	negative
Color	white or light brown	dark-brown
Stele	attached	loose
Tissues	turgid	decomposing, broken
Bubbles	absent	present

2.3.3. Rhizotrons

The rhizotron approach was readily adapted for use with mangrove seedlings. Mangroves roots grew towards the viewing face of the rhizotron, where they were easily observed and measured (Fig. 2.12). Accurate measurement of root elongation, root phenology, root density, and root distribution in the soil column was readily accomplished using this approach. A detailed description of the results obtained using rhizotrons are in chapter 4.

2.3.4. Root Morphology with MacRHIZO

The MacRHIZO system allowed rapid and accurate measurements of mangrove root morphology. Roots could be selected and scanned quickly, providing a digital image that could be analyzed at a later time. Measurements that would otherwise be extremely time-consuming (total root length, branching) were accomplished quickly. The software program superimposed a colored skeleton onto the root image and automatically calculated the morphological features, which were imported into an Excel file for analysis. Analysis of root segments using MacRhizo is explained in greater detail in chapters 3 and 4. Fig. 2.13 shows how the analysis was performed.

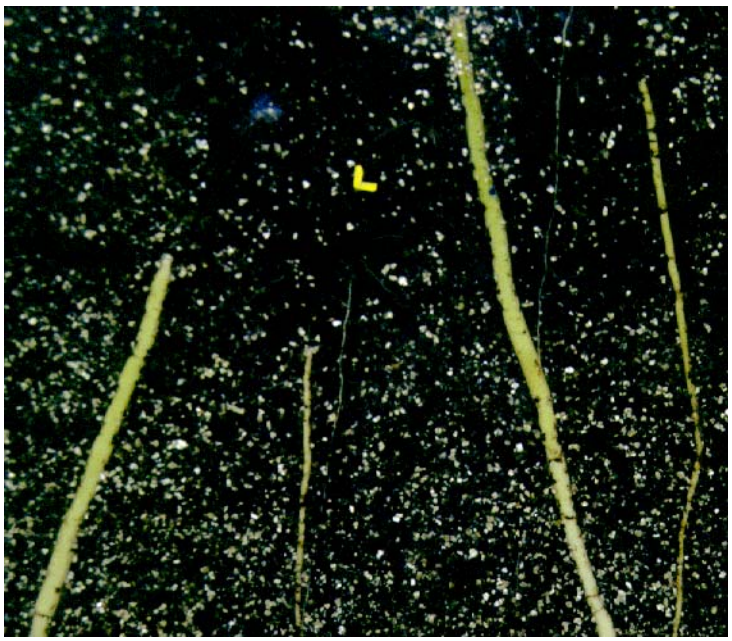

Figure 2.12. Close up of A*vicennia germinans* roots growing on the viewing face of a rhizotron.

(a)

(b)

(c)

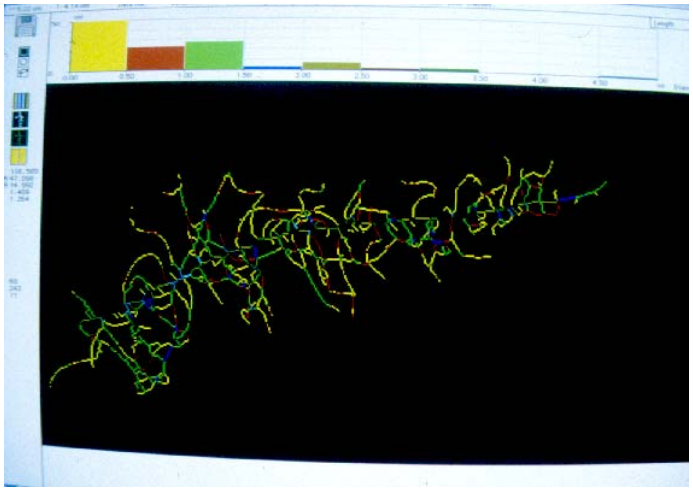

Figure 2.13. (a) A scanned image of a mangrove root is imported into MacRHIZO. (b) the root has been analyzed and a root skeleton superimposed over the image; (c) the root skeleton is shown alone.

2.4. DISCUSSION

The FDA technique was effective to identify vitality of mangrove roots. In general, live or recently dead roots fluoresced green to yellow. One drawback of the technique was that some cell structures in dead roots were still able to fluoresce (Fig. 2.11). This technique would be more accurate if intensity of fluorescence could be estimated. Observation and identification of root samples was easier under dry conditions because the green fluorescence resulted by the formation of fluorescein was more evident. In contrast, in samples that were immersed in FDA solution, the same green color dominated both root cells and the background, making it difficult to assess root cell fluorescence.

Other separation techniques based on staining analyses were attempted but discarded due to difficulties during prior testing of the technique. Acridine Orange described by Stadelmann and Kinzel (1972) was used by Robertson and Dixon (1993) to analyze the efficiency of the colloidal silica to separate roots. This method allows separation of live and dead tissues under UV light. However, identification of live and dead tissues by color was more difficult because different tissues and organelles in both living and dead roots produced different fluorescence coloration. Therefore, identification of live and dead tissues with FDA was a more straightforward method, since live roots fluoresce only green.

The sorting stage of the PF and FDA methods depends on a subjective decision made by the researcher about the vitality of any root. In contrast, TTC was totally quantitative, which may be a more desirable approach to minimize operator bias. However, calculation of proportion of live roots based on the standard curves per species was sensitive to the amount of root sample, which contributed to overall variation. Another problem that may have affected these calculations was formation of formazan by microbial activity, especially on decomposing

roots. Also, the mass of roots that could be analyzed was very small and would not be appropriate for separations of large sample sizes. TTC was been tested in several tissues including fine roots with slight variation of the technique (Table 2.3). However, more studies are needed to evaluate the accuracy of this method in quantifying proportions of live roots. Compared to other methods, the TTC method required the longest processing time, mainly due to the required 24 hr incubation period. If this incubation period is not considered, TTC would take about 16 minutes per sample, which is less analysis time compared to all other techniques. Another positive characteristic of the TTC method is the possibility of processing several samples or a set of samples at the same time, which is also desirable in terms of time saving.

Separation of live and dead roots has been used previously and successfully by inspection of physical features when studying marshes (Connor and Chmura 2000), freshwater swamp forest (Symbula and Day 1988), and mangroves (Komiyama et al. 1987, McKee 2001). In the current analysis, manual sorting of the roots based on physical features was the fastest of all methods tested and relatively accurate. It also had several advantages in comparison to the other techniques that were tested (Table 2.4). CS, TTC, and FDA techniques were limited by the sample size that could be analyzed. Also, chemical contamination of root samples using these techniques would limit further analysis of the roots. In contrast, direct methods such as PF, allowed fast and accurate separation of large samples that did not require the use of chemicals. Separation of roots based on buoyancy in water was fast and accurate also. PF would be a good choice when financial support is restricted.

Table 2.3. Comparison of procedures using the TTC separation technique in different studies. WL: Wavelength, Studies 1, Steponkus and Lanphear 1967; 2, Towill and Mazur 1975; 3, Joslin and Henderson 1984; 4, Clemensson-Lindell 1994.

Study	Samples	Preparation	Sample size (mg)	Analysis		WL (nm)
1	Stems and leaf discs from woody plants	Cut in sections 1 cm max Stems length and 0.7 cm leaf discs	100 frozen	TTC (ml) Infiltration time (min) Incubation Temperatute (°C) Time (hrs) Ethanol 95% (ml) Extraction Temperatute (°C) Time (min) Final volume (ml)	3 ? 30 15 7 100 5-10 10	530 (Max at 490)
2	Tissue cultures Root tissue *from Acer saccharum* and seeds from *Haplopappus gracilus*	Small cells and clumps planted in agar and TTC	Log-stage and late log-state not sized cultures Pelleted cells	TTC (ml) Infiltration time (min) Incubation Temperatute (°C) Time (hrs) Ethanol (ml) Extraction Temperatute (°C) Time (min) Final volume (ml)	* No 22 18-22 3 no, 60 30,5-15 3	485
3	Woody fine roots <1mm	Cut in 1 cm lengths	175-200	TTC (ml) Infiltration time (min) Incubation Temperatute (°C) Time (hrs) Ethanol (ml) Extraction Temperatute (°C) Time (min) Final volume (ml)	40 5 26 20 5 78 15 5	480
4	Coniferus fine roots <1mm	3 Vitality classes cut	200	TTC (ml) Infiltration time (min) Incubation Temperatute (°C) Time (hrs) Ethanol (ml) Extraction Temperatute (°C) Time (min) Final volume (ml)	6 15 30 20 ? 80 15 ?	520
Present	Fine mangrove roots <2 mm	Cut into root segments	200	TTC (ml) Infiltration time (min) Incubation Temperatute (°C) Time (hrs) Ethanol (ml) Extraction Temperatute (°C) Time (min) Final volume (ml)	6 15 32 24 7 80 15 10	480

* Incubation mixture of 0.8% TTC dissolved in a 2:1 Solution of 0.05 M Sodium phosphate buffer:Acer growth medium, pH 7.5

Table 2.4. Comparison of root analysis techniques used to identified root vitality.

	TTC	FDA	CS	PF
Use of chemicals	yes	yes	yes	no
Sample size	small	small	small	large
Buoyancy	-	-	>30 min	good, fast
Financial requirement	yes	yes	yes	minimum
Accuracy	sensitive to sample size	subjective	low	good
Time (min)	111	40	37	20

2.5. CONCLUSIONS

Several techniques were tested for applicability to the study of mangrove roots. To separate live and dead roots, visual analysis in combination with buoyancy in water was accurate, fast, and applicable to large root samples. Staining and fluorescent techniques were problematic when applied to mangrove root study, but with further optimization may prove useful in some specialized studies. Rhizotrons were useful to examine mangrove root elongation rates, phenology, and depth distribution patterns in greenhouse experiments. Root ingrowth bags provided a feasible approach to measure relative differences in root production in the field, especially where sequential coring or other techniques are not feasible. Image analysis based on MacRhizo provided a rapid and accurate way to quantify various morphological features of mangrove roots.

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CHAPTER 3

BELOWGROUND PRODUCTION OF MANGROVES IN SOUTH WEST FLORIDA

3.1. INTRODUCTION

In the 1990's, the importance of terrestrial forests as carbon sinks became a research interest because of their potential influence on concentrations of carbon dioxide in the atmosphere, and consequently climate change (Schimel et al. 2001). Few studies have quantified the belowground carbon accumulation in mangrove forests, and the available estimations vary spatially. In Australia estimates ranged between 120-360 t C ha^{-1} (Alongi et al. 2003), or averaged 471 t C ha^{-1} (Matsui 1998). In tropical Pacific islands, total carbon accumulation has been estimated to be around 1300 t C ha^{-1} (Fujimoto et al. 1999). Recently, studies reported that the rate of C sequestration in mangroves (38 Tg C yr^{-1}) was higher than terrestrial forests (Chmura et al. 2003). This information supports the importance of mangroves in the cycling of nutrients and influence on global climate change due to their possible role as a C sink.

In forest ecosystems fine root production may contribute to a high proportion (up to 75%) of total annual net primary production (Nadelhoffer and Raich 1992). In mangroves of Honduras this proportion has been calculated between 62-75 % (Cahoon et al. 2003). However, the available information about root production is limited due to the difficulty measuring fine root production (Symbula and Day 1988), and the few estimates are variable mainly because estimation methods have not been standardized (Nadelhoffer and Raich 1992).

In forest ecosystems, root production responds negatively to flooding and soil type (Powell and Day 1991). Seasonal changes have been observed with increasing root

production, i.e., increasing during rainy seasons (Sundarapandian and Swamy 1996). In a Maple-dominated ecosystem, root production was controlled by soil moisture, temperature, and nutrients (N and P) (Cote et al. 1998). In scrub communities, nutrients, especially phosphorus, have been found to be limiting to root production and more important than water availability (Martinez et al. 1998). In mangroves, hydrology and nutrients have been identified as important controlling factors for mangrove distribution and primary production (Thom 1967, McKee and Mendelssohn 1987, Wada and Takagi 1988, Boto 1992, Clough 1992, McKee 1996, 2001). However, there is uncertainty about how these factors control the belowground production of roots.

The spatial variability of belowground biomass of mangroves suggests that it may be controlled by global and local factors (Clough 1992), as observed for the above ground component (Saenger and Snedaker 1993). However, this variability may be reflecting non-uniformity in the methodologies used to determine belowground biomass and production. Published information on belowground biomass of mangroves suggests that it is high in comparison to other tropical, temperate (Golley et al. 1975), freshwater forested wetlands (Lugo et al. 1988), and also marsh species (range 6.95-80.44 t ha^{-1}) (Connor and Chmura 2000). In mangroves, high estimates of belowground biomass with a high proportion of fine roots (up to 69%) (Tabuchi et al. 1983) suggests an important allocation of carbon in mangrove soils.

In mangroves, the contribution of root production to soil organic matter may be more important than litterfall (Chen and Twilley 1999a). However, there is a lack of comparisons between litter and root production (Clough 1992, Ong et al. 1995). A more complete knowledge of root dynamics could contribute to understanding these ecosystem processes

(Hendrick and Pregitzer 1992). Studies of belowground biomass and productivity of mangroves forest would reveal possible correlations with the aboveground component, their responses to control factors, and possible patterns at a local scale (between forest types and species). This information is needed to evaluate the possible role of mangrove forest as carbon sinks as well as the importance of root production for the carbon global cycle and climate change.

The research questions addressed in this study were:

1) How does root-standing biomass vary between mangrove forest types and species composition?

2) How does belowground production vary between forest types and species composition?

3) How does root standing biomass and root production vary relative to abiotic factors?

4) What is the effect of nutrient amendments on root growth and production?

5) How does above-ground production of mangroves vary across forest types and species composition in relation to below-ground production?

3.2. STUDY AREA

Field studies were conducted at Rookery Bay National Estuarine Research Reserve (RBNERR), located in southwestern Florida adjacent to the Gulf of Mexico. The area has a subtropical climate with an annual mean temperature of 23.6°C (Twilley et al. 1986) and an average annual precipitation of 1,346 mm (Cahoon and Lynch 1997). Rainfall is seasonal, with 60-65% occurring during the summer months (Twilley et al. 1986). Therefore, the region experiences a six-month dry season (November through April), and a six month wet

season (May through October) (Cahoon and Lynch 1997). In this area, the fringe forest site is exposed to wave action and dominated by *Rhizophora mangle*. The basin forest, located immediately landward of the fringe forest, includes a mixed association of *Avicennia germinans* L. (black mangrove), *Rhizophora mangle* L. (red mangrove) and *Laguncularia racemosa* (L.) Gaert.f. (white mangrove). Tidal flooding in the basin forest is more limited than in the fringe forest. In the basin forest, the soil surface is not sloping (Cahoon and Lynch 1997). In the Rookery Bay area there is also an association of scrub mangrove characterized by a low, closely packed mass of stunted red mangrove trees that are 4 to 6 feet tall (Craighhead 1971). This research area has a tremendous importance because relevant mangrove research has been conducted there (Lugo and Snedaker 1974, Twilley et al. 1986, McKee 1993, Cahoon and Lynch 1997, McKee and Faulkner 2000a). Therefore, there is a valuable data base that can be used to compare and discuss the outcomes of this study. This study focused on belowground aspects such as root production, root standing biomass, and effect of nutrient amendments on root growth. Aboveground productivity was also quantified and compared between forest types and species composition in relation to belowground productivity. Study sites located in mangrove forest types (basin, fringe, and Scrub) were selected as shown in Figure 3.1 and Table 3.1.

3.3. MATERIALS AND METHODS

The in-growth core technique was used to estimate root production. Root in-growth cores were made of nylon bags approximately 30 cm long and 4.5 cm diameter

Figure 3.1. Map of study area showing location of sites in the Rookery Bay National Estuarine Research Reserve (Henderson Creek, Cat Claw, and New York Ave) and at Windstar along Naples Bay.

Table 3.1. Study sites selected at Rookery Bay National Estuarine Research Reserve, which include several mangrove forest types.

Study sites	Forest Type / Stand	Species composition	Label
Cat Claw trail	Fringe	*Rhizophora* dominated	CF
	Basin Mixed	*Rhizophora Avicennia Laguncularia*	CM
	Basin Monospecific	*Avicennia* dominated	CA
Henderson Creek	Fringe	*Rhizophora* dominated	HF
	Basin Mixed	*Rhizophora Avicennia Laguncularia*	HM
Windstar	Fringe	*Rhizophora* dominated	WF
	Basin Mixed	*Rhizophora Avicennia Laguncularia*	WM
New York Ave.	Scrub Monospecific	*Rhizophora* dominated	NS

with a flexible mesh of 3 mm^2. Bags were filled with approximately 85 g of peat soil (Hyponex, Canadian Spagnum Peat Moss, Hyponex Corporation 14111 Scottslacon Rd., Marysville OH 43041), which was root free. At each study site (CF, CM, CA, HF, HM, WF, WM, and NS), 15 core holes 30 cm deep, were randomly dug with a corer, and tagged in-growth core bags were plugged into core holes a total of 40 root in-growth cores (8 sites * 5 replicates). Roots growing into the in-growth cores were considered as new production (Symbula and Day 1988). The root in-growth cores were inserted in the soil on April 2001 and collected a year later using a sharpened corer with a slightly larger diameter. Soil and protruding roots were trimmed away from the outer surface of the bags. After collection, cores were tagged, and stored in a cold room until analysis.

At the laboratory, the in-growth cores were divided (approximately 12.5 cm each half), and the upper and lower halves were analyzed separately. Each sample was rinsed and placed in a tray with tapwater. A lamp with a magnifier was used while picking up and separating the roots from debris. Then, root samples were sorted by condition (live and recently dead, and dead roots), and then by size (fine, medium and coarse). Live and dead roots were separated by flotation (water) and physical features as described in Chapter 2. Roots were placed in labeled paper bags and dried in the oven at 70ºC until constant weight to obtain dry biomass (g). Five roots per sample were preserved in a solution of 1:10 methanol for digital analysis.

Mangrove root morphology was analyzed based on digital root analysis using MacRHIZO software (Regent Instruments, Inc.). Root samples were scanned, and the images were imported for analysis. A colored root skeleton was superimposed over the image, each color representing a different diameter class. When a detailed root analysis was

finished, data such as total root length, diameter, surface area, and branching were saved into an Excel file. The analysis was performed following the scanning protocol suggested by (Bouma et al. 2000). Finally, root samples were dried to calculate specific root length (SRL= root length/root biomass) (Eissenstat 1992), and specific root area (SRA = root surface area/dry weight).

Standing root biomass was analyzed at the eight mangrove sites selected at RBNERR: CF, CM, CA, HF, HM, WF, WM, and NS. Soil cores (8 sites * 5 replicates) were extracted at the end of the study with a 30 cm height, and 5 cm diameter corer adjacent to where the in-growth bags were removed. Cores were divided into upper and lower layers of approximately 15 cm each and stored in tagged plastic bags. At the laboratory, the roots were washed on a screen (1 mm mesh), separated from debris, placed in labeled paper bags, and dried in the oven at 70ºC until constant weight to obtain biomass (g). Roots were not separated by size class or condition; consequently, only total standing biomass was determined.

Climatic and edaphic factors that have been reported influencing mangrove distribution and primary production (Thom 1967, Wada and Takagi 1988, Boto 1992, Clough 1992) were measured to assess correlation of abiotic factors with root standing biomass and root production. Soil redox potential (Eh), pH, salinity, nutrient concentration in pore water (N and P), and water table level were measured seasonally (Spring 2001, Summer 2001, Fall-Winter 2001, and Spring 2002). Soil-extractable P and N concentrations, soil moisture, and bulk density were analyzed at the beginning and the end of the study (Spring 2001and 2002). These abiotic variables were measured near each in-growth bag (8 sites * 5 replicates = 40).

Standardized methodologies were used for these analyses. Redox potential (Eh) was measured by using platinum electrodes and a portable voltmeter with a saturated calomel reference electrode according to standard procedures (Faulkner et al. 1989). Porewater was extracted with a sipper {McKee, 1988 #3615}. Porewater pH and salinity were measured with portable meters (pH-meter and refractometer, respectively). Nutrients were analyzed using a Autoanalyzer based on Murphy-Riley and flow injection techniques (Lachat Instruments). Water table level was measured in reference to the soil surface. Soil moisture was measured by gravimetric determination by oven drying soil samples at 105°C. Percentage moisture was calculated by dividing the weight of the water by the oven-dry weight of the soil and multiplying by 100 (Faulkner et al. 1989). A sample of sediment was obtained with a piston corer (volume of 42.27 cm^3) to measure bulk density, which was calculated as the ratio between the dry weight of the soil sample divided by its known volume.

Nutrient amendments in root in-growth cores were additionally made to test the effect of nutrient fertilization on root growth and production at four RBNERR sites: CF, CM, CA, and NS. Fertilizers were Triple Super PO_4 or Urea, for P and N fertilization treatments respectively. The nutrients were added by mixing 14 g of fertilizer granules and approximately 85 g of peat soil (Hyponex, Canadian Spagnum Peat Moss, Hyponex Corporation 14111 Scottslacon Rd., Marysville OH 43041) prior to packing the cores. A total of 120 (4 sites * 2 treatments * 3 seasons* 5 reps) fertilized in-growth cores were implanted into the soil. Root production data from the in-growth cores without nutrient additions were used as controls. The collection of nutrient-amended cores occurred seasonally at the same time as the unamended cores (Summer 2001, Fall-Winter 2001, and

Spring 2002). Finally, analysis of the roots that grew in the cores followed the same methodology described previously.

Aboveground production was also estimated using mangrove litter production and dynamics proposed by (Cintron and Shaeffer-Novelli 1984). Litter traps (50 x 50 cm) made of PVC and nylon screen were installed at control points of all 8-study sites at RBNERR. Five replicates per site were placed, for a total of 40 litter traps. Samples were collected seasonally (Summer 2001, Fall-Winter 2001, and Spring 2002) due to logistical constraints. This schedule may have underestimated litterfall rates due to leaching and decomposition. However, this approach was sufficient to get an estimate of litter fall rates and patterns for comparison with root production. Items such as twigs, stipules, propagules and fruits/species, flowers/species were sorted and dried to obtain biomass data.

In general, this field study corresponded to a Randomized Block Design experiment with plots as blocks, mangrove forest types and species composition as treatments, and in-growth bags or litter traps as experimental units. One-way ANOVA (Main plot RBD) was performed to examine effects of different forest types and species composition (treatments), and depth (top and bottom) on root production (with and without nutrient amendments), and root standing biomass, and litterfall. Significant treatment effects were identified using pair-wise contrasts (Freund and Wilson 1997). Also multiple correlations were applied between physicochemical variables (salinity, pH, Redox potential, soil moisture, water level, bulk, and pore water and soil nutrients) and with response variables (root production with and without nutrient amendments, and root standing biomass, and litterfall).

3.4. RESULTS

3.4.1. Physicochemical Conditions

Table 3.2 summarizes the soil characteristics at the study sites. Annual mean salinity was highest at CA, the basin monospecific stand, and lowest at NS, the scrub mangrove stand, and intermediate at all other study sites (Fig. 3.2). Water pH was lightly acidic to neutral at all the sites, but the extremes were observed at Cat claw Trail between the fringe and the basin monospecific forest types (high and low, respectively) (Fig. 3.3). In general, redox potential (Eh) decreased with depth across sites, but it was lower at NS (all depths) and at CF (depth from 15 to 30 cm). Soil redox potential showed the highest variability between forest types at Windstar (fringe and basin mixed) where the highest Eh values (consistently with depth) were measured at WM (Fig. 3.4). The lowest Eh values were consistently measured at CF and NS (Fig. 3.4). Soil moisture (%) was lowest at NS (Fig. 3.5). During the study year, NS was continuously flooded and had the highest water level relative to the soil surface, and CA the lowest. At Windstar, water level decreased significantly from fringe to basin mixed forest, and at Cat Claw Trail and Henderson, it was similar between these forest types (Fig. 3.6). Bulk density was highest and more variable at NS and intermediate at CF, and HF. At Cat Claw Trail, bulk density decreased towards the interior showing spatial differences between the fringe and the basin forest (Fig. 3.7). Soil organic matter (%) increased from the fringe to the basin forest, and spatial differences were more evident at Cat Claw Trail and Henderson Creek. Soils at the scrub forest site had the lowest proportion of soil organic matter (Fig. 3.8). Dissolved nitrite was highest at the basin mixed forest at Henderson Creek (HM), and lowest at the fringe forest at Cat Claw Trail (CF) (Fig. 3.9).

Table 3.2. Range values of physico-chemical factors measured at study sites of Rookery Bay between spring 2001 and 2002. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; WF: Windstar, Fringe; WM: Windstar, Basin-mixed, and NS: New York Av., Scrub-monospecific.

Site	Forest Type	Flooding	Salinity	pH	Redox mV	Moisture %	Bulkdensity g cc^{-1}	Organic matter %
CF	Fringe	Frequent	17-41	7	-83, 304	55-67	0.3-0.6	9-13
CM	Basin	Infrequent	4-45	5-6	-41, 325	74-76	0.1-0.2	15-18
CA	Basin	Infrequent	20-63	5-6	-42, 373	58-74	0.2-0.3	16-20
HF	Fringe	Frequent	1-42	6-8	-74, 398	63-72	0.2-0.4	12-15
HM	Mixed	Infrequent	0-45	6-7	-9, 392	71-76	0.1-0.2	19-24
WF	Fringe	Frequent	5-43	6-7	-122, 380	69-74	0.2-0.3	10-12
WM	Mixed	Infrequent	9-52	5-6	60, 432	63-75	0.2-0.4	12-18
NS	Scrub	Continual	0-32	6-7	-89, 266	36-57	0.3-0.9	4-9

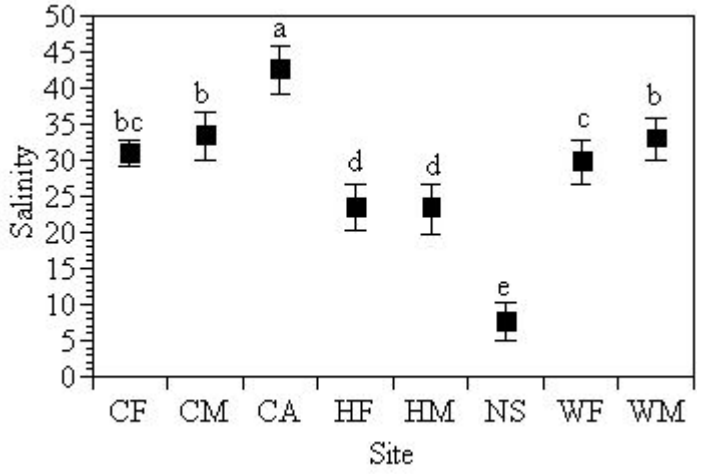

Figure 3.2. Annual mean salinity by mangrove forest site. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error, and letters indicate posterior pairwise comparisons (LSMeans with Tukey's adjustment).

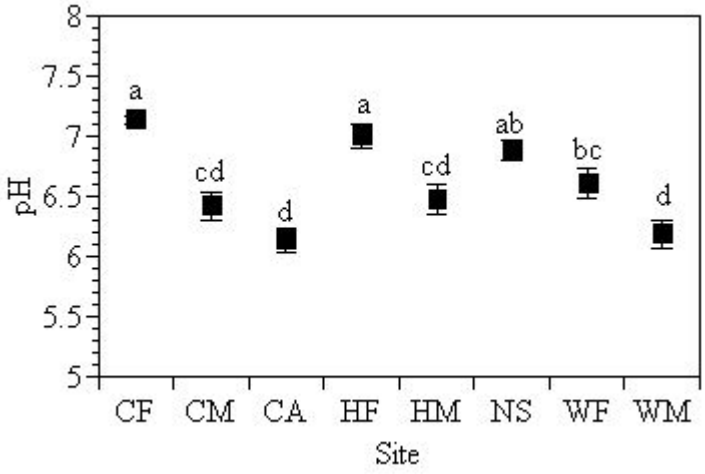

Figure 3.3. Annual mean pH by mangrove forest site. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error, and letters indicate posterior pairwise comparisons (LSMeans with Tukey's adjustment).

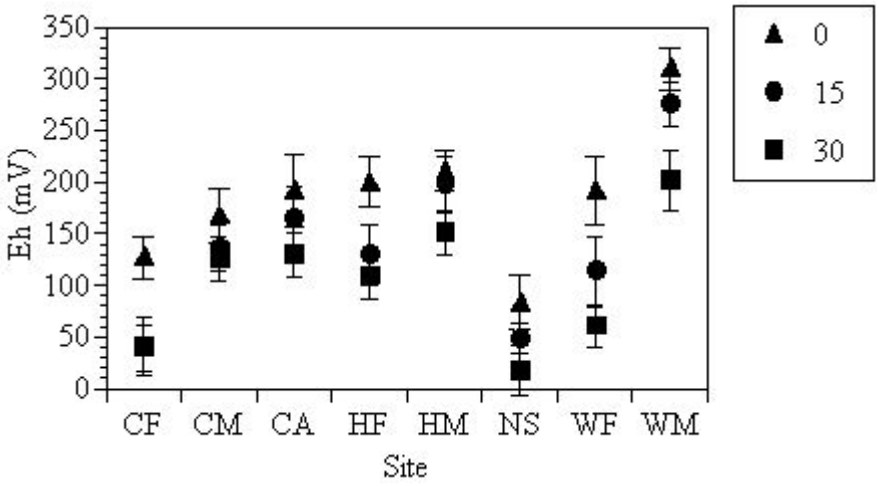

Figure 3.4. Annual mean redox potential (Eh) by depth (0, 15, and 30 cm), at the mangrove forest sites. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error.

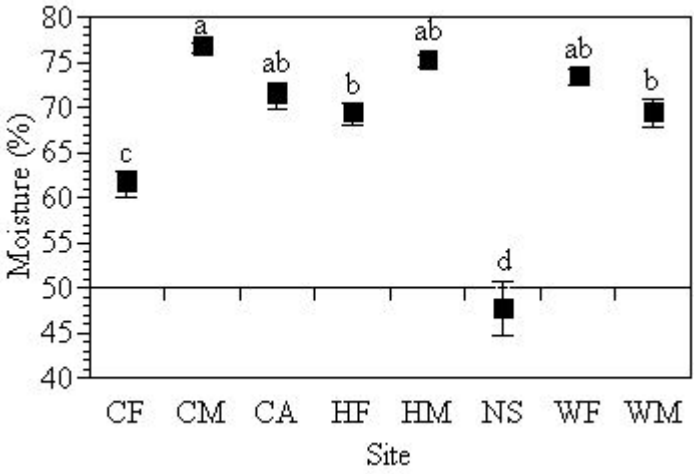

Figure 3.5. Annual mean soil moisture (%) at the mangrove forest sites. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error, and letters indicate posterior pairwise comparisons (LSMeans with Tukey's adjustment).

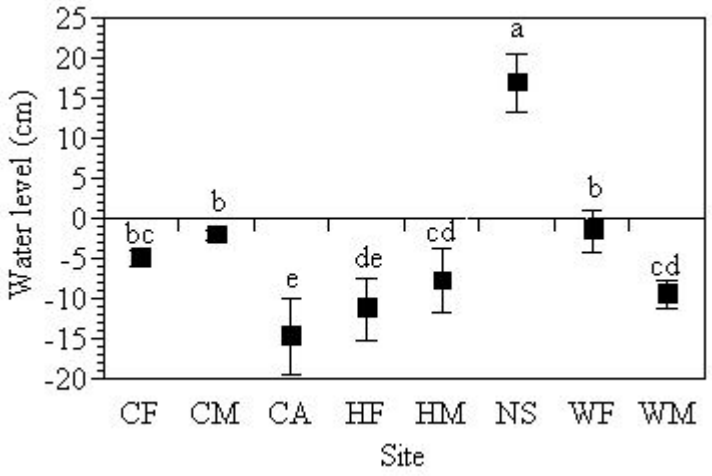

Figure 3.6. Annual mean water level at the mangrove forest sites. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error, and letters indicate posterior pairwise comparisons (LSMeans with Tukey's adjustment).

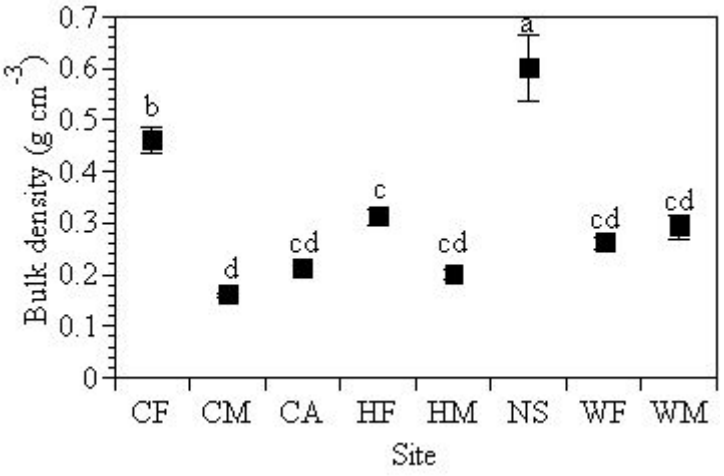

Figure 3.7. Annual mean soil bulk density at the mangrove forest sites. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error, and letters indicate posterior pairwise comparisons (LSMeans with Tukey's adjustment).

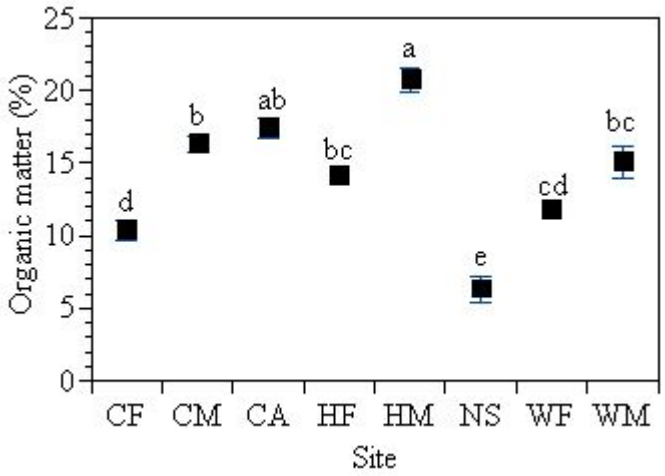

Figure 3.8. Soil organic matter (%) at the mangrove forest sites. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error, and letters indicate posterior pairwise comparisons (LSMeans with Tukey's adjustment).

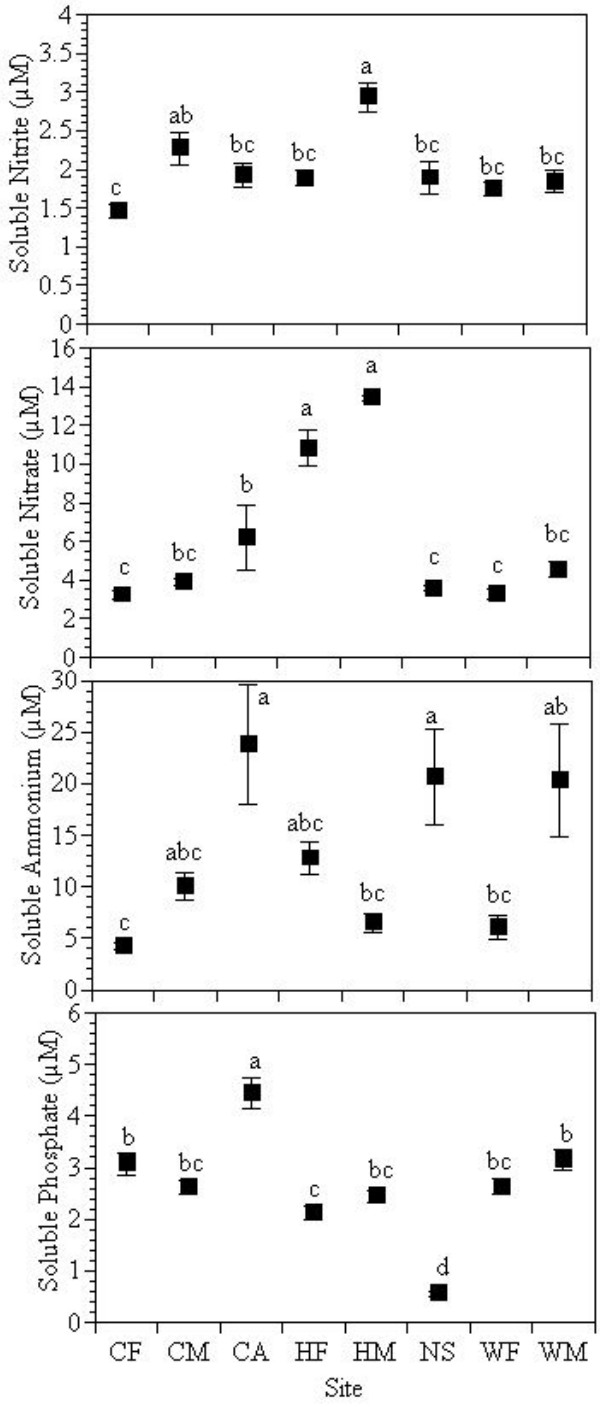

Figure 3.9. Annual mean of nitrite, nitrate, ammonium, and phosphate measured in pore water at the mangrove forest sites. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error, and letters indicate posterior pairwise comparisons (LSMeans with Tukey's adjustment).

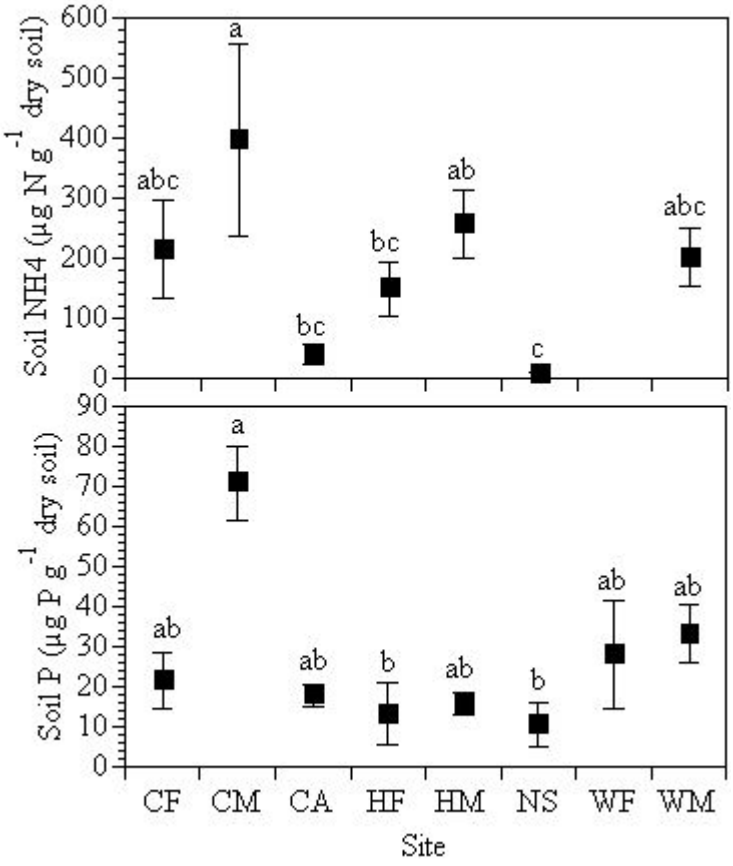

Figure 3.10. Annual mean of extracted soil ammonium and phosphate at the mangrove forest sites. CA: Cat Claw Trail, Basin-monospecific; CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Bars indicate one standard error, and letters indicate posterior pairwise comparisons (LSMeans with Tukey's adjustment).

Dissolved nitrate was highest at Henderson Creek, and lowest at the scrub (NS) and the fringe forest at Windstar (WF). At Cat Claw Trail, it was significantly higher at the interior basin monospecific stand (Fig. 3.9). The concentration of ammonium in pore water was higher than the concentration of nitrite and nitrate. Dissolved nitrogen as ammonium was highest and more variable at the basin and scrub monospecific stands (CA and NS, respectively) and the basin mixed stand at Windstar (WM) (Fig. 3.9). Phosphate availability in pore water was highest and more variable at the basin monospecific forest at Cat Claw Trail (CA), and lowest at the scrub forest site (NS) (Fig. 3.9). Soil ammonium was significantly higher and more variable at the mixed forest at Cat Claw Trail (CM), and lowest at the scrub forest (NS) (Fig. 3.10). Soil phosphate was significantly higher at the mixed forest at Cat Claw Trail (CM), and lowest at the fringe forest at Henderson Creek (HF), and the scrub forest (NS) (Fig. 3.10).

The soil properties were different between sites, showing a significant spatial variability. In an annual average, the basin mono-specific forest at Cat Claw Trail (CA) had the highest salinity possibly because of limited water flow to the interior of the forest. In contrast, the lowest salinity was observed in the scrub mangrove area (NS), which was also permanently flooded with low soil redox potentials (Fig. 3.4). The soils in the scrub area also had a high bulk density, but were limited in moisture, organic matter, and pore water phosphate (Figs. 3.5, 3.7, 3.8, and 3.9).

Table 3.3. Summary of ANOVA results for soil physicochemistry measured at eight sites. Values are the F-ratio and probability of a greater F. 1 DF contrasts compare monospecific (Mono) vs Mixed (Mix) forests, *Avicennia* (A) vs. *Rhizophora* (R) dominated forests, interior (Int) vs. shoreline (shore), fringe (Fg) vs scrub (Sb), Mixed (Mx) vs. Scrub (Sb), and Rookery Bay (RB) vs. Windstar (WS) locations; t-values are given and significance (contrasts) is indicated by P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant. N=40.

	F-ratio	Prob>F	Mono vs. Mx	A vs. R	Int vs. Shore	Fg vs Sb	Mx vs. Sb	RB vs. WS
pH	22.17	<0.0001	6.82****	-8.78****	-8.60****	0.32ns	-5.77****	4.28***
Salinity	212.66	<0.0001	-5.94****	25.04****	-0.19ns	25.06****	27.40****	-7.68****
Water level	82.12	<0.0001	4.33****	-12.06****	3.19**	-18.50****	-18.94****	1.79ns
Eh (0 cm)	13.85	<0.0001	4.91****	3.13**	1.37ns	4.91****	7.39****	4.76****
Eh (15 cm)	19.61	<0.0001	-8.27****	4.12***	5.50****	2.35*	7.79****	5.15****
Eh (30 cm)	9.17	<0.0001	-5.86****	3.08**	3.51**	2.39*	6.10****	2.74**
Moisture	31.05	<0.0001	7.62****	4.35****	-0.37ns	10.01****	13.17****	3.05**
Bulk density	26.89	<0.0001	7.18****	6.13****	-2.35*	-7.85****	-11.61****	2.07*
Organic matter	36.61	<0.0001	-9.93****	8.21****	-5.71****	6.75****	12.93****	1.40ns

Table 3.4. Summary of ANOVA results for porewater and soil nutrients measured at eight sites. Values are the F-ratio and probability of a greater F. 1 DF contrasts compare monospecific (Mono) vs Mixed (Mix) forests, *Avicennia* (A) vs. *Rhizophora* (R) dominated forests, interior (Int) vs. shoreline (shore), fringe (Fg) vs scrub (Sb), Mixed (Mx) vs. Scrub (Sb), and Rookery Bay (RB) vs. Windstar (WS) locations; t-values are given and significance (contrasts) is indicated by P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant. N=40.

	F-ratio	Prob>F	Mono vs. Mx	A vs. R	Int vs. Shore	Fg vs Sb	Mx vs. Sb	RB vs. WS
Porewater								
NH_4	5.52	0.0004	-0.53ns	3.45**	-3.71***	-3.66***	-2.37*	-0.08ns
PO_4	38.22	<0.0001	1.40ns	10.50****	-0.25ns	11.02****	11.69****	-2.63*
NO_2	8.87	<0.0001	5.08****	0.92ns	-4.18***	-0.89ns	2.90**	2.14*
NO_3	30.14	<0.0001	3.84***	2.12*	-1.73ns	3.28**	5.52****	5.93****
Soil								
NH_4	6.31	0.0002	5.13****	-0.91ns	-1.47ns	1.94ns	4.70****	1.59ns
PO_4	3.09	0.0151	3.14**	1.04ns	-1.64ns	1.18ns	2.98**	-1.49ns

3.4.2. Standing Root Biomass

Standing root biomass varied significantly from 2923 to 28,456 g m^{-2} (Fig. 3.11) ($F = 9.48$, $P<0.0001$). Standing root biomass was higher at monospecific forests (fringe, scrub, and basin, 12,512 g m^{-2}) than at basin mixed forest (6,704 g m^{-2}), with the lowest estimation observed at Henderson basin mixed (Fig. 3.11). Standing root biomass was higher at the shoreline (15,395 g m^{-2}) compared to the interior (7,297 g m^{-2}). Standing root biomass was different between the fringe (15,395 g m^{-2}) and scrub (6,185 g m^{-2}) forest types dominated by *R. mangle* (Fig. 3.11, Table 3.5).

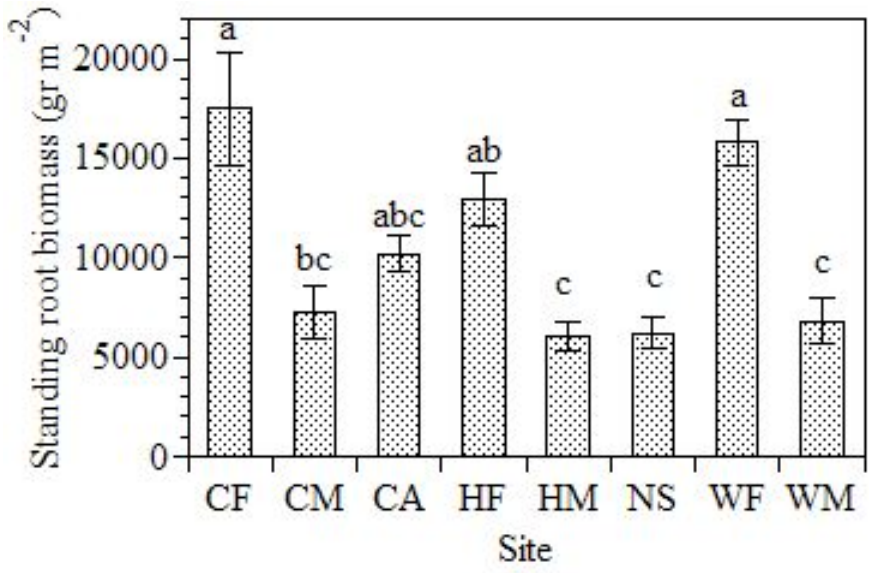

Figure 3.11. Standing root biomass across eight sites. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Values are the mean ± SE (n = 5). Letters above bars indicate significant differences ($P \leq 0.05$) (Tukey HSD).

Table 3.5. Summary of ANOVA results for standing root biomass at eight mangrove sites. Values are the F-ratio and probability of a greater F. 1 DF contrasts compare monospecific (Mono) vs Mixed (Mix) forests, *Avicennia* (A) vs. *Rhizophora* (R) dominated forests, interior (Int.) vs. shoreline (shore), fringe vs scrub, and Rookery Bay (RB) vs. Windstar (WS) locations. t-values are given and significance (contrasts) is indicated by P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant. N=40.

	Standing biomass
F-ratio	9.48
Prob>F	<0.0001
Contrasts:	
Mono vs. Mx	-5.72****
A vs. R	-1.04ns
Int vs. Shore	7.41****
Fg vs. Sb	5.48****
Mx vs. Sb	0.29ns
RB vs. WS	-0.97ns

3.4.3. Root Production by Forest Type

Total belowground production of roots varied across the eight sites from 106 to 842 g m^{-2} yr^{-1} (Fig. 3.12), but it did not change with depth (F=2.03, P value=0.159). These values fall within the range reported for other wetland and forested systems (Table 3.16). There was no consistent pattern in total root production by forest type, although there were significant differences depending on location (Table 3.6). Sites at Rookery Bay (400 g m^{-2} yr^{-1}) produced more root mass compared to sites along Naples Bay (Windstar, 202 g m^{-2} yr^{-1}) (Table 3.6). The

fringe forests dominated by *R. mangle* produced between 156 to 684 g m^{-2} yr^{-1}. The other monospecific forests dominated by *R. mangle* (scrub) or *A. germinans* (basin) produced 298 and 489 g m^{-2} yr^{-1}. Root production in the mixed basin forests varied from 138 to 618 g m^{-2} yr^{-1}. Most of the roots recovered from the in-growth bags were dead (>50%) and were not separated by size class (Fig. 3.12). The proportion of dead roots was higher at the monospecific *Avicennia* site (CA) compared to the monospecific *Rhizophora* sites (CF, HF, WF, and NS) and higher at Rookery Bay (Cat Claw Trail, Henderson Creek, and New York Ave.) compared to sites along Naples Bay (Windstar). The use of bags installed for one full year may underestimate total production due to decomposition losses. However, other work has shown that mangrove roots decompose extremely slowly under the flooded, anaerobic conditions found in mangrove soils (van der Valk and Attiwill 1984, McKee and Faulkner 2000a, Middleton and McKee 2001, Alongi et al. 2003).

In general, most of the live roots were < 2 mm in diameter (i.e., fine roots) and accounted for ~50 to 80 % of the total, depending on site. Intermediate and coarse roots each contributed another 5 to 20 % at most sites except for the basin monospecific forest dominated by *A. germinans*. At the latter site, coarse roots accounted for ~40% of the total live mass. However, live mass of coarse and intermediate roots did not differ significantly across sites (Table 3.6). Only fine roots differed between monospecific forests (*Avicennia* < *Rhizophora*) and spatial position (shoreline > interior) (Table 3.6).

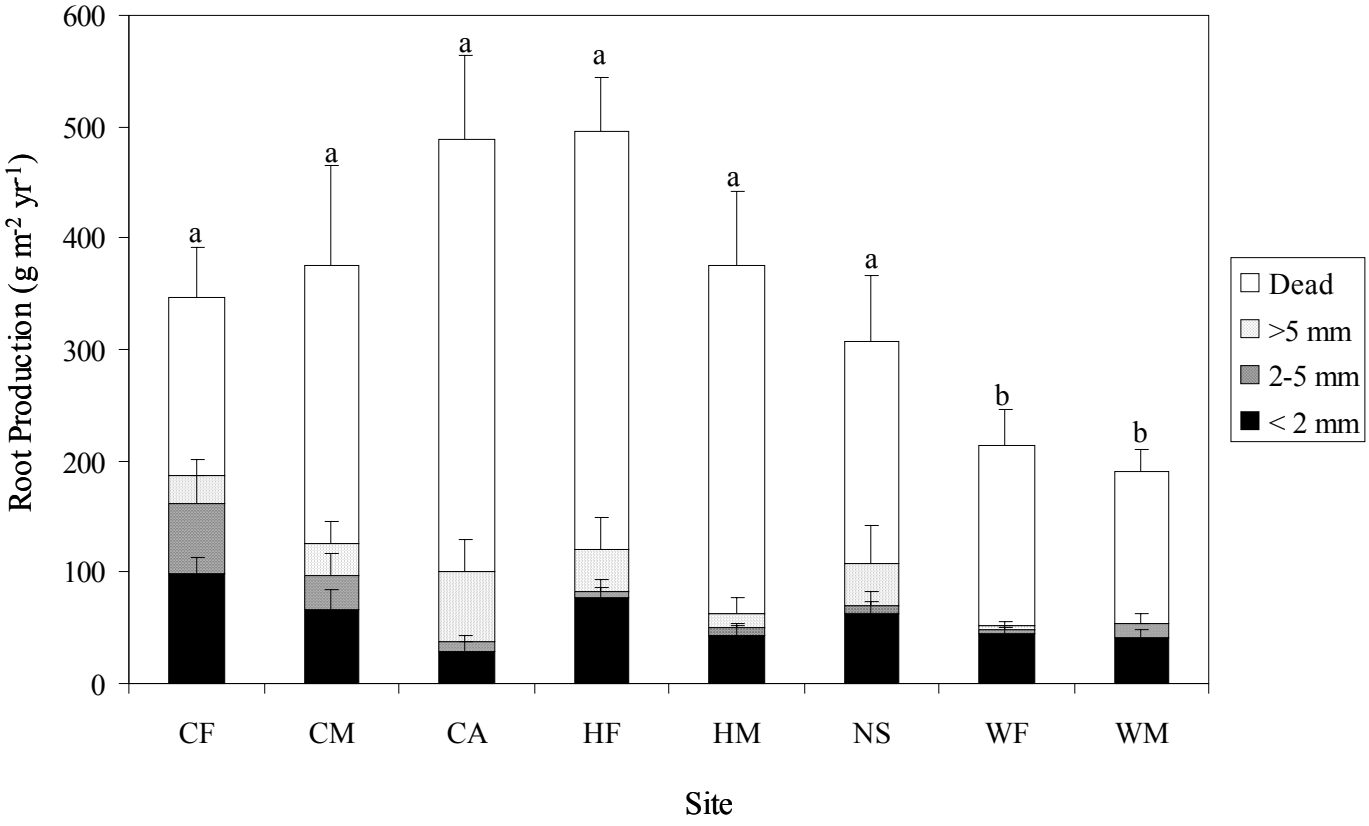

Figure 3.12. Root production measured with in-growth bags at eight sites. Components are live roots by size class (< 2 mm, 2-5 mm, and > 5 mm diameter) and dead roots. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Values are the mean ± SE (n = 5). Letters above bars indicate significant differences ($P \leq 0.05$) (Tukey HSD).

Table 3.6. Summary of ANOVA results for total, fine (< 2 mm), intermediate (2-5 mm), coarse (>5 mm) and dead mass of roots produced at eight sites. Values are the F-ratio and probability of a greater F. 1 DF contrasts compare monospecific (Mono) vs Mixed (Mix) forests, *Avicennia* (A) vs. *Rhizophora* (R) dominated forests, interior (Int.) vs. shoreline (shore), fringe vs scrub, and Rookery Bay (RB) vs. Windstar (WS) locations. t-values are given and significance (contrasts) is indicated by P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant.

		Live			
	Total	Fine	Intermediate	Coarse	Dead
F-ratio	2.81	3.36	2.91	1.40	2.82
Prob>F	0.0218	0.0088	0.0184	ns	0.0213
Contrasts:					
Mono vs. Mix	-1.11ns	1.50ns	0.16ns	-	0.61ns
A vs. R	2.00ns	-3.18**	-0.75ns	-	2.43*
Int. vs. Shore	0.12ns	2.44*	1.16ns	-	-0.61ns
Fringe vs. Scrub	0.63ns	-0.02ns	1.02ns	-	0.30ns
Mix vs. Scrub	0.18ns	-1.46ns	0.54ns	-	0.30ns
RB vs. WS	3.55**	2.02ns	0.20ns	-	2.76**

3.4.4. Root Turnover

Total root turnover rate averaged 0.04±0.004 yr^{-1}, ranging from 0.006 to 0.115 yr^{-1} (Table 3.7). Values varied spatially between the shoreline (0.02 yr^{-1}) and the interior forest (0.05 yr^{-1}), and were higher at mixed basin forests (0.05 yr^{-1}) compared to monopecific stands (0.03 yr^{-1}). Root turnover was higher in forest types dominated by *A. germinans* (0.05 yr^{-1}) compared to forests dominated by *R. mangle* (0.03 yr^{-1}). Sites at Rookery Bay had a higher root turnover rate (0.04 yr^{-1}) compared to sites at Naples Bay (Windstar, 0.02 yr^{-1}) (Table 3.8).

Table 3.7. Root turnover rate (mean± SE) calculated per study site.

Site	Root turnover (yr^{-1})
CF	0.023±0.006
CM	0.060±0.016
CA	0.050±0.013
HF	0.040±0.007
HM	0.063±0.009
NS	0.033±0.012
WF	0.014±0.002
WM	0.031±0.006

Table 3.8. Summary of ANOVA results for root turnover at eight mangrove sites. Values are the F-ratio and probability of a greater F. 1 DF contrasts compare monospecific (Mono) vs Mixed (Mix) forests, *Avicennia* (A) vs. *Rhizophora* (R) dominated forests, interior (Int.) vs. shoreline (shore), fringe vs scrub, and Rookery Bay (RB) vs. Windstar (WS) locations. t-values are given and significance (contrasts) is indicated by P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant. N=38.

Root Turnover	
F-ratio	3.36
Prob>F	0.0091
Contrasts:	
Mono vs. Mx	2.75**
A vs. R	2.05*
Int vs. Shore	-3.08**
Fg vs. Sb	-0.57ns
Mx vs. Sb	1.35ns
RB vs. WS	2.86**

3.4.5. Annual Litter Production

Annual litter production varied across sites from 101 to 263 g m^{-2} yr^{-1} (Fig. 3.13). Litter production varied significantly depending upon forest type and dominant species (Fig. 3.13, Table 3.9). Overall, total annual litter fall was highest at the Henderson Creek mixed forest and lowest at the scrub forest dominated by stunted *R. mangle* trees (Fig. 3.13). Mixed stands (244 g m^{-2} yr^{-1}) had higher litter production than monospecific stands (177 g m^{-2} yr^{-1}), regardless of dominant species. Scrub stands had lowest litter production (101 g m^{-2} yr^{-1}) compared to either fringe (213 g m^{-2} yr^{-1}) or mixed basin (219 g m^{-2} yr^{-1}) stands, which were not different (Table 3.9). The primary component of litter was leaves, which accounted for 63 to 82 % of the total (Fig. 3.13). Leaf production was higher in mixed vs. monospecific stands and was lowest in scrub stands (compared to fringe or mixed basin stands) (Table 3.9). Wood fall varied from 4 to 58 g m^{-2} yr^{-1} across sites and accounted for 4 to 16 % of the total (Fig. 3.13). Wood fall showed similar patterns to leaves across forest types and additionally was higher at Windstar sites (38 g m^{-2} yr^{-1}) compared to all sites at Rookery Bay (26 g m^{-2} yr^{-1}). Production of reproductive components (flowers, fruit, propagules) varied from 5 to 28 g m^{-2} yr^{-1} across sites, and was highest at the Henderson Creek mixed forest (Fig. 3.13, Table 3.9). In sites dominated by *R. mangle*, fringe forest had a higher production of these components than the scrub forest. Stipules accounted for 4 to 10 % of the total litter and varied from 8 to 12 g m^{-2} yr^{-1} across sites; however, differences were not significant (Fig. 3.13, Table 3.9).

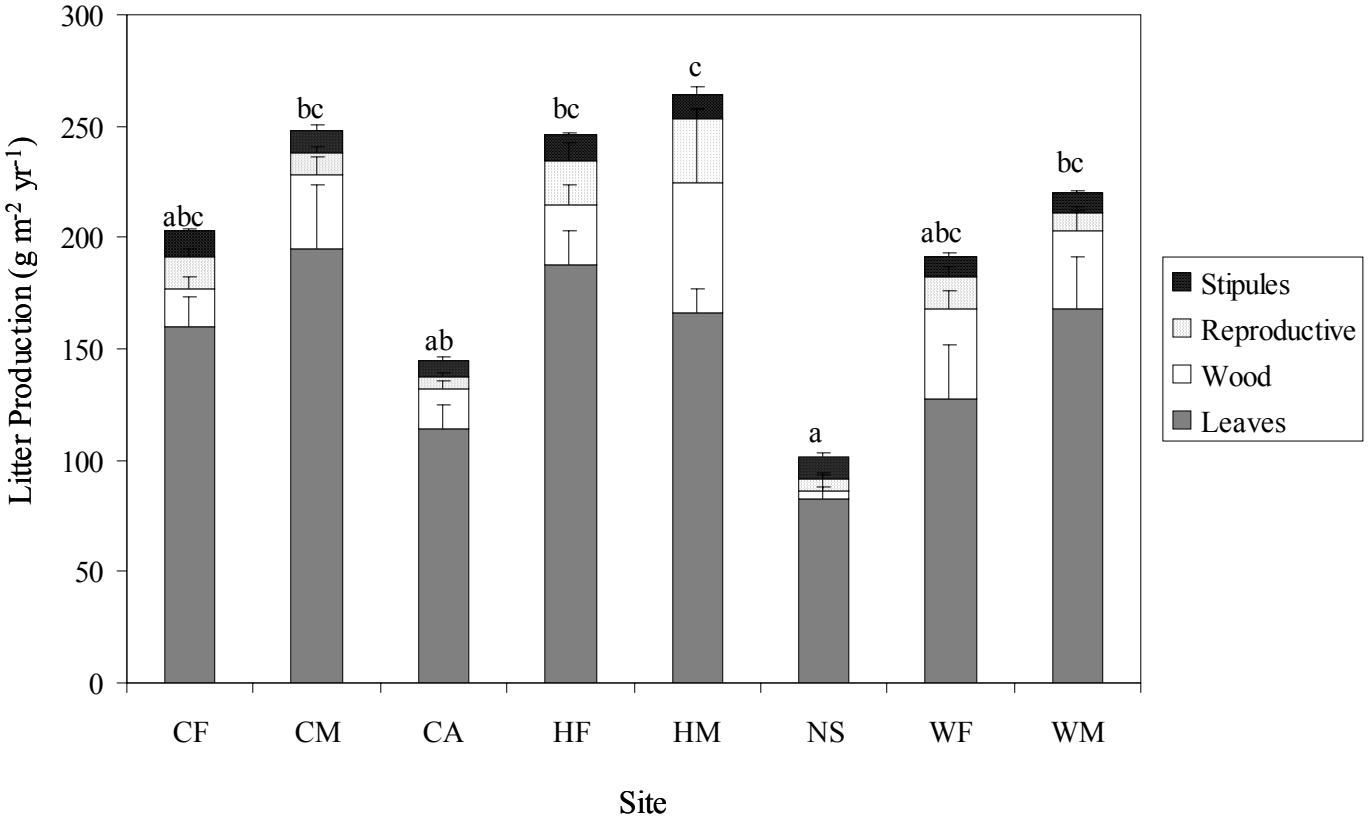

Figure 3.13. Annual litter production by component (twigs, stipules, reproductive, and leaves) across eight study sites. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Values are the mean ± SE (n = 5). Letters above bars indicate significant differences ($P \leq 0.05$) (Tukey HSD).

Table 3.9. Summary of ANOVA results for aboveground litter production at eight sites. Values are the F-ratio and probability of a greater F. 1 DF contrasts compare monospecific (Mono) vs Mixed (Mix) forests, *Avicennia* (A) vs. *Rhizophora* (R) dominated forests, interior (Int) vs. shoreline (shore), fringe (Fg) vs scrub (Sb), Mixed (Mx) vs. Scrub (Sb), and Rookery Bay (RB) vs. Windstar (WS) locations; t-values are given and significance (contrasts) is indicated by P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant.

		By Component			
	Total	Leaf	Stipules	Wood	Reproductive
F-ratio	5.66	4.35	1.44	3.26	3.09
Prob>F	0.0003	0.0018	> 0.05	0.0103	0.0131
Contrasts:					
Mono vs. Mx	3.90***	3.10**	-	2.01ns	1.09ns
A vs. R	-1.55ns	-1.22ns	-	-0.51ns	-1.60ns
Int vs. Shore	1.04ns	0.97ns	-	1.07ns	1.43ns
Fg vs. Sb	4.14***	3.53**	-	3.27**	2.06*
Mx vs. Sb	5.27****	4.37****	-	3.62***	1.92ns
RB vs. WS	-0.23ns	0.23ns	-	-3.21**	0.60ns

When combined, annual litter and root production varied significantly from 172 to 946 g m^{-2} yr^{-1} (Fig. 3.14, Table 3.10). Total production was lowest in the scrub forest and at Windstar, and highest in the fringe forest at Henderson Creek (Fig. 3.14). There were no consistent patterns, however, among forest types. In fact, the total production was most similar within a location than between locations. Sites at Rookery Bay had a higher annual total production (605 g m^{-2} yr^{-1}) compared to sites at Naples Bay (Windstar, 407 g m^{-2} yr^{-1}). The ratio of root production to litter production varied from 0.6 to 8.0 (Fig. 3.14). The basin forest dominated by *A. germinans* (CA) and the scrub forest (NS) had high allocation to root production (3.7 and 3.2 respectively) (Fig. 3.14). However, biomass allocation at the scrub site was similar to that at fringe forests, and higher to the allocation at mixed basin forests (Table 3.10). Monospecific forest had a higher root: litter production ratio (2.4) compared to mixed forest (1.3) (Table 3.10). Sites dominated by *A. germinans* had a higher root: litter production ratio (3.7) than sites dominated by *R. mangle* (2.0) (Table 3.10). The fringe and basin forests at Windstar exhibited the lowest root: litter production ratio (Fig. 3.14, Table 3.10).

3.4.6. Correlation Between Soil Variables and Response Variables

Standing root biomass was directly correlated with pH, and negatively correlated with porewater nitrite, and soil redox potential (Table 3.11). On the other hand, root turnover was positively correlated with porewater nitrite and soil organic matter, and negatively correlated with soil bulk density (Table 3.11).

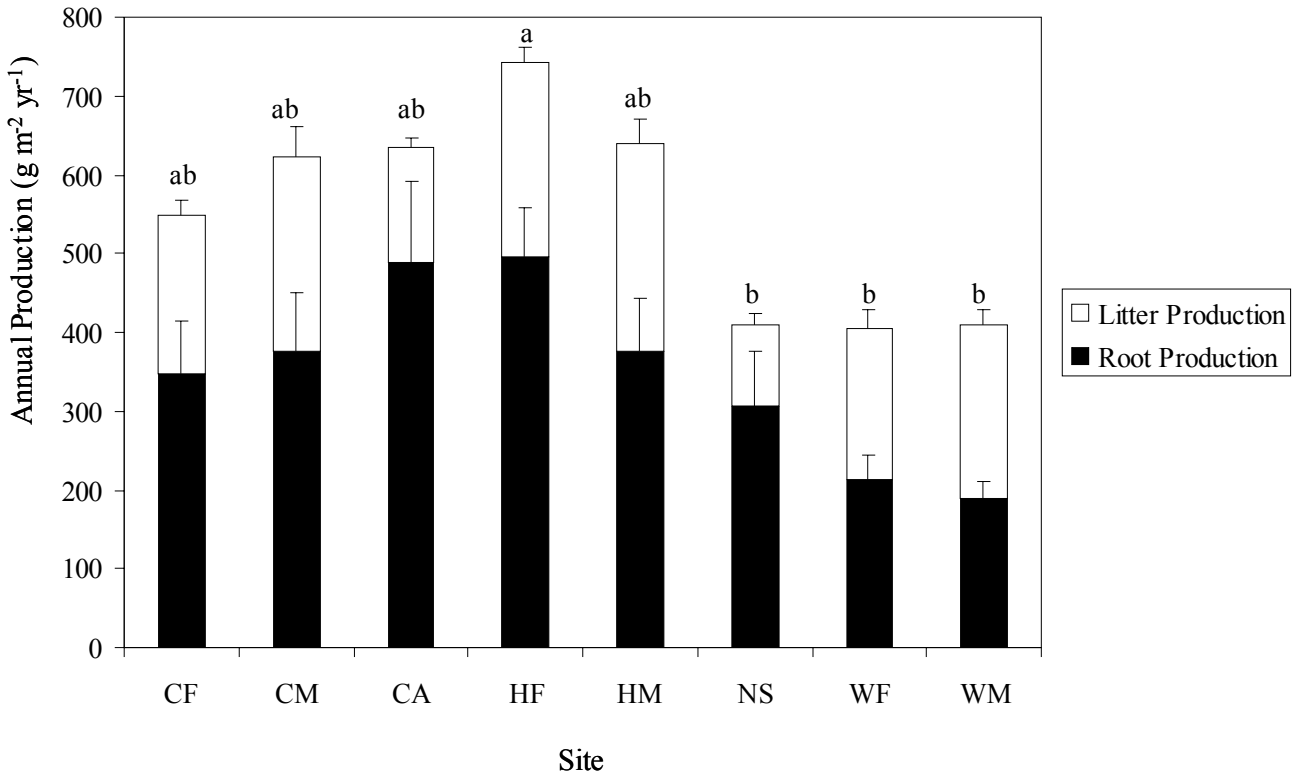

Figure 3.14. Annual total production (root production plus litter production) across eight sites. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; HF: Henderson Creek, Fringe; HM, Henderson Creek, Basin-Mixed; NS: New York Av., Scrub-monospecific WF: Windstar, Fringe; and WM: Windstar, Basin-mixed. Values are the mean ± SE (n = 5). Letters above bars indicate significant differences in the total production (root plus litter) ($P \leq 0.05$) (Tukey HSD).

Table 3.10. Summary of ANOVA results for total production and root: litter production ratio at eight mangrove sites. Values are the F-ratio and probability of a greater F. 1 DF contrasts compare monospecific (Mono) vs Mixed (Mix) forests, *Avicennia* (A) vs. *Rhizophora* (R) dominated forests, interior (Int.) vs. shoreline (shore), fringe vs scrub, and Rookery Bay (RB) vs. Windstar (WS) locations. t-values are given and significance (contrasts) is indicated by P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant. N=39.

	Production	
	Root + Litter	Root: Litter
F-ratio	3.63	3.11
Prob>F	0.0057	0.0132
Contrasts:		
Mono vs. Mx	-0.23ns	-2.73*
A vs. R	1.45ns	2.38*
Int vs. Shore	0.47ns	-0.87ns
Fg vs. Sb	1.93ns	-1.97ns
Mx vs. Sb	1.85ns	-2.65*
RB vs. WS	3.45**	3.31**

Root production was positively correlated with porewater nitrate, but no other soil variable (Table 3.12). In contrast, litter production varied positively with soil redox potential, porewater ammonium and nitrite, soil moisture, soil ammonium, and soil organic matter, and negatively with water level and bulk density (Table 3.12). The combined litter and root production positively correlated with porewater nitrate, soil moisture and organic matter, and negatively with water level and bulk density (Table 3.13). The ratio of root to litter production was negatively correlated with soil redox potential, porewater phosphate, and soil moisture (Table 3.13).

Table 3.11. Results of pairwise correlations between standing root biomass and root turnover and soil variables (n = 40). The r-value and probability are given.

	Standing Root Biomass		Root Turnover	
	r	Probability	r	Probability
pH	**0.40**	**0.0101**	-0.23	> 0.05
Salinity	0.20	> 0.05	0.11	> 0.05
Water Level	-0.16	> 0.05	-0.15	> 0.05
Eh (0 cm)	-0.26	> 0.05	0.19	> 0.05
Eh (15 cm)	**-0.36**	**0.0238**	0.14	> 0.05
Eh (30 cm)	**-0.38**	**0.0141**	0.29	> 0.05
Moisture	-0.00	> 0.05	0.24	> 0.05
Bulk density	0.14	> 0.05	**-0.33**	**0.0427**
Porewater NH4	-0.30	> 0.05	0.01	> 0.05
Porewater PO4	0.21	> 0.05	-0.03	> 0.05
Porewater NO2	**-0.49**	**0.0021**	**0.59**	**0.0002**
Porewater NO3	-0.10	> 0.05	0.29	> 0.05
Soil NH4	-0.20	> 0.05	0.32	> 0.05
Soil PO4	-0.14	> 0.05	-0.07	> 0.05
Organic matter	-0.27	> 0.05	**0.47**	**0.0025**

Table 3.12. Results of pairwise correlations between root production, aboveground litter production (total) and soil variables (n = 40). The r-value and probability are given.

	Belowground Root Production		Aboveground Litter Production	
	r	Probability	r	Probability
pH	0.09	> 0.05	0.00	> 0.05
Salinity	0.11	> 0.05	0.19	> 0.05
Water Level	-0.24	> 0.05	**-0.45**	**0.0035**
Eh (0 cm)	0.03	> 0.05	**0.36**	**0.0236**
Eh (15 cm)	-0.15	> 0.05	**0.36**	**0.0223**
Eh (30 cm)	-0.02	> 0.05	**0.42**	**0.0069**
Moisture	0.16	> 0.05	**0.47**	**0.0022**
Bulk density	-0.22	> 0.05	**-0.36**	**0.0087**
Porewater NH4	0.02	> 0.05	**0.21**	**0.0283**
Porewater PO4	0.03	> 0.05	0.38	> 0.05
Porewater NO2	0.13	> 0.05	**0.15**	**0.0211**
Porewater NO3	**0.42**	**0.0155**	0.21	> 0.05
Soil NH4	0.03	> 0.05	**0.39**	**0.0200**
Soil PO4	-0.11	> 0.05	0.04	> 0.05
Organic matter	0.23	> 0.05	**0.47**	**0.0022**

Table 3.13. Results of pairwise correlations between root + litter production, root: litter production and soil variables (n = 40). The r-value and probability are given.

	Root + Litter Production		Root: Litter Production	
	r	Probability	r	Probability
pH	0.09	> 0.05	0.09	> 0.05
Salinity	0.15	> 0.05	-0.12	> 0.05
Water Level	**-0.38**	**0.0173**	0.14	> 0.05
Eh (0 cm)	0.15	> 0.05	**-0.32**	**0.0477**
Eh (15 cm)	-0.01	> 0.05	-0.12	> 0.05
Eh (30 cm)	0.13	> 0.05	-0.15	> 0.05
Moisture	**0.32**	**0.0473**	**-0.37**	**0.0218**
Bulk density	**-0.35**	**0.0266**	0.26	> 0.05
Porewater NH4	-0.15	> 0.05	0.22	> 0.05
Porewater PO4	0.09	> 0.05	**-0.40**	**0.0144**
Porewater NO2	0.26	> 0.05	0.11	> 0.05
Porewater NO3	**0.45**	**0.0079**	0.05	> 0.05
Soil NH4	0.17	> 0.05	-0.24	> 0.05
Soil PO4	-0.09	> 0.05	-0.32	> 0.05
Organic matter	**0.38**	**0.0155**	-0.01	> 0.05

3.4.7. Effect of Nutrients on Root Production and Morphology

Nutrient enrichment affected root production at the monospecific basin forest dominated by *A. germinans* (CA) and the scrub forest dominated by *R. mangle* (NS) (Fig. 3.15). However addition of nitrogen and phosphorus resulted in an increase in root production at NS and a decrease in root production at CA (Fig. 3.15). The fringe and mixed basin forests (CF and CM) did not respond significantly to nutrient treatment.

Root length responses to nutrient treatments did not varied significantly within forest type (Fig. 3.16A, Table 3.14). In general, root length did not change with depth except at the fringe forest where it was significantly higher at the soil surface (Fig. 3.16B, Table 3.14). Root diameter was higher in the lower half of the bags (0.041cm) compared to the upper half (0.037cm) at all study sites. However, it varied across sites being highest at the monospecific basin forest, intermediate at the scrub forest, and lowest at the fringe and basin mixed forests (Fig. 3.16C, Table 3.12).

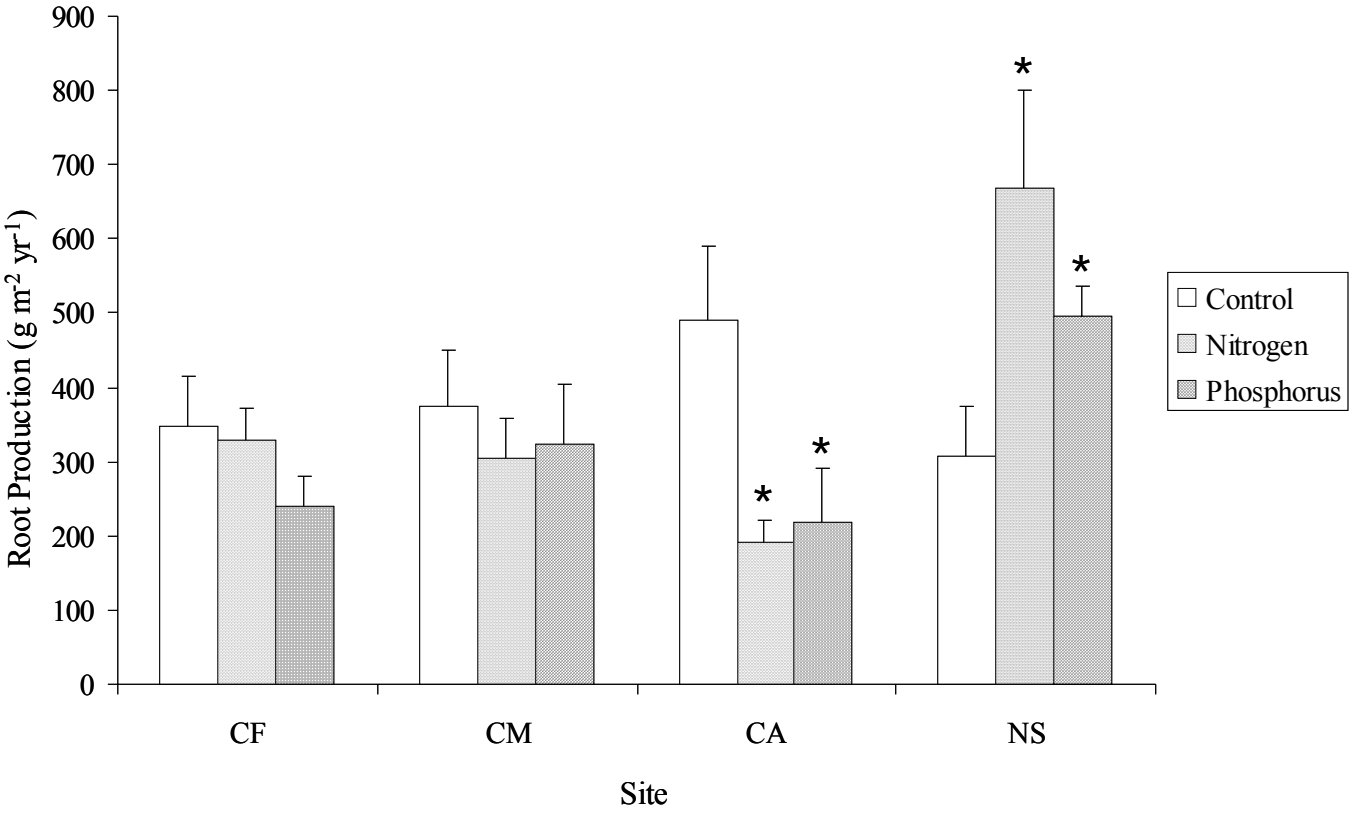

Figure 3.15. Effects of nitrogen and phosphorus additions on root production at four sites. Values are the mean ± SE (n = 5). ANOVA showed a significant interaction between site and nutrient treatment ($F = 3.82$, $P \leq 0.01$); asterisks above bars indicate significant nutrient effect relative to control (within site). CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; NS: New York Av., Scrub-monospecific.

Root surface area was highest at the scrub and basin mixed forests, intermediate at the fringe forest, and lowest at the basin monospecific forest (Fig. 3.16C, Table 3.14). Root branching and root tips increased significantly at the surface (top) at the fringe forest, but did not vary with depth at the basin mixed and monospecific, and the scrub forests (Fig. 3.17A and B, Table 3.14). Number of root tips did not vary significantly in response to nutrient additions within forest types (Fig. 3.17C, Table 3.14). The number of root branches per cm was higher at the surface (top, 1.69), than at the bottom (1.44), however, it varied between sites and was lowest (0.70) at the basin monospecific site compared to all other sites (1.79-1.93) (Table 3.14). The number of branches and root tips per cm of root (root length) varied between forest types and were lowest at the basin monospecific forest (0.70 and 1.63, respectively) (Table 3.14). In general, the number of branches per cm of root was higher at the soil surface (1.69 and 1.44, top and bottom, respectively) (Table 3.14). The number of root tips per cm of root increased significantly in response to nitrogen additions (1.90) compared to control treatments (1.69) at all forest types (Table 3.14). More evident responses to nutrients additions were observed for both specific root length (SRL), and specific surface area (SSA) (Table 3.15). SRL increased significantly with nitrogen additions in comparison to the control treatment (6818, and 3222 cm g^{-1}, respectively) at the basin monospecific forest. Also at this site, SSA increased significantly with nitrogen (1046 cm^2 g^{-1}) and phosphorus (850 cm^2 g^{-1}) additions in comparison to the control treatment (487 cm^2 g^{-1}). In contrast, at the scrub forest, SSA decreased significantly with nitrogen (884 cm^2 g^{-1}) and phosphorus (829 cm^2 g^{-1}) additions in comparison to the control treatment (2410 cm^2 g^{-1}).

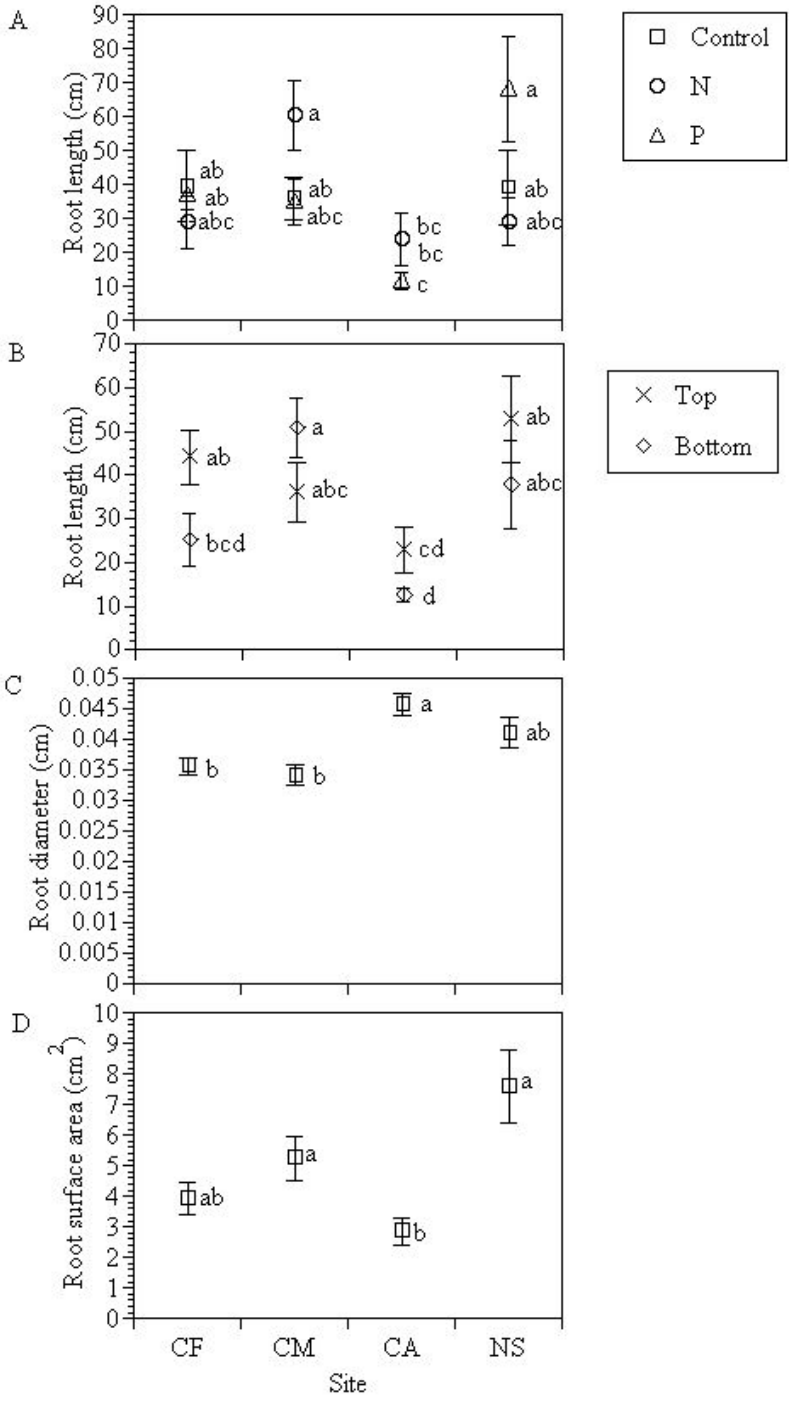

Figure 3.16. Root morphology (length, diameter, and surface area) at four sites. Values are the mean ± SE. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; NS: New York Av., Scrub-monospecific. (A) Site*Treatment interaction effect on root length, (B) Site*Depth interaction effect on root length, (C) and (D) Site effect on root diameter and surface area, respectively. Letters next to symbols indicate a posteriori pairwise comparisons (LSMeans with Tukey's adjustment).

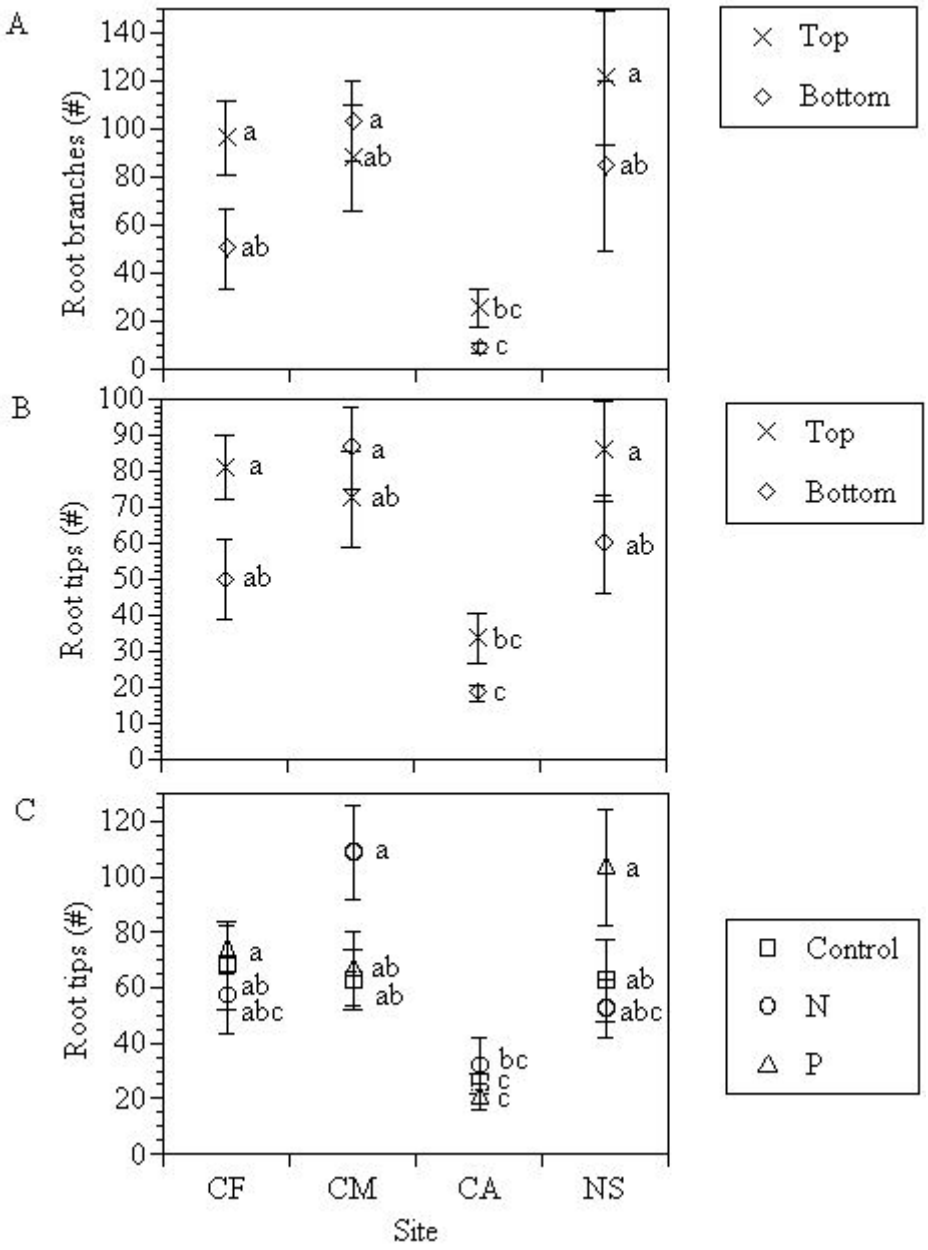

Figure 3.17. Root morphology (root branching and root tips) at four sites. Values are the mean ± SE. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; NS: New York Av., Scrub-monospecific. (A) and (B) Site*Depth interaction on root branching and root tips, (C) Site*Treatment interaction effect on root tips. Letters next to symbols indicate a posteriori pairwise comparisons (LSMeans with Tukey's adjustment).

Table 3.14. Summary of ANOVA results for effects of site, treatment (control, nitrogen or phosphorus additions), and depth on root morphology at four sites. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; NS: New York Av., Scrub-monospecific. Values are the F-ratios and probability of a greater F. P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant.

	Morphology traits						
Source	Length	Diameter	Surface Area	Branching	Tips	Branches/Length	Tips/Length
Site	**11.09******	**8.98******	**6.15*****	**17.35******	**17.53******	**22.91******	**6.22*****
Treatment	0.11ns	1.03ns	0.06ns	0.08	0.30ns	0.76ns	**3.66***
Site*Treatment	**2.98***	0.78ns	2.00ns	1.93	**2.31***	0.88ns	1.93ns
Depth	2.80ns	**5.57***	0.52ns	3.50	**4.92***	**4.30***	2.71ns
Site*Depth	**3.76***	0.37ns	1.71ns	**2.94***	**3.33***	0.92ns	1.23ns
Treatment*Depth	0.12ns	0.94ns	0.19ns	0.03	0.05ns	0.16ns	0.39ns
Site*Treatment*Depth	0.84ns	1.75ns	0.81ns	1.11	0.88ns	0.92ns	0.94ns

Table 3.15. Summary of ANOVA results for effects of site, and treatment (control, nitrogen or phosphorus additions), on root SRL (specific root length), and specific surface area (area/root dry weight ratio, SA/DW) at four sites. CF: Cat Claw Trail, Fringe; CM: Cat Claw Trail, Basin-mixed; CA: Cat Claw Trail, Basin-monospecific; NS: New York Av., Scrub-monospecific. SRL: Specific root length, SA:root surface area, and DW:dry weight. Values are the F-ratios and probability of a greater F. P < 0.05*, 0.01**, 0.001***, 0.0001****, ns = not significant.

Source	SRL	SA/DW
Site	6.66***	4.16**
Treatment	1.24ns	1.03ns
Site*Treatment	2.33*	0.78*

3.5. DISCUSSION

In this study, root responses varied spatially. Differences between forest types and species composition were more evident for standing root biomass and biomass allocation to roots (root:litter ratio) than for root production. The present root production estimates are in the higher range of reported values in the literature (Table 3.16). However, the ingrowth method may result in overestimation of root production due to use of unoccupied substrate. However, underestimation may occur due to delayed ingrowth of roots (after cutting) into bags and decomposition of roots that died during the experimental interval. The latter may be of less concern in mangrove soils since root decomposition rates are slow under the anaerobic conditions in waterlogged soils (Albright 1976, McKee and Faulkner 2000a, Middleton and McKee 2001).

Root standing biomass ranged between 29 to 284 t ha^{-1}, indicating high accumulation of roots in mangrove soils at Rookery Bay and Naples bay particularly along shorelines. The upper end of this range is high compared to estimates of belowground biomass in mangroves around

the world (Table 1.2). The accumulation of roots as organic matter in the soil also was a result of the slow root turnover rates estimated in these mangrove sites, which are lower than other herbaceous dominated wetlands but comparable to northern forested and tundra ecosystems (Gill and Jackson 2000). The turnover time calculated is about 39 years on average, which means that that roots are virtually preserved in these mangrove areas. Some studies have observed that most of the roots that accumulate in mangrove soils are dead (Alongi et al. 2003), which is consistent with the proportion of live and dead roots found in the ingrowth cores in this study since dead roots accounted for more than 50% of ingrown biomass.

In this study, high rates of root production contributed to high allocation of biomass underground compared to aboveground. The ratio root:litter production varied from 1 to 3, indicating that root production was equal to or exceeded litterfall production at all sites. This ratio was highest at sites that had more stressful environmental conditions (the monospecific basin and the scrub site), which suggest at higher allocation of biomass underground in response to nutrient limitation and anaerobic conditions at the scrub site, and to desiccation at the basin monospecific site.

Belowground production was correlated only with porewater nitrate, but allocation to roots was negatively correlated with flooding and nutrient related factors. Biomass allocation to roots increased under anaerobic soil conditions, low moisture, and low porewater phosphate. Hydrology (frequency and duration of flooding) is known to control root production in other forested systems, with lower root allocation under flooding conditions (Powell and Day 1991), which is opposite to the results at the scrub area (NS) that was continuously flooded. Nutrients (N and P) had also been reported to control root production in a Maple dominated ecosystem in combination with soil moisture and temperature, and soil nutrients (Cote et al. 1998). Other

studies in scrub communities had found phosphorus to be the most limiting factor for root production and even more important than water availability (Martinez et al. 1998).

The combined root and litter production varied by flooding and nutrient stress. The four study areas each exhibited a distinct pattern of hydro-edaphic characteristics (Fig. 3.18) that help explain the measured differences in total production. The combination of flooding, and nutrient stress indicates the following ranking of sites: New York Ave (NS) > Windstar (WF, WM) > Cat Claw (CF, CM, CA) > Henderson Creek (HF, HM). NS was characterized by low salinity, but low phosphorus availability and high flooding, which suggests that this site was the most stressful for plant growth. Fringe and basin mixed zones at Cat Claw (CF, CM) as well as both zones at Windstar (WF, WM) had low flooding and nutrient availability and intermediate salinity. CA exhibited high salinity, but low flooding and high nutrient availability. Thus, these sites were considered to be moderately stressful. The sites at Henderson Creek (HF, HM) were characterized by low to moderate salinity, low flooding and intermediate nutrient availability, which suggested the best conditions for plant growth. Mangrove production (roots plus litter) thus increased with decreasing environmental stress.

Nutrient enrichment caused different root responses between forest types. At NS, both N and P increased root production, and at CA, N and P decreased root production, but there was no response to nutrients additions at the CF and CM. At NS, the concentration of these nutrients measured in the control sites was low, so addition of N and P may have stimulated increased C allocation underground to maximized uptake of these limiting resources. In contrast, at CA with higher nutrient availability, the extra nutrients may have been used to increase aboveground production, thereby reducing the need for root exploration.

Table 3.16. Estimations of annual root production for different forest communities.

Source	Location		Community	Root production g m^{-2} yr^{-1}
Powell and Day 1991	Great Dismal Swamp, USA	31˚N 81˚ W	Mixed hardwood	354 *
			Cedar	274 *
			Maple-gum	91*
			Cypress	68*
(Sundarapandian and Swamy 1996	Kodayar, India	8˚ 29'N 77˚15'E	Moist deciduous	174-262*
			Semi-evergreen	
			Evergreen	
Nadelhoffer et al. 1985	Univ. of Wisconsin, USA	-	Black Oak	591
			Red Oak	524
			White Oak	413
			Maple	402
			Birch	324
			White Pine	257
			Mixed Pine	262
			Spruce	160
			Red Pine	198
Martinez et al. 1998	SW Coast of Spain	37˚7'N 6˚ 12'W	Scrub at sand dune	548
Symbula and Day 1988	Virginia, USA	-	Nyssa-Acer	651
Fahey and Hughes 1994	Hubbard Brook Experimental Forest, North Central New Hampshire	43˚56'N 71˚ 45'W	Hardwood Forest	254
Persson 1983	Sweden	-	Scots pine	226*
Jordan and Escalante 1980	Venezuela	-	Terra Firme	201*
Cuevas and Medina 1988	Venezuela	-	Terra Firme	1117*
			Mangroves	
McKee and Faulkner 2000a	Naples, Florida	30º91º8.6'W 23.'N	Basin	18-1,146*
Cahoon et al. 2003	Bay Islands Honduras	-	Fringe	311*
			Basin	333*
This study	**Florida**		**Fringe**	**352***
			Basin	
			Mixed	**314***
			Mono	**378***
			Scrub	**307***

* Estimation based on root in-growth core technique

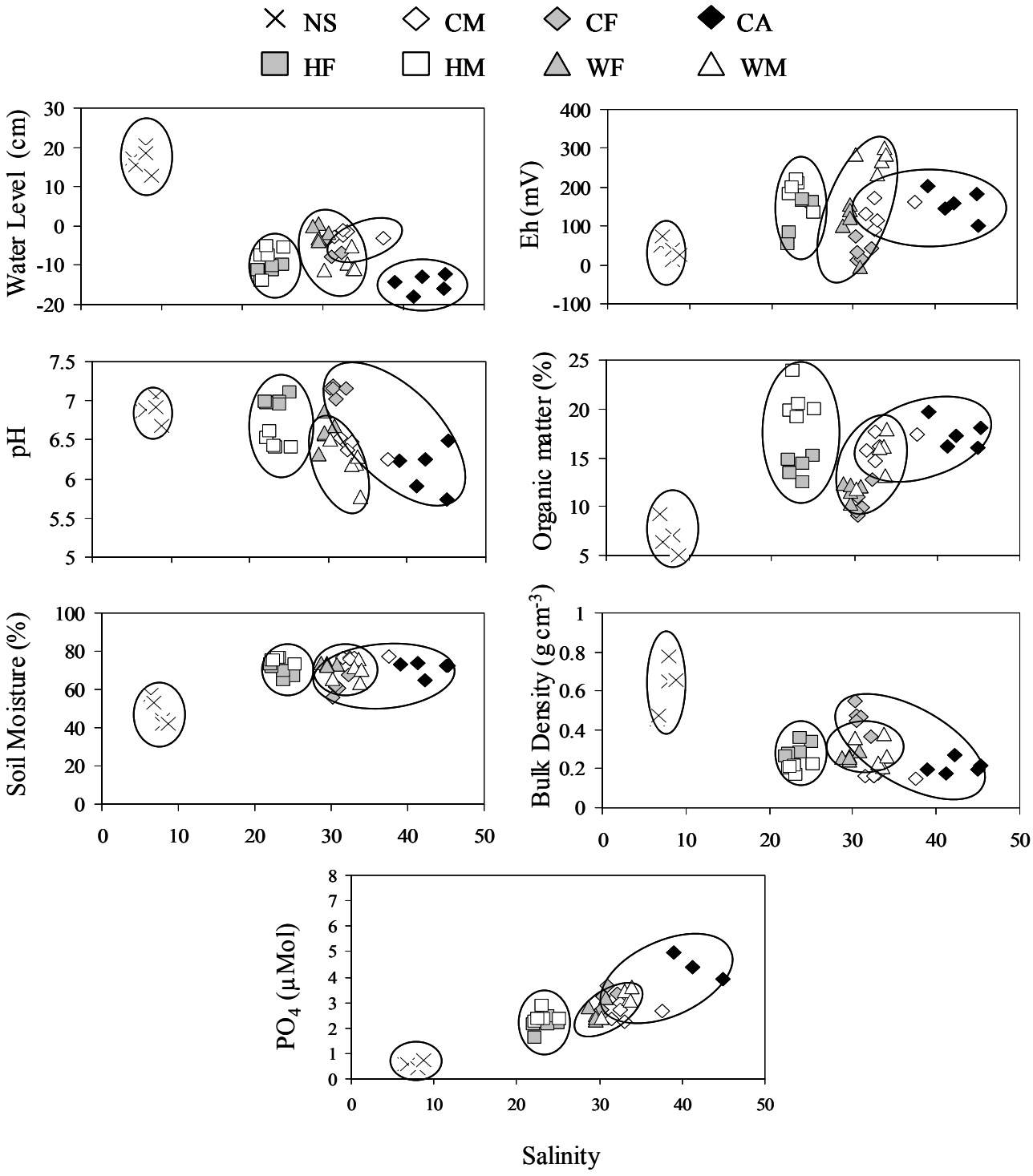

Figure 3.18. Characterization of study sites by correlation of porewater salinity with water level, redox potential, soil moisture, bulk density, soil organic matter, and porewater phosphate (annual means). Density ellipses are drawn around of the four study areas: Cat Claw (CF, CM, and CA), Henderson Creek (HF, and HM), Windstar (WF, and WM), and New York Ave (NS).

3.6. CONCLUSIONS

This study found that mangrove root biomass and production in forests of Southwest Florida varied both within and among stands. Standing biomass varied among forest types: monospecific > mixed, shoreline > interior, fringe > scrub, whereas root production varied primarily between locations: Rookery Bay > Naples Bay. These patterns reflect differences in factors controlling biomass allocation to root growth and turnover of roots produced. The ranges of root biomass and production measured in these forests indicate the importance of the belowground component to forest productivity and accumulation of organic matter. In comparison with litter production, belowground production in these tidal forests accounts for a substantial proportion of the total net primary production. In contrast to leaf litter, however, roots decay slowly and accumulate over time (Middleton and McKee 2001). Slow root turnover times calculated in this study (30-40 yr) are consistent with the high standing biomass of mangrove roots found at these sites and indicate that roots produced in the anaerobic soils are contributing substantially to soil organic matter. The results for root production and turnover, in combination with the wood production aboveground, indicate that these mangrove ecosystems are potentially large sinks for carbon.

The comparison of root and litter dynamics with environmental variables provides some insights into factors that may influence biomass production and above- to belowground partitioning. Although belowground production was not correlated with any specific environmental variable, allocation to roots was negatively correlated with flooding and nutrient related factors. These patterns were consistent with root responses to nutrient enrichment. As nutrient limitation is alleviated, new root growth may decrease because fewer roots are required to obtain nutrients. Alternatively, root growth might increase because stimulation of shoot

growth will increase plant demand for nutrients and water. Interactions between non-resource and resource limiting factors may also have quite different consequences for shoot and root dynamics. In particular, the relative responses of roots and shoots to specific nutrients may differ because of differences in tissue requirements for specific elements and different growth limiting factors. Addition of nutrients stimulated an increased production of roots at a scrub forest stand characterized by perennial flooding and low nutrient availability. In contrast, nutrient enrichment of a basin monospecific stand characterized by higher nutrient availability and less flooding caused a decrease in root production. At these sites, root morphology responses to nutrient additions were also observed. Specific root length increased in response to N additions at the basin monospecific site. Specific surface area increased in response of N and P additions at the basin monospecific forest, and decreased at the scrub forest.

The results of this study have provided some valuable information about belowground production, standing biomass, and turnover rates in relation to external factors. Further work is needed to better understand how root dynamics are controlled by nutrients and non-resource factors such as salinity and flooding.

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CHAPTER 4
EFFECTS OF FLOODING AND PHOSPHORUS ON BELOWGROUND DYNAMICS OF MANGROVE SEEDLINGS GROWN IN RHIZOTRONS

4.1. INTRODUCTION

Net primary production of mangroves varies spatially with latitude, geomorphologic setting (Thom 1967), mangrove types (Lugo and Snedaker 1974, Clough 1992), structure, and productivity (Pool et al. 1977, Chen and Twilley 1999, Day et al. 1996). This variation reflects different flooding regimes, salinities, nutrient concentrations, and H_2S levels (Day et al. 1989) that are controlled by local hydrology. Local patterns of hydrology such as tides, waves, river inputs, groundwater inputs, and surface drainage from uplands may affect the chemical and physical characteristics of the soil in mangrove habitats and the physiognomy of mangrove forest (Lugo and Snedaker 1974). Flooding depths and redox status of mangrove soils can be important controls on the distribution of *A. germimans* and *R. mangle* in the intertidal zone (McKee 1995a), which can be modified by aeration from aboveground roots (McKee 1993) and other factors such as dispersal (Rabinowitz 1978), predation (Smith 1987, McKee 1995c), and salinity (McKee 1993, 1995a). However, mangroves develop morphological and physiological adaptations to live under anaerobic conditions that include an aeration function of aerial roots (prop roots and pneumatophores) (Chapman 1940, Scholander and Scholander 1955, Curran 1985, McKee et al. 1988, McKee 1996), aerenchyma (air-space tissue) (Curran 1985), oxidized rhizosphere (Thibodeau and Nickerson 1986, McKee et al. 1988), increased capacity for alcoholic fermentation McKee and Mendelssohn (1987), and changes in biomass partitioning (Pezeshki et al. 1990, Toma et al. 1991, McKee 1993).

Soil nutrient availability may control mangrove biomass and productivity in the absence of physical stress by sulfide and salinity (Feller, 1995, Feller et al. 1999, Chen and Twilley 1999). Studies have shown that phosphorus (P) may be a limiting factor for primary productivity in some mangrove ecosystems (Feller 1995, Feller et al. 1999, McKee 2001). Inorganic P in mangrove sediments may be stored in the soil in the form of Ca, Fe, and Al phosphates or as soluble reactive phosphorus adsorbed onto, or incorporated into, hydrated Fe and Al sexquioxides and therefore unavailable to the mangrove trees (Alongi et al. 1992, Alongi et al. 2003). However, mangroves may survive low nutrient conditions with conservative mechanisms that become more efficient as nutrient availability decreases (Feller 1995, Feller et al. 1999, McKee 2001). In addition, when nutrient resources are limiting, an increased proportion of biomass may be allocated to root growth (Stitt and Scheible 1998) to support soil exploration and increased surface area for nutrient absorption. This allocation pattern could increase acquisition of the limiting resource and increase the overall rate of growth (Eissenstat 1992).

Many authors have quantified aboveground production of terrestrial and wetland ecosystems but few have explored the belowground component. Even less is known about the functioning of mangrove roots belowground in terms of nutrient acquisition. The mangrove root system has been generally described previously (Chapman, 1975, Tomlinson 1986, Gill and Tomlinson 1969, 1971, and 1977, Wada and Takagi 1988), but these descriptions have focused primarily on gross features of the adult root system and the aerial roots rather than on features controlling nutrient acquisition. Several studies conducted in mangrove forests have emphasized the importance of the belowground component in terms of root biomass (Golley et al. 1962, Briggs 1977, Komiyama et al.

1987, 1988, 1989, 2000, Mackey 1993, Saintilan 1997 a,b, Matsui 1998, Alongi et al. 2003, Sherman 2003) with a high percentage (more than 50%) allocated to fine roots (Golley et al. 1962, Komiyama et al. 1987). However, estimates of belowground biomass for mangrove species in the New World are scarce. Many biomass estimates are based on regression equations (Komiyama et al. 1987, 1988, 1989, 2000, Mackey 1993, Matsui 1998) and vary substantially between locations and mangrove species: 38.5 to 509.5 t ha^{-1} (Komiyama et al. 1987, 1988, 1989, 2000). Some workers found a direct relationship between belowground biomass and salinity stress (Saintilan 1997 a,b) or surface water (Sherman 2003), but effects of hydrology and nutrient levels on belowground biomass or production have not been reported in spite of the importance of these factors. In Florida, estimates of root production rates in basin mangrove forests (natural and restored) ranged between 0.05 to 3.14 g $m^{-2}day^{-1}$, suggesting that carbon input by roots was 60-70% of that from litter fall (McKee and Faulkner 2000). Because degradation of roots is slow, this component can make a greater relative contribution to peat formation, especially in areas with low mineral sediment inputs (Middleton and McKee 2001).

More detailed studies of mangrove root morphology and distribution may help to explain the effects of hydrology and nutrient limitation on the morphology and biomass partitioning to mangrove roots, particularly fine roots that are important for nutrient absorption. The objective of this study was to evaluate the effects of two major forcing functions, hydrology and nutrient concentration (phosphorus), on the growth, distribution, and morphology of mangrove roots under controlled conditions. To increase the scope of inference, two species were studied, *Avicennia germinans* L. and *Rhizophora mangle*.

However, due to logistical and space limitations, each experiment was conducted separately in consecutive years.

4.2. MATERIALS AND METHODS

4.2.1. Plant Material

Greenhouse experiments were performed at the facilities of Louisiana State University. The first experiment was conducted with *Avicennia germinans* from February to December, 1999 and the second experiment was conducted with *Rhizophora mangle* from April 2000 to April 2001. Propagules of *A. germinans* and *R. mangle* were collected 1 to 2 mo prior to each experiment directly from trees in Southwest Florida and examined for insect predators that could influence seedling establishment or growth. Only those propagules free of insects and showing healthy initial growth were used in these experiments. The propagules were individually planted in small pots containing peat soil (Jiffy-Mix, Jiffy Products of America, Inc., 1119 Lyon Road, Batavia IL 60510-4303, or Hyponex, Canadian Spagnum Peat Moss, Hyponex Corporation 14111 Scottslacon Rd., Marysville OH 43041), and watered regularly with tap water. Seedlings were transplanted into Plexiglas rhizotrons of 10.6 L (56 cm x 38 cm x 5 cm) at the 4 to 6-leaf stage for *A. germinans* and 0 to 4 leaf stage for *R. mangle*. The rhizotrons were filled with moistened commercial soil over a bottom layer of 4 cm of pea gravel. Rhizotrons were held in two frames at an angle of 30 ° from the vertical to promote root growth on the viewing face. To exclude light, each rhizotron was covered with a black plastic sleeve.

4.2.2. Experimental Design

The experimental design of the greenhouse experiments was a CRD with a 2 x 3 factorial treatment arrangement (2 phosphorus levels x 3 flooding levels) and five

replicates of each treatment combination for a total of 30 experimental units (planted rhizotrons). Also, six additional rhizotrons without mangrove seedlings were used as soil controls.

The rhizotrons were subjected to different hydroperiods: constantly flooded (water level at 10-15 cm above the soil surface), alternately flooded, and drained (flooded at 10-15 cm above the soil surface for about 40% of time), and drained (moistened once per week and completely flushed). To provide balanced nutrition for the plants, 3 L of quarter strength nutrient solution was added to each rhizotron (Table 4.1). All rhizotrons received the same amount of macro- and micro-nutrient elements, except phosphorus. In each experiment, this solution was regularly replaced in all rhizotrons to maintain good nutritional conditions to the seedlings. Salinity was initially set at 25 ‰ by adding synthetic sea salt (Instant Ocean, nitrate and phosphate free) and adjusted as necessary.

Phosphorus treatments for these experiments included two levels of phosphorus concentration, high (High P) and low (Low P). The high P treatment was applied as shown in Table 4.1. In contrast, the low P treatment did not have additional P. Consequently, the P added was that in the deionized water (average 0.024 μM PO_4-P) used to water the seedlings during the experiment, the sea salts, and the peat soil. Differences in porewater and soil-extractable P between the two nutrient treatments in each experiment are shown in Table 4.2. Dissolved P in water and soil-extractable P were significantly higher for high P treatment. In the first experiment, dissolved P concentration under high P treatment was 4 times higher than the low P treatment, and the concentration for extractable phosphate under high P treatment was 8 times higher than the low P treatment. P concentrations for experiment 1 were lower than P concentrations for experiment 2 because during the first 23

weeks of the experiment, the P concentration in the nutrient solution was 10 times lower. Then, it was adjusted to the strength shown in Table 4.1.

Table 4.1. Composition of nutrient solution used in rhizotron experiments. Note that these concentrations do not include elemental contributions from artificial sea salts.

Chemical	Low P Solution	High P Solution
KH_2PO_4	0	4 mM
$MgSO_4$	2 mM	2 mM
K_2SO_4	2 mM	0
$CaCL_2$	5 mM	5 mM
NH_4NO_3	5 mM	5 mM
FE-EDTA	1.8 mM	1.8 mM
Micronutrients:		
H_3BO_3	46 μM	46 μM
H_2MoO_4	0.1 μM	0.1 μM
$MnCl_2$	0.4 μM	0.4 μM
$ZnSO_4$	0.8 μM	0.8 μM
$CuSO_4$	0.3 μM	0.3 μM

Dissolved and extractable phosphate analyses were performed using Murphy-Riley and flow injection techniques (Lachat Instruments) to monitor P concentrations for each treatment (Table 4.2). Seedlings were watered with the corresponding nutrient solution (with or without P), and the 3 L volume was kept constant by regularly adding deionized water.

Table 4.2. Differences in average concentrations of porewater and soil-extractable phosphate (PO_4-P) between nutrient treatments for each greenhouse experiment.

	Porewater		Soil	
	Low P (μM PO_4-P)	High P (mM PO_4-P)	Low P (mg g^{-1} PO_4-P)	High P (mg g^{-1} PO_4-P)
Experiment #1	0.06	0.26	0.004	0.036
Experiment#2	0.04	1.41	0.025	0.317

The following growth conditions were monitored: PO_4-P concentrations in water and soil, porewater salinity and pH, and soil redox potential. Biotic variables examined were: root elongation and distribution (density at different soil depths), root morphology, and biomass production and partitioning.

4.2.3. Analytical Procedures

Root growth patterns were traced onto plastic films. This root tracing allowed analysis of root growth rates and root distribution (root density by size class (fine <2mm, medium 2-5 mm, and coarse roots >5mm) and depth). Linear equations of root growth were obtained for each species using the linear portion of the growth curve, i.e., before roots reached the bottom of the rhizotron: 9 weeks for *A. germinans*, and 32 weeks for *R. mangle*. In addition to elongation rates, change in root density by depth was also calculated. For this measurement, a ruler was placed across the width of the rhizotron at 10, 20, 30, and 40 cm depths and all visible roots (by size class) intercepting this line were counted. Changes in root density by depth were plotted over time, and the slope of the line fitted to the data was used to calculate rates of root density change.

Root tracing for *A. germinans* began two weeks after the experiment started. All emerging roots were traced weekly in the first month and then biweekly. When the new roots became more abundant and the anchor roots reached the bottom of the rhizotron (after six months), only two quadrants 10x10 cm (one at the top 0-10 cm, and one at the bottom 30-40 cm) were traced (at weeks 31 and 56, the end of the experiment). Root tracing for *R. mangle* began six weeks after the experiment started and continued at biweekly intervals for 2.5 months, then monthly thereafter. In month 7, a 10x10 cm quadrant was traced at the top of each rhizotron where root density was higher.

Stem diameter, shoot elongation, and number of leaves, nodes, and branches were recorded monthly. Shoot growth of *A. germinans* was measured using an ink mark made on the stem of each seedling as reference. Shoot growth of *R. mangle* was measured from the top of the hypocotyl.

The experiment was terminated when the plants began showing signs of being root-bound (Day and Megonigal 1993). Plants were separated into roots and other vegetative structures and the following information was recorded: number of adventitious roots; number of pneumatophores (*A. germinans*); deepest root length; total number of leaves, nodes, and branches (order was recorded); height at first pair of leaves from the plant base (*A. germinans*); and total length of stem and branches. The entire root system was washed, divided into top (22.5 cm) and bottom (anything below 22.5 cm) layers, and sorted by size classes (fine, medium, and coarse roots) and vitality (live and dead). Both belowground and aboveground tissues were oven dried to constant mass at 70ºC and weighed. The following biomass ratios were calculated:

Root biomass ratio (RBR) = Root biomass / Total plant biomass

Root:shoot ratio = Root biomass / Shoot biomass

Assuming that root initial weight was zero, relative root growth rate (RRGR) during the experiment, was calculated as:

RRGR= ln (final root dry weight)/time

4.2.4. Root Morphology Based on Root Image Analysis

A computerized root analysis program (MacRHIZO, Regent Instruments, Inc.) was used to further assess differences in mangrove root morphology under different flooding and nutrient regimes. This technique allowed rapid quantification of total root length,

diameter, surface area, and branching. Prior to drying, a root sample was scanned, and the image was imported into MacRHIZO and analyzed. The analysis was performed by following the scanning protocol suggested by Bouma et al. (2000). Validation of data obtained with MacRHIZO was done by comparison with direct measurements of root length and diameter distribution on separate root samples. After image analysis, the roots were dried to allow calculation of specific root length (SRL) (Eissenstat 1992):

SRL = total root length/root biomass

4.2.5. Statistical Analysis

Root growth and density data were analyzed with a repeated measures ANOVA with a correlated POWA errors model with nutrient and flooding treatments as the grouping factors. The covariance structure for these models was a first order autoregressive structure (spatial power), and the estimation method was the Restricted Maximum Likelihood (REML) in a mixed model analysis. For root growth analysis (root elongation rates), time was the repeated measures factor, and for root density analysis (log-transformed data), time and depth were the repeated measures factors (Moser et al. 1990 and Gurevitch and Chester 1986). Separate density analyses were performed for each root class (fine, medium, and coarse) because roots of all size classes were not observed from the beginning of the experiment (Moser personal communication, Moser et al. 1990 and Gurevitch and Chester 1986). Multiple aposteriori pairwise comparisons were performed using Least Squares Means (LSMeans) on interactions or main effects.

Root biomass (fine, medium and coarse roots) and root morphological data, were analyzed using separate MANOVA analyses (log-transformed data), with nutrient and

flooding treatments (main plot), and depth (subplot) as grouping factors (Freund and Wilson 1997, Johnson and Wichern 1999).

Variables measured once at the end of the experiment such as length, density, and biomass of pneumatophores (log-transformed data for *A. germinans* only), and biomass partitioning (aboveground, belowground, total biomass, and root biomass ratio) were analyzed with MANOVA (separate analyses), using nutrient and flooding treatments as grouping factors (Johnson and Wichern 1999). Specific root length (SRL) (log-transformed) was analyzed using MANOVA, with a split plot design, in which phosphorus and flooding were the main factors, and depth was the subplot factor.

4.3. RESULTS

4.3.1. *Avicennia germinans*

4.3.1.1. Abiotic Variables

Effects of flooding and nutrient treatments on selected soil variables are summarized in Table 4.3. Soil redox potentials indicate reducing to moderately reducing conditions in flooded treatments and were lower than that in alternately drained/flooded and constantly drained treatments, which were not different. Both soluble and extractable P concentrations were significantly higher in the high P treatment. Salinity was maintained between 26-30 ‰ in all rhizotrons, although it was slightly lower, on average, in drained compared to flooded treatments. Porewater pH was not significantly different between flooding and phosphorus treatments. Thus, the experimental treatments created differences in nutrient availability and soil reduction-oxidation status in the rhizotron experiment.

4.3.1.2. Root Elongation and Distribution

Elongation of anchor roots increased with soil flooding, but the pattern across flooding treatments differed with P treatment (Fig. 4.1, Table 4.4). The difference was mainly due to response in the alternately drained/flooded treatment. Under high P, both drained and alternately drained/flooded treatments were similar and lower than that in flooded treatment. Under low P, the alternately drained/flooded treatment was similar to the flooded treatment. Flooding caused different temporal patterns of root elongation monitored over 17 wk (Fig. 4.2, Table 4.4). Root elongation of anchor roots under the drained treatment was significantly higher than the other flooding treatments (beginning in week 4) and followed a linear pattern of growth during the first 7 weeks. Roots in the drained/flooded treatment exhibited slower initial root extension. However, by week 7, they had elongated significantly more than roots under the flooded treatment, and by nine weeks, they reached similar depths to that in the drained treatment. The roots exposed to flooded conditions grew even more slowly, but at a linear rate. Roots under all flooding treatments reached similar depths at the bottom of the rhizotron by week 17 (Fig. 4.2). Linear equations for root growth of *A. germinans* under the different flooding regimes were calculated for the initial 9 weeks period (Table 4.5). Root growth rate was lower under flooding conditions (2.7 cm/week) in comparison to drained and drained/flooded conditions (4.7 and 4.6 cm/week, respectively).

Table 4.3. Soil variables measured in rhizotrons containing *A. germinans.* The values are the mean ± SE (n = 5).

	Drained		Drained/Flooded		Flooded	
Variable	Low P	High P	Low P	High P	Low P	High P
Dissolved phosphate ($\mu g\ ml^{-1}\ PO_4$-P	1.5±0.1	8.3±1.6	1.7±0.2	10.1±2.3	2.5±0.3	6.1±1.0
Extractable phosphate (Soil P) $\mu g\ ml^{-1}\ PO_4$-P	0.1±0.0	0.6±0.1	0.0±0.0	0.5±0.1	0.0±0.0	0.2±0.0
Redox Potential (Eh, mV)						
0-5 cm	383±12	408±15	439±16	421±16	180±20	193±19
30-35 cm	388±23	403±25	393±31	373±27	113±24	167±29
Salinity ‰	26±1	27±1	27±1	30±1	29±1	30±1
pH	7.3±0.1	6.6±0.1	7.2±0.1	6.7±0.1	7.2±0.1	7.1±0.1

Table 4.4. Results of a repeated measures ANOVA for anchor root growth of *A. germinans* in response to phosphorus and flooding treatments over time. Significance: P≤0.05*, 0.01**, 0.001***, 0.0001****.

Source	F Ratio	P Value
Phosphorus	0.31	0.5796
Flooding	11.33	0.0002
Phosphorus*Flooding	3.59	0.0393*
Week	92.07	<0.0001
Phosphorus*Week	0.36	0.8763
Flooding*week	4.61	<0.0001***
Phosphorus*Flooding*Week	0.82	0.6124

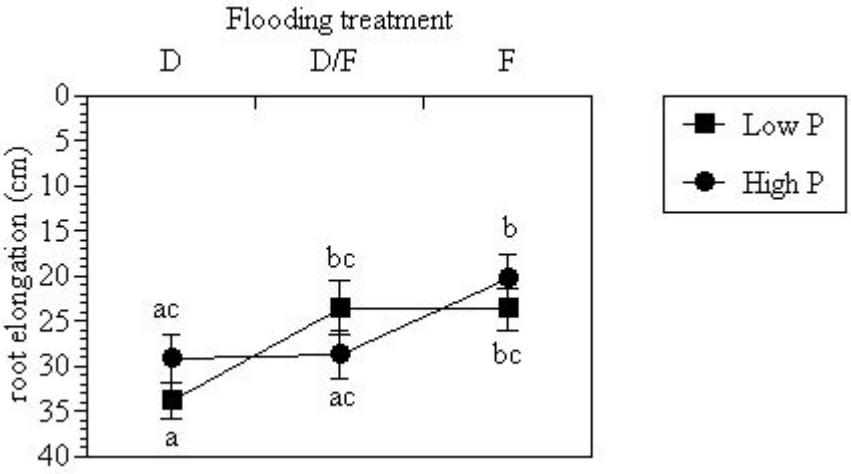

Figure 4.1. Anchor root elongation of *A. germinans* under flooding (D: drained, D/F: alternatively drained and flooded, and F: flooded) and P (Low P and high P concentration) treatments. A posteriori pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). Bars indicate one standard error. N=5.

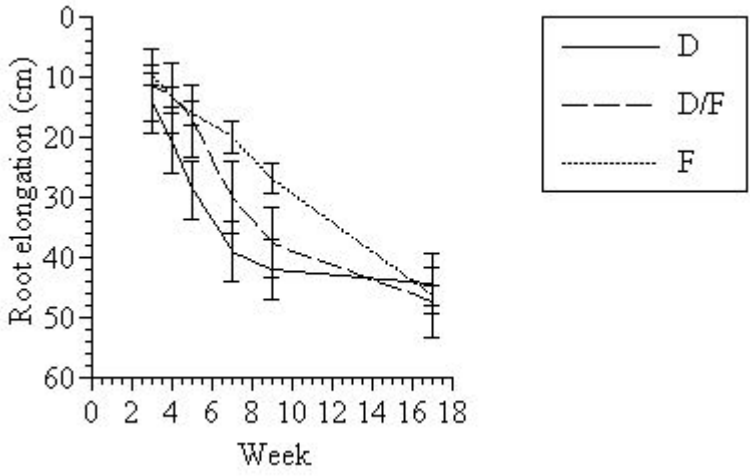

Figure 4.2. Anchor root growth of *A. germinans* under flooding treatments (D: drained, D/F: alternatively drained and flooded, and F: flooded) over time. Bars indicate one standard error. N=10.

Table 4.5. Linear equations for root growth of *A. germinans* under different flooding treatments. The curve was fitted for the root growth observed during the first nine weeks of the experiment.

Flooding	Root growth linear equation Y=mX±b	R^2
Drained	y = 4.7105x + 2.6631	0.9406
Drained/Flooded	y = 4.6539x - 4.1117	0.9826
Flooded	y = 2.7259x + 1.9307	0.9889

Cumulative root density of *A. germinans* was highest for fine roots, intermediate for medium size roots, and lowest for coarse roots (Figs. 4.3, 4.4, and 4.5, respectively). Fine root density of *A. germinans* was increased by high phosphorus treatment at the surface (depths 10 and 20 cm) under drained/flooded conditions, but decreased under drained conditions (Fig. 4.3, Table 4.6). Phosphorus treatment had no effect on fine root density under flooded treatment (Fig. 4.3, Table 4.6). At depths of 30 to 40 cm, fine root density was decreased by flooding, but the effect was greatest for the low P treatment (significant three-way interaction between phosphorus, flooding, and depth) (Fig. 4.6, Table 4.6). Examined over time, fine root density increased rapidly in the drained and drained/flooded treatments and more slowly in the flooded treatment (Fig. 4.7, Table 4.6). Differences among depths were most apparent in the drained and drained/flooded treatments (significant three-way interaction between flooding, date, and depth) (Fig 7, Table 4.6).

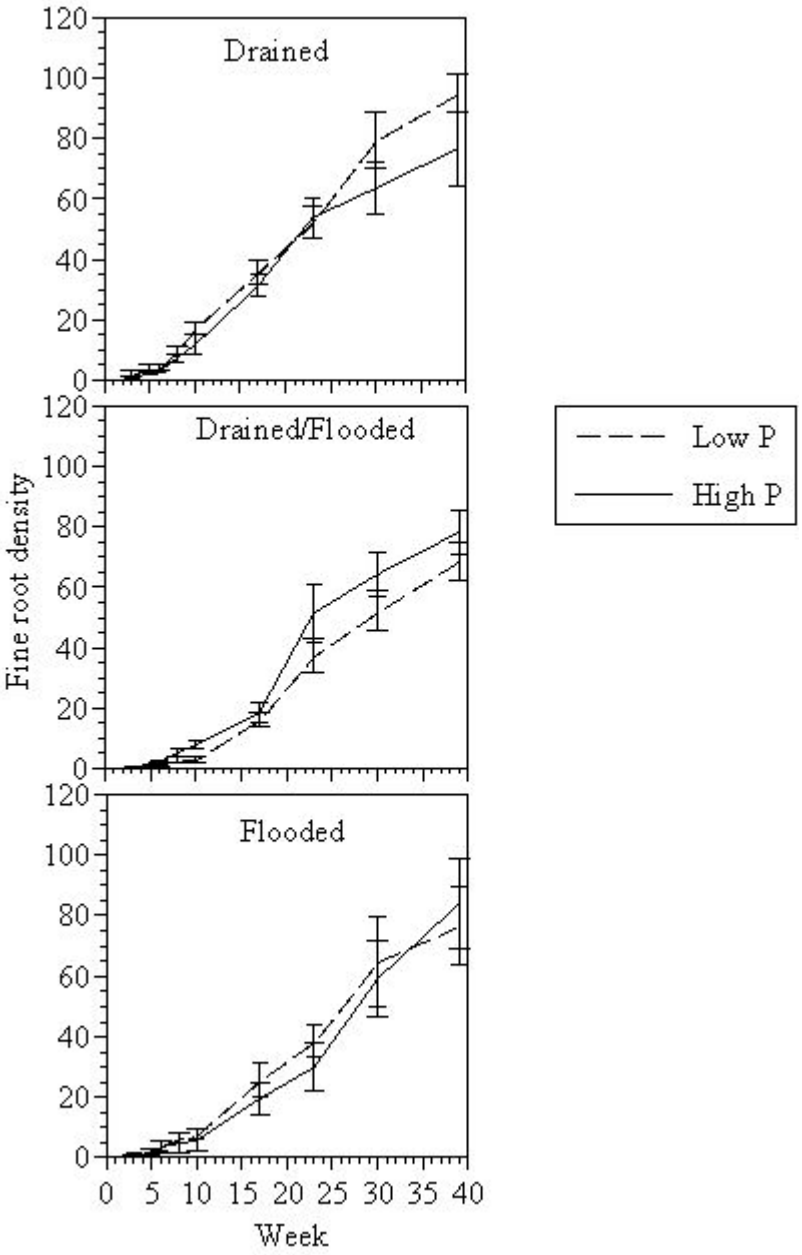

Figure 4.3. Temporal variation of fine root density of *A. germinans* under the experimental treatment combinations. Phosphorus concentration: Low P and High P, and flooding treatment: drained, alternately drained/flooded, and flooded. Bars indicate one standard error. N=5.

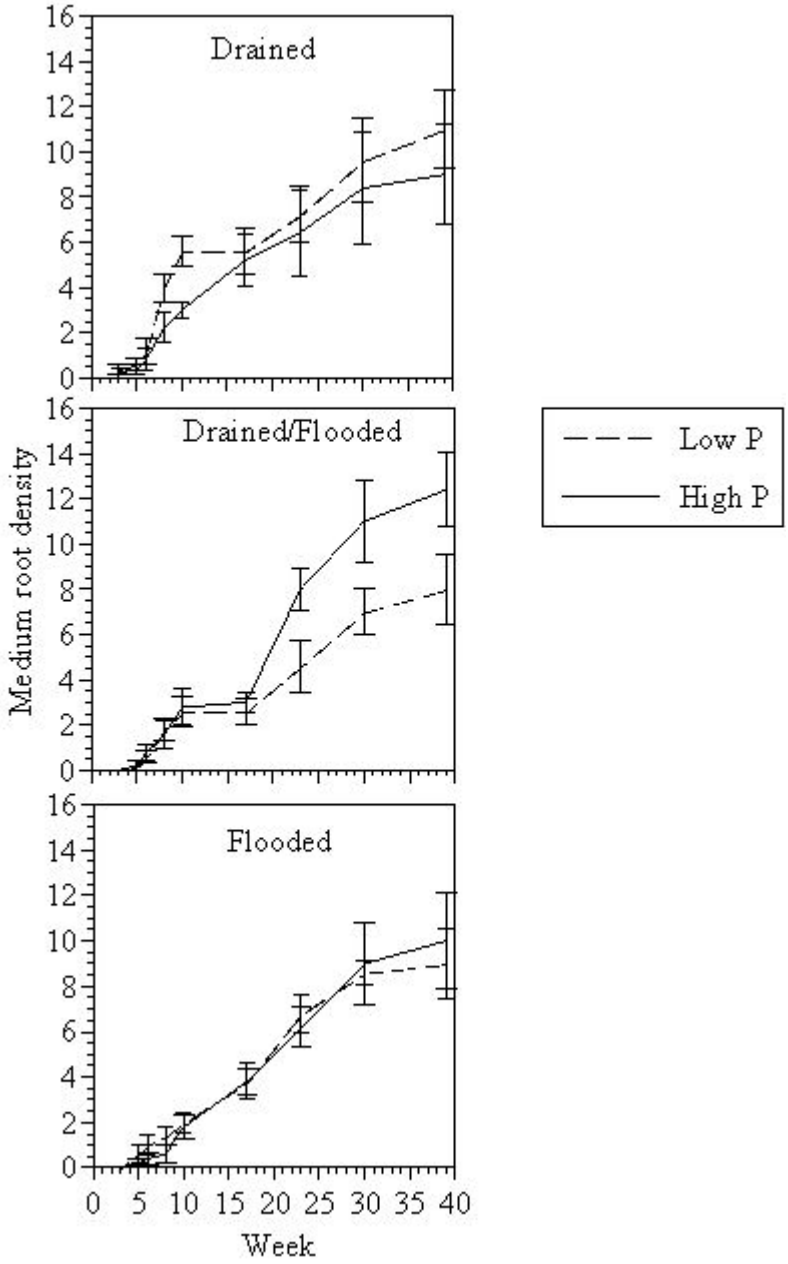

Figure 4.4. Temporal variation of medium root density of *A. germinans* under the experimental treatment combinations. Phosphorus concentration: Low P and High P, and flooding treatment: drained, alternately drained/flooded, and flooded. Bars indicate one standard error. N=5.

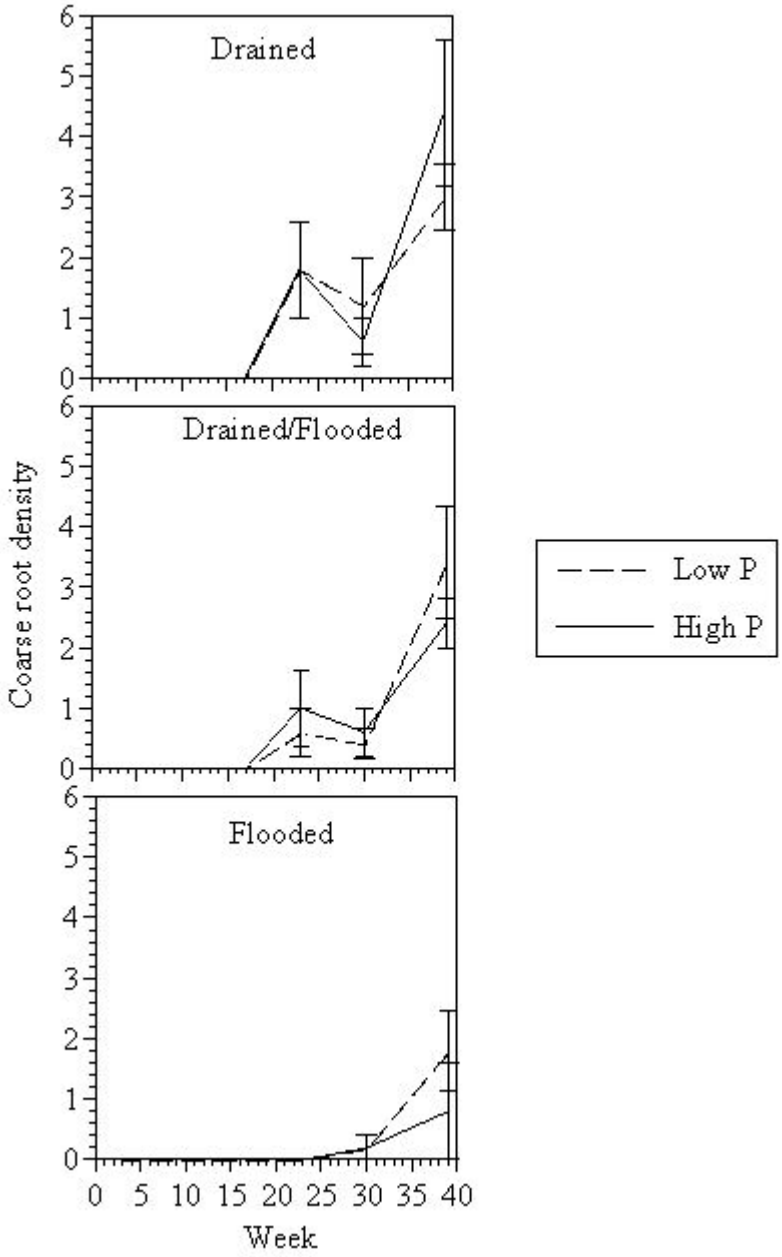

Figure 4.5. Temporal variation of coarse root density of *A. germinans* under the experimental treatment combinations. Phosphorus concentration: Low P and High P, and flooding treatment: drained, alternately drained, and flooded. Bars indicate one standard error. N=5.

Table 4.6. Results of the repeated measures ANOVA for root density of *A. germinans* for each size class in response to phosphorus and flooding treatments and depth over time. The table provides F-ratios and their significance is indicated as: P≤0.05*, 0.01**, 0.001***, 0.0001****.

Source	Fine	Medium	Coarse
Phosphorus	0.03	0.07	0.06
Flooding	7.25**	1.27	6.26**
Phosphorus*Flooding	2.66	1.51	0.14
Date	281.11***	71.52***	22.89***
Phosphorus*Date	0.75	0.81	0.28
Flooding*Date	4.09***	1.90*	1.46
Phosphorus*Flooding*Date	1.25	0.57	1.05
Depth	6.53***	10.48***	6.20***
Phosphorus*Depth	5.95***	0.33	0.56
Flooding*Depth	19.59***	3.04**	1.44
Phosphorus*Flooding*Depth	2.36*	2.98**	0.33
Date*Depth	11.66***	3.82***	1.36
Phosphorus* Date*Depth	0.57	0.65	1.32
Flooding* Date*Depth	2.31***	1.47*	1.12
Phosphorus* Flooding* Date*Depth	0.89	0.90	0.70

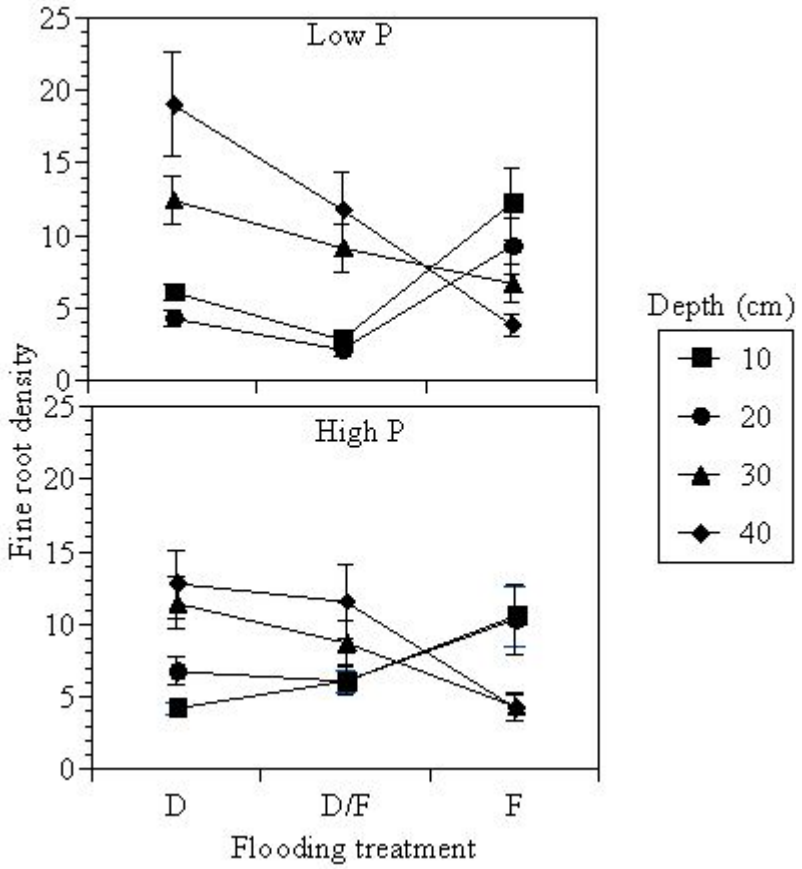

Figure 4.6. Response of fine root density of *A. germinans* to phosphorus, flooding, and depth. Phosphorus concentration: Low P and High P. Flooding treatment: drained (D), alternately drained/flooded (D/F), and flooded (F). Bars indicate one standard error. N= 5.

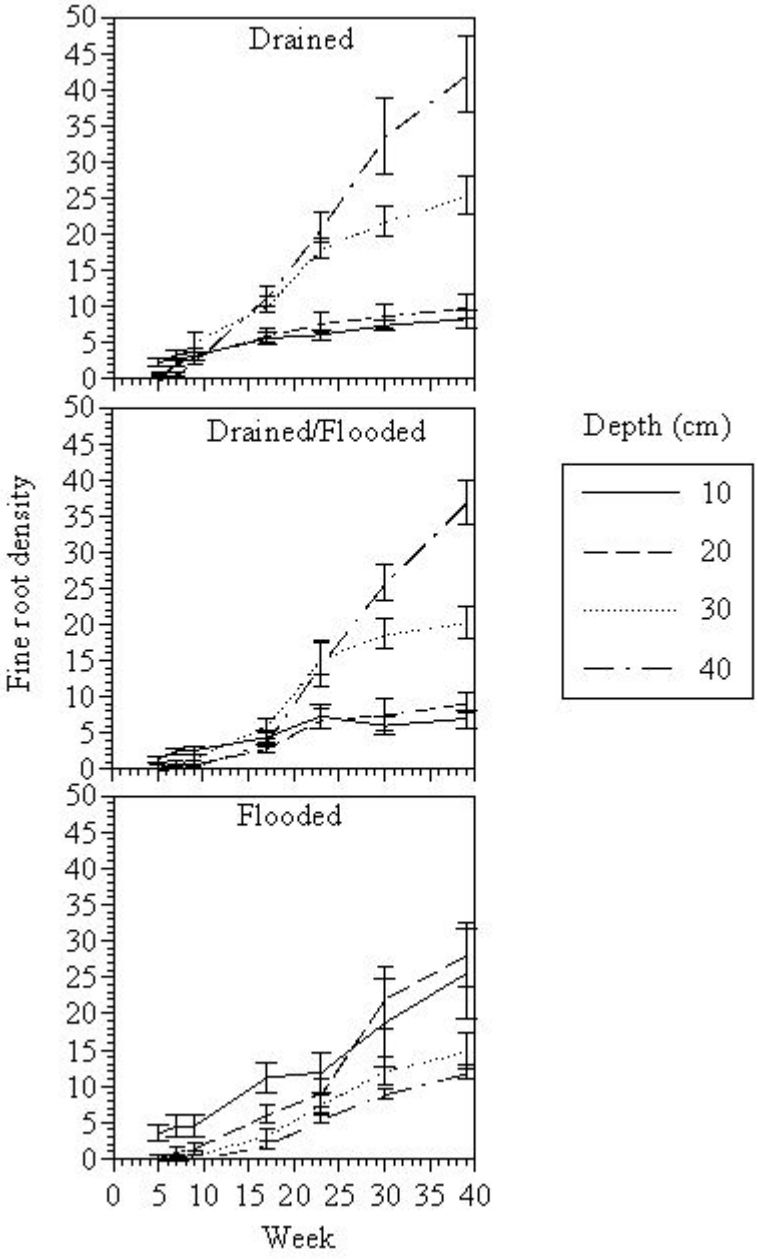

Figure 4.7. Density of fine roots of *A. germinans* by flooding and depth over time. Flooding treatment: drained (D), alternately drained/flooded (D/F), and flooded (F). Bars indicate one standard error. N= 5.

Significant differences in root density of medium roots of *A. germinans* between flooding regimes were observed in the bottom layers (depths 30 and 40 cm), where root density was significantly higher under drained conditions at low P concentration (Fig. 4.8, Table 4.6). Abundance of these roots decreased significantly with depth under drained/flooded conditions at low P concentration and under flooded conditions at both P levels. Significant differences between P treatments were observed at the deepest layer of the rhizotron: under drained/flooded conditions, root density of medium size roots of *A. germinans* was highest under high P concentration, and lowest under low P concentration (significant three-way interaction between phosphorus, flooding, and depth, Table 4.6).

There were significant differences in medium root density between flooding regimes (Fig. 4.9, Table 4.6). For seedlings growing under flooded conditions for 9 wk, root density was still near zero at depth (30 and 40 cm), while roots were already visible in other flooding treatments and depths. By 23 wk, root density was still significantly lower under flooded conditions at the deepest layer (40 cm). However, by the end of the experiment, no differences in medium root biomass of this species were found between flooding regimes over depth.

Significant differences in medium size root density between depths were observed. Under drained conditions, medium root density was higher at surface layers of the rhizotron early in the experiment (7 wk). Subsequently, no differences in density by depth occurred until the end of the experiment, when it was significantly higher at deep layers of the drained rhizotons. Initially, medium size roots appeared only at the surface (10 cm depth) of rhizotrons under drained/flooded treatment. Two weeks later, similar root density was observed up to 20 cm depth. By nine weeks, roots were evenly spread with depth, and

root density continued to increase at the surface layers up to 17 wk. At depth (30 and 40 cm), density increased linearly ultimately reaching twice that at the surface. Under flooded conditions, root density was lower at depth from 7 to 23 wk, then was similar for all depths (significant three-way interaction between flooding, date, and depth, Table 4.6).

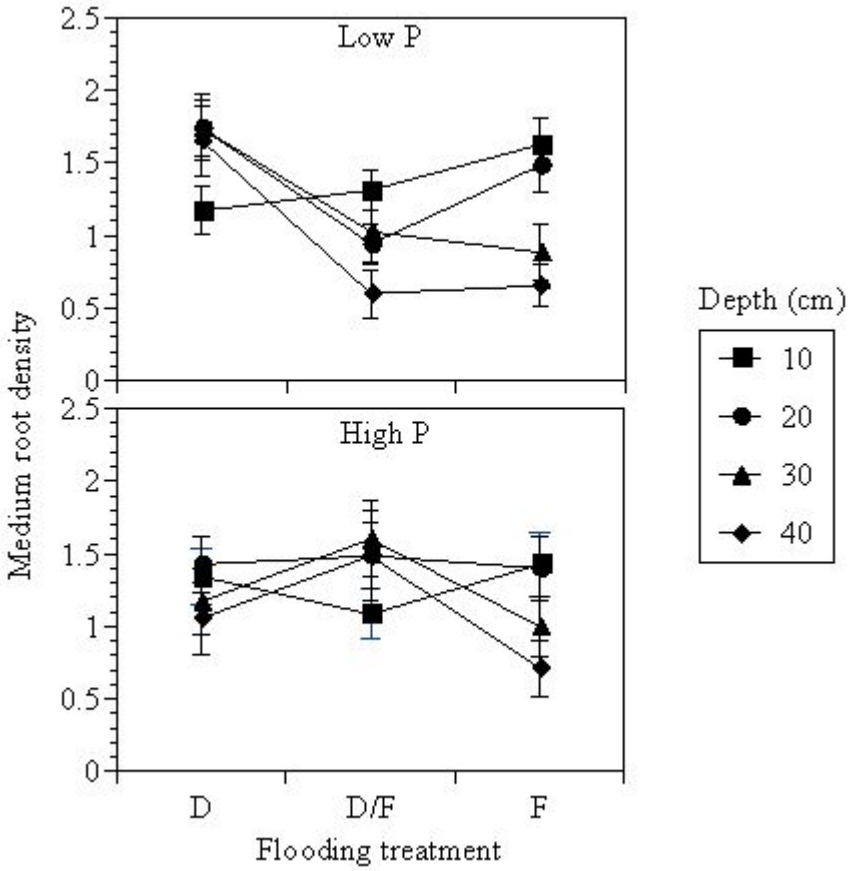

Figure 4.8. Density of medium size roots of *A. germinans* by phosphorus, flooding, and depth. Phosphorus concentration: Low P and High P. Flooding treatment: drained (D), alternately drained/flooded (D/F), and flooded (F). Bars indicate one standard error. N= 5.

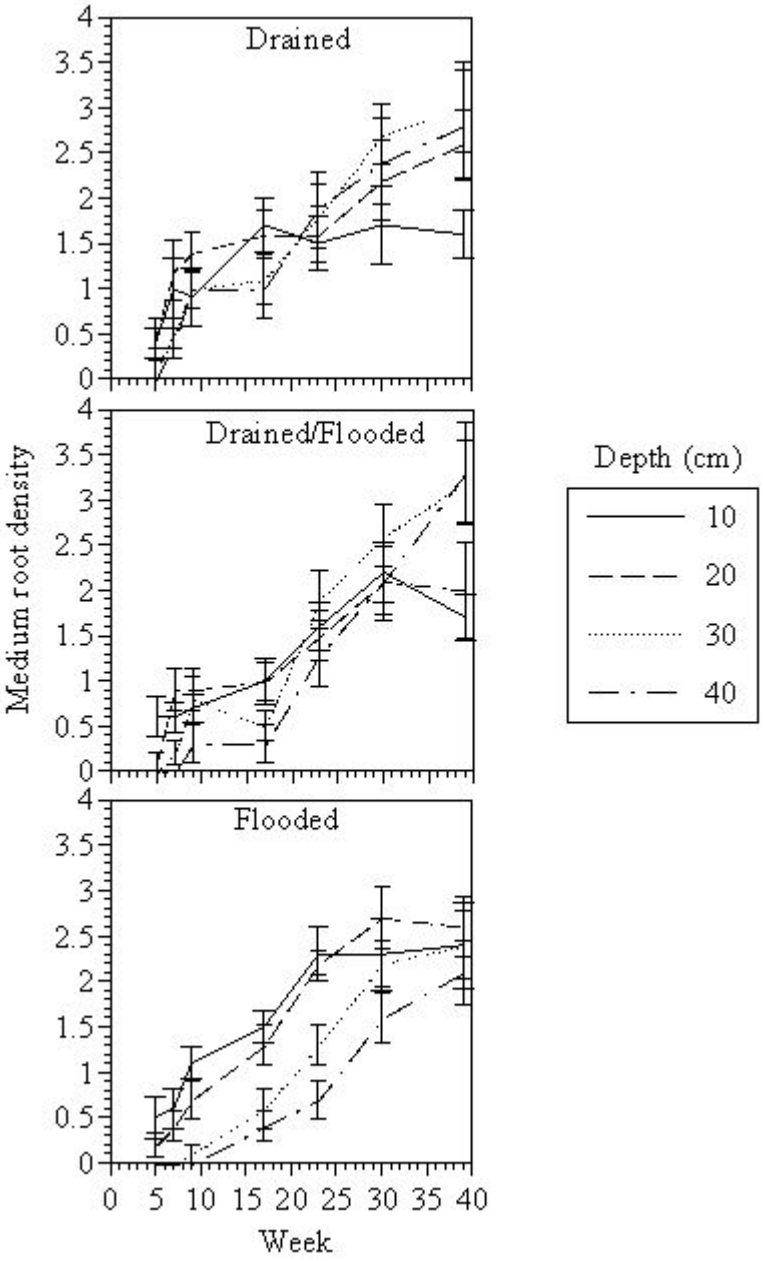

Figure 4.9. Density of medium size roots of *A. germinans* by flooding and depth over time. Bars indicate one standard error. N= 5.

Root density of coarse roots was significantly higher under drained conditions, intermediate under drained/flooded conditions, and lowest under flooded conditions (Fig. 4.10, Table 4.6). Density increased significantly over time (Fig. 4.11, Table 4.6) and also

was significantly higher at the surface (depths 10 and 20 cm) (Fig. 4.12, Table 4.6). Changes in density of fine, medium, and coarse roots of *A. germinans* by treatment combination (different levels of phosphorus and flooding regimes) and depth for are shown in Table 4.7.

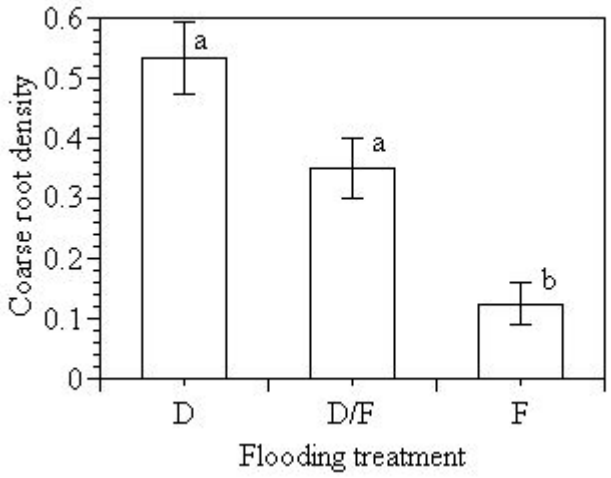

Figure 4.10. Density of coarse roots of *A. germinans* by flooding treatment. D: drained, D/F: alternatively drained and flooded, F: flooded. A posteriori pairwise comparisons (Least Squares Means) represented by letters ($P \leq 0.05$). Bars indicate one standard error. N=10.

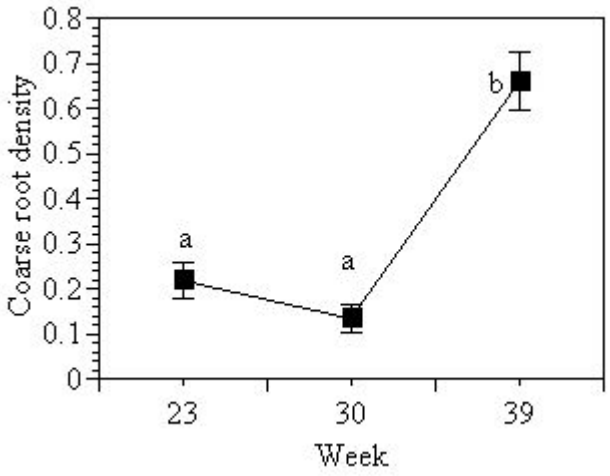

Figure 4.11. Density of coarse roots of *A. germinans* over time. A posteriori pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). Bars indicate one standard error. N=30.

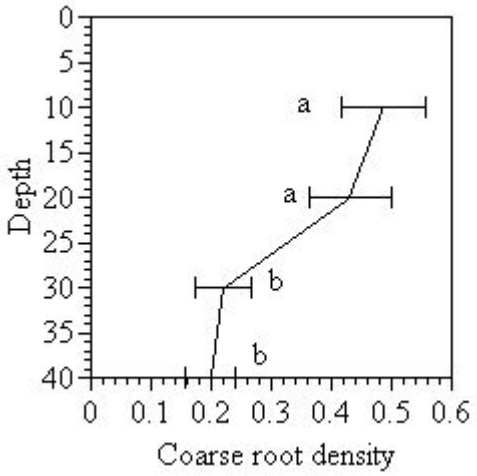

Figure 4.12. Density of coarse roots of *A. germinans* by depth. A posteriori pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). Bars indicate one standard error. N=30.

Table 4.7. Rates of change in *A. germinans* root density (# roots visible on viewing face of rhizotron at each depth) by treatment for fine, medium, and coarse roots. Data are average of the slopes of lines fitted to the data. Values in bold represent means by group. N=5.

Phosphorus	Flooding	Depth	Fine (#roots wk^{-1})	Medium (#roots wk^{-1})	Coarse (#roots wk^{-1})
Low	Drained	10	0.20	0.04	0.02
		20	0.20	0.06	0.03
		30	0.78	0.09	0.01
		40	1.52	0.09	0.01
			0.68	**0.07**	**0.02**
	Drained/Flooded	10	0.12	0.05	0.03
		20	0.16	0.04	0.02
		30	0.69	0.07	0.01
		40	1.03	0.06	0.01
			0.50	**0.06**	**0.02**
	Flooded	10	0.63	0.06	0.01
		20	0.73	0.08	0.01
		30	0.54	0.07	0.01
		40	0.32	0.06	0.01
			0.55	**0.07**	**0.01**
			0.58	**0.06**	**0.02**
High	Drained	10	0.17	0.04	0.03
		20	0.38	0.06	0.03
		30	0.76	0.08	0.01
		40	0.97	0.07	0.02
			0.57	**0.06**	**0.02**
	Drained/Flooded	10	0.27	0.05	0.03
		20	0.40	0.08	0.02
		30	0.63	0.11	0.00
		40	1.03	0.12	0.00
			0.58	**0.09**	**0.01**
	Flooded	10	0.68	0.07	0.01
		20	0.85	0.09	0.00
		30	0.36	0.08	0.00
		40	0.37	0.06	0.00
			0.56	**0.08**	**0.00**
			0.57	**0.08**	**0.01**

4.3.1.3. Root Morphology

Total root length was significantly lower at the bottom of the rhizotron under flooded conditions, but there were no significant differences in root length between the top and bottom layers under drained conditions (Fig. 4.13, Table 4.8). The number of root branches was significantly higher at the top of the rhizotrons under flooded conditions, but did not differ significantly between the top and bottom layers under drained conditions. The number of root tips was significantly lower at the bottom of the rhizotrons under flooded conditions. Root surface area was significantly lower at the bottom layer of the rhizotrons under flooded conditions. The number of root branches was the only morphological trait that responded to the phosphorus treatments (Fig. 4.14, Table 4.8).

Table 4.8. Results of the MANOVA analysis for root morphology of *A. germinans* (root length, root branching, # tips, root diameter, and root surface area) in response to phosphorus and flooding treatments by depth. Significance: * P≤0.1, *** P≤0.001.

Source	F Ratio	P Value
Phosphorus	2.22	0.0586*
Flooding	21.26	<0.0001***
Phosphorus*Flooding	0.73	0.6047
Depth	9.18	<0.0001***
Phosphorus*Depth	0.94	0.4588
Flooding*Depth	5.03	0.0004***
Phosphorus*Flooding*Depth	0.79	0.5593

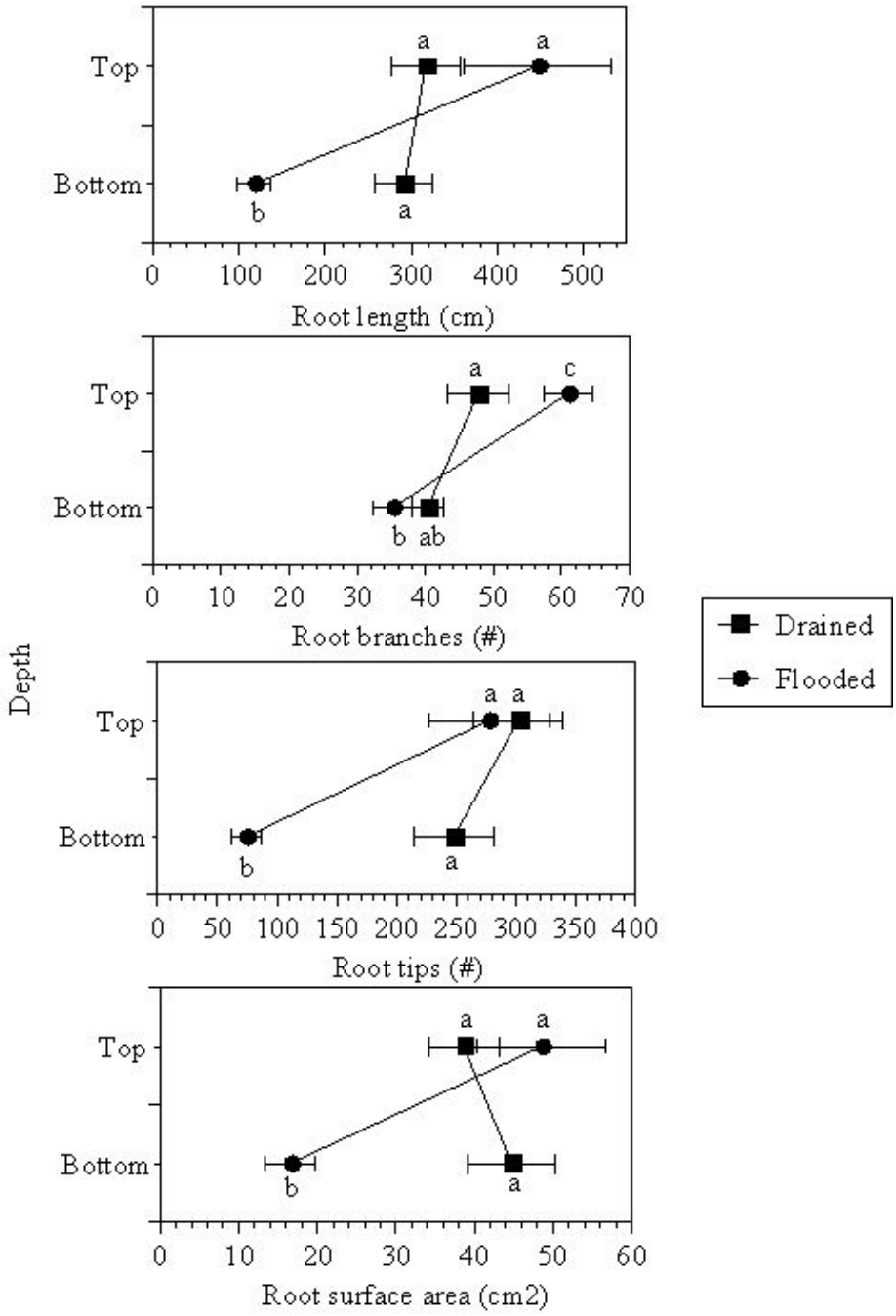

Figure 4.13. Root length, root branching, # root tips, and root surface area of *A. germinans* by flooding and depth. A) Under drained conditions, B) under flooded conditions. A posteriori pairwise comparisons (Least Squares Means) represented by letters ($P \leq 0.05$). Bars indicate one standard error. N=10.

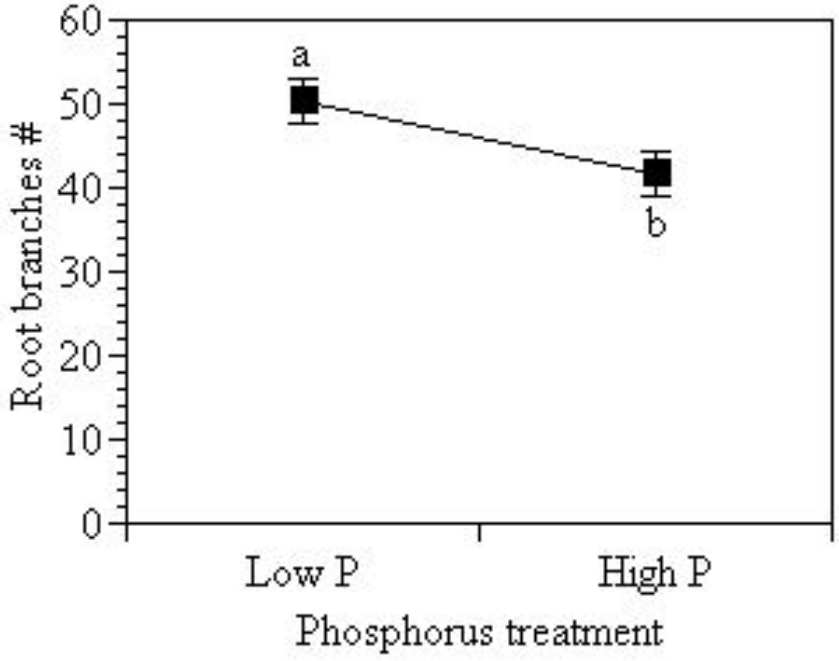

Figure 4.14. Root branching of *A. germinans* given by the number of forks. Data averaged over flooding treatments. A posteriori pairwise comparisons (Least Squares Means) represented by letters ($P \leq 0.05$). Bars indicate one standard error. N=15.

Specific root length (SRL) was higher for fine roots than medium-coarse roots averaging 4.7and 0.1 cm mg^{-1}, respectively. Under drained conditions, SRL of fine roots was higher at the bottom (6.3±0.9) than top (2.9±0.5) layers, and under flooded conditions, it was higher at the top (7.1±0.2) than at the bottom (2.3±0.9) (significant two-way interaction between flooding and depth, $F=23.27$, $P<0.0001$). In contrast, SRL for medium size roots did not differ between treatments or depth (Table 4.9).

Table 4.9. Specific root length (SRL cm mg^{-1}) for fine and medium size roots of *A. germinans* (mean ± SE).

Phosphorus concentration	Flooding	Depth	Fine Roots	Medium and coarse roots
Low	Drained	Top	6.0±1.7	0.3±0.1
		Bottom	2.6±1.0	0.2±0.1
	Flooded	Top	1.2±0.2	0.2±0.0
		Bottom	6.8±2.6	0.3±0.1
High	Drained	Top	6.6±1.1	0.4±0.1
		Bottom	2.7±0.4	0.2±0.1
	Flooded	Top	3.7±1.8	0.3±0.1
		Bottom	7.6±2.7	0.2±0.1

4.3.1.4. Biomass Responses

Root relative growth rate (RRGR), total biomass, and aboveground biomass were similar under drained and drained/flooded conditions, but significantly lower under flooded conditions. Belowground biomass was highest under drained/flooded conditions, intermediate under drained conditions, and lowest under flooded conditions (Fig. 4.15, Table 4.10). Flooding also significantly affected biomass allocated to roots (RBR) and the root:shoot ratio (R/S): 36, 42, and 49% RBR, and 0.57, 0.76, and 0.97 R/S, respectively. RBR and R/S were the only biomass-related traits that significantly responded to phosphorus treatment (Table 4.10). Low availability of P resulted in an increased allocation of biomass to roots (45%) compared to high P (40%). Similarly, root:shoot ratio was significantly higher under low P concentration (0.84) compared to high P concentration (0.69).

Table 4.10. Results of the MANOVA analysis for root final traits of *A. germinans* (above and below-ground biomass, total biomass, RRGR, RBR, root:shoot) in response to phosphorus and flooding treatments. Significance: * P≤0.05, *** P≤0.001.

Source	F Ratio	P Value
Phosphorus	3.09	0.0278*
Flooding	6.96	<0.0001***
Phosphorus*Flooding	1.03	0.4463

A posteriori pairwise comparisons based on LSMeans are explained in the text, P≤0.05.

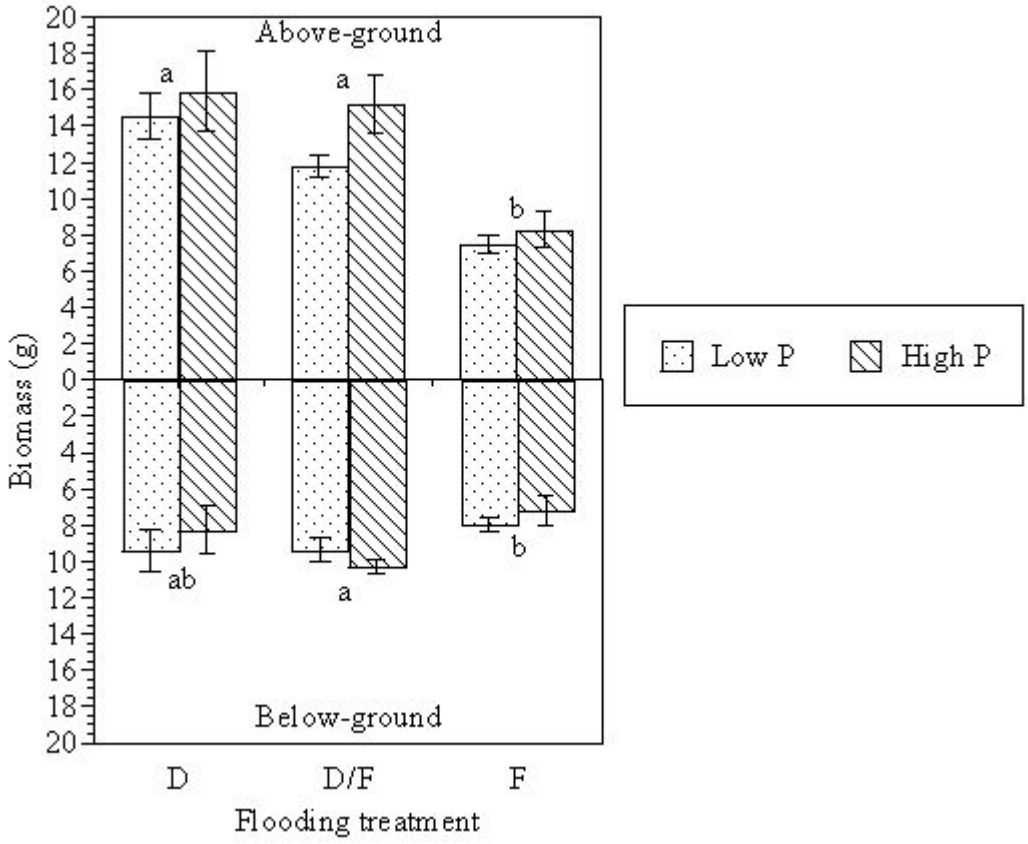

Figure 4.15. Biomass production of *A. germinans* showing aboveground and belowground partitioning. Treatments: Phosphorus: L:Low, and H:high; and flooding: D: drained, D/F: alternately drained and flooded, and F: flooded. Posterior pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). Bars indicate one standard error. N=5.

The percent of total root biomass of *A. germinans* by class size was 20, 25, 55 % for fine, medium, and coarse roots, respectively. Flooding effects differed by root size class (Table 4.11). Biomass of fine and medium roots was significantly higher under drained/flooded conditions, intermediate under drained conditions, and lowest under flooding conditions. In contrast, biomass of coarse roots did not vary between flooding treatments (Fig. 4.16). Biomass of fine, medium, and coarse roots differed in the upper soil layers, but were similar in the lower half of the rhizotrons (Fig. 4.17).

Table 4.11. Results of the MANOVA analysis for root biomass of *A. germinans* by class size (fine, medium, and coarse roots) in response to phosphorus and flooding treatments by depth. Significance: * P≤0.05, *** P≤0.001.

Source	F Ratio	P Value
Phosphorus	0.61	0.6111
Flooding	2.80	0.0151*
Phosphorus*Flooding	0.93	0.4792
Depth	29.86	<0.0001***
Phosphorus*Depth	1.60	0.2021
Flooding*Depth	1.78	0.1122
Phosphorus*Flooding*Depth	0.45	0.8400

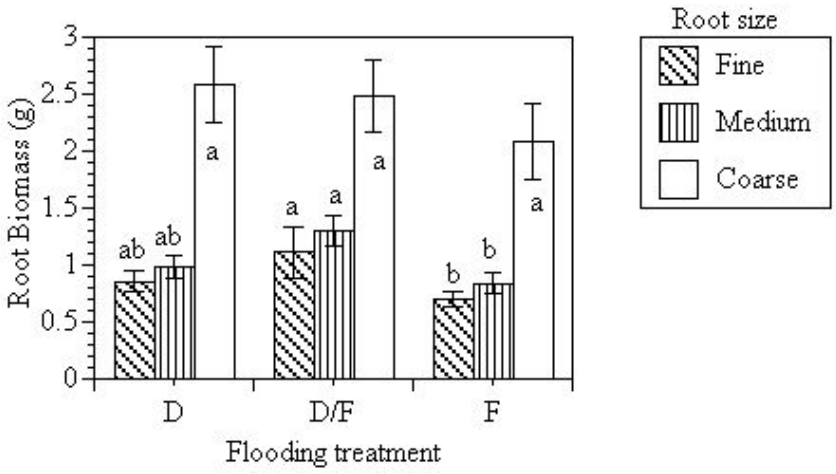

Figure 4.16. Root biomass (g) of *A. germinans* by root size under different flooding treatments, D: drained, D/F: alternately drained and flooded, F: flooded. A posteriori pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). Bars indicate one standard error. N=5.

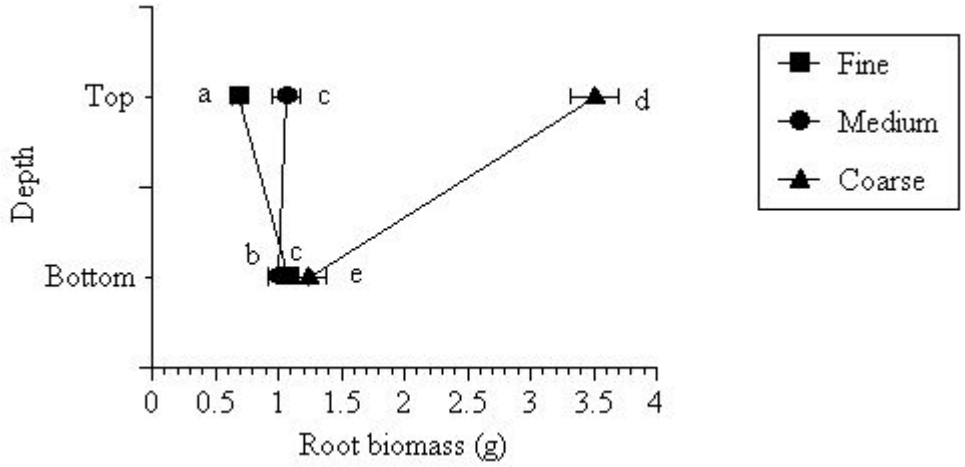

Figure 4.17. Biomass (g) of fine, medium, and coarse roots of *A. germinans* by depth. A posteriori pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). A posteriori pairwise comparisons (Least Squares Means) represented by letters within each size class (P≤0.05). Bars indicate one standard error. N=5.

Density, length, and biomass of pneumatophores increased significantly with increased flooding (Fig. 4.18, Table 4.12).

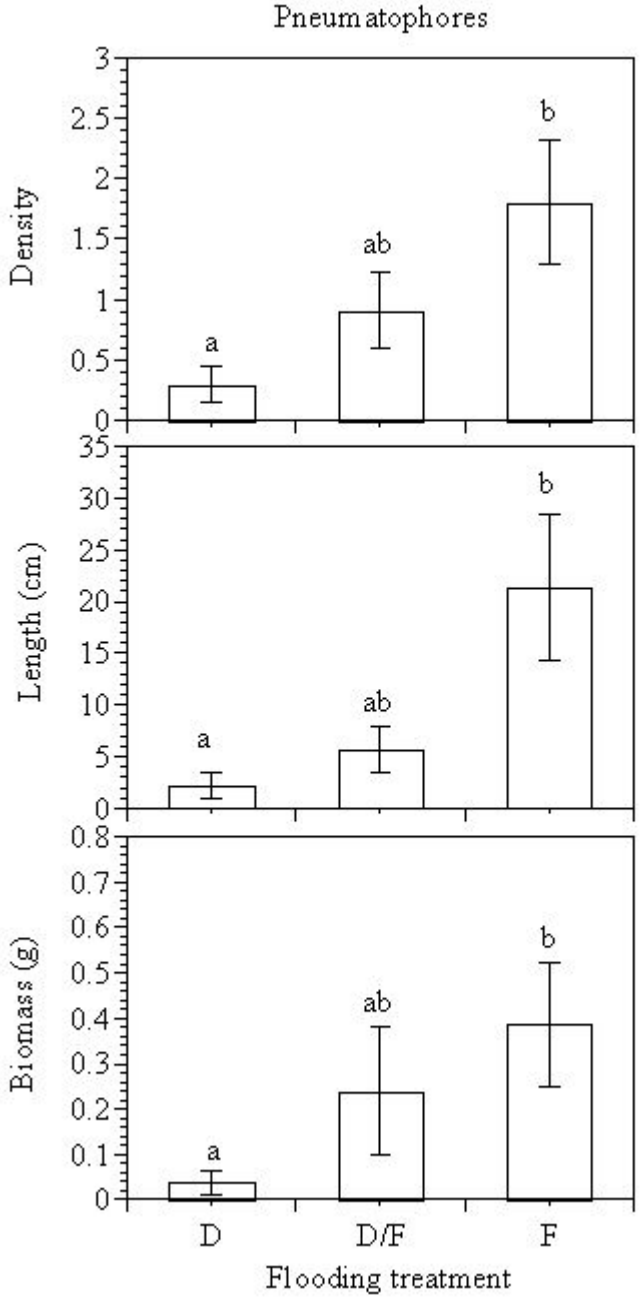

Figure 4.18. Density, length, and biomass of pneumatophores of *A. germinans* under different flooding treatments: D: drained, D/F: alternately drained and flooded, and F: flooded. A posteriori pairwise comparisons (Least Squares Means) represented by letters ($P \leq 0.05$). Bars indicate one standard error. N=5.

Table 4.12. Results of the ANOVA analysis for pneumatophores of *A. germinans* in response to phosphorus and flooding treatments. Table shows F Ratio * P≤0.05.

	Pneumatophores		
Source	Length	Density	Biomass
Phosphorus	4.29 NS	1.02 NS	1.16 NS
Flooding	4.77 *	4.35*	4.01 *
Phosphorus*Flooding	0.85 NS	0.29 NS	0.24 NS

4.3.2. *Rhizophora mangle*

4.3.2.1. Abiotic Variables

Effects of flooding and nutrient treatments on selected soil variables are summarized in Table 4.13. There were significantly higher concentrations for both soluble and extractable P in the high P treatment. Salinity was maintained between 19-25 ‰ in all rhizotrons, although slightly higher in drained versus flooded treatments. Porewater pH was not significantly different between flooding and phosphorus treatments. Soil redox potential was similar between the drained/flooded and the drained treatments and indicated oxidized to slightly reducing conditions. Redox potentials were lower under the flooding treatment, indicating moderately reduced conditions.

4.3.2.2. Root Elongation and Distribution

Root elongation of *R. mangle* anchor roots during 56 wk of the experiment did not differ significantly between phosphorus or flooding treatments. The roots of this species grew linearly up to week 32 until reaching the bottom of the rhizotron (Fig. 4.19, Table 4.14). Linear equations of root growth under the different flooding regimes were calculated with data for the first 32 weeks (Table 4.15).

Table 4.13. Growth conditions for the greenhouse experiment with *R. mangle.* Values are the mean ± SE.

Variable	Drained		Drained/Flooded		Flooded	
	Low P	High P	Low P	High P	Low P	High P
Dissolved phosphate (Water P) μg ml^{-1} PO_4-P	1.3±0.4	49.8±5.4	1.7±0.4	45.5±4.9	0.7±0.1	35.9±4.0
Extractable phosphate (Soil P) μg ml^{-1} PO_4-P	0.4±0.1	9.1±1.3	0.3±0.0	3.5±0.4	0.3±0.1	4.3±0.9
Redox Potential (Eh, mV) 0-5 cm depth	484±22	404±23	466±22	419±24	209±16	200±23
Salinity ‰	24±1	25±1	20±1	24±4	20±1	19±1
pH	3.1±0.1	3.1±0.1	3.2±0.0	3.1±0.0	3.3±0.1	3.3±0.1

Table 4.14. Results of the repeated measures ANOVA for anchor root growth of *R. mangle* in response to phosphorus and flooding treatments over time. Significance: * P≤0.05, ** P≤0.01, *** P≤0.001.

Source	F Ratio	P Value
Phosphorus	0.02	0.8962
Flooding	0.78	0.4684
Phosphorus *Flooding	0.43	0.6572
Week	31.43	<0.0001***
Phosphorus *week	0.47	0.8538
Flooding*Week	0.92	0.5355
Phosphorus*Flooding*Week	2.13	0.0128***

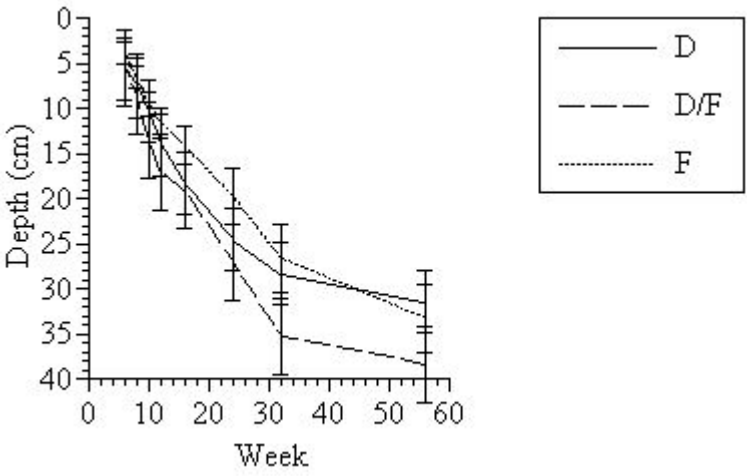

Figure 4.19. Anchor root growth of *R. mangle* over time in response to flooding treatments: drained (D), alternately drained and flooded (D/F), flooded (F). Bars indicate one standard error. N=10.

Table 4.15. Linear equation of root growth of *R. mangle* under different regimes of hydrology. The curve was fitted for the root growth observed during up to week 32 of the experiment.

Flooding	Root growth linear equation Y=mX±b	R^2
Drained	y = 0.8944x + 1.7627	0.9598
Drained/Flooded	y = 1.0843x + 1.3223	0.9725
Flooded	y = 0.8301x + 0.3736	0.9856

Cumulative root density over time was highest for fine roots, and medium and coarse roots showed similar densities (Figures 4.20, 4.21, and 4.22, respectively). Flooded and drained/flooded conditions were related to highest fine root density of *R. mangle* (Fig. 4.23, Table 4.16). At week 24 of the experiment, density was highest under flooded conditions, and at week 33, it was highest under both flooded and drained/flooded

conditions (significant two-way interaction between flooding and time, Table 4.16). Fine root density increased at depth over time (up to 35 cm) (Fig. 4.24, Table 4.16). At 45 cm depth, however, there were not significant changes of fine root density over time (significant two-way interaction between time and depth, Table 4.16).

Density of medium roots of *R. mangle* varied with depth between flooding regimes over time (significant three-way interaction between flooding, time, and depth, Table 4.16, Figure 25). In general, density was higher under drained/flooded conditions at 5 cm depth (except at week 24), and also at deeper layers (25 and 35 cm) by the end of the experiment. In contrast, the density of these roots was constant over time at the surface (0 cm depth) under drained and drained/ flooded conditions; at 5 cm depth, under flooded conditions; and at the bottom (45 cm depth) under all flooding conditions (Fig. 4.25). Coarse roots of *R. mangle* were more visible over time as roots began growing and concentrating at shallow soil depths. At low phosphorus concentration, root density of coarse roots of *R. mangle* increased the most in the upper soil layers (from 0 to 25 cm) mainly under drained/flooded conditions. At high phosphorus concentration, root density of coarse roots of this species increased under flooding conditions from depths 15 to 35 cm and under drained conditions at 15 and 25 cm depth (significant four-way interaction between phosphorus, flooding, time, and depth Table 4.16, Fig. 4.26).

Root density rate (RDR = rate of appearance) of fine, medium, and coarse roots of *R. mangle* by treatment combination and depth are shown in Table 4.17. RDR of coarse roots growing under low P concentrations was highest under drained/flooded conditions, intermediate under flooded conditions, and lowest under drained conditions. RDR of coarse roots growing under high P concentrations increased with increased flooding

(significant two-way interaction between phosphorus and flooding, F=5.12, P<0.0001).

Additionally, RDR for all root sizes decreased with depth (F=13.94, P<0.0001).

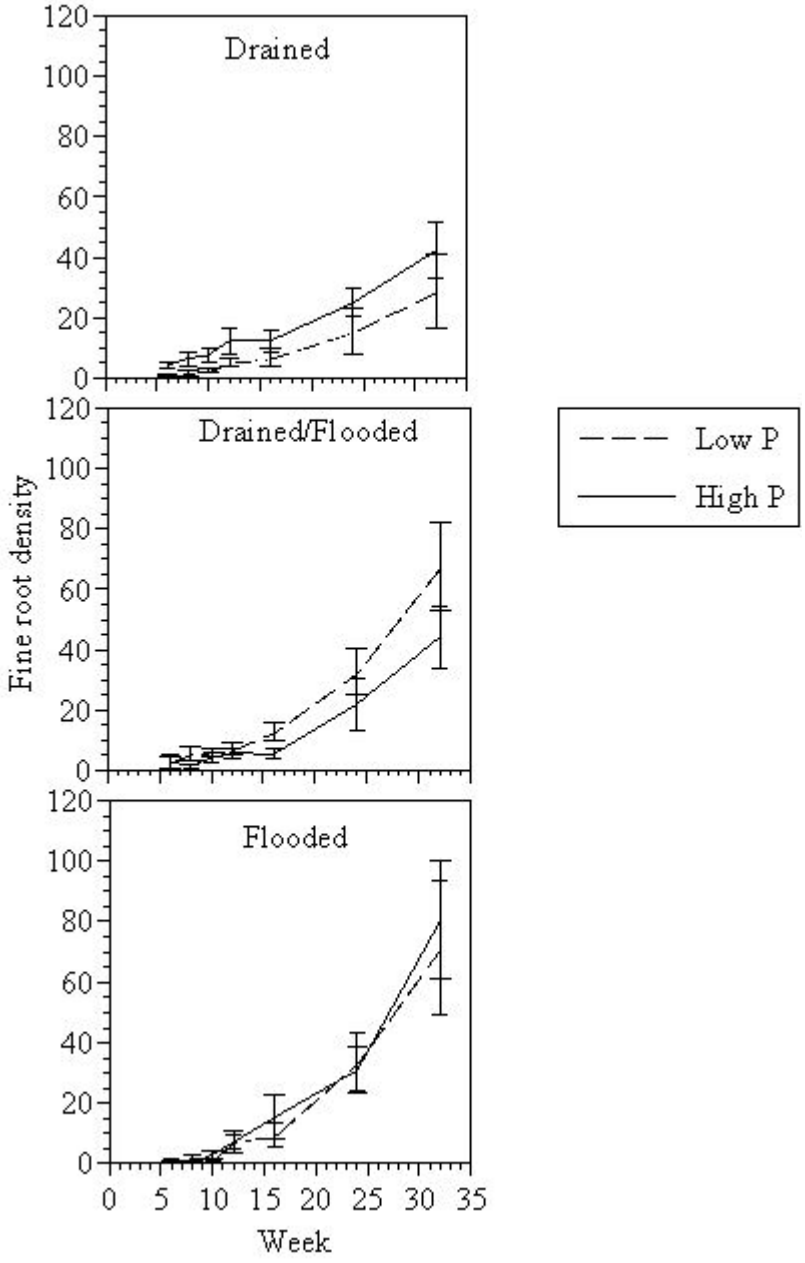

Figure 4.20. Temporal variation of fine root density of *R. mangle* under the experimental treatment combinations. Phosphorus concentration: Low P and High P, and flooding treatment: drained, alternately drained, and flooded. Bars indicate one standard error. N=5.

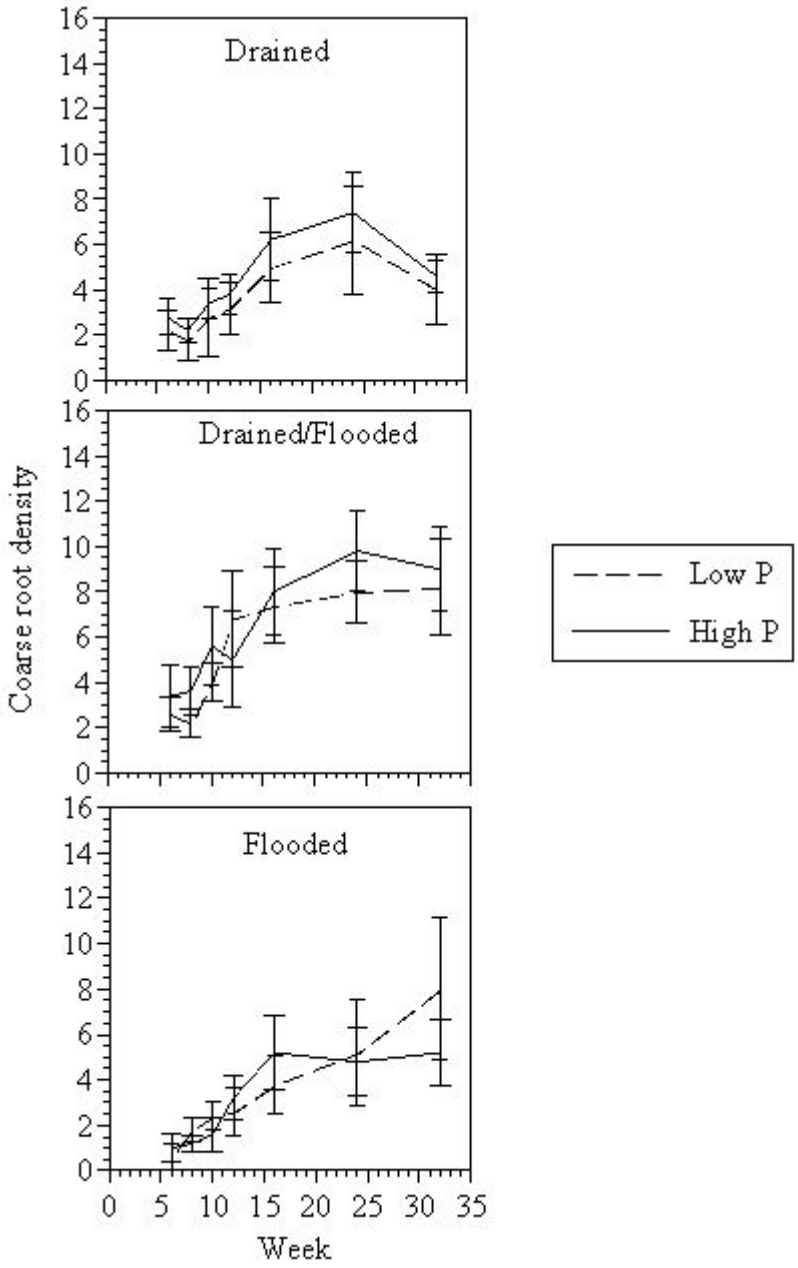

Figure 4.21. Temporal variation of medium root density of *R. mangle* under the experimental treatment combinations. Phosphorus concentration: Low P and High P, and flooding treatment: drained, alternately drained, and flooded. Bars indicate one standard error. N=5.

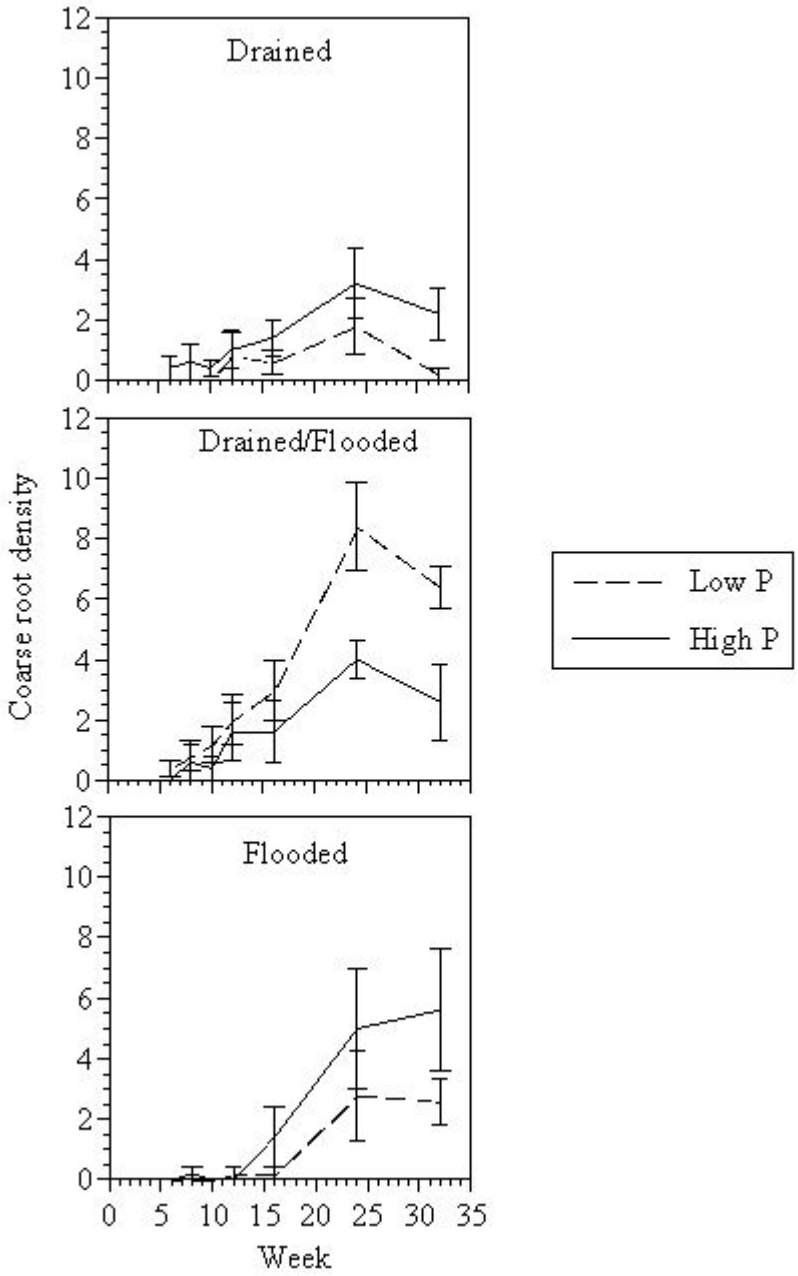

Figure 4.22. Temporal variation of coarse root density of *R. mangle* under the experimental treatment combinations. Phosphorus concentration: Low P and High P, and flooding treatment: drained, alternately drained, and flooded. Bars indicate one standard error. N=5.

Table 4.16. Results of a repeated measures ANOVA for root density of *R. mangle* for each root size class in response to phosphorus and flooding treatments and depth over time. The table provides F-ratios and their significance is indicated as: * P≤0.05, ** P≤0.01, ***P≤0.001.

Source	Fine	Medium	Coarse
Phosphorus	0.12	0.09	0.03
Flooding	0.38	3.22	4.77*
Phosphorus*Flooding	1.54	0.28	4.46*
Date	102.55***	22.19***	28.74***
Phosphorus*Week	0.09	0.11	0.23
Flooding*Week	1.96*	1.01	2.51*
Phosphorus*Flooding*Week	0.86	0.86	1.72
Depth	42.14***	81.97***	37.68***
Phosphorus*Depth	0.33	0.41	0.06
Flooding*Depth	0.41	1.03	3.44***
Phosphorus*Flooding*Depth	0.57	0.53	1.86
Date*Depth	33.23***	16.57***	19.06***
Phosphorus* Week*Depth	0.35	1.28	0.56
Flooding* Week*Depth	1.39	1.74**	3.00***
Phosphorus*Flooding*Week*Depth	0.74	1.21	1.95***

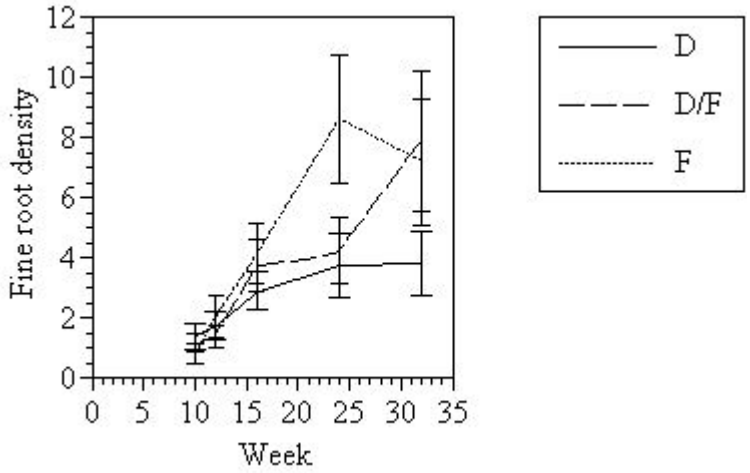

Figure 4.23. Density of fine roots of *R. mangle* by flooding treatment over time. drained (D), alternately drained and flooded (D/F), flooded (F). Bars indicate one standard error. N=10.

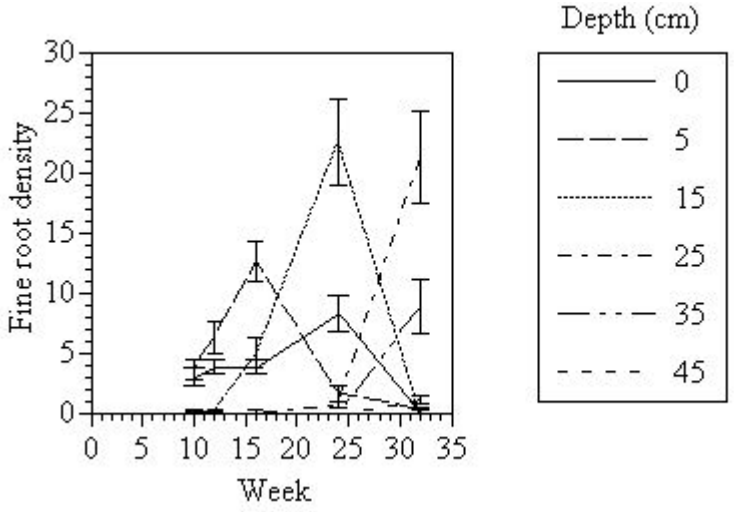

Figure 4.24. Fine root density of *R. mangle* by depth over time. Bars indicate one standard error. N=30.

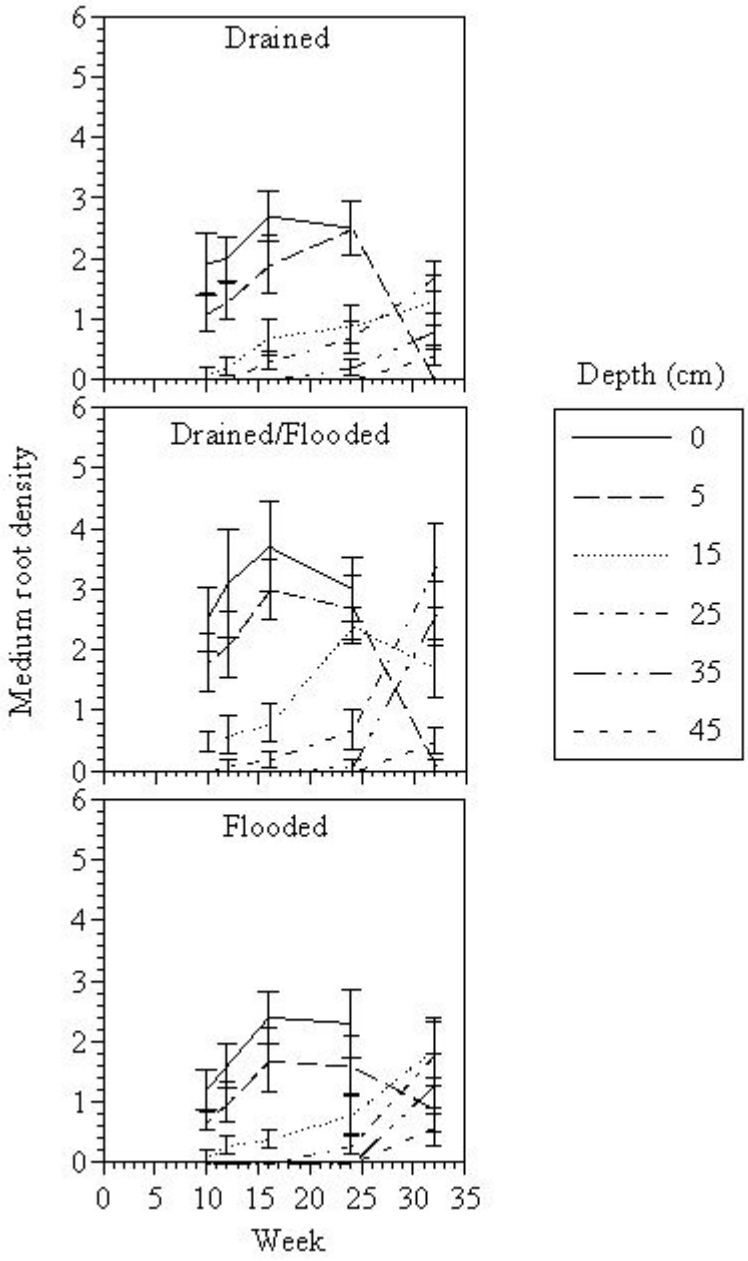

Figure 4.25. Density of medium size roots of *R. mangle* by flooding and depth over time. At 0, 5, and 15 cm depth. Flooding treatment, D: drained, D/F: alternately drained and flooded, F: flooded. Bars indicate one standard error. N= 5.

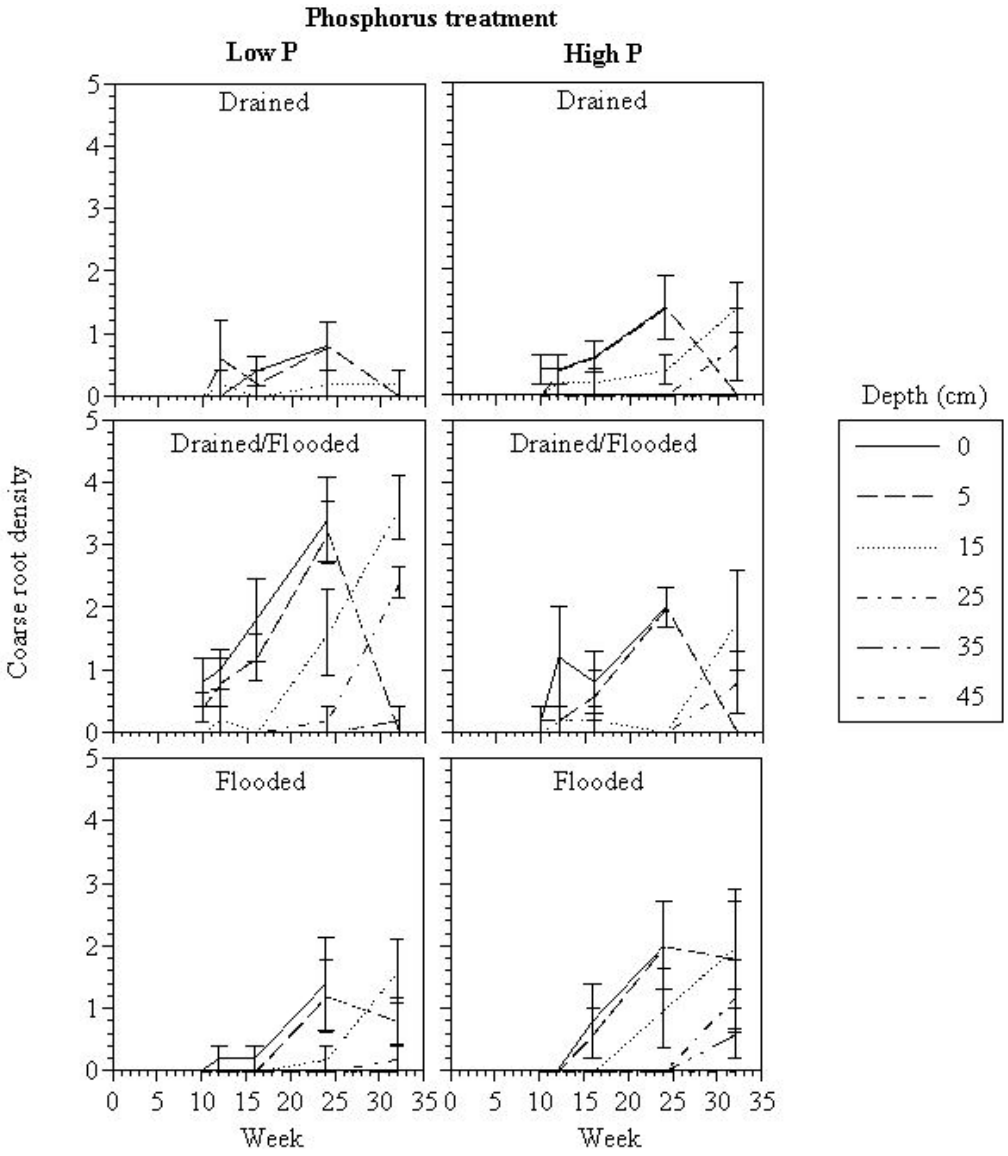

Figure 4.26. Density of coarse roots of *R, mangle* under low P concentration by flooding and depth over time. Depths: 0, 5, and 15 cm (A, B, and C, respectively). Flooding treatment, D: drained, D/F: alternately drained and flooded, F: flooded. Bars indicate one standard error. N= 5.

Table 4.17. Rates of change in *R. mangle* root density (# roots visible on viewing face of rhizotron at each depth) by treatment for fine, medium, and coarse roots. Data are average of the slopes of lines fitted to the data. Values in bold represent means by group. N=5.

Phosphorus	Flooding	Depth	Fine (#roots wk^{-1})	Medium (#roots wk^{-1})	Coarse (#roots wk^{-1})
Low	Drained	0	0.13	0.06	0.02
		5	0.12	0.06	0.02
		15	0.23	0.04	0.01
		25	0.21	0.04	0.00
		35	0.05	0.02	0.00
		45	0.02	0.01	0.00
			0.13	**0.04**	**0.01**
	Drained/Flooded	0	0.20	0.10	0.06
		5	0.22	0.08	0.06
		15	0.29	0.07	0.08
		25	0.63	0.07	0.04
		35	0.16	0.04	0.00
		45	0.05	0.01	0.00
			0.26	**0.06**	**0.04**
	Flooded	0	0.21	0.07	0.03
		5	0.26	0.07	0.04
		15	0.55	0.05	0.03
		25	0.36	0.04	0.00
		35	0.10	0.03	0.00
		45	0.01	0.01	0.00
			0.25	**0.04**	**0.02**
			0.21	**0.05**	**0.02**
High	Drained	0	0.16	0.08	0.03
		5	0.19	0.16	0.03
		15	0.30	0.04	0.03
		25	0.19	0.05	0.01
		35	0.18	0.01	0.00
		45	0.03	0.01	0.00
			0.18	**0.06**	**0.02**
	Drained/Flooded	0	0.13	0.09	0.04
		5	0.12	0.08	0.03
		15	0.26	0.07	0.03
		25	0.34	0.07	0.01
		35	0.18	0.05	0.00
		45	0.00	0.01	0.00
			0.17	**0.06**	**0.02**
	Flooded	0	0.22	0.07	0.04
		5	0.30	0.06	0.06
		15	0.45	0.05	0.05
		25	0.52	0.03	0.02
		35	0.23	0.01	0.01
		45	0.00	0.01	0.00
			0.29	**0.04**	**0.03**
			0.21	**0.05**	**0.02**
Total Mean			**0.21**	**0.05**	**0.02**

4.3.2.3. Root Morphology

Root length and root branching, number of root tips, and root surface area were significantly higher under flooded conditions (Fig. 4.27, Table 4.18) and at the top (surface layer) of the rhizotron (Fig. 4.28, Table 4.18). In contrast, the average diameter of *R. mangle* roots was not affected by phosphorus, flooding treatments, or depth. Phosphorus treatment did not have any effect on the morphology of this species.

Table 4.18. Results of the MANOVA analysis for root morphology of *R. mangle* (root length, root branching, # root tips, root diameter, and root surface area) in response to phosphorus and flooding treatments by depth. Significance: * P≤0.1, *** P≤0.001.

Source	F Ratio	P Value
Phosphorus	0.97	0.4386
Flooding	7.03	<0.0001***
Phosphorus*Flooding	1.67	0.1502
Depth	7.28	<0.0001***
Phosphorus*Depth	0.17	0.9724
Flooding*Depth	0.80	0.5492
Phosphorus*Flooding*Depth	0.66	0.6540

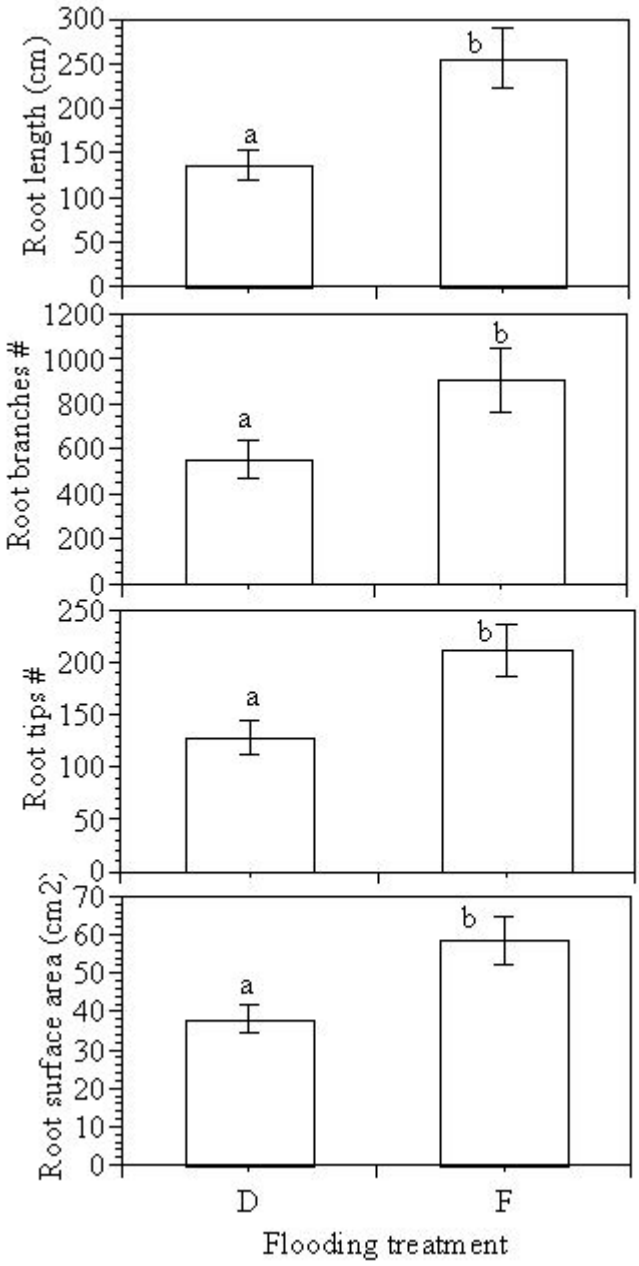

Figure 4.27. Root length, root branching, # root tips, and root surface area of *R. mangle* under drained (D), and flooded (F) conditions. A posteriori pairwise comparisons (Least Squares Means) represented by letters ($P \leq 0.05$). Bars indicate one standard error. N=20.

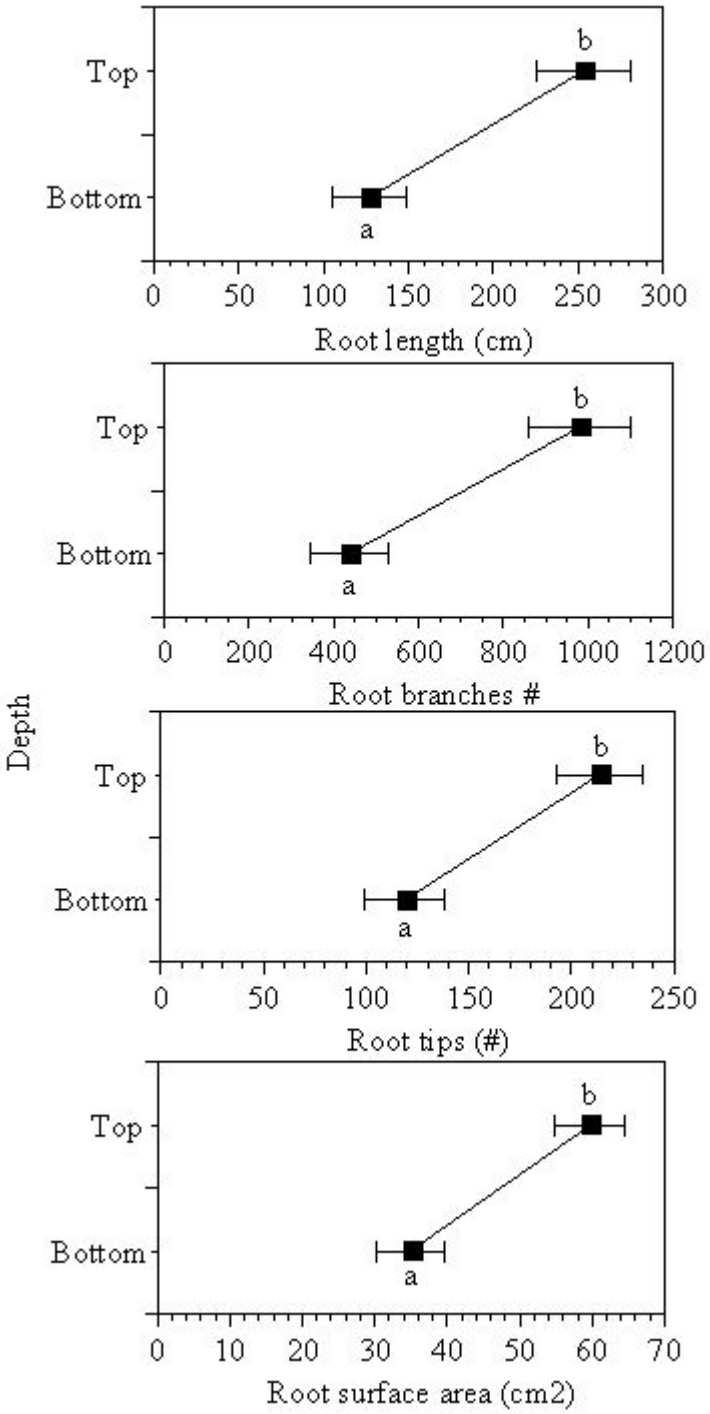

Figure 4.28. Root length, root branching, # root tips, and root surface area of *R. mangle* by depth. A posteriori pairwise comparisons (Least Squares Means) represented by letters ($P \leq 0.05$). Bars indicate one standard error. N=20.

Specific root length (SRL) was higher for fine roots than medium-coarse roots, averaging 3.1 and 0.1 cm mg^{-1}, respectively. However, fine and medium-coarse size roots did not change between phosphorus or flooding treatments, and depth (Table 4.19).

Table 4.19. Specific root length (SRL cm mg^{-1}) for fine and medium size roots of *R. mangle* (mean ± one standard error).

Phosphorus concentration	Flooding	Depth	Fine Roots	Medium and coarse roots
Low	Drained	Top	2.2±0.8	0.2±0.2
		Bottom	4.3±0.2	0.1±0.1
	Flooded	Top	4.0±0.3	0.1±0.1
		Bottom	3.8±0.2	0.1±0.1
High	Drained	Top	1.1±0.3	0.0±0.0
		Bottom	2.9±0.3	0.1±0.0
	Flooded	Top	3.5±0.3	0.1±0.1
		Bottom	1.5±0.3	0.0±0.0

4.3.2.4. Biomass Responses

Total biomass and root relative growth rate (RRGR) were lowest under drained conditions, intermediate under flooded conditions, and highest under drained/flooded conditions (Fig. 4.29, Table 4.20). Aboveground biomass was similar under drained and flooded conditions, and it was significantly highest under drained/flooded conditions. Belowground biomass was significantly lower under drained conditions (Fig. 4.29). Biomass allocation to roots (RBR) was significantly different for all flooding regimes: drained<drained/flooded< flooded treatments, with 28, 37, and 48%, respectively. Root:shoot ratio was higher under flooding conditions (0.96) than under drained (0.39), and drained/flooded (0.58) conditions (Table 4.20).

Table 4.20. Results of the MANOVA analysis for final root traits of *R. mangle* (above- and belowground biomass, total biomass, RRGR, RBR, root:shoot) in response to phosphorus and flooding treatments. Significance: *** P≤0.001. A posteriori pairwise comparisons based on LSMeans are explained in the text, P≤0.05.

Source	F Ratio	P Value
Phosphorus	1.12	0.3983
Flooding	4.95	0.0002***
Phosphorus*Flooding	0.79	0.6569

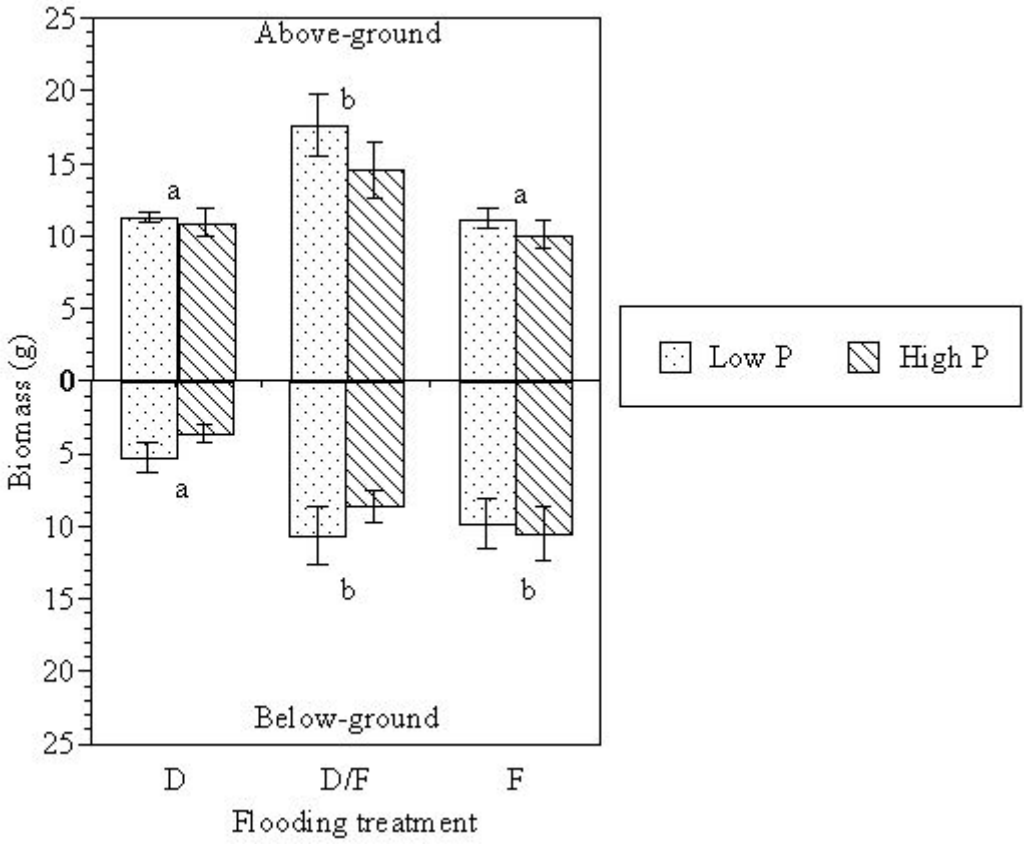

Figure 4.29. Biomass allocation of *R. mangle* showing the aboveground and belowground partitioning, and the total biomass allocation (aboveground+belowground). Treatments: Phosphorus: L:Low, and H:high, and flooding: D: drained, D/F: alternately drained and flooded, and F: flooded. A posteriori pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). Bars indicate one standard error. N=5.

Biomass of fine and medium roots was significantly higher under drained/flooded conditions, intermediate under flooded conditions and lowest under drained conditions. Biomass of coarse roots was similar between drained/flooded and flooded conditions, and lowest under drained conditions (Fig. 4.30, Table 4.21). Root biomass of all root sizes was significantly higher in upper soil layers (Fig. 4.31, Table 4.21). Considering total root biomass of *R. mangle*, the percentage by class size was 23, 29, and 48 % for fine, medium, and coarse roots, respectively.

Table 4.21. Results of the MANOVA analysis for root biomass of *R. mangle* (fine, medium, and coarse roots) in response to phosphorus and flooding treatments by depth. Significance: ** P≤0.01, *** P≤0.001.

Source	F Ratio	P Value
Phosphorus	0.53	0.6672
Flooding	3.30	0.0055**
Phosphorus*Flooding	0.66	0.6824
Depth	17.52	<0.0001***
Phosphorus*Depth	0.70	0.5543
Flooding*Depth	0.53	0.7812
Phosphorus*Flooding*Depth	0.98	0.4464

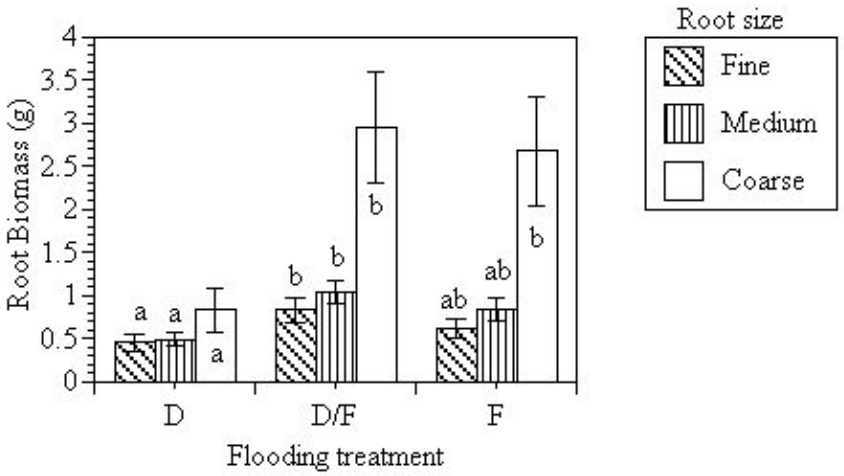

Figure 4.30. Biomass (g) of roots of *R. mangle* by class size under different flooding treatments, D: drained, D/F: alternatively drained and flooded, F: flooded. A posteriori pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). Bars indicate one standard error. N=5.

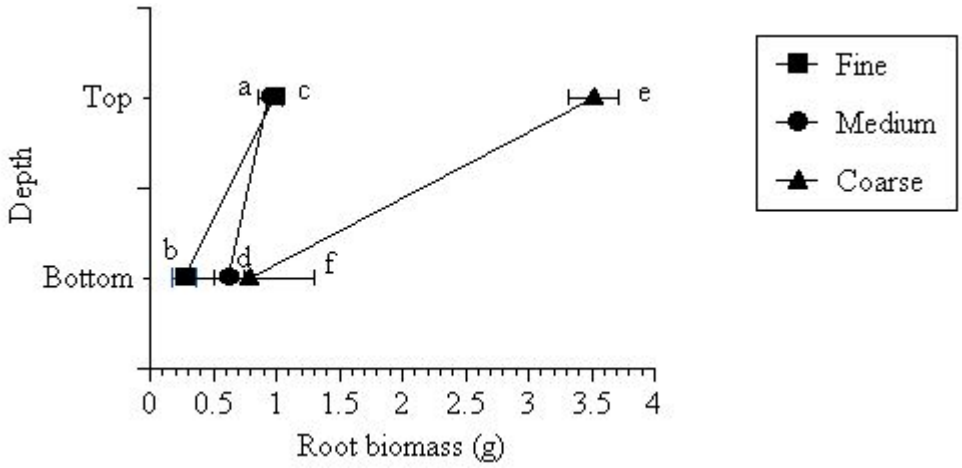

Figure 4.31. Biomass (g) of fine, medium, and coarse roots of *R. mangle* by depth. A posteriori pairwise comparisons (Least Squares Means) represented by letters (P≤0.05). Bars indicate one standard error. N= 5.

4.4. DISCUSSION

4.4.1. Root Elongation

Root elongation rates of *R. mangle* were not affected by flooding treatments, wheras *A. germinans* exhibited slower anchor root growth under flooded conditions. Root extension of the latter species decreased 43% from drained to flooded treatments. This result supports the hypothesis that *A. germinans* is more sensitive to continuous flooding. Differential flood tolerance between seedlings of these two species has been demonstrated with field and greenhouse experiments (McKee 1993, 1995a, 1996). Similarly, McKee (1996) found that hypoxia caused a 38% decrease in the root extension rate of *A. germinans* compared to aerated controls; a similar effect was not observed in *R. mangle*. This differential tolerance to flooding regimes has been considered an important factor contributing to species distribution across the intertidal zone. *Rhizophora mangle* dominates the lower intertidal zone, areas characterized by deeper or continuously waterlogged conditions, whereas *A. germinans* dominates the upper intertidal zone that is less frequently inundated (Odum et al. 1982, McKee 1995a). However, both mangrove species *A. germinans* and *R. mangle* are able to oxidize the surrounding rhizosphere when soil anaerobic conditions exist (McKee et al. 1988). Several studies have observed a decrease in the internal root aeration of *A. germinans* under anaerobic stress (McKee and Mendelssohn 1987, McKee 1993, 1996) that does not occur in *R. mangle*. These observations indicated that although loss of oxygen from roots to surrounding soil may create an oxidized buffer (protecting against toxic reduced compounds such as sulfide), oxygen leakage affects internal aeration of the roots, which would affect energy-requiring processes such as nutrient uptake.

Anchor roots of *A. germinans* and *R. mangle* responded differently to the combination of nutrient and water levels. Anchor roots of *R. mangle* grew slower than *A. germinans*. Anchor roots of *A. germinans* reached depths of 40 cm in ten to fifteen weeks, wheras *R. mangle* roots took over 50 weeks to reach similar depths. These interspecific differences in root growth rates may be related to differential response to nutrient availability or may be an inherent trait. Previous work showed that *R. mangle* exhibits low potential growth rates compared to other species such as *A. germinans* and *L. racemosa* (McKee 1995). However, mangroves may also respond differently to nutrient availability, which reflects their relative growth strategies (e.g., slow-growing vs. fast-growing). A slow-growth strategy would require less nutrients, whereas a fast-growth strategy would increase demand for nutrients. Anchor roots of *A. germinans* responded to the P treatment combinations in interaction with flooding conditions. Anchor roots of this species, elongated the most when P concentration was limited, and soil aeration was not a stress factor (e.g., under drained conditions). This finding further suggests that *A. germinans* and *R. mangle* have different strategies relative to nutrients. Under P stress, *A. germinans* produced a root system that explored the surrounding soil rapidly, but invested more C in roots only when soil aeration was optimal. However, when anaerobic conditions became dominant, anchor roots of this species were distributed at shallow depths and were more limited by low nutrient conditions because of a higher metabolic demand. These interspecific differences in root growth rates and responses to the interaction of different flooding and P regimes may account for differences in root distribution with depth in natural settings.

4.4.2. Root Distribution

Cumulative root density of both *A. germinans* and *R. mangle* was similar and varied by size class. For both species, fine root density was the highest-- exceeding medium root density by ten times; coarse root density was about half that of medium roots. Komiyama et al. (1989) found that in proportion, fine roots of *B. gymnorrhiza* were more abundant than medium and coarse roots (the abundance of medium size and coarse roots was about half). In contrast, *R. stylosa* roots showed higher densities of coarse and medium size roots. However, seedlings (this study) may exhibit different root strategies to that of mature trees (Komiyama et al. 1989). Root proliferation by mature mangroves growing under natural conditions may occur when roots find appropriate conditions, e.g., in nutrient-rich microsites (McKee 2001).

The only clear response of fine root density by *A. germinans* to nutrients was observed under high P treatment at the soil surface (depths 10 and 20 cm). Thus, when aeration conditions are good, this species may use an opportunistic strategy to proliferate in response to high nutrient availability. When this species is under a combination of nutrient and flooding stress, it seems to be advantageous to increase the fine root density in more aerated surface layers to minimize oxygen lost. Under highly reducing conditions given by the combination of flooding at deeper layers of the soil, fine root density is affected the most. On the other hand, flooding and drained/flooding conditions and therefore the aeration status of the soil were related to higher fine root density of *R. mangle* over time up to 35 cm depth. Field observations also indicate high root density in the upper 30-40 cm of soil in mangrove forests (Komiyama et al. 1988, 2000).

Medium roots of *A. germinans* distribute deeper when they are under optimal soil aeration conditions. However, as reducing conditions become stronger with depth especially in the least aerated flooding regimes a significant decrease in medium root density occurs (under flooded conditions at both P levels and drained/flooded conditions at low P concentration). Medium roots responded positively to nutrient treatments at the deepest layer of the rhizotron but only in well aerated (drained/flooded) conditions and no nutrient stress. Under well-aerated soil conditions, medium to coarse roots of *A. germinans* concentrated near the surface initially, but subsequently foraged deeper over time. Under flooded conditions, it took longer for anchor roots of *A. germinans* to increase their density and to explore deeper. Thus soil exploration by *A. germinans*, especially of deeper layers, was limited by flooding in comparison to *R. mangle*. Differential ability to explore the soil for nutrients will obviously affect growth and distribution of these species, as hypothesized by McKee et al. (2002).

4.4.3. Root Morphology

Root morphology of *A. germinans* was also affected by continuous flooding. Under this condition, root length, number of root tips and forks, and root surface area of this species decreased with depth. Effects on morphology of mangrove roots under oxygen stress have been reported in previous studies. McKee (1996) observed a decreased in the number of lateral roots of *A. germinans* and *R. mangle* under anaerobic conditions. In contrast, root morphology of *R. mangle*, root length, number of root tips and forks, and root surface area, were significantly higher under flooded conditions and at shallow depths. Root diameter of both *A. germinans* and *R. mangle* species did not respond to depth or phosphorus or flooding treatments. Similarly, McKee 1996 did not observe any response in

root diameter of these species to anaerobic conditions although there were species differences.

In this study, phosphorus treatment did not have strong effect on the morphology of these species. However, branching of *A. germinans* roots increased in response to low levels of phosphorus. In oligotrophic environments, fine root production, which determines absorbing surface area, is higher for *R. mangle* than *A. germinans* (McKee 2001). Salt marsh studies have shown that flooding limitations on root growth and metabolism impairs plant ability to acquire nutrients (reviewed in Mendelssohn and Morris, 2000). Some work has shown that oxygen stress may decrease the P uptake rates by *Typha domingensis* under high soil oxygen demand (Delaune et al. 1999). Changes in root morphology (e.g., increased branching or allocation to fine versus coarse roots) without a change in root biomass may be a strategy to conserve carbon. Root proliferation in high-nutrient microsites may improve nutrient acquisition, particularly phosphorus (McKee 2001). In general, roots of *A. germinas* showed some morphological plasticity, which would increase this species ability to obtain resources when they are limited.

Specific root length (SRL), which shows root extension per unit biomass, is an indication of how efficient a plant is at acquiring nutrients and water. Fine roots of both *A. germinans* and *R. mangle* had high SRL, but there were interspecific differences in the response to experimental treatments. Although patterns were not completely consistent across treatments, both *A. germinans* and *R. mangle* exhibited changes in SRL in response to flooding. In *A. germinans*, a high production of fine roots at the top of the rhizotron in response to flooding resulted in higher SRL. This suggests that this species may reallocate fine roots at shallow and more aerated layers under stressful anaerobic conditions.

4.4.4. Biomass Production and Allocation

In general, total biomass production and relative growth rate (RGR) of *A. germinans* seedlings were higher than those of *R. mangle*. However, RGR and total biomass production of *A. germinans* were lowest under flooded conditions (fine and medium roots), whereas these rates were lowest under drained conditions for *R. mangle*. The opposite response in RGR and total biomass production between species to hydrology may be explained by the differential tolerance to flooding regimes discussed previously. Also, previous studies have demonstrated that under anaerobic stress *A. germinans* decreases its root biomass (McKee 1993, 1996), and root aeration (McKee and Mendelssohn 1987, McKee 1993, 1996), which does not occur in *R. mangle*.

Root biomass of both species was higher at the top for all root sizes except fine roots of *A. germinans*. Similar observations have been made in natural conditions for *R mangle* (Komiyama et al. 1988). However, Matsui (1998) observed that biomass allocation to roots of *R. stylosa* was 30% average in Japan, and for roots of *Rhizophora and Ceriops* it was 11% average, in Australia. The data obtained in Japan are similar to the mean percentages obtained in this study from 28 to 48% for *R. mangle*, but the data obtained in Australia are low possibly because they calculated biomass proportions based on empirical equations applicable to the conditions in Japan. Ogino and Chihara 1988 found that the biomass allocation to prop roots in a *Rhizophora* zone was 13%, which is low, but they also estimated the biomass of the aerial portion (aboveground) of the entire root system.

Table 4.22. Root/shoot and shoot/root ratios for mangrove forests from different studies around the world.

Source	Location	Species	Root/Shoot
Golley et al. 1962	Puerto Rico	*R. mangle*	0.8[a]
Briggs 1977	Australia	*A. marina*	1.02-1.41
Komiyama et al. 1988	Indonesia	*Sonneratia*	0.23 [a]
		Bruguiera	0.29-0.44 [a]
		Rhizophora	0.53-0.67 [a]
Komiyama et al. 1989	Japan	*Bruguiera*	1.38
		Rhizophora	1.39
Pezeshki et al. 1990	Greenhouse	*R. mangle*	0.38 [a]
		A. germinans	0.42 [a]
Mackey 1993	Queensland	*A. marina*	0.58 [a]
McKee 1995b	Greenhouse exp.	*R. mangle*	0.1 [a]
		L. racemosa	0.4 [a] -1.5
		A. germinans	0.12 [a] -0.5
Saintilan 1997a	Australia	*A. marina*	4.1
		A. corniculatum	1.9
Saintilan 1997b	Queensland	*A. marina*	0.4-3.1
		A. corniculatum	0.4-1.4
		R. stylosa	1.2-1.7
Matsui 1998	Japan	*R. stylosa*	0.44 [a]
	Australia	*Rhizophora*	0.42 [a]
		Ceriops	0.42[a]
Komiyama et al. 1989	Thailand	*C. tagal*	1.05
Sherman et al. 2003	Dominican	*R. mangle*	
	Republic	*L. racemosa*	<0.5
		A. germinans	
This study	Greenhouse exp.	*A. germinans*	>0.5-1
		R. mangle	>0.5-1

[a]Ratio calculated based on data or figures provided

In this study, both mangrove species, *A. germinans* and *R. mangle*, increased their biomass allocation to roots under the flooded treatment. For both species root:shoot ratios were higher than 0.5 and increased with flooding (except for *R. mangle* under drained treatment) up to 1 under constantly flooded conditions. Pezeshki et al. (1990) in a greenhouse study with seedlings found that *R. mangle*, and *A. germinans* under saline (50% seawater) and flooding conditions (Eh –92 mV) had a higher total and root biomass that

resulted in increased root/shoot ratios (Table 4.11). Although our data agree, the responses in root partitioning to flooding obtained in this study are stronger.

The highest root/shoot ratios and the lowest shoot/root ratios in Table 4.11 are related to an increased biomass allocation to roots and decreased biomass allocation to the photosynthetic parts aboveground due to stressful conditions, most commonly hypersalinity (Saintilan 1997a and b). The ratio reported by Golley et al. 1962 does not include peat biomass which was about 40,000 g m^2, but such a large value may include a large proportion of dead and decomposing roots. When mangroves are under stress, root/shoot ratios give an idea about how they are investing more efficiently their production of C as biomass to cope with flooding stress or to improve nutrient acquisition. Considering the results of the present study, it seems clear that biomass allocation to roots of *A. germinans* and *R. mangle* was strongly controlled by flooding regime. These results support the hypothesis that mangroves can allocate higher biomass to roots to improve the aeration status and support to the plant given the anaerobic and unstable characteristics of the substrate. However, further field studies should analyze variation of these ratios under natural conditions between species, forest type, or if there are more intrinsic changes during the life stages. For example, Matsui (1998) for young trees obtained lower root/shoot ratios (0.32) than the average shown on Table 4.11).

Another biomass component needed to survive in flooded conditions is an aeration pathway, served by aerial roots such as pneumatophores and prop roots (Gill 1977). For example, *A. germinans* increased the density, length, and biomass of pneumatophores with increased flooding. Chapman (1940) demonstrated the importance of pneumatophores of *A. nitida* Jacq.(=*A. germinans*) in the gaseous exchange with the atmosphere and

respiration. In a greenhouse study, Toma et al. (1991), investigated the effect of flooding and plant density in the development of pneumatophores of *A. marina*. They observed that under the reduced flooded treatment (Eh 118±28mV), the density and height of pneumatophores per pot and seedling was higher. These results offer further support to the hypothesis that under anaerobic stress, mangroves will allocate higher biomass to aerial roots to provide more oxygen and better support to the plant.

Under low P concentration, *A. germinans* increased its biomass allocation to roots and the root:shoot ratio. In contrast, *R. mangle* did not change either of these traits in response to different nutrient regimes. McKee (1995b) analyzed how different light and nutrient regimes affected the growth and biomass partitioning of these species. She observed that under high nutrient concentration, RGR increased for both species, and it was higher for *A. germinans*. She also observed that under low nutrient concentrations biomass allocation to roots of both species increased from 22 to 41% for *R. mangle*, and 14 to 36% for *A. germinans*, and root: shoot ratio increased to 0.5 only for *A. germinans*. In this study, root:shoot ratios under both low (0.84) and high (0.69) nutrient regimes were higher than those reported by McKee (1995b). Also, in the present study RGR of both species did not respond to nutrient levels. Although, in this study I have observed some responses to nutrients levels, the stronger responses observed by McKee (1995b) may be due to the lack of the effect of natural stressors such as flooding in her study. The results of the rhizotron experiments suggest that the root system of *A. germinans* has greater plasticity than the root system of *R. mangle*, which would improve the efficiency of C investment in roots when P is a limiting factor.

In this study, the percent of total root biomass by class size for *A. germinans* was 20, 25, 55 % for fine, medium, and coarse roots, respectively, and for *R. mangle* it was 23, 29, and 48 %. Thus, for both species, greater root biomass was allocated to coarse roots, at least in young plants. Komiyama et al. (1987) found a high percentage of fine root biomass in forest stands that ranged from 46.4-66.4% (diameter classes higher than 55 mm). Their data agree with the results in this study since their class for fine roots would correspond to coarse roots in this study. Komiyama et al. (2000) estimated that fine root (0-2 mm) biomass of *C. tagal* was 2.2% of the total root biomass with similar percentages for medium and coarse roots. These proportions are 10 to 25 times lower than that obtained in this study for biomass allocation to roots. These differences may be attributable to different growth stages (seedlings versus mature trees). Also, Komiyama et al. (2000) studied a secondary mangrove stand, which may have influenced the biomass partitioning.

4.5. CONCLUSIONS

Mangroves are vital ecosystems, providing numerous goods and services along tropical and subtropical coastlines (Ewel et al. 1998, Rivera-Monroy et al. 2004). A better understanding of how resource and non-resource factors interact to affect plant growth is essential to conservation and management of these vital resources. Mangroves generally thrive under conditions that would be stressful for other species: flooded soils, high salinity, variable nutrients and interactions of these factors. However, extremes of these variables result in growth limitations of mangrove species such as those studied here. Mangrove root responses indicate interesting strategies that are species-specific and that allow acquisition of nutrients under stressful conditions. Nutrients such as phosphorus are relatively immobile in the soil, so that roots must essentially intercept ions through

development of an extensive root system with a high surface area. Soil anoxia is a well-known stress factor that restricts root growth (e.g., (Mendelssohn and Morris 2000). Also, lower redox potentials (higher soil oxygen demand) promote oxygen leakage from roots, leading to internal oxygen deficits (Chabbi et al. 2000, McKee 1996).

The results of the two rhizotron experiments indicate that flooding has an important impact on root growth, biomass production and partitioning, root morphology, and root distribution depth of the root systems of both *A. germinans* and *R. mangle*. Furthermore, *A. germinans* has a relatively plastic response in growth, biomass allocation and root morphology to nutrient limitation. In contrast *R. mangle* has a more conservative growth strategy that is less influenced by flooding and low nutrients. Similar findings have been reported for herbaceous species inhabiting the Florida Everglades, e.g., *Cladium jamaicense* (sawgrass) (Lissner et al. 2003). Such insights may help in understanding growth and distribution of wetland species and to predict potential effects of changes in nutrient regimes and/or hydroperiod on these important plant communities.

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CHAPTER 5

OVERALL CONCLUSIONS

This dissertation examined mangrove root dynamics and factors influencing these processes. The main objectives of this research were to assess several root measurement techniques and to test them in field and greenhouse settings as well as to determine mangrove root response to resource (nutrients) and non-resource (flooding and salinity) stress factors.

Root separation techniques to identify and quantify live and dead roots were compared. Colorimetric and fluorescence techniques were compared to colloidal silica separation by flotation as well as visual assessment of vitality, using live and killed roots as controls. All techniques were assessed for their advantages and disadvantages, including accuracy, ease of use, time required, and other considerations. In general, the colorimetric and staining methods were problematic and could only assess small quantities of roots, whereas the traditional method of visual assessment combined with root buoyancy was accurate, fast, and applicable to larger samples. Additionally, techniques such as rhizotrons, root ingrowth cores, and root image analysis were useful to study mangrove root dynamics.

Once root separation techniques were established and tested, a field study was initiated to examine natural variation in root standing biomass, production, turnover, and morphology. Field sites were selected in eight different mangrove forest types including fringe, basin, and scrub forest types in four different locations in Southwest Florida. Along with belowground dynamics, aboveground litter (leaf, wood, reproductive) production and soil physicochemistry were determined seasonally for one year. The findings showed wide

variation in subsurface production, especially compared to aboveground productivity. Root production was equal or greater than aboveground litter production. The combined root and litter production was a good predictor of flooding and nutrient stress. This field study of root production and root morphology and correlations with environmental conditions not only showed spatial and temporal variation in belowground production, but indicated that above- and belowground processes respond differently to environmental conditions. A nutrient enrichment experiment (additions of nitrogen or phosphorus) conducted in four forest types showed different patterns of root responses. Mangrove root production and morphology responded to nutrient enrichment depending upon forest condition and stress factors interacting with resource acquisition.

Finally, rhizotrons were utilized to examine in more detail root growth rates, biomass allocation, and morphology. Two separate experiments were conducted with *Rhizophora mangle* (red mangrove) and *Avicennia germinans* (black mangrove) in response to phosphorus availability and flooding regime. The two species showed different inherent root growth rates and patterns as well as plasticity of response to nutrients and flooding. The more flood tolerant species, *R. mangle*, was slower growing and root growth was not substantially restricted by flooding. The faster-growing species, *A. germinans*, exhibited flooding limitations to root growth and soil exploration for nutrients. *A. germinans* exhibited changes in root morphology that altered the amount of surface area for absorption of nutrients. These results indicate that there is a trade-off between root strategies to tolerate flooding and strategies to acquire nutrients and water. *Rhizophora mangle* appears to tolerate stressful conditions by slow growth and turnover of roots and other components, thus decreasing internal demand for nutrients. *Avicennia germinans*,

reallocates root biomass to fine root production and greater branching to ensure sufficient nutrient absorbing area, but this strategy is less successful under more flooded conditions.

This research contributes to a better understanding of how mangrove ecosystems function, specially their role in the global C (as carbon sinks), and nutrient cycles. Studies like this are needed to elucidate local and global patters of belowground dynamics in mangroves and other wetlands. Comparison of different root separation techniques as well as developing and testing some new methods for use in mangrove research will contribute to further work on belowground productivity and a better understanding of structure and function in these and other coastal ecosystems.

VITA

Beatriz Eugenia Giraldo was born in Santiago de Cali, Colombia, in January 5, 1970. She is the second daughter of Jairo Giraldo and Lucila Sánchez, both high school teachers. Beatriz's parents have constantly encouraged her to seek education and she admires the way her parents dedicate time and effort to educate the youth of the nation. She attended Eustaquio Palacios High School in Cali, from where she graduated in 1986. In 1987, she enrolled Universidad del Valle (UV). As an undergraduate, she demonstrated a unique interest, dedication, and hard work towards biology. She worked as assistant in several classes obtaining a great experience in teaching and research. She graduated in September of 1995 with a bachelor's degree in biology and emphasis in marine biology. The title of her bachelor's thesis is: "Natural Regeneration of Mangroves in the West Zone (Salamanca Island – Pajarales System) of the Cienaga Grande de Santa Marta, Colombian Caribbean." Soon after graduation, Beatriz started working at INVEMAR (Institute of Marine and Coastal Research) as a research assistant under the GTZ program. There, she gained a wide knowledge on mangrove research, but she wanted to expand her education. So, she applied for a very competitive fellowship from the Colombian government agency, COLCIENCIAS (Colombian Institute of Science and Technology), to pursue doctoral studies in the United States. She was offered the fellowship in 1996. In January of 1997, Beatriz married Guillermo Duque, her long term boyfriend and best friend. In the fall of 1997, she moved with her husband to Baton Rouge, Louisiana, to enroll the doctoral program in the Department of Oceanography and Coastal Sciences (DOCS) at Louisiana State University (LSU) under the supervision of Dr. Karen Lee McKee. In this department, she took coastal-related courses and minor

courses in statistics and biology. In May of 1998, Beatriz and her husband were blessed with a beautiful baby girl named Maria del Mar. In 1999 and 2000, Beatriz worked on her greenhouse experiments while taking classes. Then, in spring of 2001, she began her field study, which was finished in spring of 2002. She gained teaching experience assisting professors in the undergrad class "Introduction to Oceanography" and also being a laboratory instructor in the class "Biology for Science Majors I." In January of 2004, she gave birth to her second child, Estefania Beatriz. Since then, she has dedicated her time to analyze data from her research and write her dissertation. Beatriz will receive the degree of Doctor of Philosophy in December of 2005 from the Department of Oceanography and Coastal Sciences at Louisiana State University, and the Wetland Biogeochemistry Institute. She will then move back to Colombia to share her knowledge in coastal sciences and wetlands.