Brazilian Atlantic Rainforest Remnants and Mycorrhizal Symbiosis – Implications for Reforestation
A case study in Sergipe, Northeast Brazil

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1. Gutachter: Prof. Dr. W. Heyser (Universität Bremen)
2. Gutachter: Prof. Dr. M. Diekmann (Universität Bremen)

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Eu não tenho filosofia; tenho sentidos...
Se falo na Natureza não é porque saiba o que ela é,
Mas porque a amo, e amo-a por isso
Porque quem ama nunca sabe o que ama
Nem sabe por que ama, nem o que é amar...

Alberto Caeiro,
em "O Guardador de Rebanhos",
8-3-1914

A meus pais,
Iza e Samuel

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Summary

This work presents results of several aspects of the ecology of two Brazilian Atlantic rainforest remnants. Both forests are situated in the Sergipe state of the Brazilian northeast region. The region is characterized by a marked rainfall seasonality, which is likely to affect soil nutrient dynamics and plant reproduction and recruitment. Sampling was therefore carried out in the two periods. One of the forest fragments (Crasto forest) was a coastal tableland forest on Red-Yellow Podzols, the other, a *restinga* forest (Caju forest) on quartziferous sands. Soils, as typical tropical soils, are moderately to strongly acidic, with relatively high Al levels and nutrient-poor. The Crasto site presented generally higher nutrient levels, maybe due to the higher clay content in its soils, but in both, nutrients were generally concentrated in the soil upper layers. Collections were also carried out in the adjacent region of each fragment, occupied by a coconut plantation in both sites. With some minor variation, these regions presented lower nutrient levels than the forest plots. Soil properties are, therefore, probable to hinder tree seedling establishment and, consequently, forest restoration in these areas. The mycorrhizal symbiosis is also equally important to plant growth, particularly during the seedling phase, and may have been similarly affected by disturbance. Aiming to investigate the potential of native mycorrhizal species on forest regeneration, the assessment of mycorrhizal symbiosis in roots of seedling and trees was performed as well as the analysis of the spatial distribution of inoculum (spores and roots). Arbuscular mycorrhizal fungi (AMF) was, like in all tropical ecosystems, dominant. AMF inoculum was not, contrary to the expectation, excluded from the plantation plots. In fact, spore species richness was higher than in the forest plots. Disturbance in these areas was therefore not enough to eliminate the occurrence of the mycorrhizal symbiosis. However, both sites differed in its response. In the Caju forest, roots in plantation sites presented higher colonization levels than in the Crasto site. Difference in soil properties and/or management practices may be responsible for these results and will be further examined. AMF spore species richness and root colonization levels were higher in the upper layers of the soil, similarly to the nutrient and root biomass. Disturbance that involve active removal of the top soil or increasing of soil erosion will therefore remove also the native AMF inoculum important for plant growth and survival, particularly under stressed conditions. Seedling recruitment was lower in the plantation than in forest plots. However, forest fringe plots presented also similarly low values. Seedling root mycorrhizal colonization was spatially uneven. Analysis of seedling roots from four different native tree species revealed a very variable picture of colonization type and intensity between species. An important finding was the high colonization levels of roots of some of these seedling species by dark septate endophyte (DSE), group of fungi supposed to have diverse phylogenetic origins and at present being tentatively placed in the Deuteromycotina, or Fungi Imperfecti. Both pathogenic and positive effects have been reported for plants in culture with these fungi, and it is not clear whether the analyzed seedlings benefit from them. As all the seedlings seemed to be healthy, without any noticeable signal of disease or nutrient deficiency, and as this root endophyte was also found in roots of some tree species analyzed, it is probable that these unexpected root partner may have a positive effect in enhancing plant growth and survival. This is an important issue for tree seedling recruitment and will be considered in subsequent studies. The analysis of native tree roots

revealed also that ectomycorrhizal inoculum, although rare, is present in these forests, as one of the selected species, *Cocoloba rosea* (Polygonaceae), presented ectomycorrhizal roots. The family is already described in the literature as presenting ectomycorrhizal tropical species, suggesting an important taxonomic, more than biogeographical, component determining the distribution and occurrence of ectomycorrhizas in tropical regions. For recomposition of these tropical forests, however, arbuscular mycorrhizas are far more important. Contrary to some studies relating mycorrhizal colonization and host successional status, roots from pioneer species, *Cecropia pachystachya* and *Vismia guianensis*, were strongly colonized and will be subject of future investigations. Preliminary experiments intending to test the ability of native AMF inoculum in promoting seedling growth and survival were performed with a native legume tree species, *Bowdichia virgilioides*. These experiments aimed to compare native mixed inoculum from plantation and forest plots as well as pure inoculum (*Glomus clarum*) under different P levels or sources (KH_2PO_4 and phytate). The observed positive effects of AMF inoculation on plant growth, even with native mixed inoculum, suggests native inoculum can be successfully used on restoration of these deforested regions. Moreover, the high AMF colonization of the pioneer species, *C. pachystachya* and *V. guianensis*, should be managed to maximize the effects of inoculation of *B. virgilioides* seedlings in field conditions.

Resumo

Este trabalho apresenta resultados de vários aspectos da ecologia de dois remanescentes de mata atlântica. Ambas as florestas estão situadas no estado de Sergipe, na região nordeste do Brasil. A região costeira de Sergipe é caracterizada por uma acentuada sazonalidade na precipitação, o que deve afetar a dinâmica de nutrientes do solo e a reprodução e recrutamento vegetal. Por esse motivo, amostragens foram feitas na estação seca e chuvosa. Um dos fragmentos florestais (mata do Crasto) é uma floresta em tabuleiro costeiro de planalto litoral sobre solo podzólico vermelho-amarelo, o outro, uma floresta de restinga (Fazenda Caju) sobre areias quartsosas. Os dois solos, como típicos solos tropicais, são de moderadamente para fortemente ácidos, com conteúdo de Al relativamente alto e pobres em nutrientes. A área do Crasto apresentou níveis de nutrientes geralmente mais altos, talvez devido ao conteúdo de argila mais alto, mas em ambas as áreas de estudo, os nutrientes estão geralmente concentrados na terra camadas superiores do solo. Coletas também foram realizadas na região adjacente de cada fragmento, ocupada por uma plantação de coco em ambos os locais. Com alguma variação, estas regiões apresentaram mais baixos níveis de nutriente que as parcelas na floresta. Portanto, é provável que essas características do solo dessas áreas desflorestadas representem uma dificuldade para o estabelecimento de plântulas de especies arbóreas e, consequentemente, para a recomposição florestal nestes áreas. A simbiose micorrízica é igualmente importante para o crescimento vegetal, particularmente durante a fase de plântula, e pode ter sido semelhantemente afetada pela remoção da cobertura floretal. De modo a investigar o potencial de espécies micorrízicas nativas para a regeneração florestal, foi realizada uma avaliação da colonização micorrízica em raízes de plântulas e árvores, assim como uma análise da distribuição espacial de inóculo micorrízico (esporos e raízes). Fungos micorrízicos arbusculares (FMA) foram, como usual em ecossistemas tropicais, dominantes. Inóculo de FMA não foi, ao contrário do esperado, excluído das áreas desflorestadas, ocupada pela plantação de coco. Na realidade, a riqueza de espécies de esporos foi mais alta nessas áreas que nas parcelas de floresta. Portanto, o grau de perturbação nestas áreas não parece ter sido suficiente para eliminar a ocorrência da simbiose micorrízica. Porém, ambos os locais apresentaram diferenças em suas respostas. Na Fazenda Caju, raízes em parcelas na plantação apresentaram mais altos níveis de colonização que na área do Crasto. Diferenças em propriedades do solo e/ou práticas de manejo podem ser responsáveis por estes resultados, o que será examinado em estudos subsequentes. Riqueza de espécies de esporos de FMA e níveis de colonização de raízes foram mais altos nas camadas superiores do solo, semelhantemente ao encontrado para a concentração de nutrientes e biomassa de raízes. Portanto, perturbações que envolvam a remoção direta da camada superficial do solo ou que aumentem a sua erosão, removerão também o inóculo de FMA nativo, importante para o crescimento e sobrevivência vegetal, particularmente sob condições ambientais adversas. O recrutamento de plântulas foi menor nas parcelas de plantação do que em parcelas de floresta. Porém, parcelas na borda da floresta também apresentaram valores igualmente baixos. A colonização micorrízica em raízes de plântulas apresentou variações espaciais. A análise de raízes de plântulas de quatro espécies arbóreas nativas revelou também um quadro variável de tipo e intensidade de colonização entre espécies. Uma observação importante foi o alto nível de colonização de raízes de

algumas destes espécies de plântulas por endófitos septados escuros (DSE), grupo de fungos possivelemente de origem filogenética diversa, sendo presentemente classificados junto aos Deuteromycotina, ou fungos imperfeitos. São registrados na literatura efeitos patogênicos e positivos para plantas cultivadas com estes fungos, e não está claro se as plântulas analisadas no presente estudo beneficiam-se desses endófitos. Como todas as plântulas pareciam saudáveis, sem qualquer sinal evidente de doença ou deficiência nutricional, e como esses fungos também foram achados em raízes de algumas espécies arbóreas analisadas, é provável que este outro tipo de endófitos, assim como os FMA, também possa ter um efeito positivo sobre o crescimento e sobrevivência vegetal. Este é um tema importante para o recrutamento de plântulas de espécies arbóreas e será considerado em estudos subseqüentes. A análise de raízes de árvores nativas também revelou que inóculo de espécies ectomicorrízicas, embora raro, também está presente nestas florestas, com uma das espécies selecionadas, *Cocoloba rosea* (Polygonaceae), apresentando raízes ectomicorrízicas. Essa família já é descrita na literatura como apresentando espécies ectomicorrízicas tropicais, o que sugere um importante componente taxonômico, mais que biogeográfico, determinando a distribuição de espécies ectomicorrízicas em regiões tropicais. Para a recomposição destes fragmentos florestais, no entanto, micorrizas arbusculares são mais importantes. Ao contrário de alguns estudos relacionando colonização micorrízica e estados successionais da planta hospedeira, raízes de espécies pioneiras, como *Cecropia pachystachya* e *Vismia guianensis*, apresentaram alta colonização e serão assunto de estudos futuros. Foram também realizados experimentos preliminares pretendendo testar a capacidade de inóculo de FMA nativos para promover o crescimento e sobrevivência de plântulas de uma espécie leguminosa arbórea nativa, *Bowdichia virgilioides*. Estes experimentos visavam comparar inóculo mixto nativo proveniente de parcelas na plantação e na floresta, como também inóculo puro (*Glomus clarum*) sob diferentes de níveis ou fontes de P (KH_2PO_4 e fitato). Os efeitos positivos observados da inoculação de FMA no crescimento das plantas, mesmo com inóculo mixto nativo, sugere que este pode ser usado com sucesso na restauração destas regiões desflorestadas. Além disso, a alta colonização por FMA em algumas espécies pioneiras, como *C. pachystachya* e *V. guianensis*, deveria ser manejada para maximizar os efeitos da inoculação de plântulas de *B. virgilioides* em condições de campo.

Zusammenfassung

In dieser Arbeit werden die Ergebnisse verschiedener Aspekte der Ökologie zweier atlantischer Regenwaldreste in Brasilien dargestellt. Beide Wälder liegen in der nordöstlichen Region Brasiliens im Bundesstaat Sergipe. Diese Region ist durch eine ausgeprägte Regen-Saisonalität charakterisiert, die auch die Nährstoffdynamik im Boden, die Reproduktion der Pflanzen und deren Aufwuchs beeinflusst. Die Probennahme wurde aus diesem Grund zu zwei ausgewählten Zeitpunkten durchgeführt. Bei einem der Regenwaldreste (*Crasto* forest) handelt es sich um einen küstennahen Wald auf einer Hochebene mit rötlich-gelbem Podsol, bei dem anderen um einen sogenannten „Restinga"-Wald (*Caju* forest) auf Quarz-Sand. Die Böden sind, wie für tropische Regionen üblich, schwach bis stark sauer, nährstoffarm, mit relativ hohen Al-Gehalten in der Bodenlösung. Der Standort *Crasto* ist im Allgemeinen durch ein höheres Nährstoffniveau ausgezeichnet, welches wahrscheinlich auf die höheren Tongehalte im Boden zurückzuführen ist, an beiden Standorten ist das Nährstoffvorkommen allerdings auf die obersten Bodenschichten begrenzt. Untersuchungen und Probennahmen wurden auch in den an die Regenwaldreste angrenzenden Gebieten durchgeführt, die beidseitig aus Kokosnuss-Plantagen bestehen. Mit geringen Abweichungen zeichneten sich diese Regionen an beiden Standorten durch ein geringeres Nährstoffvorkommen als die Waldstücke aus. Diese Bodeneigenschaften erschweren an diesen Standorten den Aufwuchs von Baumsämlingen und damit auch die Regenerationsfähigkeit der Regenwälder. Weiterhin ist das Vorkommen von Mykorrhiza-Symbiosen ein sehr wichtiger Faktor für das Pflanzenwachstum insbesondere während der Etablierungsphase der Sämlinge, wo vielfältige Störungen Einfluss nehmen. Um das Potenzial von natürlich vorkommenden Mykorrhiza-Pilz-Arten auf die Regenwald-Regeneration abschätzen zu können, wurden Untersuchungen an Wurzeln von Sämlingen und Bäumen durchgeführt. Weiterhin wurde versucht, die räumliche Verteilung von Inokulationsmaterial (Sporen und Mykorrhiza-Wurzeln) zu erfassen. In den Untersuchungsgebieten dominieren die für tropische Ökosysteme typischen arbusculären Mykorrhiza-Pilze (AMF). AMF-Inokulate lagen entgegen den Erwartungen auch in den Plantagengebieten vor. Dabei war die Vielfalt der Sporenarten sogar höher als in den Waldgebieten. Das heißt, trotz der Abholzungen in diesen Gebieten ist das Potenzial für Mykorrhiza-Symbiosen weiterhin erhalten geblieben. Allerdings unterschieden sich beide Standorte deutlich bezogen auf das Mykorrhizavorkommen. Die Wurzeln der Plantagenstandorte in *Caju* zeigen höhere Kolonisierungsraten als in *Crasto*. Die Unterschiede in den Bodeneigenschaften und/oder dem Bodenmanagement nach der Abholzung sind wahrscheinlich für diese Ergebnisse verantwortlich und werden in Zukunft weiter untersucht. Die Vielfalt der AMF-Sporenarten und die Wurzelkolonisierungsraten waren wie die Nährstoffgehalte und die Wurzelbiomasse in den oberen Bodenschichten höher. Störungen wie zum Beispiel die aktive Beseitigung des oberen Erdreiches oder zunehmende Bodenerosion müssen sich deshalb negativ auf das natürliche AMF-Inokulum, das unter den genannten Bedingungen wichtig für das Pflanzenwachstum und den –fortbestand ist, auswirken. Das Sämlingsaufkommen war auf den Plantagen-Pflanzungen niedriger als auf den Waldstandorten. Jedoch zeigten Standorte am Waldrand ähnlich geringe Werte. Die Mykorrhizakolonisierung der Sämlingswurzeln war je nach Gegebenheiten ungleichmäßig. Untersuchungen der Sämlingswurzeln von vier verschiedenen einheimischen Baumarten zeigten ein

sehr variables Bild des Kolonisierungstyps und der -intensität zwischen den Arten. Ein wichtiges und neues Ergebnis war die hohe Kolonisierungsrate von Wurzeln einiger dieser Sämlinge durch dunkle septierte Endophyten (DSE), einer Gruppe von Pilzen mit vermutlich mehrfachem phylogenetischem Ursprung, die derzeit in die Gruppe der Deuteromycotina, oder *Fungi imperfecti,* eingeordnet werden. Sowohl pathogene als auch positive Wirkungen sind aus Kulturen von Pflanzen mit diesen Pilzen bekannt. In Verbindung mit den hier vorgelegten Untersuchungen bleibt offen, ob die untersuchten Sämlinge von ihnen wirklich profitiert haben. Da jedoch alle Sämlinge gesund und ohne merkliche Anzeichen von Krankheit oder Nährstoffunzulänglichkeit zu sein schienen und Wurzelendophyten derselben Art auch in Wurzeln einiger der untersuchten Baumarten gefunden wurden, ist es eher wahrscheinlich, dass diese unerwarteten Wurzelpartner eine positive Wirkung auf das Wachstum und Überleben der Pflanzen haben. Dies ist ein für die Sämlingsanzucht in Baumschulen wichtiger Aspekt und wird in nachfolgenden Studien berücksichtigt werden. Die Untersuchung von Baumwurzeln an den Standorten belegte auch das, wenn auch seltene, Vorkommen von Ektomykorrhizen, da eine der ausgewählten Spezies, *Cocoloba rosea* (Polygonaceae), solche Wurzeln zeigte. Diese Familie wird in der Literatur bereits als ektomykorrhizabildend beschrieben, was nahegelegt, dass die Verbreitung und das Vorkommen von Ektomykorrhizen mehr durch taxonomische als durch biogeographische Komponenten bestimmt wird. Für die Wiederherstellung der in dieser Arbeit untersuchten tropischen Wälder sind jedoch arbuskuläre Mykorrhizen von viel größerer Bedeutung. Im Gegensatz zu anderen Untersuchungen, in denen die Mykorrhizierung mit dem Sukzessionsstatus der Wirtspflanze verknüpft wurde, waren die Wurzeln von Pionierarten wie z.B. *Cecropia pachystachya* und *Vismia guianensis* stark kolonisiert, was weiterhin verfolgt wird. Vorläufige Experimente, welche die Fähigkeit der einheimischen AMF-Inokulate zur Förderung des Sämlingswachstums und ihrer Überlebensfähigkeit zum Ziel hatten, wurden mit einer einheimischen Leguminosen-Baumart *Bowdichia virgilioides* durchgeführt. Diese Experimente zielten darauf ab, den Beitrag zur Phosphatversorgung der Pflanzen von einheimischen gemischten Inokulaten aus Pflanzungen und Waldstandorten gegenüber dem eines ausgewählten Pilzpartners (*Glomus clarum*) aus unterschiedlichen Angebotsquellen zu untersuchen (KH_2PO_4 und Phytat). Die beobachteten positiven Wirkungen auf das Pflanzenwachstum besonders mit gemischten einheimischen Inokulaten deuten darauf hin, dass einheimisches Inokulum erfolgreich zur Wiederherstellung dieser entwaldeten Regionen genutzt werden kann. Darüber hinaus sollte die starke AMF-Kolonisierung der Pionierarten *C. pachystachya* und *V. guianensis* genutzt werden, um die Wirkungen der Inokulation von *B. virgilioides* unter Feldbedingungen zu maximieren.

1 Introduction

1.1 Tropical forests

About 33% of the total world's land surface are covered by forests (Rumney 1968), and the tropical rainforests comprise about 30% of the tropical forests (Sanchez 1976). Most of them are found in South America (Lieth & Werger 1989).

Although tropical rainforests are usually found in regions with mean annual precipitation of 2000-3000mm, the frequency and intensity of dry periods has important effects on its structure and dynamics (Lauer 1989). Temperature present generally small variation and allows do not limits plant growth as in temperate regions (Lauer 1989). But the most typical, and ecologically important, feature of these forests is their high species diversity (Prance 1989, Richards 1996). Figures may differ, however, between different groups of organisms (Lieth & Werger 1989). Although these tropical forests can present about two thirds of the world's species, only *circa* 500,000 species from tropical and subtropical regions have been identified (Raven 1997), and much of their species are still not properly described (Richards 1996).

Besides the generally high species diversity, the high levels of endemism is also a typical feature of tropical rain forests (Lieth & Werger 1989). The restricted distribution area of some species (Prance 1989) is an important characteristic, as it makes these species particularly vulnerable to habitat disturbance. In fact, disturbance of these ecosystems had lead to local and complete extinction of some taxa (Lieth & Werger 1989).

The extent of area occupied with tropical forests has varied with natural periodic climatic changes (Richards 1996). For instance, in the dry and colder periods of the Quaternary, the total forest area was restricted to small refuges, which expanded during subsequent more humid phases (Richards 1996). However, human impact on these ecosystems has led to more extreme, non-cyclic changes. Indeed, the European colonization throughout the tropics represented a drastic impact on these forest regions, much of it being cleared for the establishment for monocultures such as sugar cane, coffee, and oil palms, among other, as well as for wood extraction (Richards 1996). Today, pressures on what is left from these rich, and in great part unknown, ecosystems are due to socio-economic causes (growth and impoverishment of the population), but also to the insufficient knowledge on how to manage them in an sustainable form (Raven 1997).

Brazil is the country with larger forested areas, or 357.480.000 ha (Mittermeir 1997), most of it comprised by the Amazon forest. However, other forest formations are also present, some of them still more endangered than the Amazon forests.

1.1.1. The Brazilian Atlantic rainforest

A long and narrow strip along the Brazilian coast is occupied by Atlantic forest, which is disjointed from the Amazon forest by a large extension of savannas (the Brazilian *cerrados*) and dry deciduous forests (Richards 1996). This forest formation extended originally from the State of Rio Grande do Norte to the State of Rio Grande do Sul. The Atlantic ocean is the main source of humidity of these forests. In fact, the occurrence of Atlantic tropical forests even southern of the Tropic of Capricorn are due to particularly favorable climatic conditions, which include orographic rains at the east coast of the continents (Lauer 1989).

Along this coastal area, not only a wide latitudinal range, but also a consequent great variation in climates, besides relief and soil types, is found. These correspond also to the many vegetation forms found within this area. Actually, several many different forest formations are present within this region and are included in the broad denomination 'Atlantic forest'. Forest formations range from the typical evergreen to semideciduous, but also herbaceous vegetation occur in higher altitudes, as well coastal vegetation forms, such as mangroves, sand dunes and *restingas* (Rizzini, 1963; Romariz, 1972). These latter, being a characteristic vegetation form on Quaternary sand deposits of marine origin (de Lacerda, de Araújo & Maciel 1993), in some regions developing into forest formations.

The Atlantic rain forest is certainly one of the most endangered Brazilian ecosystems, with great rates of human disturbance since colonization by the Portuguese settlers after 1500 (Figure 1.1). A comprehensive review of the human settlement in the Brazilian coastal region, and the consequent forest removal has been done by Dean (1996). Of the covered area when of the arrival of the Portuguese to Brazil, it remains today very a little. In some parts, it was completely eliminated and in another just remain small forest fragments, very altered by the human action.

The history of the colonization of this region helps to understand this situation. Great part of the coastal forest was destroyed, initially, for exploration of the wood, being substituted soon afterwards by the agriculture and livestock. The coastal region was not only the first part to be colonized, meaning a long-term history of forest disturbance, but is also where the agricultural and industrial activities are presently mostly concentrated, with the largest cities being found tin this region (Mittermeir 1997). The crescent increase in their numbers and densities is one more pressure factor on those ecosystems. In fact, the conservation of what remains from the Atlantic forest is, "probably, the greatest and most urgent problem of conservation in the country" (Câmara 1983).

In the South and Southeast regions, the mountainous areas close to the sea restricted somewhat the human penetration and occupation. Nowadays, the largest remains of Atlantic forest are found in these areas. In the Brazilian Northeast, on the contrary, the less steep relief allowed man's easy access. The report of occupation of the coast for the predatory extraction of the Brazil wood (*Caesalpinia echinata*), initially, and for the culture of the cane of sugar, that still persists in vast extensions, propitiated the almost complete elimination of that ecosystem. Presently, with some few exceptions, they are only found dispersed fragments, varying in size and conservation level. Though also located along the Atlantic coast, in the Northeast this ecosystem presents a more marked seasonality, with a

well defined dry season. This climatic feature has important implications for the species composition and structure of these forests.

Many researchers have emphasized its great diversity of animal and plant species, the high frequency of endemic species, and the risk of extinction due to the continuous and increasing use of these areas and of its resources. The Brazilian coast region is a regional center of endemism (Prance 1989) and high endemism levels in the Atlantic forests are common (Mori *et al*. 1981, Prance 1989, Thomas *et al.* 1998). However, two distinct groups, in northern and southern sectors, can be recognized regarding endemic species (Prance 1989). Studies in the southern Bahian forests revealed that about 40% of the species are endemic to the coastal forests, and approximately 27% are endemic to this area and the northern region of the neighbor State Espírito Santo (Thomas *et al.* 1998). Interestingly, about 7% of the species are disjunct between these coastal forests and the Amazonia forest. Mori *et al*. (1981) presented even higher estimates of endemism for the Atlantic forests, of about 53%.

High species diversity and endemism levels are presented not only by plant species, but also by animal species. Brazil is one of the most important countries for the conservation of primates, with 53 species of the world (or 27%), of which 35 are endemic (Mittermeir 1997). Within Brazil, the area of grater priority for primate conservation is the Atlantic forest, with 80% of its primate species, and 40% of the non-flyer small mammals, being endemic (Mittermeir 1997). Consequently, deforestation affects also these endemic animal populations. For instance, extinction rates of endemic bird species from the Atlantic forest region have been related to the rates of deforestation of these habitats (Brooks & Balmford 1996).

Although the deforestation of the Atlantic forest has happened since the beginning of the colonization of the region, the main damage occurred after 1950 (Myers 1997). Originally, the Atlantic forests covered an area of more than one million km^2, or 15% of the Brazilian territory (Fundação SOS Mata Atlântica/INPE/ISA 1998). Estimates of Atlantic forest remaining areas vary from 1% (Wilson 1997), 1-5% (Mittermeir 1997), or about 8% (285,000 ha in 1990) (SOS Mata Atlântica/INPE 1993). Variation in these estimates are probably due to the different methods used, and date of satellite images upon which these analysis were made. What is impressive in these estimates is the almost complete elimination of this ecosystem.

The conservation status of the forests situated in the northern sector of the Atlantic forest is even worse, having been almost completely cleared by uncontrolled logging, agriculture and cattle raising. Only a limited number of forested remnants exits and the extent area protected under governmental or private reserves is smaller than in the southern sector (Câmara 1983). Here, only one national park is found (in the south of the Bahia State), whereas most of the conservation areas is situated in the southeast and south regions (Câmara 1983).

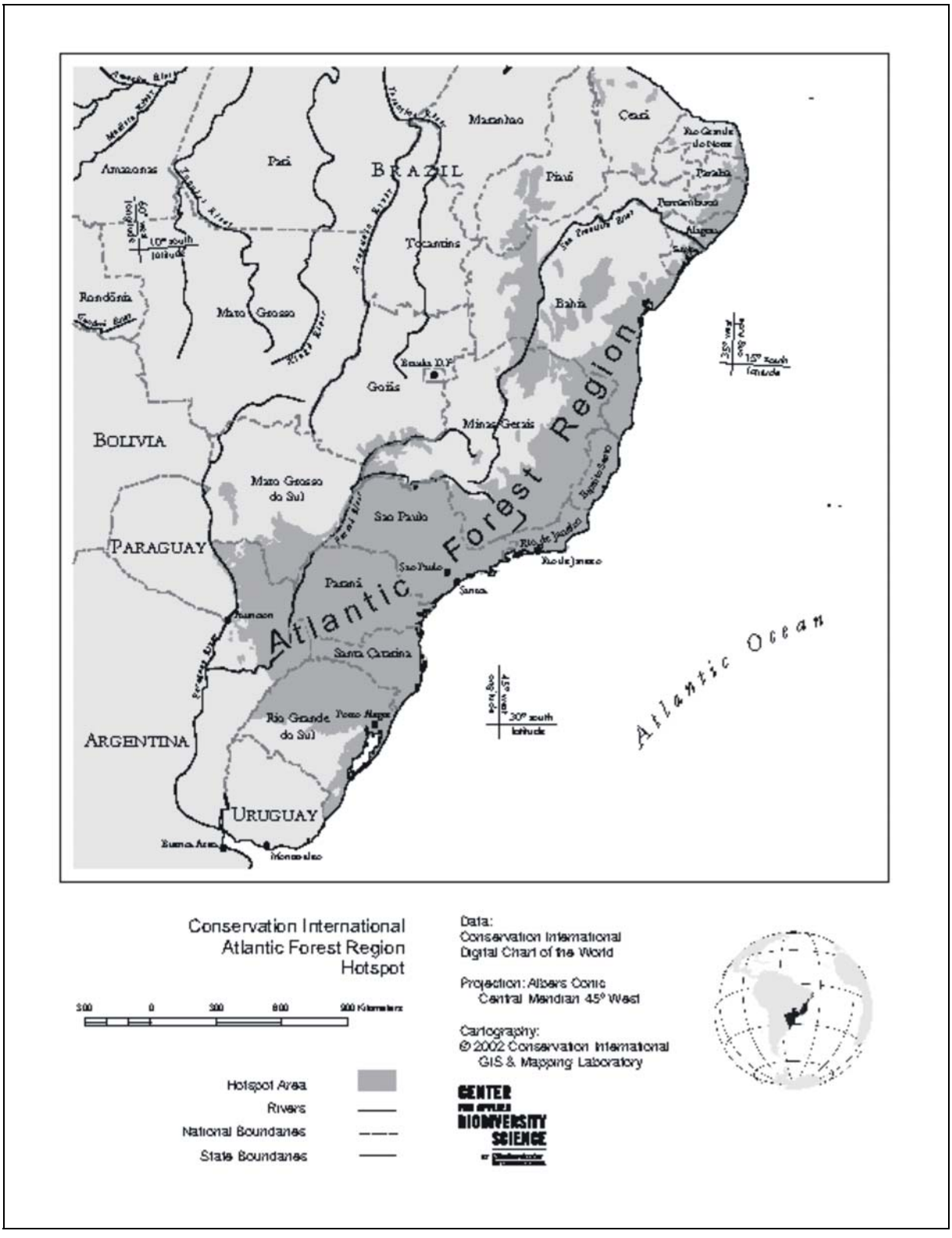

Figure 1.1 Distribution of the Brazilian Atlantic rain forest: yellow, delimitation of the Atlantic rainforest region. (source: Conservation International)

In Sergipe, the smallest Brazilian State, the rain forest extended originally within an area of approximately 40 km from the coast, with the precipitation ranging between 1.100 mm and 1.500 mm. Nearly 41,07% of the Sergipe area were then covered by forests (Campos 1912). Nowadays, estimates of the rain forest in Sergipe are of 1 to 5%. Few information is available on the forests of Sergipe. A rather general description, without information of tree species, of some forest areas is provided by Franco (1973). More recently, efforts to assess plant species diversity in the forest remnants have been carried out (Landim & Siqueira 2001a) and are still underway.

If not much information is available about the flora, faunal inventories are even more scarce. However; absence of published data does not mean absence of a significant diversity in the forests remnants still existent. Indeed, a new snake species from the Atlantic forests of Sergipe and Alagoas has been described (Fernandes 1995). The genus from this new species, *Attractus*, is described as having a more restricted range, what makes this species particularly more sensitive to disruption of its habitat (Fernandes 1995). Furthermore, a survey on small mammals in the probably larger continuous Atlantic forest fragment of Sergipe, the "Mata do Crasto", in the Santa Luzia do Itanhy county, revealed nine species, from three families, none of them having being captured in the coconut plantations surrounding this forest (Stevens & Husband 1998). These authors also showed significant edge effects on the distribution of these populations, which were increasingly more abundant outside the forest border region.

Although many other potential economic resources and uses of the tropical forests exist, in addition to wood, this may be, presently, one of the main causes of destruction of the rain forest fragments in Sergipe. Besides continuing the floristic inventories, necessary to assess the actual diversity of these forests, studies providing information strategies to maximize reforestation programs in these regions are, therefore, urgent.

1.2. Forest fragmentation and constraints for reforestation

Much of the tropical forests has been substituted by secondary formations, ranging from grassland to forests almost similar to mature climax forests (Richards 1996). Atlantic forest remnants are typically small, isolated and highly disturbed (Viana *et al*. 1997). This high fragmentation, poses important problems to their conservation. Some effects of this fragmentation are decrease in tree species recruitment and reduction of the populations size of several species (Viana *et al*. 1997). A decline of zoochorous plant families in smaller fragments has been reported (Tabarelli, Mantovani & Peres 1999), and may be a two-way process, as these low fruit producer species populations may have been caused by diminished activity of frugivores in these smaller fragments, but on the other side, the smaller availability of resources would consequently lead to the reduction of frugivores populations.

A reduction of species richness in fragments isolated from continuous forest have been reported (Turner 1996a), and small fragments may be unable to maintain animal and plant species

diversity (Tabarelli, Mantovani & Peres 1999). This is in great part caused by the so-called 'edge effects', resulting from the increased proportion of the forest border exposed to non-forest habitats (Kapos *et al*. 1997). For instance, these edge effects have been shown to affect markedly microclimate and vegetation structure of forest fragments (Didham & Lawton 1999).

In fact, although small, isolated forest fragments can subsist for many years, their composition and structure will progressively degenerate by processes such as wind and fire damage and invasion by weedy species (Richards 1996), these latter being more successful in colonizing the disturbed regions of these fragments than forest species. Populations of rare, endemic species can be faded to extinction, at least locally, as these are the most endangered species as consequence of the fragmentation of their habitat (Richards 1996).

The natural secondary succession in deforested areas is highly variable and partly influenced by the history of disturbance (Richards 1996). In fact, if left undisturbed, a slow recovery from these communities to a state much comparable to the original conditions can take place (Richards 1996). If the region has been subjected to cultivation, as in much of the regions surrounding these forest fragments in Sergipe, the use of management practices such as slash-and-burn, and the soil erosion level will affect the initial recover of native vegetation (Richards 1996). This is a very slow process, even when disturbance has completely ceased, and its speed will depend on factors like the distance from seed sources, among others (Richards 1996).

The highly fragmented landscape of the Atlantic rainforest region makes necessary the use of these fragments as sources to the recovering of much of the original diversity. In fact, secondary forests may also posses an important role in maintaining species diversity, and their role in conservation should not be forgotten (Lugo 1997). Actually, despite the species impoverishment reported to isolated forest fragments, much of the diversity fragments less than 100ha can be preserved for decades after isolation had taken place (Turner 1996b). The increase of forested area from subsisting forest fragments, until they coalesce (Turner 1996b), seems to be a viable approach to restore Atlantic forests.

However, a great problem to reforestation of these areas is the oligotrophic character of the soils from most of the humid tropics (Baillie 1996). In fact, 63% of the world's humid tropics, and 81% of the humid tropical America, possess acid, unfertile soils. Due to the moderate to high acidity of much of these soils, Al is commonly the dominant cation present is soil solution, leading to a reduction of P availability to plants (Baillie 1996). These three basic features, low fertility, Al toxicity, and high phosphorus fixation are the main constraints to plant growth in these regions (Sanchez 1989). Furthermore, deforestation affects important microclimate features (Walsh 1996) and soil physical and chemical properties, such as organic matter and nutrient content (Baillie 1996). Furthermore, the existence of a well defined dry season in the northern sector of the Atlantic forests must represent a critical period for the seedling establishment, particularly where the forest was total or partially removed.

All these characteristics may restrain the natural regeneration of forest tree seedlings in these areas, and diminish the success of reforestation plans, if suitable strategies are not performed to assure

greater survivorship and growth rates of these seedlings. In this context, the management of the mycorrhizal symbiosis was considered to aid restoration of Sergipe's Atlantic forest remnants, and is the subject of the present study

1.3 Mycorrhizas

1.3.1 Definition

Mycorrhizas are symbiotic relationships between different fungi groups and plant roots. This is a rather general definition, as many different types of relationships, including variation between fungus and plant host species are included under this term. This term also do not include, necessarily, any evaluation of the nature of this relationship, what will be discussed below.

1.3.2 Types of mycorrhizas

Among the different mycorrhizal types existent (see Smith & Read 1997), the arbuscular mycorrhizas and the ectomycorrhizas are focused in the present study, as they can be more significant to tropical forest ecosystems, being shortly described below. Another group of fungal endophytes, the "dark septate endophytes", recently discussed in the literature as possibly mycorrhizal is also presented.

1.3.2.1 Arbuscular mycorrhizas

Arbuscular mycorrhizas are formed by fungi from the Glomales order from the Zygomycetes (Morton & Benny 1990). These species are contained in two sub-orders, Glomineae and Gigasporineae. More recently (Morton & Redecker 2001), two new families were described, Archaeosporaceae, with the new genus *Archaeospora*, and Paraglomaceae, with *Paraglomus*.

Species are described by the mode of spore formation what leads to difficulties and errors in species identification. No more than 200 species have been described (Morton & Benny 1990), but the use of recently developed molecular methods of study has revealed a great diversity of arbuscular mycorrhizal fungi (AMF) in plant roots, suggesting that a significant part of it has still not been described (Helgason *et al*. 2002). Molecular methods have also allowed a new revision of the phylogenetic relationships between groups presently described, revealing a new systematic structure for this group (Schüßler A, Schwarzott D, Walker C 2001). This new classification system, presents a new fungal phylum, the ***Glomeromycota.***

In general, mycorrhizas are widespread among plant groups (Trappe 1987), being sometimes said it is easier to list non-mycorrhizal, than mycorrhizal species, so abundant they are. AMF are all obligately biotrophic (Ho & Trappe 1973, Warner & Mosse 1980), what means they depend on the

plant host to obtain their carbon supply. AMF were generally considered to be non-specific regarding their plant hosts, but some level of specificity occurs (Ravnskov & Jakobsen 1995), and may turn out to be more significant than presently recognized, with increasing knowledge on the ecology of individual AMF species.

Fungal colonization occupies predominantly the root cortex. Structures formed are depicted in the figure. Internal hyphae can from intracellular arbuscules, cited as interface of nutrient transfer between fungi and plant occurs, or hyphal coils. Hyphae can colonize both inter and intracellular root spaces.

1.3.2.2 Ectomycorrhizas

Ectomycorrhizas are formed by a larger group of fungal species, most of them, basidiomycetes. Characteristics of this symbiotic relationship are a fungal mantle surrounding the rootlets, a Hartig net formed by hyphae growing within epidermal and cortical cells, and the hyphal strands by which these fungi explore soil and are connected to fruit bodies (Smith & Read 1997).

Compared to the arbuscular mycorrhizas, ectomycorrhizas present a much more restricted group, although important, of host plants (Smith & Read 1997). Reports on the occurrence of ectomycorrhizas in plants from tropical ecosystems are mostly limited to some African monospecific woody formations (e.g. Högberg & Nylund 1981, Newbery et al. 1988, Thoen & Ba 1987). However, the diversity of fungi in tropical natural ecosystems is not necessarily low. Surveys of ectomycorrhizal fungal species in Brazil are comparatively fewer. Ectomycorrhizal fungi species were found in Amazonian forests (Singer & Araújo 1979), and in a general survey of the Agaricales in different ecosystems in the State São Paulo, in the southeast region of Brazil (Pegler 1997). Thomazini (1974) also reported ectomycorrhizal roots in some of the *cerrado* plants.

However sampling is still insufficient to present a picture of ectomycorrhizal fungi diversity, distribution and host plants. This can take several years of systematic sampling (Hunt & Trappe 1987). Furthermore, it is possible that initiation of ectomycorrhizal fruit bodies may be inhibited under unfavorable conditions (Redhead 1982)

No register of systematic mycological collections in the forests of Sergipe could be found. The Herbarium of the Federal University of Sergipe (ASE, in the Index Herbariorum) does not have any mycological collection and no report could be found in the literature. It is possible that there are collections made in Sergipe deposited in other herbaria, but it is not very probable. In the broad revision of Batista Vidal's work (Silva & Minter 1995), a great mycologist and collector in the northeast region of Brazil, only two species collected Sergipe are mentioned: *Leptosphaeria eustomoides* and *Sorosporium cryptum*. However, the absence of information on the occurrence of possibly ectomycorrhizal species may not by any means that these fungi are absent from Sergipe forests. Sampling of roots and fruit bodies is highly necessary, particularly due to the great disturbance of these forest remnants.

1.3.2.3 Dark septate endophytes as a mycorrhizal partner?

More recently, a group of uncertain taxonomic status, the dark septate endophytes (DSE) has been characterized as possibly mycorrhizal. Dark septate fungi have been recently subjected to a comprehensive review (Jumpponen & Trappe 1998). This term (or a variation of this, like 'dark septate', 'septate endophytes', 'dark septate fungi') has been indiscriminately used for dark, septate hyphae colonizing roots, either intercellularly or intracellularly (Jumpponen 2001). Differently from the AMF, root colonization by DSE have been found to include not only the root cortex, but also phloem sieve elements (Barrow & Aaltonen 2001).

Although they are more frequently described in artic and alpine ecosystems, they have also been reported in tropical and subtropical regions (see references in Jumpponen & Trappe 1998). These fungi are reported for several species of angiospermous families, including mono- and dicotyledons, as well as for members of gymnosperms and Lycopsida, Polypodiopsida, Equisetopsida and Psilotopsida (Jumpponen & Trappe 1998). However, no reliable comparison on frequency of occurrence of DSE throughout both regions can be made, as sampling effort has been much more intensive in temperate ecosystems. As Jumpponen & Trappe (1998) put it, "DSE appear to be found whenever they are thought".

Jumpponen & Trappe (1998) tentatively include these fungi in the Deuteromycotina, *Fungi imperfecti*. Indeed, DSE fungi are considered to have diverse phylogenetic origins, although a high similarity between isolates from diverse geographical origins and a very broad host range potential is reported (Schadt, Mullen & Schmidt 2001).

If isolation and identification of these fungi is still in a initial phase, their role on root physiology is much more unclear. The usual argument that DSE fungi are found in roots of apparently healthy plants should not be considered necessarily proof of their beneficial status (Newsham 1999). In the same way, the common occurrence of DSE throughout plants within ecosystems or habitats does not necessarily mean that these fungi are not pathogenic, as suggested for arctic and alpine sites (Väre, Vestberg & Eurola 1992). Conversely, a light pathogenicity by unspecialized fungi could also be widespread and stable in a plant community.

Reports on DSE effects on host plants vary from negative to positive (see references in Jumpponen 2001) and, just like with mycorrhizal studies, they are probably influenced by the experimental conditions (Jumpponen 2001). However, inconsistent results on DSE effects on host plants are also partially due to differences between the various fungus taxa and strains involved, and it is possible that only some of them may be able to form mutualistic associations (Jumpponen 2001). Actually, the absence of a clear taxonomic definition of the fungi encompassed in the DSE designation hinders any conclusive understanding on the functioning of this relationship.

Although DSE form interfaces different from the typical mycorrhizal interfaces, they have been found to form root associations functionally similar to mycorrhizas (Jumpponen 2001). The occurrence of a continuum between mycorrhizal symbiosis and pathogenic infection is suggested, at least in some groups as the dematiaceous fungi (Wilcox & Wang 1987). In his review on DSE

Jumpponen (2001) states that they must be considered mycorrhizal, at least under some conditions. Indeed, increased host P concentration and shoot growth after DSE infection has been reported (Haselwandter & Read 1982). However variable the host responses obtained with different DSE strains, the range of host responses seems similar to those obtained when considering mycorrhizal fungi (Jumpponen 2001). Although enhanced shoot P concentration was observed in two *Carex* species, only one species showed a correspondent growth response (Haselwandter & Read 1982), suggesting the existence of some degree of specificity.

DSE infection seems to be more important in increasing stressed environments than AMF colonization (Read & Haselwandter 1981), and these fungi are suggested to be better adapted to arid ecosystems than aseptate fungi (Barrow & Aaltonen 2001). In contrast, distinct ecological roles are suggested for DSE and AMF in populations of *Vulpia ciliata* (Newsham 1999). Furthermore, the coexistence of two or more fungi in the same host root system could provide greater variability in the responses to environmental stress (Johansson 2001).

1.3.3 Effects of the mycorrhizal symbiosis on plant fitness

The main physiological characteristic of the mycorrhizal symbiosis is the nutrient transfer between both partners. Basically, the host plant provides organic compounds to the fungus, receiving in return an increased uptake of some nutrients. The differences in plant growth and nutrient uptake between plants inoculated with AMF and control plants is called "plant responsiveness", and different indexes have been used (Menge *et al*. 1978, Plenchette *et al*. 1983). This responsiveness is affected by environmental conditions (Brundrett 1991).

Differences also exist between different mycorrhizal types and even between fungi species in their ability to provide equally high nutrient levels. For instance, within the AMF, different effects have been found between species (Mosse, Hayman & Arnold 1973, Schubert & Hayman 1986). Actually, plant responses to inoculation with AMF are affected by fungal species, plant host and soil conditions (Hayman 1982, Plenchette, Fortin & Furlan 1983a). In fact, variation between different endophytes are also related to soil characteristics like nutrient status and soil pH. Different responses have been reported between fungi species growing in different soils, being the soil pH one of the factors responsible for the observed variability in fungi performance. Soil phosphorus levels, particularly, plays an important role on determining the ability of AMF to colonize plant roots. Higher soil P levels leads to low root colonization by AMF species (Powell 1980a, Schubert & Hayman 1986), although responses may vary between different fungi species (Plenchette , Fortin & Furlan 1983a, Schubert & Hayman 1986). In tropical regions, where soil P levels are usually low, significant positive effects of AMF on plant growth and P nutrition are reported (Hayman, 1982, Howeler, Cadavid & Buckhardt 1983, Howeler, Sieverding and Saif 1987).

However, some other non-nutritional effects of the mycorrhizas are also frequently reported, being equally important for plant growth and survival. The most frequent cited, the increased resistance to pathogens, is of great importance to forest regeneration. These have been reported not

only in laboratory experiments (Yao, Tweddell & Désilets 2002), but also in natural ecosystems (Carey, Fitter & Watkinson 1992, Newsham, Fitter & Watkinson 1994, 1995a).

1.3.4 Role of mycorrhizas in forest ecology and reforestation

The above described effects of AMF on plant nutrition are, particularly, of great importance to understanding relationships in natural ecosystems, and to managing them for enhancing forest restoration. Forest environments present great spatial and temporal variation in the biotic and abiotic conditions, as well as the mycorrhizal inoculum source. The composition and structure of the vegetation results, partly, from the interaction between these factors.

As the seedling stage is a critical phase for the permanence of one individual plant or species under determined environmental conditions, the mycorrhizal association should also be particularly critical during the initial establishment. Mycorrhizal seedlings usually present higher growth rates (Mosse & Hayman 1971). In natural ecosystems, a seasonal variation in AM fungal colonization may happen (Daniell *et al*. 2001, Helgason, Fitter & Young 1999). Furthermore, fungal species colonizing seedling roots in a tropical forest have been found to vary with maturing of seedlings (Husband, Herre & Young 2002).

As the environment conditions arising from anthropic disturbances are not favorable to the germination and recruitment of seedling from species of late successional stages of forest ecosystems, this should reinforce the importance of mycorrhizal infection to the surviving and growth of seedlings planted in deforested sites. Furthermore, the local differences in the biotic and abiotic environment, reinforce the importance of regionalized research programs for the mycorrhizal use in the restoration from forest areas (Perry, Molina & Amaranthus 1987).

1.4 Objectives of this work

This work intended to investigate some features of two forest remnants in the coastal area of Sergipe. Some basic aspects of floristic composition and soil properties were studied in the forest fragments and in the adjacent deforested area, occupied in both sites by coconut plantations.

The objective of this study was to determine in which ways the cutting of forest may have implications on physical, chemical as well as biological properties of soils of two rain forest remnants on different soil types. The basic question is whether there is any potential for restoration of these areas with arbuscular AMF inoculum. This would greatly increase effectiveness of reforestation practices as well as implying in reduced costs.

The present study is a co-operation between Bremen University and the "Universidade Federal de Sergipe" (home university in Brazil). The National Council for the Scientific and Technologic Development (CNPq) is the Brazilian agency responsible for the PhD grant.

1.5 Outline of this work

A short description of the general structure of this work is presented below.

Chapter 2 - Study area

Characterization of the geological, pedological and climatologic context where both forest remnants are situated.

Chapter 3 - Soil properties and vegetation structure

Analysis of temporal and spatial variation in soil physical and chemical parameters in two forest remnants were performed. Comparison were always performed between forested and deforested (plantations) plots. Root and litter biomass distribution was also assessed, as well as plant cover also was analyzed through the method of parcels

Chapter 4 Spatial and temporal distribution of AMF in forest and adjacent plantation sites

Analysis of the distribution of the occurrence of spores of arbuscular mycorrhizal fungi and of the roots mycorrhizal colonization from the superficial soil profile (0-30 cm) was carried out.

Chapter 5 - Analysis of the AMF colonization in seedling roots

Analysis of spatial and temporal variation in mycorrhizal colonization in seedling roots were performed.

Chapter 6 - Analysis of the root system of some native tree species

From the analysis of previous papers dealing with tropical mycorrhizas, some species pertaining to families or genus cited as potentially ectomycorrhizal were selected. Roots from these species were collected for anatomical characterization, and the presence and quantification of ectomycorrhizas, arbuscular mycorrhizas and/or nodules were assessed.

Chapter 7 - Effectiveness of native AMF inoculum on Bowdichia virgilioides seedlings growth

Experiments aiming to test the effectiveness of native mycorrhizal inoculum on a native tree species, *Bowdichia virgilioides* were carried out. *B. virgilioides* is a common species in the area of study, with potential for use in reforestation programs.

Chapter 8 - General discussion

A general analysis for the results found in the different chapters is performed, being discussed implications for enhancing reforestation of the coastal region of Sergipe.

2 Study area

2.1 Geology and geomorphology

The present study was carried out on the coastal area of the Sergipe state, located in the Northeast region of Brazil (36°24'27" to 38°11'20"W and 9°31'54" to 11°34'12"S), and comprising an area of 21,994 km^2 or 2,26 % of the Brazilian territory (Figure 2.1). Brazil is the largest country of South America (8.5 million km^2), being situated in the South American Platform, the crystalline core of this continent (Almeida *et al*. 1981).

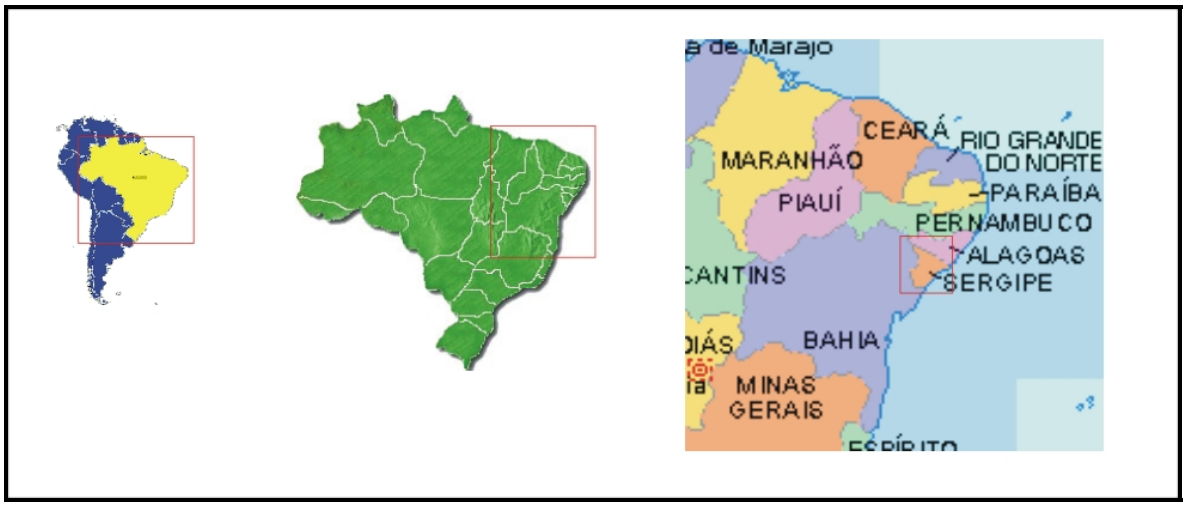

Figure 2.1 Location map of the Sergipe state in the northeastern region of Brazil.

Four main geological units are found within the Brazilian Northeast region (Almeida 1964, *in* Mabesoone 1966):

- the Precambrian basement with local covers of Cretaceous and younger sediments;
- the Paleozoic-Mesozoic (Devonian-Cretaceous) sedimentary Parnaíba basin;
- the Mesozoic sedimentary Recôncavo basin in the state of Bahia; and
- the younger coastal deposits, locally also occurring more to landward.

These coastal deposits are the substrate of the region in which the studied forest fragments are located. In a more recent and comprehensive classification (Almeida *et al*. 1981), this region is called Coastal Province (Figure 2.2). It is the youngest of the Brazilian geological provinces, having developed from the late Jurassic on, during the separation of the African and American continents at the ocean border, where rift-valleys and small sedimentary coastal basins can be commonly found (Almeida *et al*. 1981). It extends through more than 8,000 km along the Brazilian coast and can be subdivided in three zones, according depositional processes of basins during the continental drift

(Almeida *et al*. 1981). The region in which the Sergipe state is included, the intermediate part within the southern and northern sections, is the more recent zone, being formed during the late Cretaceous.

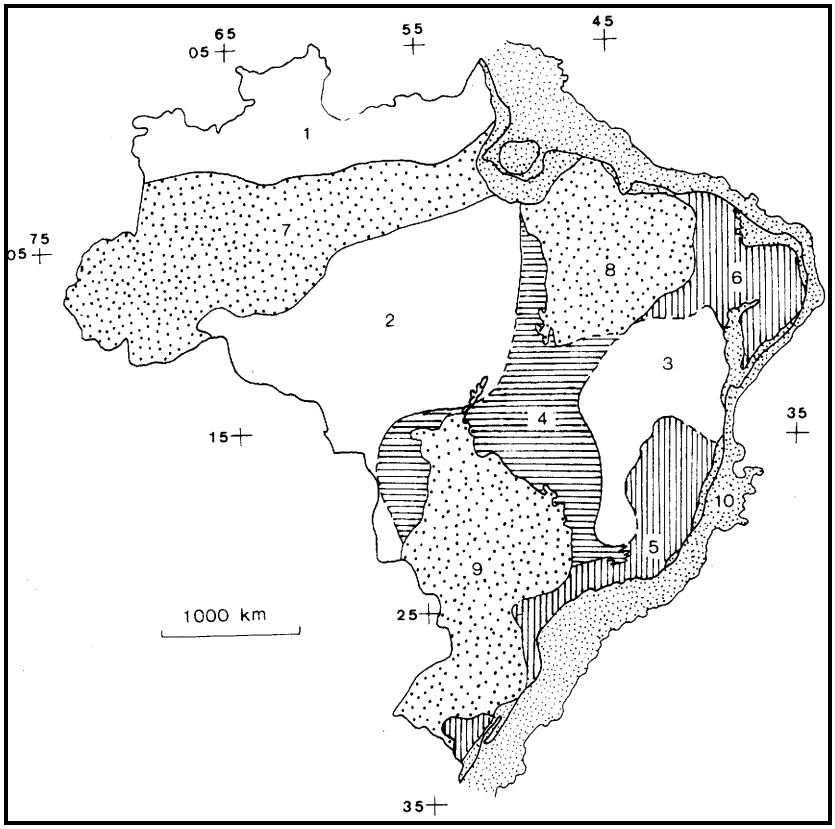

Figure 2.2 Structural provinces of Brazil (Almeida *et al*. 1981). (1) Rio Branco; (2) Tapajós; (3) São Francisco; (4) Tocantins; (5) Mantiqueira; (6) Borborema; (7) Amazonian; (8) Paranaíba; (9) Paraná; (10) Coastal Province and Continental Margin.

The physiographic feature of the emerged part of the Coastal Province is the coastal plain, a surface of low altitudes, composed of alluvial and marine sediments, which can be locally remobilized by wind action (Almeida *et al*. 1981). Particularly in the east-northeastern coast, being entirely within the southern Atlantic trade wind belt, wind (as well as sea-level history, and Holocene climatic change) had a preponderant role in controlling Quaternary coastal sedimentary processes in this area (Dominguez, Bittencourt & Martin 1992). The coastal plain is typically a narrow and long strip, sometimes discontinuous, along the Brazilian coast, extending landward as low tablelands of Cretaceous and Tertiary continental and marine sediments (Almeida *et al*. 1981). Along the east-northeastern coast of Brazil, sediment dispersal patterns have not changed since Pleistocene.

The east-northeastern coastal region of Brazil is divided in three physiographic units (Dominguez, Bittencourt & Martin 1992). This study presents the analysis of two the forests fragments which belong to two of these units: 1) the Quaternary strandplains consisting of Pleistocene and Holocene beach-ridges terraces among other coastal formations; and 2) the Tertiary Barreiras Formation, consisting of an almost flat surface, formed by unconsolidated and coalescing alluvial sediments. The Barreiras Formation (Figure 2.3), also locally called *tabuleiros costeiros* (coastal tablelands),

comprises an almost continuous strip along the entire coastal zone varying in width (10-40 km) and altitude (10-100 m), forming active cliffs when reaching the coastline (Dominguez, Bittencourt & Martin 1992). In contrast to the tablelands, the Quaternary plains have a more discontinuous distribution along the coast (Leão & Fominguez 2000). In Sergipe, tablelands cover about two thirds of the coastal zone, while Quaternary beach-ridges terraces varies from one to three kilometers wide (Bender 1959).

On the coastal tablelands composed by sediments of the Barreiras Formation grows a type of Atlantic rainforest (*mata de tabuleiro* or tableland forest). On the Quaternary beach-ridges terraces the *restingas*, vegetation type on sand deposits, are found (see 2.4 Vegetation).

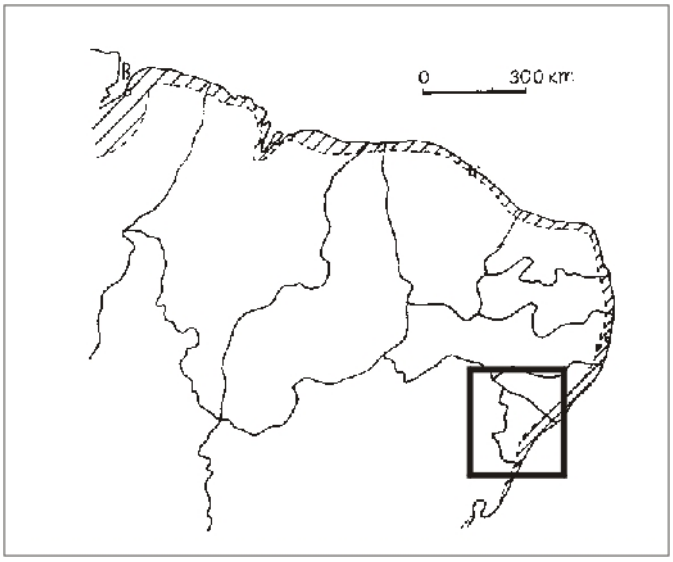

Figure 2.3 Distribution of the Barreiras Formation along the Northeast coast of Brazil (modified from Mabesoone 1966). Abbreviations refers to the names of states.

2.2 Soil types

The coastal zone presents the nutrient-poorer soils from Sergipe (Reis 1972). On the Quaternary beach-ridges, soils are quartziferous sands (*areias quartzosas*), developing from the sandy sediments from the Holocene (Reis 1972). These are, in a more recent classification (EMBRAPA 1999), called "Neosoils" (*Neossolos*). These include mineral soils, sometimes organic in the surface, usually deep, essentially composed of quartz, and so nutrients are virtually absent. Soil profiles are poor developed, with little differentiation between horizons. Sand grains of different textural classes may be present. This sandy nature implies in good drainage, small nutrient content (macro and micronutrients) and low cation retention capacity (Oliveira, Jacomine & Camargo 1992). Even in the superficial layers, this can rarely reach values higher than 2 meq/100g dry soil (Oliveira, Jacomine & Camargo 1992). These are acid to strongly acid soils, very low base saturation, and high saturation for exchangeable Al (Jacomine 1996). Although nutrient poor, adequate texture and depth allied to favorable climatic

conditions have made these sands suitable for extensive *Cocos nucifera* L. plantation in the *restingas* of Sergipe (Reis 1972).

The dominant soils in the Barreiras Formation from the south region of Sergipe are the Red-Yellow Podzols, with great variation in color and characteristics along its distribution (Reis 1972). These, together with the Red-Yellow Latosols, are the predominant soil classes in Brazil (Oliveira, Jacomine & Camargo 1992), with similar chemical and mineralogical properties (Jacomine 1996). Red-Yellow Podzols are non-hygromorphic mineral soils, with Fe_2O_3 values lower than 11% and soil horizons highly distinctive, but with great variation in morphologic and analytic features. Depth and texture also vary greatly (Oliveira, Jacomine & Camargo 1992). Soils from the southern region of Sergipe have been recently described as dystrophic Red-Yellow Podzols (Nogueira & Nogueira 1996). Jacomine (1996) describes the Yellow Podzols as a soil class formerly included in the Red-Yellow Podzols, mostly found in the coastal tablelands from East Brazil. Chemical and mineralogical properties are similar to the ones of the Yellow Latosols (Jacomine 1996). These soils present high bulk density (Souza 1996) and the hardened layers are found just bellow the A horizon and can reach greater depth of the B horizon. Quartz is the predominant component in the sand fraction, and Kaolinite is the main constituent from the clay fraction (Jacomine 1996).

Soils of the coastal tablelands are usually deep, with low natural fertility and reduced capacity of storage of water (Souza 1997). Another important feature from these soils is the presence of the *horizontes coesos* (hardened layers near the soil surface), an subsuperficial horizon hard to extremely hard when dry, and crumbly when wet (Jacomine 1996), with elevated bulk density, which can be higher than 1.5 kg dm^{-3} (Souza 1997). It is found both in Latosols and Podzols (Jacomine 1996). Smaller sand particles (< 0.05) predominate in the sand fraction of soil, and soil micropores (< 0.05mm) predominate over macropores (> 0.05mm), corresponding to 55-88% of the total soil porosity (Souza 1997). While micropores are mainly responsible for water retention, macropores act on drainage and soil aeration, and facilitate root growth (Kiehl 1979, *in* Souza 1997). Furthermore, these horizons show a low aggregation of clay particles and/or stability of aggregates, what causes the obstruction of soil pores, reducing infiltration rates and soil aeration and making these soils vulnerable to erosion (Souza 1997). This author suggests that the successive wetting and drying cycles may be responsible for the observed compact soil structure.

Although the presence of this hardened layer has been though to be the result of anthropogenic activity, reports on this layer under native vegetation are now abundant, and it seems to be a natural process occurring in soils of the coastal tablelands (Ribeiro 1998). In Yellow Latosols under primary forest this layer is present under the A horizon, reaching 50-60 in depth (horizons AB and/or BA). In Yellow Podzols, it is usually found at greater depths, and can comprise the b horizon (Jacomine 1996). Hardened layers have been found also in other undisturbed forest soils, approximately at 40cm (Ribeiro 1998), and around the AB (A3) and/or BA (B1) horizons (Fonseca 1986, *in* Jacomine 1996). Silva (1989, *in* Jacomine 1996) also found a high grade of density in virgin and cultivated soils, and Silva (1996, *in* Jacomine 1996) showed that under forest the global density in the BA (B1) horizon was greater than in the other horizons.

The acidity, small nutrient content and the presence of these hardened layers are problems when considering the agricultural use of the soils of the coastal tablelands (Rezende 2000) and many practices have been proposed in order to override them (Souza 1996). Ribeiro (1998), comparing a soil under agriculture and forest, showed that the three superficial horizons in the forest do not appear in the cultured soil, some cultures being carried out already on the hardened layers. Whether they have also the same effect on reforestation attempts is not very clear, due to the scarcity of controlled experiments in the region, but probable, and hence the importance of further comparative sampling under forest and deforested sites in the coastal tablelands.

2.3 Climate

Tropical climates with precipitation in winter (As) and autumn (As'), respectively A and As' in the Köppen classification system, occur in the eastern-northeastern coast of Brazil (Mabesoone 1966). Along the coast climate ranges from hot super-humid to hot semi-arid, with annual rainfall usually exceeding 1,250 mm, and maxima around 2,000 mm. Means annual temperatures along the coast varies from 24 to 26^{o}C (Nimmer 1989). Along this region, two basic elements are important in determining the general atmospheric circulation pattern: 1) air masses originating in the South Atlantic high pressure-cell, and 2) periodic advances of air masses of polar origin (Bigarella 1972, *in* Dominguez, Bittencourt & Martin 1992).

The localization of the South Atlantic high pressure cell (Figure 2.4) is remarkably stable, although it experienced small shift during the Holocene in response to climate changes (Dominguez, Bittencourt & Martin 1992). It is the main center of circulation and is situated at a rather fixed position, with only slightly seasonal variations, thus the extreme constancy in speed and direction of the trade winds. These constitutes the southeasterly and easterly winds dominant in the north-northeastern coast of Brazil (Dominguez, Bittencourt & Martin 1992, Leão & Dominguez 2000). The seasonal variation of the South Atlantic high pressure cell causes the zone of divergence between the Equatorial Atlantic and the Tropical Atlantic air masses (Nimmer 1989) to move northward during the summer and southward during the winter, oscillating between 10^{o} and 20^{o} S (Leão & Dominguez 2000). This seasonal migration of the divergence zone is related not only to the seasonal latitudinal shift of the South Atlantic high-pressure cell, but also to a thermal depression during the summer, caused by the heating of the adjacent land, forcing the trade winds to tangent the east coast of Brazil (Dominguez, Bittencourt & Martin 1992).

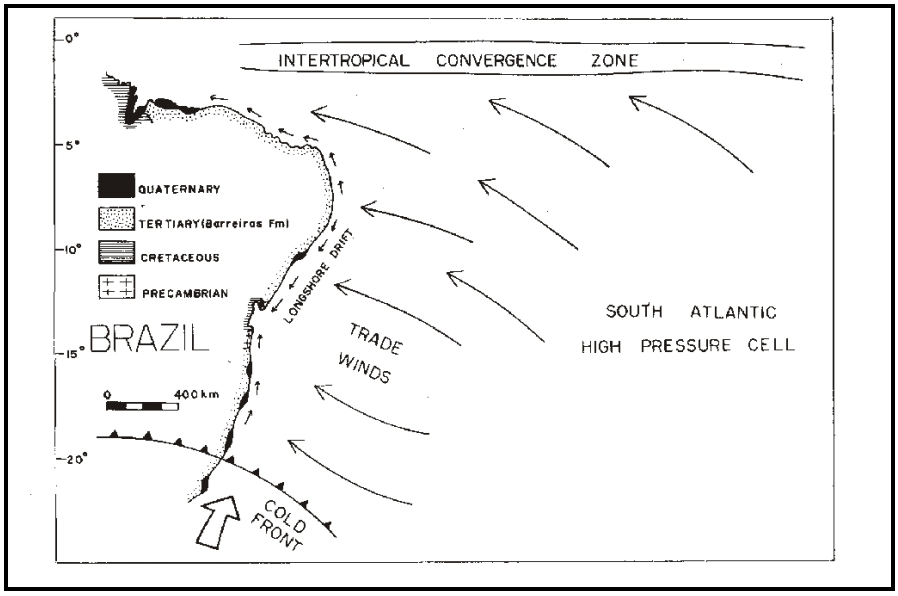

Figure 2.4 Schematic geological map of the Brazilian Northeast coast and main elements in atmospheric circulation in the region (Dominguez, Bittencourt & Martin 1992, after Martin *et al*. 1979).

In tropical coastal regions weather suffers the influence of the relatively regular sea breeze. In tropical America the Atlantic is the major source of its warm and humid air, brought with the trade winds (Rumney 1968). A strikingly climatic features in northeastern Brazil is the great uncertainty of annual rainfall (Rumney 1968). Not only the yearly total rainfall, ranging between 1,524 and 2,032mm (Rumney 1968), but also its distribution within the year (length of the dry season) show a considerable spatial variation along the coast (Figure 2.5).

The presence of mountains near coastlines aligned normal to the trades can cause an orographic uplift of the onshore winds, which, added to the thermal effects over the heated land surface, lead to a considerable winter rainfall in these regions (Rumney 1968, Walsh 1996). Ever-wet rainforest climates can be therefore found in regions relatively far from the equator (Walsh 1996). However, a temperature inversion produced by subsiding air of the subtropical anticyclones can prevent substantial vertical cumulus development, leading to dry weather periods, except where orographic uplift occurs (Walsh 1996). Where no markedly mountainous topography is found near the coast, as in Sergipe, this effect may not be enough to hinder a dry season. Sea breeze can thus penetrate far deeper inland (Rumney 1968).

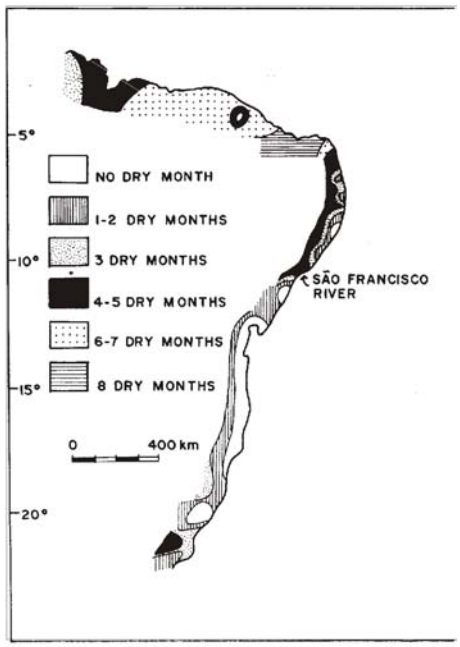

Figure 2.5 Distribution of the length of the dry season along the northeast coast of Brazil (modified from Dominguez *et al.* 1992).

Therefore, in Sergipe, as in the whole northeastern coast of Brazil, yearly rainfall is generally high enough to support tropical rainforest, as the humid winds coming landward brings rainfall with them (Weischet 1996). Precipitation in the coastal region, where the studied Atlantic rainforest fragments are located, is not only much more abundant than in the inner land (Figure 2.6), but also comparatively more regular.

Long-term climatic data with temperature (Figure 2.7) were found from the Global Historical Climatology Network (version 1, US National Climatic Data Center) for Aracaju, the capital of Sergipe state, also in the coastal zone (10°54S and 37°03' W, 3m above the sea level). Yearly mean temperature from the period between 1961 and 1990 was 25.9°C, with no month having mean temperature values bellow 20°C, as reported for tropical lowland climates (Walsh 1996). The mean annual precipitation for this time series (1961-1990) was 1570.5 mm, and rainfall being mostly concentrated in the winter, with approximately 80% happening in a period of six months. This rainy season in the winter months is characteristic of the eastern sector of Northeast Brazil, as the southeasterly winds blow perpendicular to the coast during this period (Rao, Lima & Franchito 1993). About 60% of the annual precipitation falls from April to July, while only 10 % falls during the September through December (Rao, Lima & Franchito 1993).

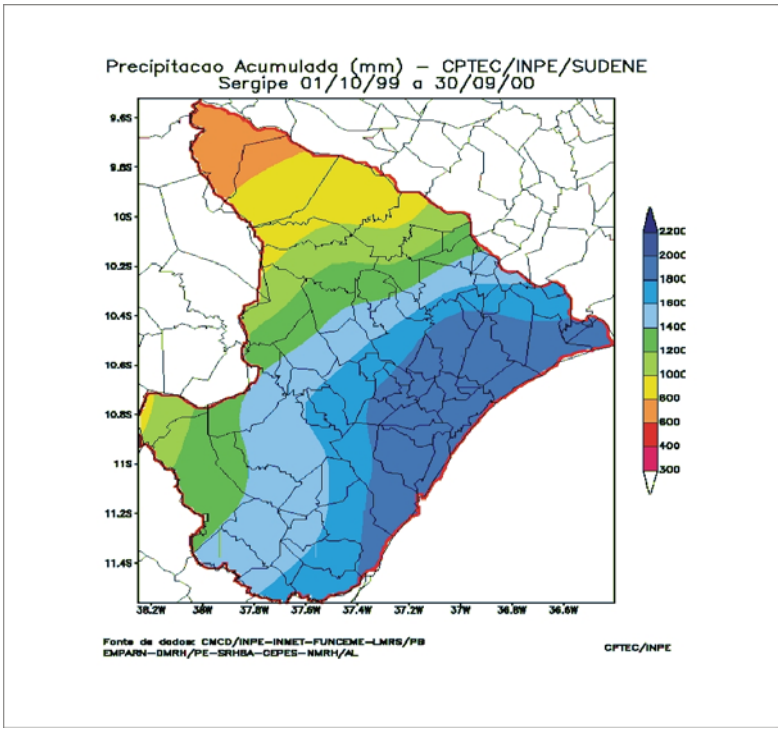

Figure 2.6 Rainfall zones in the Sergipe State (Source CPTEC/INPE/SUDENE).

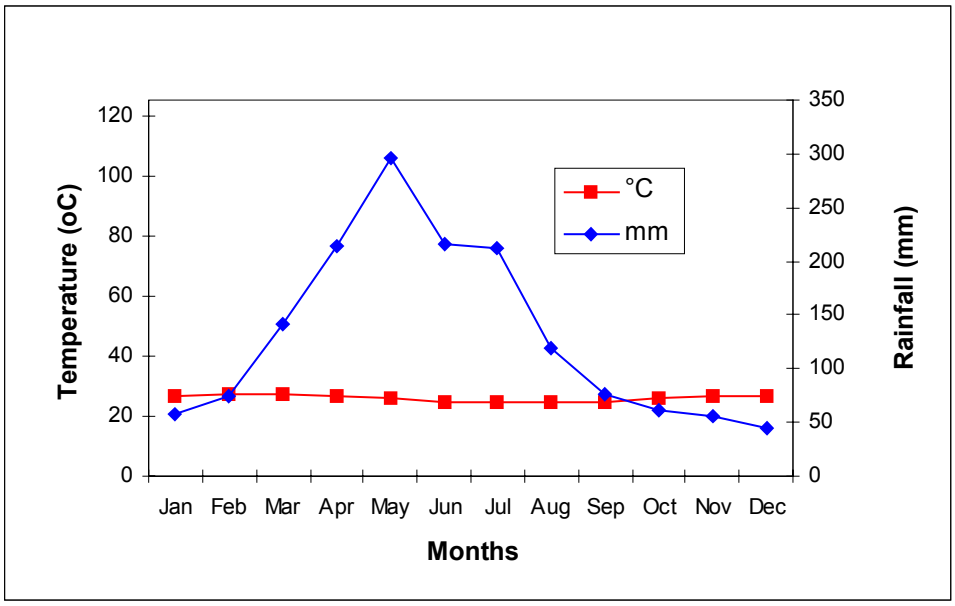

Figure 2.7 Mean rainfall and temperature for Aracaju between 1961-1990 (Source: GHCN 1 - The Global Historical Climatology Network, version 1- US National Climatic Data Center)

Long-term data series (1965-1984), though without temperature data, was found also for other meteorological stations along the coastal zone of Sergipe: Santa Luzia do Itanhy (11°21S and

37°27'W, 50m above the sea level), São Cristóvão (11°01S and 37°12'W, 20m elevation), as well as for Aracaju, showing a noticeably spatial variation in rainfall pattern within this region (Figure 2.8). The same peak in rainfall during winter is found, but the shape and intensity vary somewhat. A markedly between-year variation is nevertheless observed in all regions. Daily and yearly rainfall patterns vary within a broad range throughout tropical rain forest areas (Rumney 1968) and between-year differences were found to be greater, the greater the yearly totals. Areas with a definite dry season can face 25-40% variability (Rumney 1968), and between-year rainfall variability in rainforest areas can be higher, in absolute terms, than in the seasonal tropics, and is of both direct and indirect ecological significance, as the leaching potential is higher under higher annual rainfall (Walsh 1996).

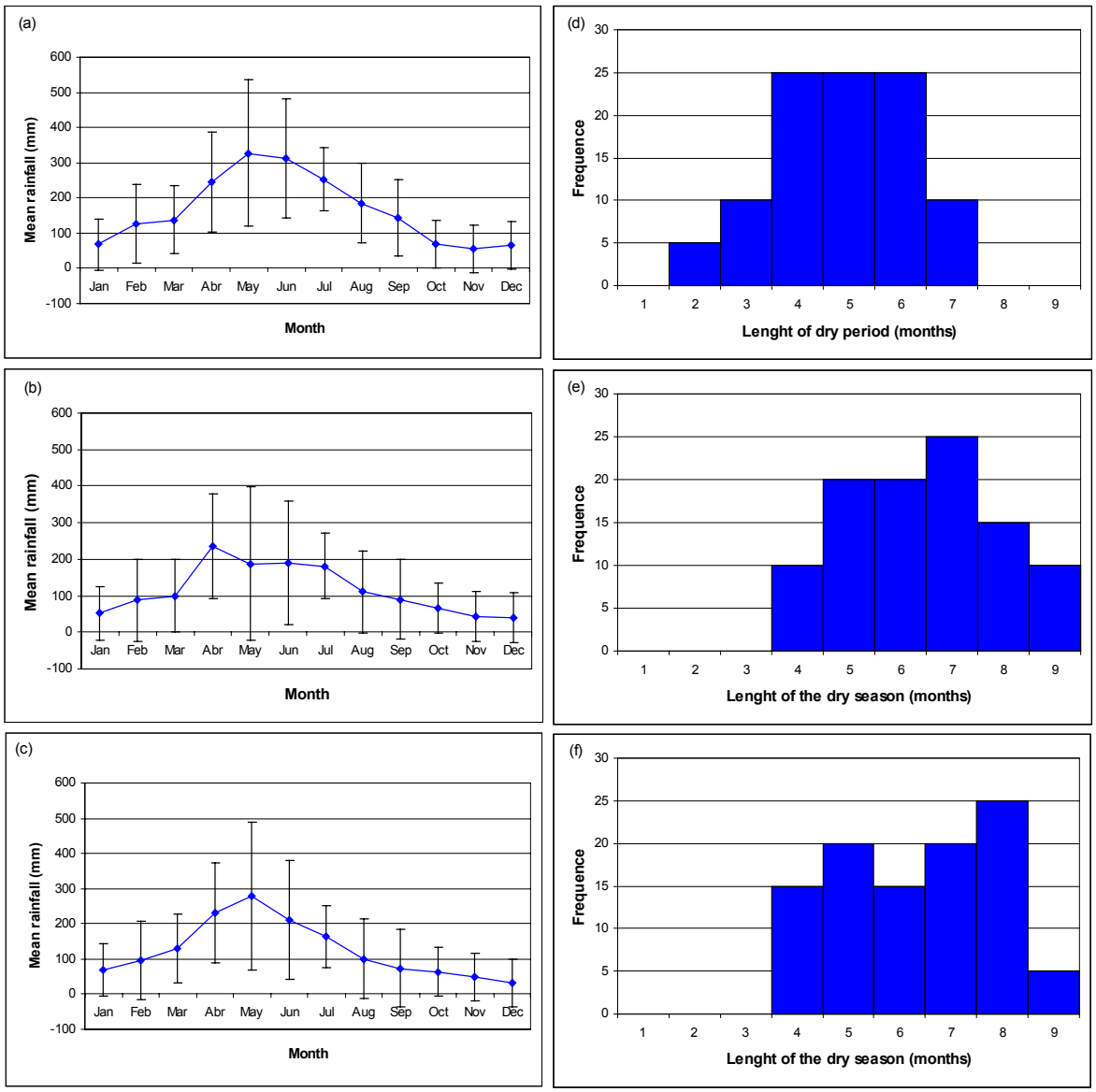

Figure 2.8 Mean rainfall (and standard deviation) and length of the dry season in Santa Luzia do Itanhy (a, d), São Cristóvão (b, e) and Aracaju (c, f) between 1965-1984 (Source: SUDENE).

Annual rainfall mean values were approximately between 1,400 and 2,000mm for all three meteorological stations. Santa Luzia do Itanhy, the southernmost from these stations, shows consistently higher mean, maximum and minimum rainfall figures (Table 2.1) than the data reported for the two other sites. Actually, although rainforests are thought to have usually high precipitation rates, areas with more than 3,000mm per year are relatively few, being less than 2,500mm over most of the tropical rainforest zone (Walsh 1996). Noticeably wetter years (in total rainfall amount and/or with longer wet season) may well be correlated with the occurrence of a major El Nino-Southern Oscillation event, as it was found to produce anomalously high rain for a year or more over the eastern Pacific and in northeast Brazil (Walsh 1996).

Table 2.1 Variation in yearly rainfall figures (mm) between Santa Luzia do Itanhy, São Cristóvão and Aracaju between 1965-1984 (Source: SUDENE). (Dry months: < 100mm; drought months. < 50mm; PI: perhumidity index).

	Sta Luzia do Itanhy	***São Cristóvão***	***Aracaju***
Mean	1985.28	1389.84	1489.96
Maximum	3438.5	2222.3	2359.3
Minimum	1452.3	866.9	803
No. dry months	4	4	4
No. drought months	0	2	2
PI	8.5	-0.5	1.5

Considerably spatial variation in temperatures and rainfall patterns are found throughout the tropics (Rumney 1968) and "this climatic diversity has important ecological implications and is responsible for some of the differences in species composition, structure, productivity, and dynamics found between rain forests" (Walsh 1996). Tropical climates may have well distributed or seasonal rainfall, being this condition (rainy periods alternating with dry or drought ones), more often found (Rumney 1968). Dry months are described as possessing less than 100mm rainfall, while drought months receive less than 50mm (Walsh 1996). Within rainforests, length and timing of dry seasons vary greatly, being its length greater with increasing distance from the equator (Walsh 1996).

Three to four (occasionally five) dry months can occur in evergreen seasonal rainforests (Walsh 1996). The Brazilian northeast coastal region, where a dry season of three to four months with rainfall between 1 and 2 inches (25.4 and 50.8mm) is found at the end of the year, is in the "limit of rainforest distribution", according to Rumney (1968), and rainfall figures in four to six months can lay bellow 2 inches (50.8mm). However, length of the dry season in Sergipe seems to be greater than it is usually reported for other tropical forest regions. Considering the mean value for the 20 years time-series (1965-1984), four dry months are found in the three stations, but no drought month in Santa Luzia do Itanhy (Table 2.1), which presented the shortest (four months) dry season (< 100mm), comparing to São Cristóvão and Aracaju (both with six months).

However, occasional extreme droughts may be of great importance in influencing rainforests, and these can remain unnoticeable when considering monthly averages. Thus a more comprehensive picture of the occurrence, length and distribution of dry periods is achieved through the analysis of individual years within long time-series rather than mean values (Walsh 1996). Consequently, analyzing individual years (Figure 2.8), as much as nine dry months (< 100mm) could be found in the two northern meteorological stations considered (São Cristóvão and Aracaju), although ranging from four to nine months, in these stations, and from two to seven months, in Santa Luzia do Itanhy.

Tropical rainforests have hot and wet climates, with little or no dry season, with mean rainfall ranging from 1,700 to over 10,000mm. Enough water, heat and light are thus available during the whole year, and do not limit productivity (Walsh 1996). Tropical rainforest climate can be defined as having "monthly mean temperatures of at least 18^{o}C throughout the year, an annual rainfall of at least 1,700mm (and usually above 2,000mm) and either no dry season or a short one of fewer than four consecutive months with less than 100mm" (Walsh 1996). However, with increasing distance from the equator, the dry season, whenever present, can becomes progressively longer (Walsh 1996).

Several attempts were made build a comprehensive classification of climate and vegetation (e.g. Holdridge 1947, Thornthwaite 1948, Walsh 1992). Thornthwaite's classification system, although widely used, do not fits well for the tropics, where other factors than rainfall and the potential evapotranspiration (estimated from rainfall and day-length), like humidity and wind are also important (Walsh 1996). According to Holdridge's system, tropical forests with annual precipitation between 1,000 and 2,000mm should be considered dry forests, while moist forests should receive between 2,000 and 4,000mm per year. However, criteria used for classifying the different divisions used by Holdridge (1948) is questioned by Walsh (1996).

The perhumidity index (PI) (Walsh 1992, Walsh 1996) represents another tentative classification of tropical climates relating to vegetation. Tropical lowland rainforest climates are thus subdivided in superwet, wet and wet seasonal, with PI values ranging from 5 (margin) to 24 (perennially very wet areas) (Walsh 1996). Although it is suggested the use of monthly rainfall series covering individuals years, PI values for the three above mentioned stations were calculated with the mean rainfall data for the time-series available (1965-1984) for comparison with the data provided (Walsh 1996), giving surprisingly results (Table 2.1). Santa Luzia do Itanhy has a PI value characteristic of wet seasonal climate (PI = 5-9.5), which lies at the seasonal margin of the tropical rainforest zone. It is characterized by a more or less regular short to moderate dry season and moderate to high annual rainfall, the longer or more intense the dry season in this zone, the higher the annual rainfall that is needed to help offset it. This area is characterized by evergreen seasonal rainforest or allied formations) Walsh (1996). However, PI values for Sao Cristóvão and Aracaju lay well bellow this range, and could be considered tropical wet-dry, bearing semi-evergreen seasonal forest (Walsh 1996). The results are particularly interesting, as the three localities are not very far from each other (approximately 50km) and it would be important to know whether forests in these regions also differ in species composition and deciduousness grade. Unfortunately, not much is left from the coastal forests in the central part of Sergipe (see 2.4 Vegetation).

Rumney (1968) describes the forest occurring in the northeast coast of Brazil as a "dominantly semideciduous forest, although the variety of vegetation and climatic types ranges from thorn scrub woodland to selva in scattered patches" (Rumney 1968). From Bahia southward rainfall is more uniformly distributed, allowing the occurrence of rainforest (Rumney 1968). So, following Rumney's description, coastal forests in Sergipe should be considered semideciduous, since "in tropical rainforest no really dry season normally occurs". But although no consistent work has been already published about forest tree phenology in this region, the absence of deciduous species as dominant component in the phytosociological survey done in the *Mata do Crasto* (see 3.2.1. Study sites), Santa Luzia do Itanhy (Landim *et al*., unpublished), and the occasional and sparse occurrence of deciduous tree species (mostly members of Bombacaceae and Bignoniaceae) in forest fragments in this region (pers. obs.), makes this characterization inaccurate. Köppen's Am climate regions present a dry season which can be "partly offset by very high wet season rainfall", thus allowing the growing of a "evergreen seasonal forest or monsoon forest" (Walsh 1996). Due to the annual rainfall amounts prevailing in the coastal zone of Sergipe, at least in the southern region, and to the nature of the forest remnants present, this definition ("evergreen seasonal forest") seems to be more adequate to describe it. Furthermore, dry periods are an important factor in rainforests (Walsh 1996), and their impact depends not only on its intensity but also with forest type composition and structure, where selection for species less vulnerable to a longer dry season may have taken place.

However, any classification of vegetation based on climatic averages is subject to error or as other climatic and non-climatic factors may have impact on soil moisture, and therefore, on consequences of dry season. Also, means do not reflect periods with extreme values, that may be more important in defining vegetation characteristics and distribution, and rainfall means can minimize the true frequency of dry periods. Last but not least, these means may not represent long-term processes, as climatic events have showed to vary significantly "even within the past century in some rain-forest regions" (Walsh 1996). Therefore, the existence (and grade) of man-induced changes on climate, is of great importance when considering the future of tropical forests. Although the appropriate time-scales of climatic change relevant to rainforests is not clear, significant changes have been registered in tropical regions, including some rainforest areas, and rainforest in marginal areas in the trade wind belt are said to have suffered important changes (Walsh 1996).

2.4. Vegetation

Despite its small area, many different vegetation types are present in Sergipe, like the *cerrados* (savanna-like neotropical formation), tropical sub-evergreen forest, tropical dry forest, *caatinga* (xerophytic vegetation form), mangroves, *restingas* (see definition bellow) and dunes (Reis 1972). *Capoeiras*, or secondary forests, were also found, being then described as a dominant secondary vegetation form growing in the regions with higher precipitation.

On the low fertility soils of the coastal tablelands, much of the natural vegetation was removed in order to establish different kinds of cultures (sugarcane, corn, bean, cassava, coconut and fruit culture). The most important are the sugarcane, due to the great extension it occupies, and fruit culture,

mostly citrus in the southern region, and coconut (*Cocos nucifera* L.) along the coastal zone. Actually, extensive coconut plantations occupy a great part of the original area of the *restingas* in the state. Aviculture and livestock (cattle) are also economic activities in the coastal region, this last having most impact on soil structure, due to soil compaction. Already in the seventies (Reis 1972) eroded areas were observed in the state, as consequence of inadequate use.

The Atlantic forest region, a narrow fringe of tropical rainforest along the coast, comprises several different vegetation types, mainly evergreen- and semi-evergreen forests, but also *restingas* and mangroves (Rizzini, 1963, Romariz, 1972). In the present work, coastal forest remnants of two types, evergreen seasonal rainforests (*sensu* Walsh 1996) and *restinga* forest, were studied. In the State of Sergipe, in the beginning of the century, 41,07% of the area of Sergipe were occupied by forests (Campos, 1912). The fragments of Atlantic forest are located in the coastal area comprising a strip of approximately 40 km of width, located around 10°30' to 11°30' S and 37° to 38°30' W.

Not much information is available on Sergipe rainforests, as most of the research already done remains unpublished. Franco (1973) described the structure of Atlantic forests in the state through indirect methods, analyzing tree trunks after slash-and-burn in forest regions. More recently, some information has been gathered (Siqueira *et al*. 1999, Siqueira & Ribeiro 2001) aiming to characterize ecologically these fragments and to establish conservation measures. Coastal forests in the Sergipe comprise 53.182 ha (Siqueira *et al.* 1999), but images (TM - LANDSAT – 5) on which this quantification was based date from 1988. This figures may be even lower, as these areas, although protected by law, still faces deforestation.

Floristic inventories in these remnants have been conducted since 1995 (Landim, unpublished). Some preliminary results have been published (Landim & Siqueira 2001a, Landim & Landrum 2002), and show that much of the floristic diversity remains to be found out. Four *Campomanesia* (Myrtaceae) species not previously reported as occurring in Sergipe were recently registered (Landim & Landrum, 2002). One of them, *C. viatoris*, formerly cited as a rare species (Landrum 1986), is actually locally abundant and widely spread. Studies considering centers of plant endemism along the Brazilian Atlantic rainforest (Thomas *et al*. 1998), may be somewhat biased, due to the little available information on the flora of regions not so extensively collected (Gentry 1992). Thus, an increase in sampling efforts is essential to understand plant biogeographical patterns, to estimate diversity in forests along the entire coast, and last but not least, to establish conservation guidelines.

A 1-ha phytosociological survey was carried out in the greater forest fragment in Sergipe, the *Mata do Crasto*, in Santa Luzia do Itanhy (Landim *et al.*, unpublished). A total of 2121 trees, pertaining to 80 species and 20 families, were found. However this figure does not represent the real diversity of this fragment, as a great number of trees could not be properly identified, being unfertile. A characteristic feature of this forests is the interruption of the canopy by emergent trees of *Sclerolobium densiflorum* (Leguminosae Caesalpinioideae). The most abundant families were Celastraceae Myrtaceae, Sapotaceae, Leguminosae Caesalpinoideae, Lecythidaceae, Lauraceae, Annonaceae, Leguminosae Mimosoideae, and Rubiaceae. As in other coastal Brazilian forests (Silva & Leitão Filho 1982, de Jesus 1987, Pexoto & Gentry 1990, Tabarelli & Mantovani 1999),

Leguminosae and Myrtaceae are important families both in number of species and trees. A remarkable feature of this fragment is the great importance of the Celastraceae, mainly due to the high abundance of *Maytenus* cf. *obtusifolia* Mart., what is not reported in other surveys (Silva & Leitão Filho 1982, Tabarelli & Mantovani 1999).

Not much information is available on the fauna of these forest remnants, but this does not mean an absence of animals inhabiting these areas, but an scarcity of faunal inventories within them. For example, if reptilians of the Brazilian northern region are poorly known, no comprehensive herpetological study had been carried in the northern coastal region (Fernandes 1995). However, a new species of snake was found in the Sergipe and Alagoas coast (Fernandes 1995). Species of this genus are reported to show a more restricted home range than other rainforest snakes, and may be more subjected to face local extinction following habitat disturbance. More recently, Stevens & Husband (1998) revealed the presence of nine species of small mammals in the *Mata do Crasto*, none of them had been captured outside the forest patches. Despite the high fragmentation and the small forested areas, the forest remnants in Sergipe still retain important faunal, besides floristic, diversity.

Restingas are Quaternary sand deposits of marine origin laying parallel to the littoral (de Lacerda, de Araújo & Maciel 1993). Due to the extensive Brazilian coastline (over 7000km) and the tropical climate prevailing in most of it, *restingas* are a vegetation form commonly found, although the preferential human occupation in coastal areas have already destroyed much of this ecosystem.

As well as being a geomorphologic concept, it is also a floristic one, as defining the vegetation type growing on these deposits. Its flora is still in great part unknown (de Lacerda, de Araújo & Maciel 1993) but it seems to be related to the Atlantic rainforest, being some species common to both systems (Rizzini 1979 *in* de Lacerda, de Araújo & Maciel 1993), despite differences in soil types. However, it does not defines a floristically and structurally homogeneous system. Local differences in environmental factors lead to the formation of a complex mosaic of plant communities (Ormonde 1960 *in* de Lacerda, de Araújo & Maciel 1993). Although much of the studies in *restingas* are concentrated in the southeastern coast, with some few and scattered data from the northeast, regional differences, and local endemisms have been cited (de Lacerda, de Araújo & Maciel 1993). Although only periodically or permanently flooded forests were described by these authors as restinga formations, non-flooded forests can also be present.

Conservation status of *restingas* in Sergipe is even worst. Not only a smaller area is left (47,380 ha, according to Siqueira *et al.* 1999), but also these areas may face an even greater anthropogenic pressure, being of high value for the expansion of urban areas bordering the beaches, and/or for the establishment of touristic facilities. Its flora, although with some elements common to the forests on the coastal tablelands (Landim, unpublished), presents also components exclusive to it. As in other areas (de Lacerda, de Araújo & Maciel 1993), *Restingas* along Sergipe coast present a varied habitat structure, ranging from bare or almost bare sand dunes to forests. The latter seems to cover a relatively smaller area from Sergipe's *restingas*, however no reliable data is still available. This diversified habitat structure may be responsible for a greater species diversity, but further sampling within the whole coastal zone must be carried until a reliable assessment can be made.

3 Soil properties and vegetation structure in rainforest remnants and adjacent degraded areas in Sergipe, Northeast Brazil

3.1 Introduction

The idea that tropical forests grow on nutrient rich soils is since long substituted for a more realistic picture of tropical soils, where nutrient is frequently a limiting factor (Jordan 1985). This fact had not, however, prevented the establishment of frequently impressive forest ecosystems throughout the tropics. Much of these forests grows actually on very poor soils, being the nutrient pool mostly concentrated in the living biomass. If this fact has theoretical implications for the nutrient cycling in these ecosystems, it has also practical implications for conservation and reforestation. These forests may be not only sensitive to disturbance, but, after disturbance, may not easily recolonize these areas. Furthermore, removal of plant cover may lead to important soil changes, due to erosion and nutrient leaching, that in tropical areas, due to intensive rainfall, may be rather high.

The elevated rates of deforestation in tropical areas in general, and the low forested area left in Sergipe (see 2.4 Vegetation) makes forest restoration a subject of extreme importance. In order to maximize resources (human and material) allocated to reforestation programs and avoid unsuccessful results, any reforestation plan should begin with an complete and accurate assessment of the soil features in the studied region. Even if more ecological based programs (e.g. with native tree species, and managing the native mycorrhizal inoculums) are to be established, it is of primary importance to assess how deforestation have affected these properties, and, therefore, in which substrate these tree species, and the mycorrhizal inoculum, are intended to grow in.

Much of the literature on impact of deforestation for logging, agroforestry or pasture establishment in tropical areas are concentrated in Amazonian ecosystems. However, due to the wide latitudinal range within the Brazilian coastal rainforests, even the few information on this subject available for Atlantic rainforests can only points to some processes and trends, which may differ somewhat locally. Soil changes following deforestation are dependent not only on soil type, topography, and climate, but also on type of land use after forest removal.

The present chapter aims to compare soil and plant cover in two forest remnants on different soil types in Sergipe, Northeast of Brazil, and to determine in which extent forest removal affects physical, chemical, as well as biological properties of these soils. It is the first step of part of a greater project on the role of the arbuscular mycorrhizal interactions in this ecosystem aiming to subside plans of forest recovery. The results presented here will, therefore, be used to explain spatial and temporal patterns of distribution of mycorrhizas species and mycorrhizal root colonization in the following chapters.

3.2 Methods

3.2.1 Study sites

The present study was carried out in two fragments of the Atlantic rain forest of Sergipe, North Eastern Brazil: 1) the *Mata do Crasto* (11°22'47" S, 37°24'51" W), in the vicinity of the village of Crasto, in the Santa Luzia do Itanhy county, possibly the greater rainforest fragment in Sergipe (approximately 1000 ha), and 2) the *Fazenda Caju* (11°07'3" S, 37°11'02" W), an experimental research station of the "Centro de Pesquisa Agropecuária dos Tabuleiros Costeiros" (Center of Agricultural Research of the Coastal Tablelands - CPATC), in ltaporanga D'Ajuda, with a small fragment of *Restinga* forest (Figure 3.1).

Floristic inventories have been conducted in this area (with greater sampling effort in the Crasto site) since 1996, and an floristic and phytosociological survey was carried in this site on 50 10x20m plots, amounting 1ha (Landim *et al.*, unpublished). Data from inventories were used for choosing tree species and sampling study areas.

The medium annual precipitation varies from 500 to 2,000 mm. Precipitation is greatly seasonal, with about 80% happening in a period of six months (See 2.3 Climate). Due to this rainfall seasonality, two sampling periods were defined: 1) end of the dry season (February), and 2) end of the rainy season (August). However, atypical rainfall patterns caused this sampling scheme to be slightly changed (see bellow, 3.3.1. Climate and microclimate).

3.2.2 Sampling design

Sampling was done in the end of the dry and rainy season in two sites: *Mata do Crasto* ("Crasto" from now on) and *Fazenda Caju* ("Caju"). Sampling was done within transects, one in each site, ranging from the cocoa plantations adjacent to the forest ("plantation" plots) towards the forest ("fringe forest" and "forest" plots). Each plot measured 20 x 10 m, being located 50 m apart from each other along the transect.

3.2.3 Sampling parameters

3.2.3.1 Climate and microclimate

Data on precipitation and temperature variation during the sampling year were obtained from the weather station in Aracaju (Ministério da Aeronáutica, Diretoria de Eletrônica e Proteção ao Vôo, Aracaju), 75 and 25 km north from Santa Luzia do Itanhy and Itaporanga D'Ajuda, respectively, also in the coastal region.

Soil (upper five centimeters) and air temperatures (10 centimeters above soil surface) in the three plots were recorded hourly during the sampling days using mercury thermometers.

3.2.3.2 Soil chemical and physical parameters

Soil physical and chemical parameters were taken from five soil cores randomly distributed in each plot. Each core was divided in five depths: 0-5, 5-10, 10-15, 15-20 and 20-30 cm, and transported in plastic bags kept in ice until processing in laboratory. In the February sampling, the five soil samples were mixed in a composite sample. In the November sampling, three samples were obtained per plot, being analyzed separately.

Analysis of the physical soil properties were made by the Laboratory of Soil Physics of the Brazilian Company of Agricultural Research (EMBRAPA) unit in Sergipe, CPATC, under the coordination of Dr. Fernando Cintra. The distribution of the size of the mineral particles of the soil (texture), bulk density, the retention of water under 10 kPa (field capacity) and 1500 kPa (wilting point) were determined and the available water in the soil was calculated (as the difference between the field capacity and wilting point), following the manual of methods of physical soil analysis of EMBRAPA/ SNLCS (1979).

Soil chemical analysis were performed by the Laboratory of Environmental Chemistry, of the Federal University of Sergipe (UFS), by the supervision of Prof. Dr. José do Patrocínio Hora Alves. All analysis follow the methods described in Silva (1999). Soil pH was obtained in water (1:2.5). Exchangeable calcium, magnesium and aluminum values (me/100 g, which is numerically equal to the SI unit cmol(+)/kg [Anderson & Ingram 1998]) were obtained after extraction in 1M KCl. Calcium and magnesium were determined by complexiometric methods using EDTA. Aluminum was determined after titration with NaOH, using bromothymol blue as indicator.

Available phosphorus (mg/kg), sodium and potassium were extracted with Mehlich I (0.05 M HCl + 0.0125 M H_2SO_4) in the proportion 1:10 soil:solution. After extraction, the absorbance of the phosphomolybdate compound, produced by the reduction of the ammonium molybdate with ascorbic acid, was read at 660 nm in a Micronal B 380 spectrophotometer. Exchangeable sodium and potassium (me/100 g) was determined after reading the extract in a Micronal B462 flame photometer, with the appropriate filters. Potential acidity (H^+ and Al^{3+}) was determined using a calcium acetate solution as extractor and after titration with NaOH, using phenolphthalein as indicator.

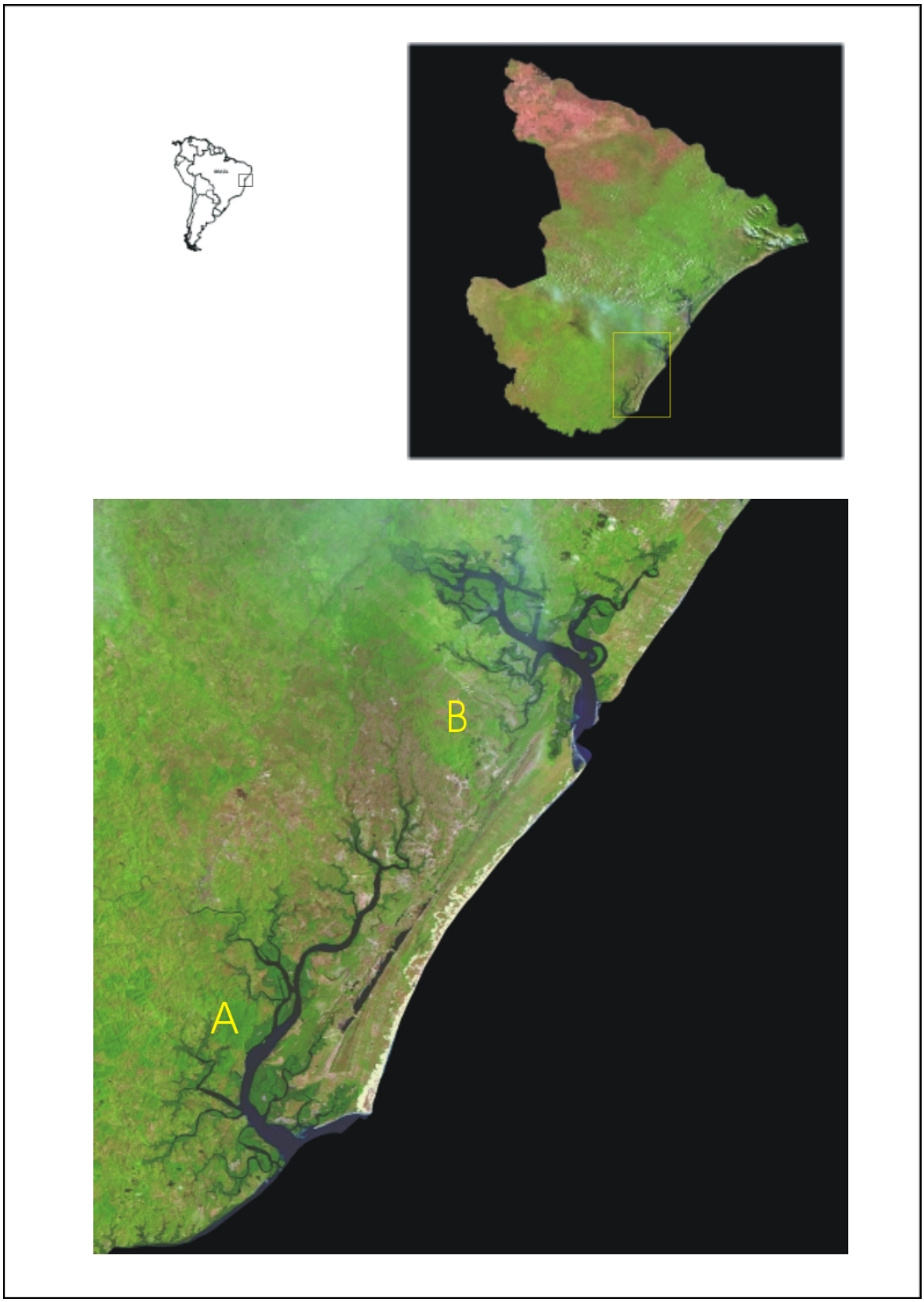

Figure 3.1 Localization of the study sites in the Sergipe sate (figure above right): (A) Crasto d (B) Caju sites (Landsat images, EMBRAPA-CNPM, 2002).

3.2.3.3 Litter biomass and nutrient content

In February the sampling was done in $1m^2$ quadrats randomly distributed in each plot (data not presented). In November five $1m^2$ quadrats were used for sampling in each plot. Litter biomass was oven dried at 60^oC for at least 72h, until constant weight, and expressed in dry weight.m^{-2}.

3.2.3.4 Root biomass

Root biomass was collected in five soil cores randomly distributed in each plot. Each core was divided in five depths: 0-5, 5-10, 10-15, 15-20 and 20-30cm and transported in plastic bags kept in ice until subsequent processing in laboratory. Soil from each bag was wet-sieved through a 1-mm mess screen and roots recovered were oven-dried at 60^oC for at least 72h. As some mineral particles were still attached to roots, the final root biomass values were obtained as ash-free dry weight after burning these samples in a muffle furnace at 550^oC for 4 hours.

3.2.3.5 Floristic composition and vegetation structure

All trees with diameter at breast height (D.B.H.) equal or greater than 5 cm in each plot were measured. Plant species, height and D.B.H. were registered and, whenever possible, samples were taken to make exsiccates for further species identification in the Herbarium (ASE) of the Universidade Federal de Sergipe. With the field collected data, quantitative parameters (species abundance, frequency, and basal area, or dominance), which relative counterparts were summed to provide an Importance Value (I.V.) as described by Mueller-Dombois & Ellenberg (1974). This importance value presents a maximum value of 300, in monospecific stands. Ecological indices (Table 3.1) were also calculated for each plot in each sampling site.

Table 3.1 Ecological indices used to characterize vegetation present in plots sampled.

Indices	***Author***
Similarity (S_s)	Sorensen (1948)
Richness (R1)	Margalef 1958
Diversity (H')	Shanon & Weaver 1949
Evenness (E5)	Alatalo 1981

3.2.4 Statistical analysis

A non-parametric one-way ANOVA (Kruskal-Wallis *H*-test; Sokal & Rohlf 1998) was carried on each data set to check for significant differences between sites, plots, depths or seasons. When variables differed significantly between groups, pairwise comparisons were then performed using the Mann-Whitney U-test. Significance level was $\alpha = 0.05$. The relationship between variables were tested using Spearman's non-parametric correlation by ranks (Zar 1999). Statistical analysis were performed with the statistical program SPSS (SPSS Inc., 1989-2001).

3.3 Results

3.3.1 Climate and microclimate

Sampling in February should represent conditions at end of the dry season (see Chapter 2.3. Climate), but this February (2000) had an extremely atypical high rainfall (Figure 3.2). Therefore, the next sampling (intended to represent the rainy period and initially thought to take place in August) was postponed to November, in order to sample somewhat drier conditions. As shown below, the two sampling periods represented, nevertheless, respectively, drier and wetter conditions.

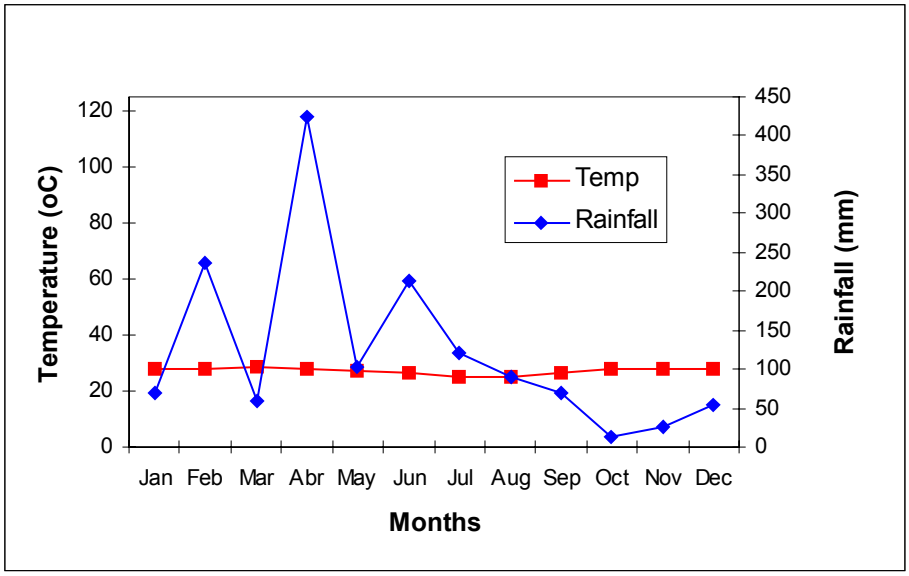

Figure 3.2 Climatic diagram for Aracaju during the study year (Source: Ministério da Aeronáutica, Diretoria de Eletrônica e Proteção ao Vôo, Aracaju, Sergipe).

As expected, due to the absence of significant plant cover, plantation plots presented in both sites (Crasto and Caju) and sampling periods, the greater soil and air temperature values and amplitudes (Table 3.2, Table 3.3). The fringe and forest plots in both sites showed a somewhat similar pattern with lower temperature peaks even in the hottest time of day. Some of these variations may be caused by small gaps in forest cover allowing a variable amount of light incidence on the soil along the day.

As it was said above, higher soil temperature amplitudes were observed in the plantation sites, but none so strikingly as the one obtained in the Caju site in February (6^{o}C). In the fringe and forest sites, it varied between 0.5 and 2^{o}C. Outstandingly was also the air temperature amplitudes obtained in the plantation plot of the Crasto site (13^{o}C). The air temperatures in the Caju site were not assessed in this sampling period but it may have been so high as this. Although the Caju fringe

plot presented air temperature amplitude value (7°C) so high as the plantation plot (6°C) in November, this was not reflected in the soil temperatures which showed an amplitude value of only 0.5°C.

Table 3.2 Soil temperature variation during sampling (February and November /2000) in the Crasto and Caju sites (from 10 to 17:00).

		Caju				*Crasto*			
Plot	***Month***	***Min.***	***Max.***	***Mean***	***Amplitude***	***Min.***	***Max.***	***Mean***	***Amplitude***
Plantation	Febr.	26	32	29.4	6	25	27	26	2
	Nov.	30	33	31.4	3	29	31	30.3	2
Fringe	Febr.	25	26	25.6	1	24	25	24.5	1
	Nov.	24.5	25	24.9	0.5	24	26	25.3	2
Forest	Febr.	24	26	24.8	2	24	24.5	24.1	0,5
	Nov.	25.5	27	26.3	1.5	25	26	25.4	1

Table 3.3 Air temperature variation during sampling (February and November /2000) in the Crasto and Caju sites (from 10 to 17:00). (data for the February sampling in the Caju site not available)

		Caju				*Crasto*			
Plot	***Month***	***Min.***	***Max.***	***Mean***	***Amplitude***	***Min.***	***Max.***	***Mean***	***Amplitude***
Plantation	Febr.	-	-	-	-	23.5	36.5	28.4	13
	Nov.	28	34	31.8	6	30	37	35.0	7
Fringe	Febr.	-	-	-	-	23	25.5	24.2	2.5
	Nov.	25	32	27.6	7	27	31.5	29.4	4.5
Forest	Febr.	-	-	-	-	23	24.5	23.8	1.5
	Nov.	26	30	27.9	4	27	30	29.0	3

No attempt was made to assess temperature variation along the soil profile, but it may be possible that the higher temperatures in the plantation plots would not represent a stress factor for the soil microflora and fauna, although it may be to young seedlings. Seedling establishment in both sites and sampling periods will be discussed elsewhere (Chapter 6).

3.3.2 Soil physical and chemical parameters

Differences in soil type and physical and chemical properties of soil were found between sites, plots and sampling season. As all data on soil physical features in each plot were obtained through compound samples, no statistical test could be performed to assure the significance of the differences found. This information will be firstly presented graphically, to illustrate the general trends, but the corresponding data are presented in the Appendix A. Chemical parameters, in the November sampling, were done through five random samples per plot, and therefore tests for this sample time could be conducted and are presented bellow.

3.3.2.1 Texture

While in the Caju site soil is sand in all plots, in the Crasto site, it ranges from loamy sand (plantation) to sandy loam (fringe and forest plots), in February (Figure 3.3). In November, a slightly difference was found, and plantation plots presented a sandy loam soil, while in fringe and forest plots a vertical variation was found along the soil profile (loamy sand prevailed in the fringe, and sandy loam in the forest plot).

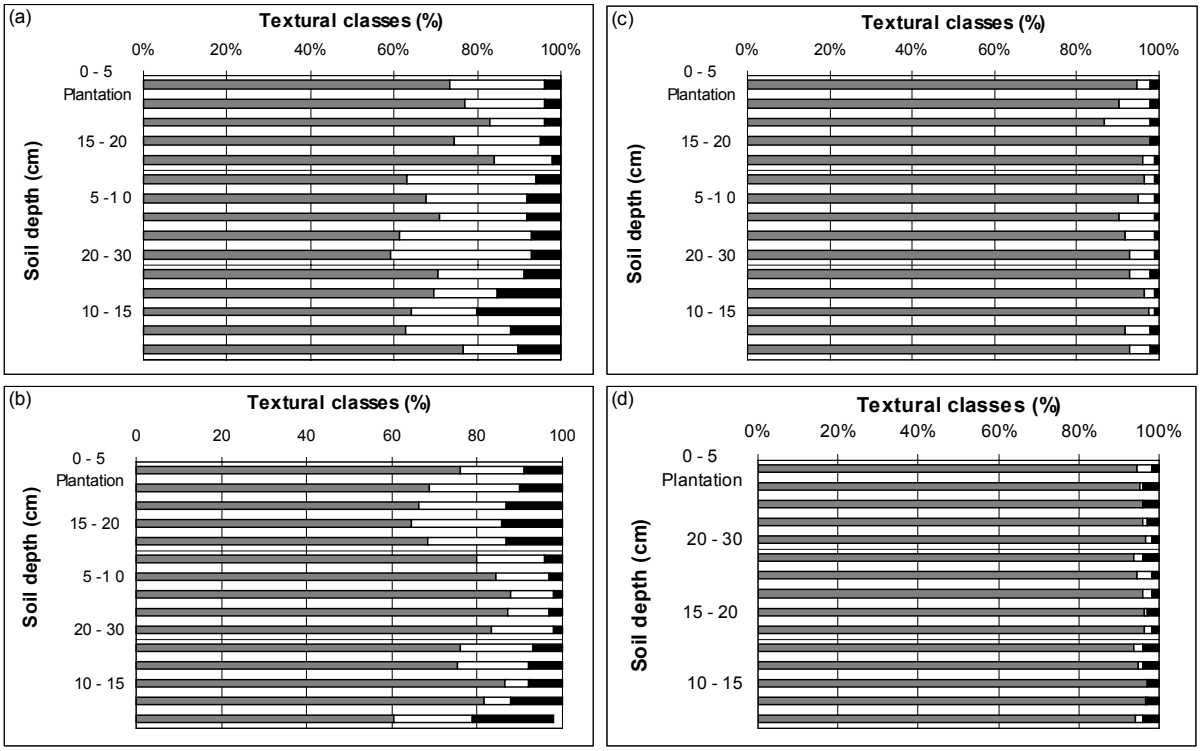

Figure 3.3 Distribution of textural classes along the soil profile (0-30 cm) in all sites (a-b: Crasto; c-d: Caju), plots and sampling periods (a,c: February; b,d: November) studied. (gray: sand; white: silt; black: clay).

Greater clay content was found from plantation to forest plots in the Crasto site and a slight growth was also found in deeper samples. This pattern was not found in the Caju site, much more homogeneous in soil textural properties between plots and along soil profile.

No marked differences were found between samples collected in both sampling periods. However, the Crasto fringe plot showed slightly smaller clay content in November than in February. Although this plot was located in a slope, which suffered great superficial water runoff in the February sampling, it does not seem possible that erosion rates in this plot could be greater than in the other ones, as the diminishing was also showed in the deeper samples.

3.3.2.2 Particle density

Figures for soil particle density varied between 2.25 and 2.54 g.cm-3, respectively, in the fringe plot from Caju in February, and in the forest plot form Crasto in November (Figure 3.4). Lower values were usually found in the upper layers, mostly in the plantation pots. but no clear pattern could be found relating to distribution of particle density along the soil profile, or between sites or sampling time.

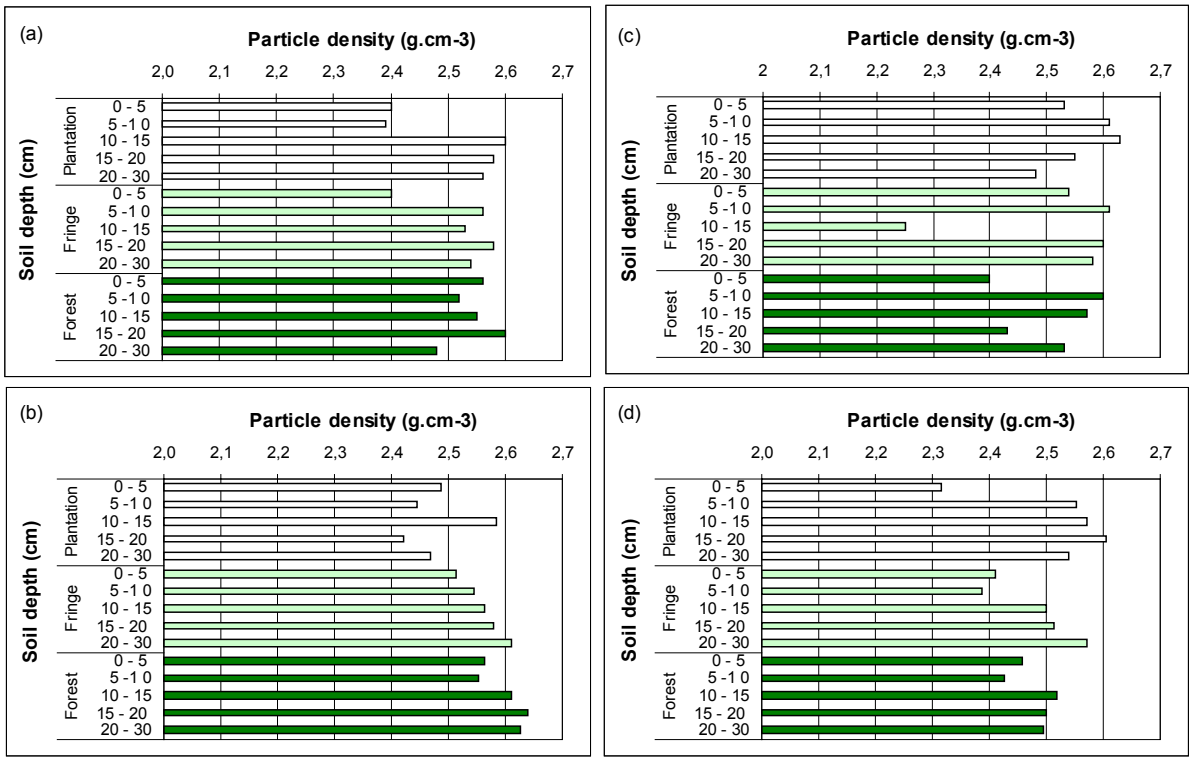

Figure 3.4 Soil particle density (g/cm3) distribution along soil profile (0-30 cm) in all sites (a-b: Crasto; c-d: Caju) and sampling periods (a,c: February; b,d: November) studied.

3.3.2.3 Water content

On the other side, regarding soil water content, a great difference was found between the two sampling times, with November presenting the higher figures (Figure 3.5).

Water content was higher in the forest plot from the Crasto site in February, but not in the Caju site. In the Crasto site, greater differences were found between plots, mostly in November. Strikingly higher figures were found in the deeper soil layers in the forest plot in November. In the Caju site, the three plots did not differed greatly from each other. In November, the forest plot from

the Caju site presented higher values than the fringe and plantation site, which presented both similar figures.

In general, comparing both sampling times, forest and fringe plots presented a more evenly distribution of water content, while the plantation plots showed a more concentrated in the upper layers.

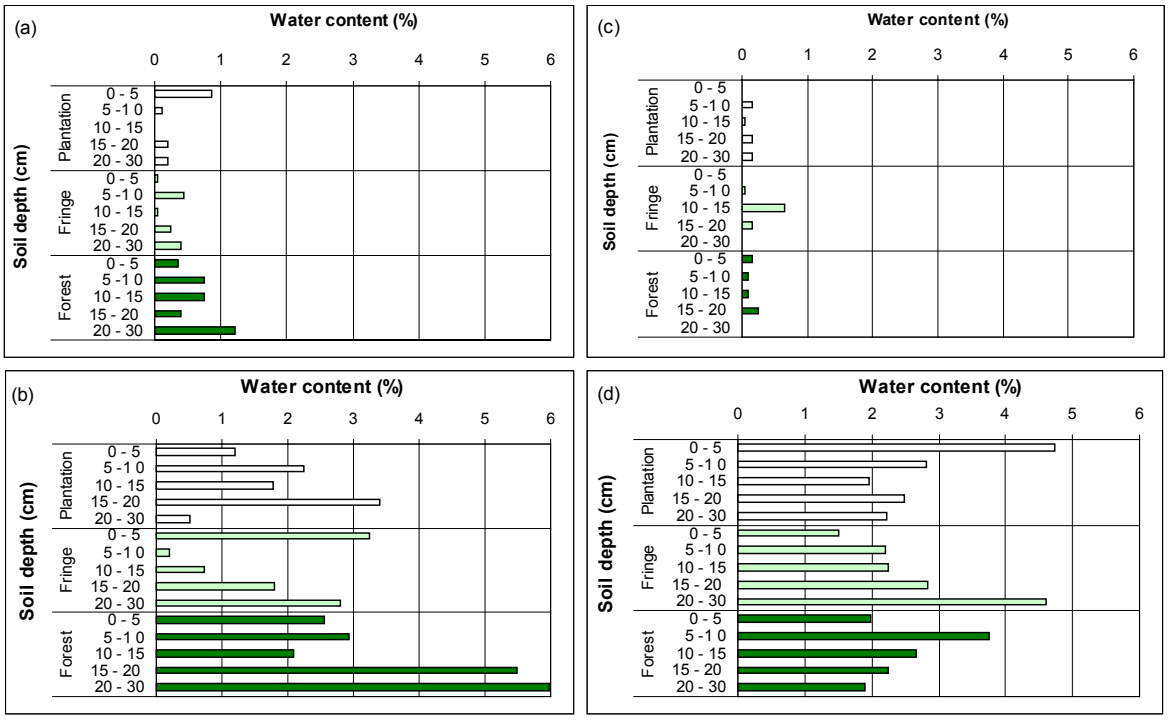

Figure 3.5 Distribution of water content (%) along the soil profile (0-30 cm) in all sites (a-b: Crasto; c-d: Caju) and sampling periods (a,c: February; b,d: November) studied.

3.3.2.4 Soil pH

The soil pH was moderately to strongly acidic, varying from 4.29 to 5.5, in the Crasto site, and between 4.17 and 5.6, in the Caju site (Figure 3.6). Although both sites did not differed much in these outer limits, the Crasto site showed in general lower pH values than the Caju site, but differences between both sites were not significant (testing was done only with data collected in November, when five cores where taken per plot).

No significant difference was found between plots, when considering the two sites together. However, Crasto's fringe and forest plots were in general more acidic than the plantation. Differences between plots were not so markedly within the Caju site in February but in November, plantation's pH values were lower than in fringe and forest plots. Indeed, pH values in the plantation differed significantly from fringe and forest plots in the Crasto site (respectively, $U = 20$, $p < 0.01$; $U = 23$, $p < 0,01$), but fringe and forest did not differed from each other. The same was

also true in the Caju site, with plantation differing from fringe ($U = 41.5$, $p < 0.01$) and forest plots ($U = 21$, $p < 0.01$), but no significant difference being found between these two.

A decrease in soil pH along the soil profile could be observed in most of the cases. Difference was significant when analyzing both plots together ($H = 11.085$; $p < 0,01$), but not individually. Although a marked difference can be observed between values obtained from both sampling periods, with more acid values being found in November, no testing could be carried out.

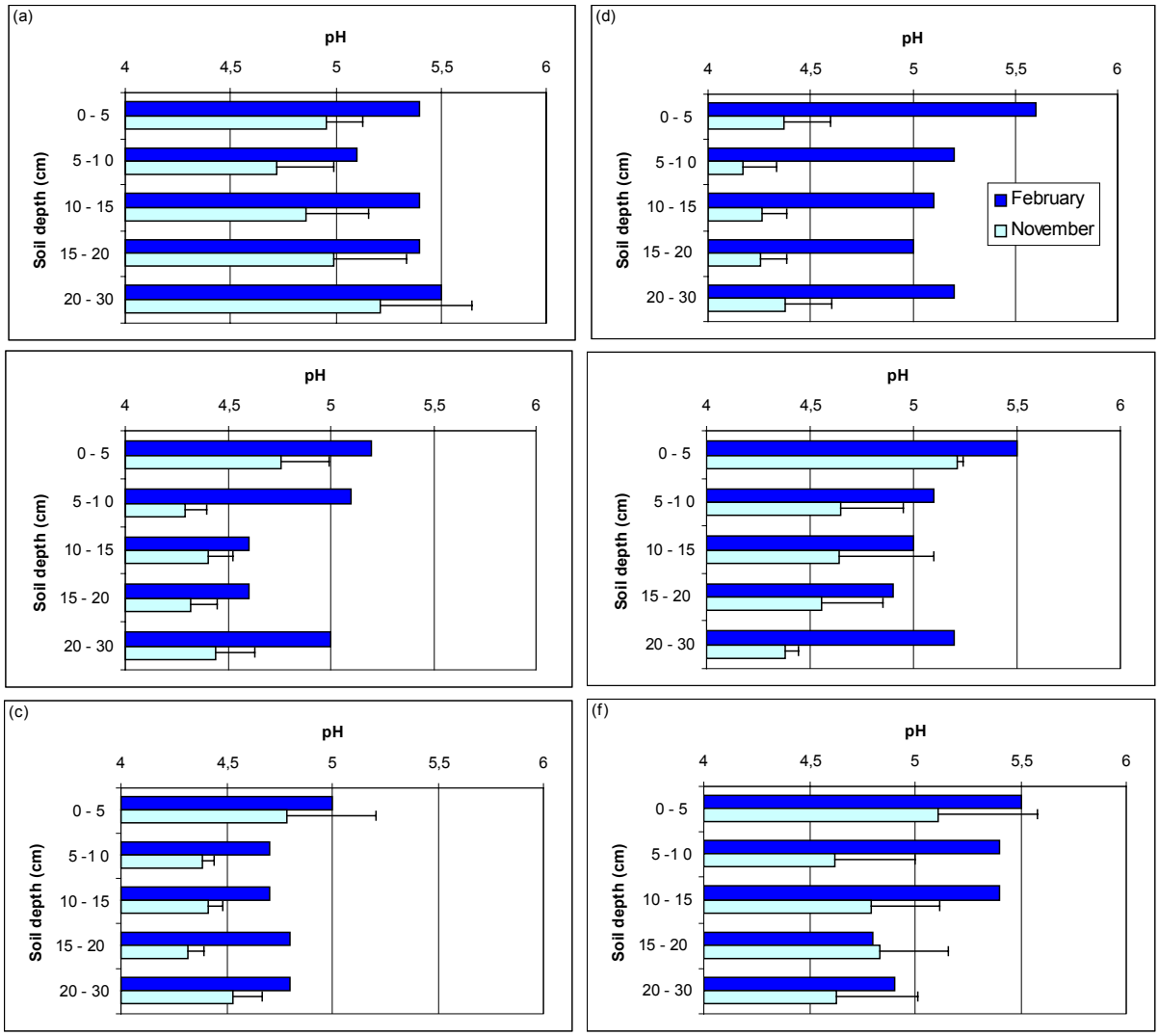

Figure 3.6 Variation of soil pH (water) along the soil profile (0-30 cm) in all sites (a-c: Crasto; d-f: Caju), plots (a,d: plantation; b,e: fringe; c,f: forest) and periods studied.

3.3.2.5 Aluminum

Greater values of soil aluminum were generally found in the Crasto site ($U = 334$; $p < 0.001$), and particularly in the forest plot from this site (Figure 3.7). On the contrary, in the Caju site, slightly higher values were found in the plantation plot. The exceptionally high value in the 10-15 soil layer

in the fringe plot in February (Figure 3.7e) may result from an analytical error, but as no replicates were collected in this month, this cannot be checked. Samples collected in November in the Crasto site were not always enough to analyze all parameters, and that is the reason why values and standard deviation bars for some soil depths are missing (Figure 3.7a-c). Differences between the two sampling times were also significant ($U = 644.5$; $p < 0.05$).

Variation along the soil profile was not significant in both sites. However, in the Crasto site, both fringe and forest plots show, respectively, a slight or more marked increase with increasing depth. In contrast, plantation plot from this site showed higher Al concentration in the two upper soil layers.

Differences between plots were also found, but while in the Caju site plantation differed significantly from the fringe and forest plots (respectively, $U = 3$, $U = 0$; $p < 0.001$), in the Crasto site distribution within plantation and forest plots were significantly different ($U = 1$; $p < 0.01$), but not between plantation and fringe plots.

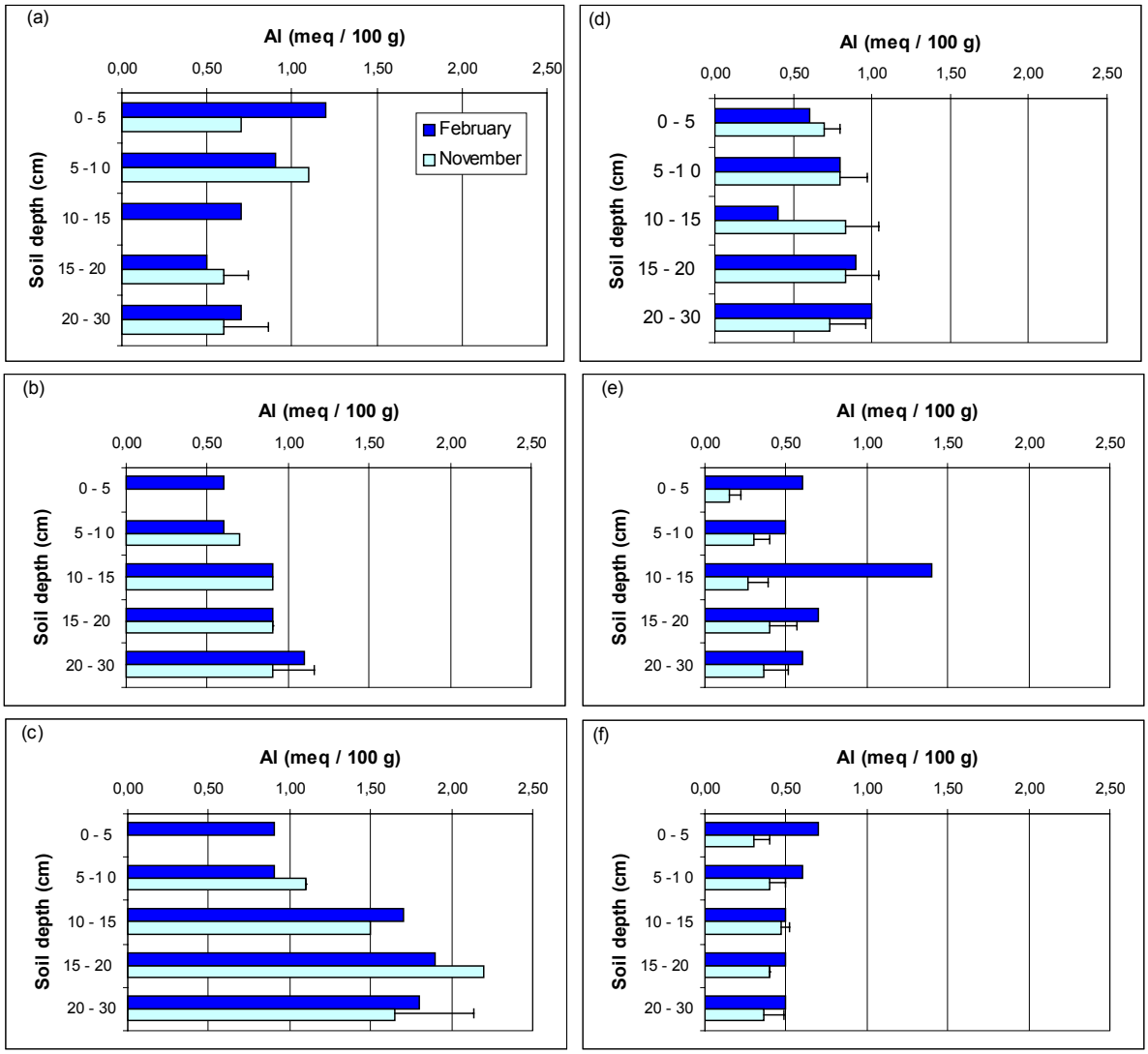

Figure 3.7 Variation of soil aluminum content (me/100g) along the soil profile (0-30 cm) in all sites (a-c: Crasto; d-f: Caju), plots (a,d: plantation; b,e: fringe; c,f: forest) and periods studied.

3.3.2.6 Phosphorus

Soil phosphorus concentration ranged from 9.18 to 0.95 mg/kg in the Crasto site and between 6.84 and 0.52 mg/kg in the Caju site). No significant difference was found between the two sites regarding the distribution of soil phosphorus content (Figure 3.8). In general, higher mean values were found in November.

Phosphorus values in plantation plots differed significantly from fringe plots ($U = 54$, $p < 0.05$) in the Crasto, but not in the Caju site. In the latter, however, plantation differed significantly from forest plots ($U = 50$, $p < 0.01$), while no significant difference was found between these two plots in the Crasto site. Nevertheless, P values in fringe and forest plots differed significantly in the Crasto ($U = 49$, $p < 0.01$) and Caju ($U = 55$, $p < 0.05$) sites.

Distribution of mean P values varied significantly through soil profile in the Crasto and Caju sites (respectively, $U = 20.085$, $p < 0.01$ and $U = 27.316$, $p < 0.01$), with the higher figures being generally found in the soil upper layers.

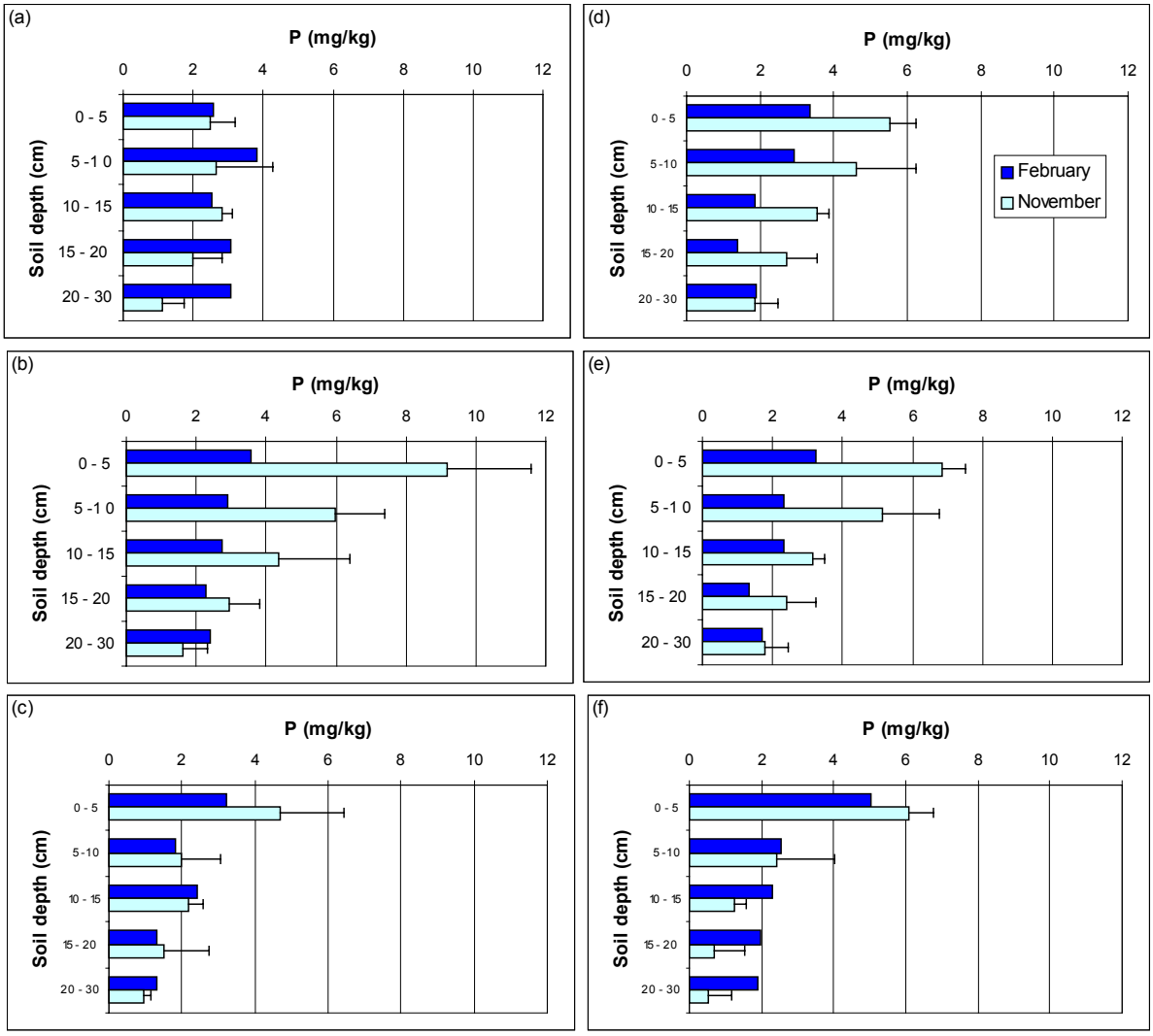

Figure 3.8 Variation of soil phosphorus content (mg/kg) along the soil profile (0-30 cm) in all sites (a-c: Crasto; d-f: Caju), plots (a,d: plantation; b,e: fringe; c,f: forest) and periods studied.

3.3.2.7 Sodium

Sodium soil content showed a much more diverse behavior (Figure 3.9). The Caju site showed markedly higher values than the Caju site ($U = 3.5$, $p < 0.01$), but no significant differences were found between plots in both sites. Mean Na values varied significantly through soil profile in the Crasto ($H = 12.102$, $p < 0.05$) and Caju ($H = 12.583$, $p < 0.05$) sites. With the exception of the fringe plot in the Crasto site, values did not differed greatly between the two sampling periods, although, as explained above, no statistical test could be performed here.

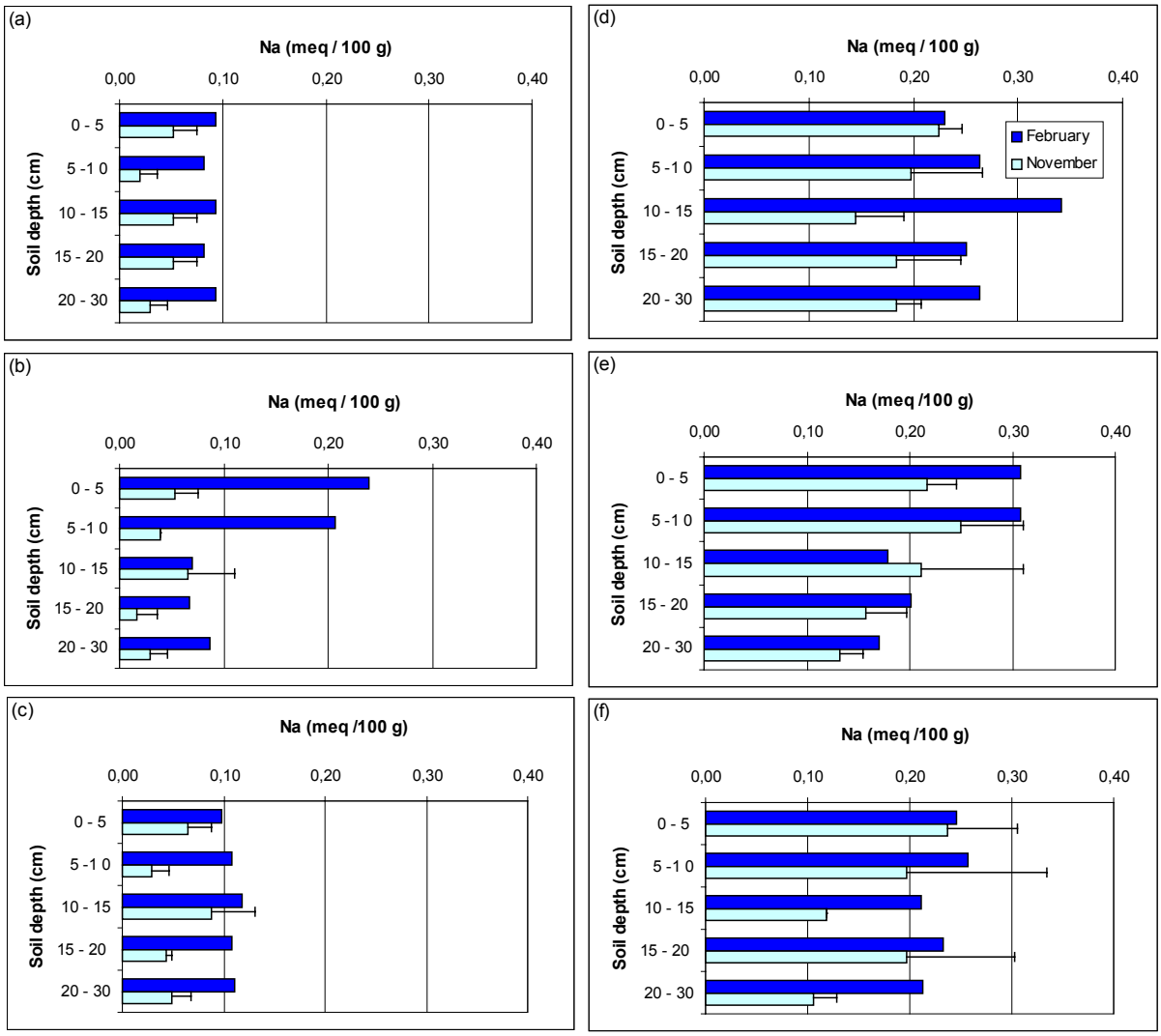

Figure 3.9 Variation of soil sodium content (me/100 g) along the soil profile (0-30 cm) in all sites (a-c: Crasto; d-f: Caju), plots (a,d: plantation; b,e: fringe; c,f: forest) and periods studied.

3.3.2.8 Potassium

Both sites differed significantly ($H = 303$, $p < 0.01$) in the distribution of soil potassium values, with the greater figures being generally found in the Caju site (Figure 3.10). In the Crasto site, plantation plots did not differed significantly from fringe plots, but from the forest plots ($U = 45$, $p < 0.05$). Fringe and forest plots also differed significantly ($U = 52$, $p < 0.05$) in this site. However, no significant difference in soil potassium concentration between plots was found in the Caju site. A more or less constant decrease along soil profile was also observed, specially in the Caju site, although not significant.

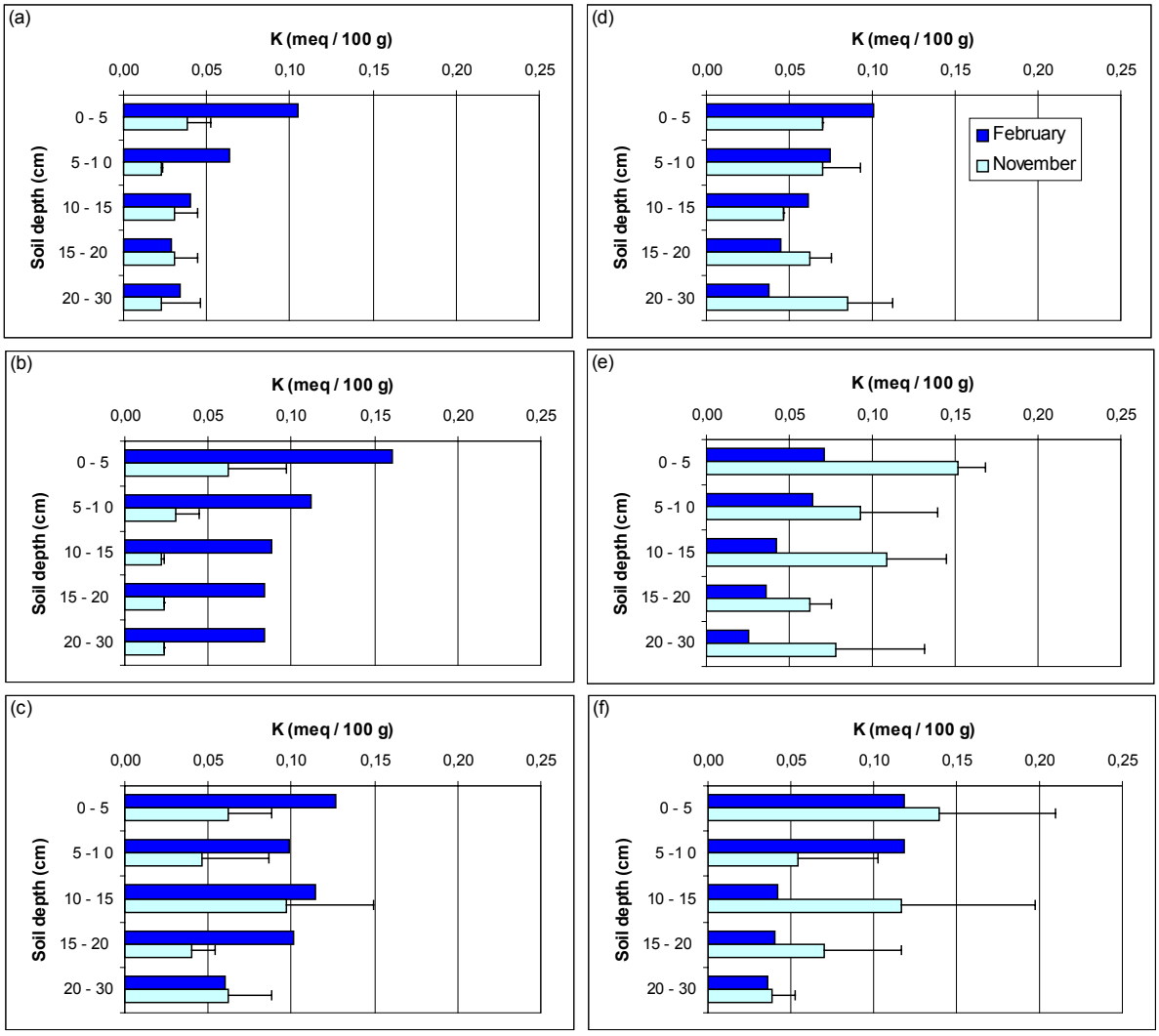

Figure 3.10 Variation of soil potassium content (me/100g) along the soil profile (0-30 cm) in all sites (a-c: Crasto; d-f: Caju), plots (a,d: plantation; b,e: fringe; c,f: forest) and periods studied.

In the Caju site, in February, the fringe plot showed values somewhat smaller than the plantation plot, but in November values from this plot were even higher than the ones found in the forest plots. In general, values obtained from the two sampling periods differed most in the fringe plots from both sites. While in the Crasto site higher values predominate in February, in the Caju site they were generally found in November. However, the outer limits in both sites did not differed

much, ranging from 0.02 to 0.16 and from 0,03 to 0,15 me/100g, in the Crasto and Caju sites, respectively.

3.3.2.9 Calcium

Calcium values were generally greater in the Caju site but differences were not significant (Figure 3.11). However, Ca mean values along the soil profile differed significantly in the Crasto ($H = 21.178$, $p < 0.01$), and Caju ($H = 10.537$, $p < 0.015$ sites. Soil upper layers from fringe and forest plots in both sites showed strikingly higher values than the underneath layers.

No significant differences were found between plots in the Crasto site. In the Caju site, soil calcium in the plantation differed significantly ($U = 38$, $p < 0.01$) from the fringe plot. Mean Ca values in the plantation did not differ from the forest plots, neither fringe and forest showed any significant difference in this site.

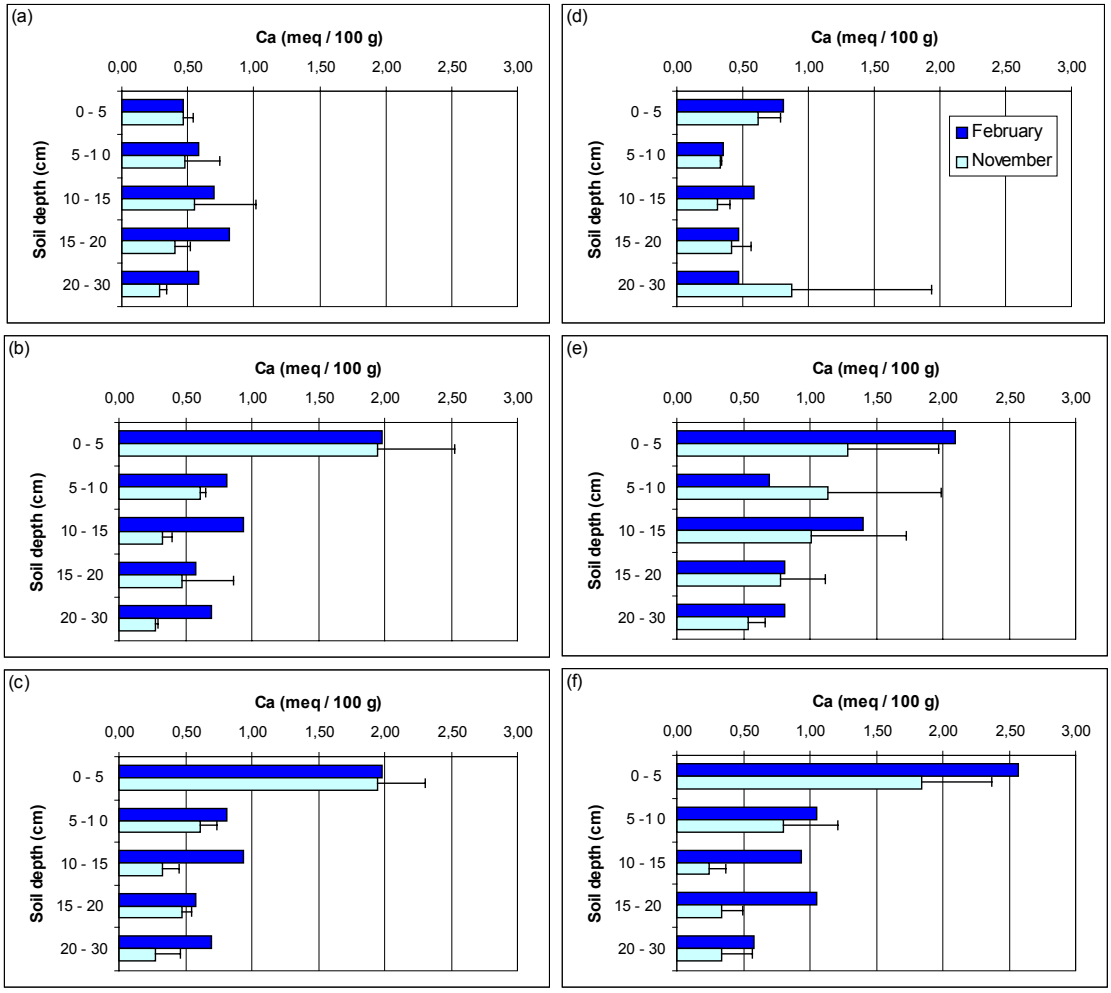

Figure 3.11 Variation of soil calcium content (me/100g) along the soil profile (0-30 cm) in all sites (a-c: Crasto; d-f: Caju), plots (a,d: plantation; b,e: fringe; c,f: forest) and periods studied.

Higher Ca values were also generally found in February (peaking at 1.98 and 2.56 meq 100 g^{-1}, respectively, in the Crasto and Caju sites). The lowest values were found in November (0.27 and 0.24 me/100g, in the Crasto and Caju sites, respectively).

3.3.2.10 Magnesium

As to the magnesium concentration in soils (Figure 3.12), the Caju site showed figures generally higher than the Crasto site, although differences were not significant. In the former, values ranged between 0.24 and 1.9 me/100g. In the Crasto site, they ranged between 0.14 and 3.09 me/100g. Higher values were generally found in February.

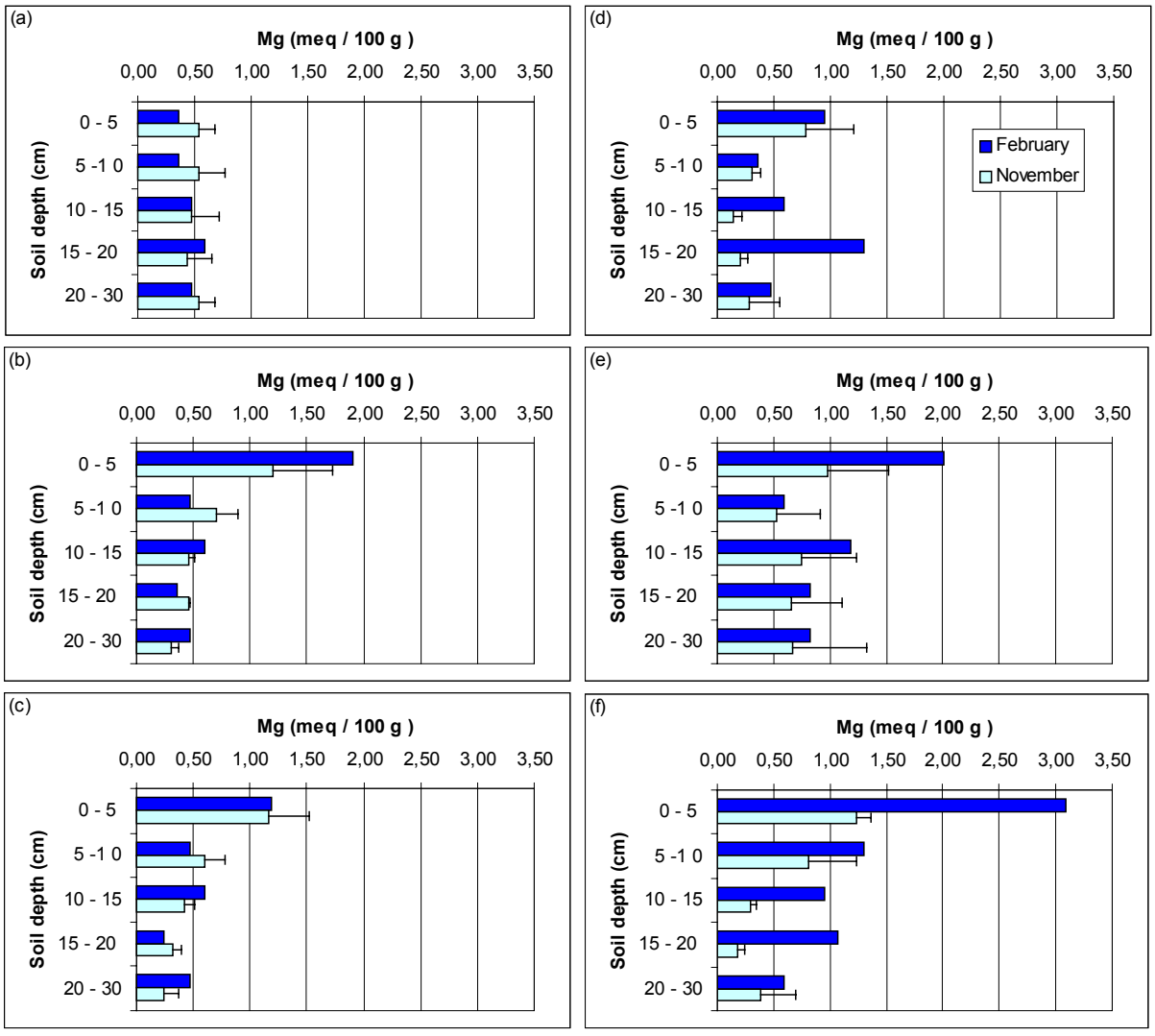

Figure 3.12 Variation of soil magnesium content (me/100g) along the soil profile (0-30 cm) in all sites (a-c: Crasto; d-f: Caju), plots (a,d: plantation; b,e: fringe; c,f: forest) and periods studied.

No significant differences were found on mean Mg values between plots in the Crasto site. In the Caju site, values in the plantation were significantly different from fringe plots ($U = 53.5$, $p < 0.05$) but significant differences were not found between plantation and forest plots, nor between fringe and forest plots.

Magnesium distribution differed significantly along the soil profile in the Crasto ($H = 20.460$, $p < 0.01$) and Caju ($H = 12.132$, $p < 0.05$). As found for calcium distribution, fringe and forest plots showed also a marked higher Mg values in the upper layers.

3.3.2.11 Correlation between soil parameters

Correlation between the soil nutrients and pH showed some positive significant correlations (Table 3.4). Because even slight associations rendered significant ($p < 0.05$) results, only r_s significant at $p < 0.01$ will be considered.

Significant positive correlation was found between pH, Ca and Mg values. Although P values showed a slightly negative correlation with pH, P was also positively correlated with Ca and Mg. Another group could be defined with the behavior of Na, K and Ca, all of them positively correlated to each other. Aluminum concentration showed negative correlation with Na.

Table 3.4 Spearman Rank Order Correlations (2-tailed) between pH and nutrient values (N = 118; ** $p < 0.01$; * $p < 0.05$)

	pH	***P***	***Na***	***K***	***Ca***	***Mg***
P	-0.066					
Na	0.187*	0.174				
K	0.132	0.188*	0.589**			
Ca	0.367**	0.436**	0.358**	0.287**		
Mg	0.430**	0.370**	0.216*	0.066	0.790**	
Al	-0.227*	- 0.094	-0.445**	-0.233*	-0,231*	-0,260*

3.3.3 Litter biomass

A continuous litter layer on soils of fringe and forest plots of both sites were found (Figure 3.13). On plantation plots it was irregularly distributed, most of its biomass depending on fallen leaves of the sparse coconut trees present is this site, as litter production from grass and herb layer is largely insignificant compared to fringe and forest plots.

Figure 3.13 Litter layer in the Crasto (a-b) and Caju (c-d) sites.

The greater values of litter biomass were found in the Caju site and in the November sampling (Figure 3.14). In both sites, the border plots presented greater values (varying from 747.16 to 1046 g . m^{-2} in November) than the forest ones (468.72 to 616.4 g . m^{-2}, in the same sampling period). The high values obtained for the Caju plantation plot in November may have reflected the random sampling of areas where coconut leaves had fallen, and so the correspondent high standard deviation (Table 3.5). However, relatively high standard deviation values were also found, in November, in the fringe plot from the Caju site.

Both sites were not significantly different in litter biomass production ($H = 0.01$; $p > 0.05$), but the two sampling times ($H = 12.381$; $p < 0.001$) and the three plots were ($H = 17.614$; $p < 0.01$). Mann-Whitney pairwise tests detected significant differences between plantation and fringe plots ($U = 68$; $p < 0.001$), fringe and forest plots ($U = 112$; $p < 0.05$) and plantation and forest plots ($U = 90$; $p < 0.001$).

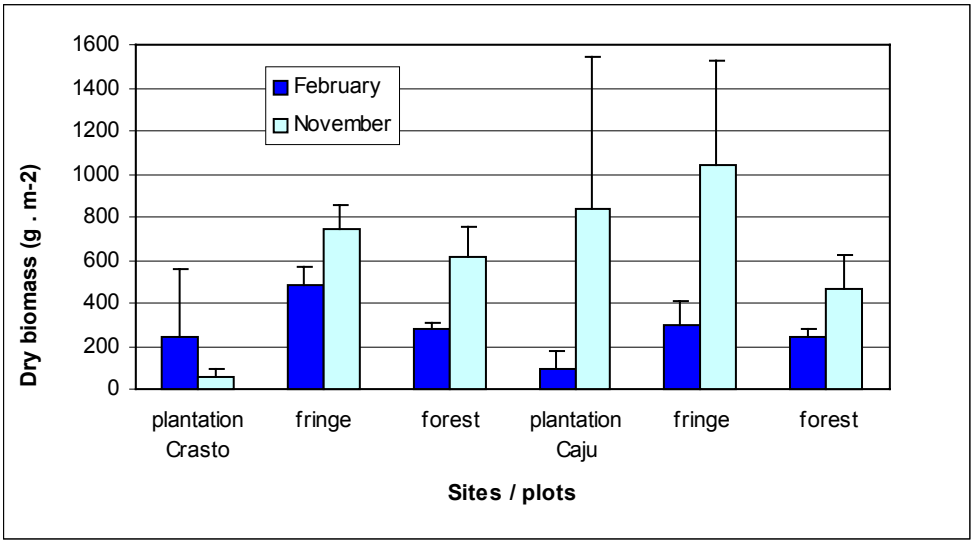

Figure 3.14 Litter dry biomass distribution in all plots and sites studied, in both sampling times.

Table 3.5 Litter biomass mean values (and standard deviation) for each site, plot and sampling time.

		Month			
Site	***Plot***	***February***	***n***	***November***	***n***
Caju	plantation	92.56 ± 84.44	5	837.93 ± 710.15	5
	border	296.90 ± 109.06	5	1046.11 ± 483.03	5
	forest	237.99 ± 45.71	5	468.72 ± 157.07	5
Crasto	plantation	240.92 ± 317.29	5	59.9 ±29.27	5
	border	481.25 ± 84.91	5	747.16 ± 110.46	5
	forest	283.23 ± 24.53	5	616.4 ± 137.96	5

Significant correlation between litter biomass and nutrient content of the upper (0-5 cm) soil layer was only found for phosphorus (Table 3.6). No correlation between litter biomass and vegetation structure (see 3.3.6 Vegetation structure) was found (Table 3.7). Litter nutrient content is being currently analyzed and will be subsequently correlated to soil nutrient values, in order to determine which of these components is the main reservoir for different nutrients in the two sites studied.

Table 3.6 Spearman's Rank Order correlations between litter biomass data and soil nutrient content in the upper layer (0-5 cm). (N = 12; ** = p < 0,01; ns = non significant)

	pH	Al	P	Na	K	Ca	Mg
Litter biomass	-0.501 ns	-0.21ns	0.748**	-0.035 ns	0.161 ns	0.217 ns	0.098 ns

Table 3.7 Spearman's Rank Order correlations between litter biomass data and plot vegetation structure. (N = 12; ns = non significant)

	Litter biomass
Basal area (m^{-2} ha^{-1})	0.311 ns
Tree abundance	0.481 ns
Tree richness	0.488 ns

3.3.4 Root biomass

Sampling methods and unities of expressing results in root biomass studies vary greatly between different authors in different geographic regions, ecosystems and scales. As the deepest soil fraction used (20-30 cm) consists of a 10 cm layer in contrast with the four upper layers (0-5, 5-10, 10-15, and 15-20 cm), root biomass values were calculated as root density (kg . m-3) along the soil profile, but as biomass (g . m^{-2}) regarding the cumulative root biomass in each site and plot, for more easy comparison between this and other studies.

The Caju site presented a marked greater root cumulative biomass than the Crasto site, although also greater standard deviations (Figure 3.15) The values in the three plots (plantation, fringe and forest) in that site did not differ much, as well as in the Crasto site. It is surprising, due to the almost complete absence of tree cover in the plantation sites, with just some scattered *Cocos nucifera* plants in it (see 3.3.5 Floristic Composition and 3.3.6 Vegetation Structure below). Also surprising is the considerable higher cumulative root biomass values found in February, markedly in the Caju site. Sampling in February reveled higher values than in November, but this difference was not so strikingly in the Crasto as in the Caju site.

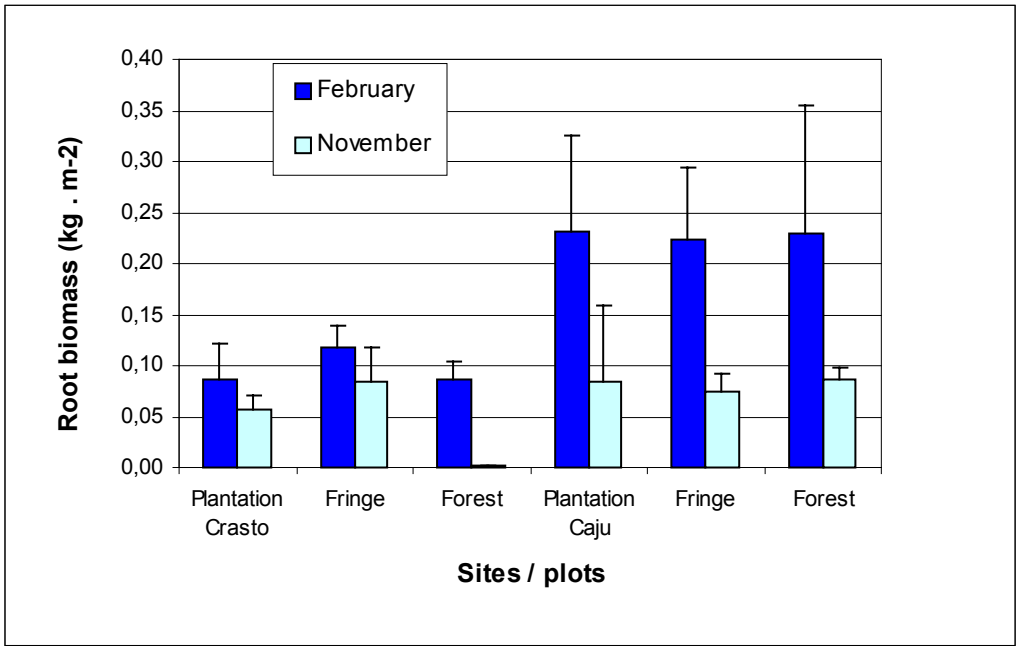

Figure 3.15 Cumulative root biomass (sum of the upper 30 cm) distribution in all sites, plots, and sampling times studied.

In both sites the upper layers present a greater root density than the deeper ones (Figure 3.16), but in the Caju site this tendency is much more marked as in the Crasto site. In fact, removal of the litter layer revealed a conspicuous root mat above mineral soils of both sites (Figure 3.17). Plantation plots also showed a greater root density in the five upper centimeters of soil (57,78 and 38,37%, respectively, in the Caju and Crasto sites) than the adjacent fringe and forest plots. In general, forest plots showed a more evenly root biomass distribution along the soil profile.

The greater standard deviations in the plantation plot of the Caju site could be explained by the irregular distribution of *C. nucifera* roots, but the same pattern is not observed in the plantation of the Crasto site. Even more, the fringe and forest plots of Caju presented also great standard deviations values.

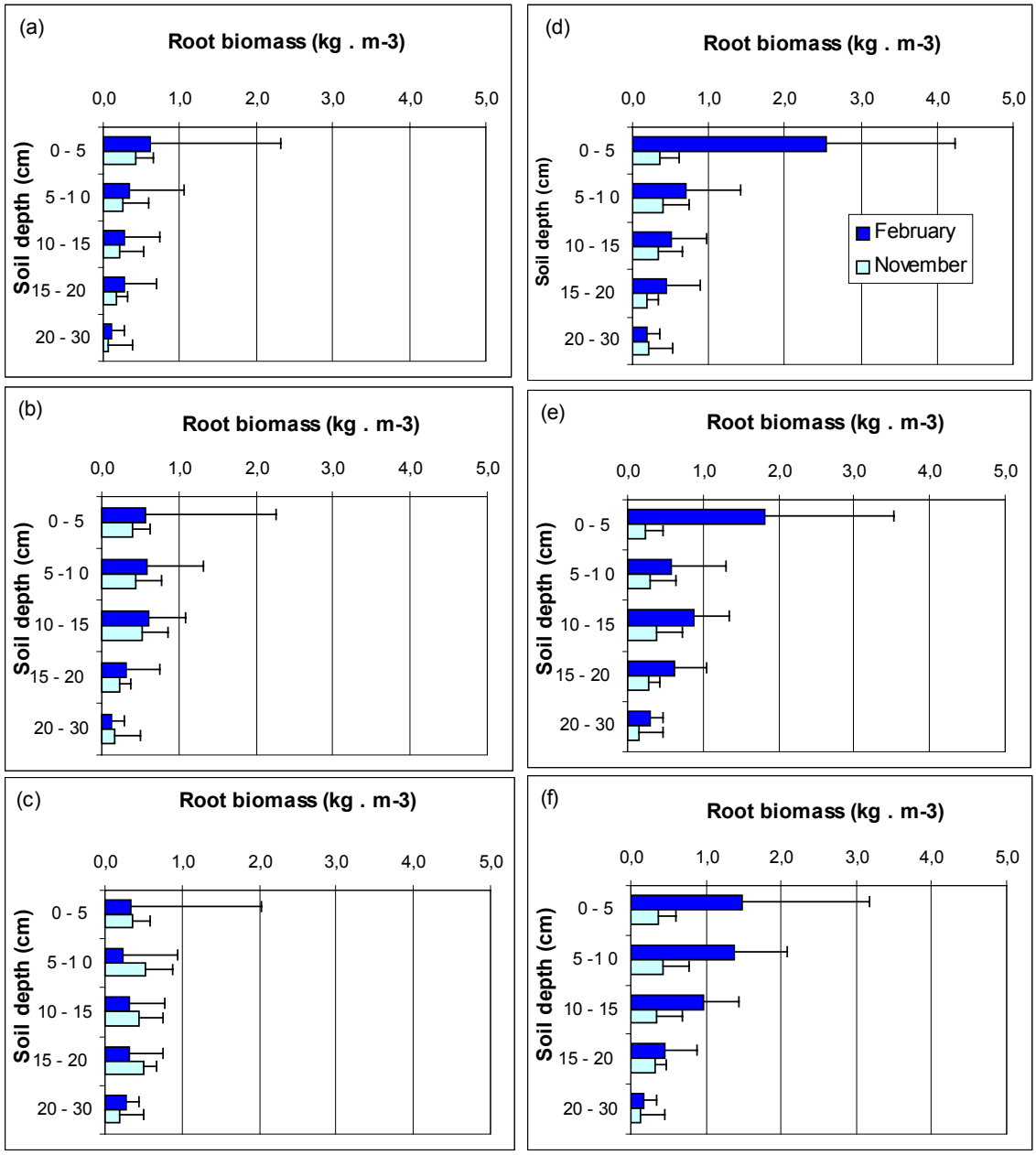

Figure 3.16 Root biomass (kg/m-3) distribution along the soil profile (0-30 cm) in all sites (a-c: Crasto; d-f: Caju), plots (a,d: plantation; b,e: fringe; c,f: forest) and periods studied.

Figure 3.17 Roots exposed on the soil surface after removal of litter layer in the Crasto (a-b) and Caju (c-d) sites.

The two sites differed significantly in mean root biomass distribution ($U = 9{,}695$, $p < 0.05$), as well as between the two sampling periods ($U = 8{,}172.5$, $p < 0.01$). Values from February in the Caju site were remarkably high. Mean root biomass in the plantation plot differed significantly from fringe and forest plots ($U = 845.5$, $p < 0.01$ and $U = 907$, $p < 0.05$, respectively), in the Crasto, but not in the Caju, where no differences were found between the three plots.

Root biomass varied significantly distribution along the soil profile ($U = 63.189$, $p < 0.01$). A greater proportion of biomass in the soil cores examined was localized in the superficial layer in the Caju site. In the Crasto site, a more even distribution was found, although the upper layer showed also proportional higher standard deviation values. Positive significant relationship (Table 3.8) was found between root biomass and Ca and Mg ($p < 0.01$), and pH, P and Na ($p < 0.05$).

Table 3.8 Spearman's Rank Order correlations between root biomass data and soil nutrient content in the upper layer (0-5 cm). (N = 119 (94 for Al); ** = p < 0,01; ns = non significant)

	pH	Al	P	Na	K	Ca	Mg
Root biomass	0.221*	0.150ns	0.192*	0.180*	0.131ns	0.292**	0.331**

3.3.5 Floristic composition

A total of 59 tree species, pertaining to 23 families, were found in the two studied sites, being only 22,03% of the species (13 species) common to both (Table 3.9). Further effort is still needed in order to achieve a more accurate identification of the material, most infertile, specially in the Myrtaceae family. This family presented the greater number of species considering both sites together (15 species or 25% of the total), or separately (10 species - or 35,7% - in the Caju site, and 8 species - or 18,2% - in the Crasto site).

Table 3.9 Families and species found, with correspondent tree numbers in each site and plot.

		Crasto			***Caju***		
Family	***Species***	***Plant.***	***Fringe***	***Forest***	***Plant.***	***Fringe***	***Forest***
Anacardiaceae	*Anacardium occidentale* L.					2	
Anacardiaceae	*Schinus terebenthifolius* Raddi					1	
Anacardiaceae	*Tapirira guianensis* Aubl.		2	2			1
Annonaceae	*Annona salzmannii* A. DC.			1			
Annonaceae	*Annona* sp1		1	1			
Annonaceae	*Annona* sp2		1				
Annonaceae	*Annona* sp3		1	1			
Annonaceae	*Xylopia brasiliensis* Spreng.		1			1	1
Arecaceae	*Bactris* sp		1				
Arecaceae	*Cocos nucifera* L.	2			5		
Arecaceae	*Syagrus* cf. *schizopylla* (Mart.) Glass.			1		40	12
Boraginaceae	*Cordia sellowiana* Cham.					2	
Burseraceae	*Protium heptaphyllum* (Aubl.) March.		3			1	1
Burseraceae	*Protium* sp		1	1			
Capparaceae	*Capparis flexuosa* L.					1	
Celastraceae	*Maytenus* cf. *opaca* Reiss.		5	10			
Chrysobalanaceae	*Hirtella* cf. *ciliata* Mart. et Zucc.		4				
Chrysobalanaceae	*Licania* sp						1
Erythroxylaceae	*Erythroxylum* sp		1	2		2	8
Guttiferae	*Rheedia brasiliensis* Planch. & Triana						5
Lauraceae	*Ocotea glomerata* (Nees) Mez.		4			2	2
Lecythidaceae	*Eschweilera ovata* (Camb.) Miers		8	12			
Lecythidaceae	*Lecythis* cf. *pisonis* Camb.		1				
Leg. Caes.	*Bowdichia virgilioides* Kunth			4			
Leg. Mim	*Abarema filamentosa* (Benth) Pittier		1	1			
Leg. Mim.	*Inga* sp					1	
Leg. Pap.	*Swartzia apetala* Raddi					8	
Leguminosae	Leguminosae N.I.		1				
Malpighiaceae	*Byrsonima* cf. *sericea* DC.		6	1		3	1
Moraceae	*Ficus* sp			1			

Myrtaceae	*Campomanesia viatoris* Landrum				1
Myrtaceae	*Campomanesia aromatica* (Aublet) Grisebach	2			
Myrtaceae	*Campomanesia dichotoma* (Berg) Mattos	1	3	1	
Myrtaceae	*Eugenia* sp 1	2	1		
Myrtaceae	*Gomidesia blanchetiana* O. Berg				2
Myrtaceae	*Myrcia* cf. *fallax* (Rich.) DC.			1	2
Myrtaceae	*Myrciaria floribunda* (West ex Willd.) O.Berg			6	10
Myrtaceae	Myrtaceae sp 1	13	5	2	3
Myrtaceae	Myrtaceae sp 2	2	12		
Myrtaceae	Myrtaceae sp 3	10	48		
Myrtaceae	Myrtaceae sp 4			1	
Myrtaceae	Myrtaceae sp 5	2			2
Myrtaceae	Myrtaceae sp 6				3
Myrtaceae	Myrtaceae sp 7			10	
Myrtaceae	Myrtaceae sp 8	1	1		
Ochnaceae	*Ouratea* cf. *fildingiana* Engl.	1			
Rubiaceae	*Alseis* cf. *floribunda* Schott		2		
Rubiaceae	*Guettarda viburnoides* Muel.. Arg.	4	2		
Rutaceae	*Fagara* sp		1		
Sapindaceae	*Allophylus* cf. *edulis* (A. St.-Hil.) Radlk.	2	1		1
Sapindaceae	*Cupania revoluta* Radlk	8	2		
Sapotaceae	*Manilkara* cf. *rufula* (Miq.) Lam.	1		7	2
Sapotaceae	*Pouteria* sp			8	2
Theaceae	*Bonnetia anceps* Mart.		2		
Tiliaceae	*Luehea divaricata* Mart.	2	1		
N.I.	N.I. 1	1			
N.I.	N.I. 2	2			
N.I.	N.I. 3	5			
N.I.	N.I. 4	3	1		
Dead		9	19		

Plant. = Plantation site

* N.I. Non identified families/species

In the Crasto site 42 species, pertaining to 20 families were found. This site presented not only the greater number of species and families, but also of trees. Only eight species corresponded to 50% pf the community's total importance value (I.V.), four of them, from the Myrtaceae family (Table 3.10).

In the Caju site both the number of species and families were lower, respectively, 28 and 15. Four species represented half of the community I.V., only one of them, a Myrtaceae member (Table 3.11).

Table 3.9 Phytosociological indices for Crasto site (sample size: 400m^2). (I.V.: importance value).

Species	Density (N)	Relative density	Frequency (%)	Relative frequency	Dominance (m^2)	Relative Dominance	I.V.
Myrtaceae sp 3	58	25.9	100	4.7	0.3	9.5	40.1
Eschweilera ovata	20	8.9	100	4.7	0.6	21.5	35.1
Myrtaceae sp 1	18	8.0	75	3.5	0.1	4.6	16.1
Maytenus cf. *opaca*	15	6.7	100	4.7	0.1	2.9	14.3
Cupania revoluta	10	4.5	75	3.5	0.1	4.4	12.4
Myrtaceae sp 2	14	6.3	75	3.5	0.1	2.1	11.9
Bowdichia virgilioides	4	1.8	50	2.4	0.2	6.8	10.9
Campomanesia dichotoma	4	1.8	100	4.7	0.1	3.7	10.2
Byrsonima cf. sericea	7	3.1	50	2.4	0.1	4.3	9.8
Tapirira guianensis	4	1.8	100	4.7	0.1	3.2	9.7
Leguminosae N.I.	1	0.4	25	1.2	0.2	7.7	9.4
Ocotea glomerata	4	1.8	100	4.7	0.1	2.8	9.2
Guettarda viburnoides	6	2.7	75	3.5	0.1	2.1	8.3
N.I. 4	4	1.8	50	2.4	0.1	2.6	6.7
Abarema filamentosa	2	0.9	50	2.4	0.1	3.0	6.2
Hirtella cf. *ciliata*	4	1.8	50	2.4	0.0	1.5	5.6
Luehea divaricata	3	1.3	50	2.4	0.0	1.7	5.4
Erythroxylum sp	3	1.3	75	3.5	0.0	0.3	5.2
Annona sp1	2	0.9	50	2.4	0.0	1.5	4.7
Allophylus cf. *edulis*	3	1.3	50	2.4	0.0	0.8	4.5
Eugenia sp 1	3	1.3	50	2.4	0.0	0.8	4.5
N.I. 2	2	0.9	50	2.4	0.0	0.9	4.1
Alseis cf. *floribunda*	2	0.9	50	2.4	0.0	0.8	4.1
N.I. 3	5	2.2	25	1.2	0.0	0.6	4.0
Myrtaceae sp 8	2	0.9	50	2.4	0.0	0.8	4.0
Annona sp2	1	0.4	25	1.2	0.1	2.3	3.9
Annona sp3	2	0.9	50	2.4	0.0	0.5	3.8
Protium sp	2	0.9	50	2.4	0.0	0.3	3.6
N.I. 1	1	0.4	25	1.2	0.1	1.9	3.5
Bonnetia anceps	2	0.9	50	2.4	0.0	0.3	3.5
Protium heptaphyllum	3	1.3	25	1.2	0.0	0.7	3.2
Campomanesia aromatica	2	0.9	25	1.2	0.0	0.7	2.8
Myrtaceae sp 5	2	0.9	25	1.2	0.0	0.3	2.4
Annona salzmannii	1	0.4	25	1.2	0.0	0.7	2.4
Manilkara cf. *rufula*	1	0.4	25	1.2	0.0	0.4	2.0
Lecythis cf. *pisonis*	1	0.4	25	1.2	0.0	0.3	1.9
Ficus sp	1	0.4	25	1.2	0.0	0.3	1.9
Fagara sp	1	0.4	25	1.2	0.0	0.3	1.9
Syagrus cf. *schizopylla*	1	0.4	25	1.2	0.0	0.1	1.8
Bactris sp	1	0.4	25	1.2	0.0	0.1	1.7
Ouratea cf. *fildingiana*	1	0.4	25	1.2	0.0	0.1	1.7
Xylopia brasiliensis	1	0.4	25	1.2	0.0	0.1	1.7

Table 3.11 Phytosociological indices for Caju site (sample size: 400m^2). (I.V.: importance value).

Species	Density (N)	Relative density	Frequency (%)	Relative frequency	Dominance (m^2)	Relative dominance	I.V.
Xylopia brasiliensis	2	1.3	50	3.7	96375.4	68.3	73.3
Syagrus cf. *schizopylla*	52	32.5	100	7.4	3194.7	2.3	42.2
Myrciaria cf. *floribunda*	16	10	100	7.4	8354.7	5.9	23.3
Pouteria sp	10	6.3	75	5.6	3979.4	2.8	14.6
Erythroxylum sp	10	6.3	75	5.6	2974.2	2.1	13.9
Manilkara cf. *rufula*	9	5.6	75	5.6	2070.9	1.5	12.6
Myrtaceae sp 7	10	6.3	50	3.7	2173.5	1.5	11.5
Myrtaceae sp 1	5	3.1	75	5.6	1992.8	1.4	10.1
Byrsonima cf. *sericea*.	4	2.5	50	3.7	5389.3	3.8	10.0
Swartzia apetala	8	5.0	50	3.7	819.7	0.6	9.3
Ocotea glomerata	4	2.5	75	5.6	520.2	0.4	8.4
Myrcia cf. *fallax*	3	1.9	75	5.6	463.3	0.3	7.8
Myrtaceae sp 6	3	1.9	50	3.7	2277.1	1.6	7.2
Rheedia brasiliensis	5	3.1	50	3.7	456.9	0.3	7.2
Anacardium occidentale	2	1.3	25	1.9	4217.3	3.0	6.1
Cordia sellowiana	2	1.3	50	3.7	220.2	0.2	5.1
Protium heptaphyllum	2	1.3	50	3.7	75.5	0.1	5.0
Myrtaceae sp 5	2	1.3	25	1.9	384	0.3	3.4
Gomidesia blanchetiana	2	1.3	25	1.9	44.7	0.0	3.1
Myrtaceae sp 4	1	0.6	25	1.9	651.8	0.5	2.9
Licania sp	1	0.6	25	1.9	484.1	0.3	2.8
Allophylus cf. *edulis*	1	0.6	25	1.9	305.9	0.2	2.7
Inga sp	1	0.6	25	1.9	272.3	0.2	2.7
Campomanesia dichotoma	1	0.6	25	1.9	267.7	0.2	2.7
Schinus terebenthifolius	1	0.6	25	1.9	219.3	0.2	2.6
Tapirira guianensis	1	0.6	25	1.9	161.1	0.1	2.6
Campomanesia viatoris	1	0.6	25	1.9	143.7	0.1	2.6
Capparis flexuosa	1	0.6	25	1.9	42.1	0.0	2.5

The two sites presented 12 families in common, Myrtaceae and Leguminosae included. Myrtaceae was the most important family, in both areas, when considering the sum of the importance values of all species in each family (Table 3.12).

The fringe and forest plots did not present great species similarity, being 42,2 and 33,3% of the tree species common to both plots in the Crasto and Caju sites, respectively. However, Sorensen's similarity index was greater between fringe and forest plots in each site, than between fringe (or forest) plots from the two sites (Table 3.13).

Table 3.12 Sum of the I.V.s (importance values) of all members of each species in both sites.

Crasto		***Caju***	
Family	***I.V.***	***Family***	***I.V.***
Myrtaceae	91.9	Myrtaceae	74.6
Lecythidaceae	37.0	Annonaceae	73.3
Leguminosae	26.5	Arecaceae	42.2
N.I.	18.4	Sapotaceae	27.3
Sapindaceae	16.9	Erythroxylaceae	13.9
Annonaceae	16.5	Leguminosae	12.0
Celastraceae	14.3	Anacardiaceae	11.3
Rubiaceae	12.4	Malpighiaceae	10.0
Malpighiaceae	9.8	Lauraceae	8.4
Anacardiaceae	9.7	Guttiferae	7.2
Lauraceae	9.2	Boraginaceae	5.1
Burseraceae	6.8	Burseraceae	5.0
Chrysobalanaceae	5.6	Chrysobalanaceae	2.8
Tiliaceae	5.4	Sapindaceae	2.7
Erythroxylaceae	5.2	Capparaceae	2.5
Theaceae	3.5		
Arecaceae	3.5		
Sapotaceae	2.0		
Moraceae	1.9		
Rutaceae	1.9		
Ochnaceae	1.7		

Table 3.13 Sorensen Index (CC) between plots and sites.

Plots / sites	***CC***
fringe x forest Crasto	0.645
fringe x forest Caju	0.579
fringe Crasto x Caju	0.291
forest Crasto x Caju	0.267
Crasto x Caju	0.343

Fringe and forest plots from the Crasto site presented higher values of richness than its counterparts in the Caju site (Table 3.14). Regarding plant diversity, Crasto's fringe and Caju's forest plots showed the higher diversity. These two plots showed also the higher values of evenness.

Table 3.14 Ecological indices for each studied plot.

	Crasto			*Caju*		
Indices	***plantation***	***Fringe***	***forest***	***plantation***	***fringe***	***forest***
Richness (R1)	-	7.32	5.43	-	4.13	4.15
Diversity (H')	-	3.19	2.34	-	2.22	2.55
Evenness (E5)	-	0.89	0.47	-	0.53	0.84

3.3.6 Vegetation structure

The fringe and forest plots in both sites showed no clear patter on tree abundance, species number or basal area (Table 3.15). The Crasto site showed greater values of trees and species number (Figure 3.18a) although the Caju site had the greater basal area values (Figure 3.18b). The Caju site presented the greater basal areas due to the presence of more individuals in the higher D.B.H. classes (greater then 30 cm), which were less frequent in the Crasto site (Table 3.15).

Table 3.15 Vegetation structure indexes of each study site and plot.

Site / Plot	***Number of trees***	***Number of species***	***Average D.B.H. (cm)***	***Basal area (m^2 ha^{-1})***	***Average height (m)***	***Maximal height (m)***
Crasto						
Plantation	2	1	28.8	6.56	22.5	23
Fringe	113	35	10.8	74.31	9.9	20
forest	139	27	9.84	70.87	9.19	17
Caju						
Plantation	5	1	25.1	12.54	12.9	15
Fringe	100	20	12	94.81	6.79	13
forest	60	18	17.6	116.41	8.3	20

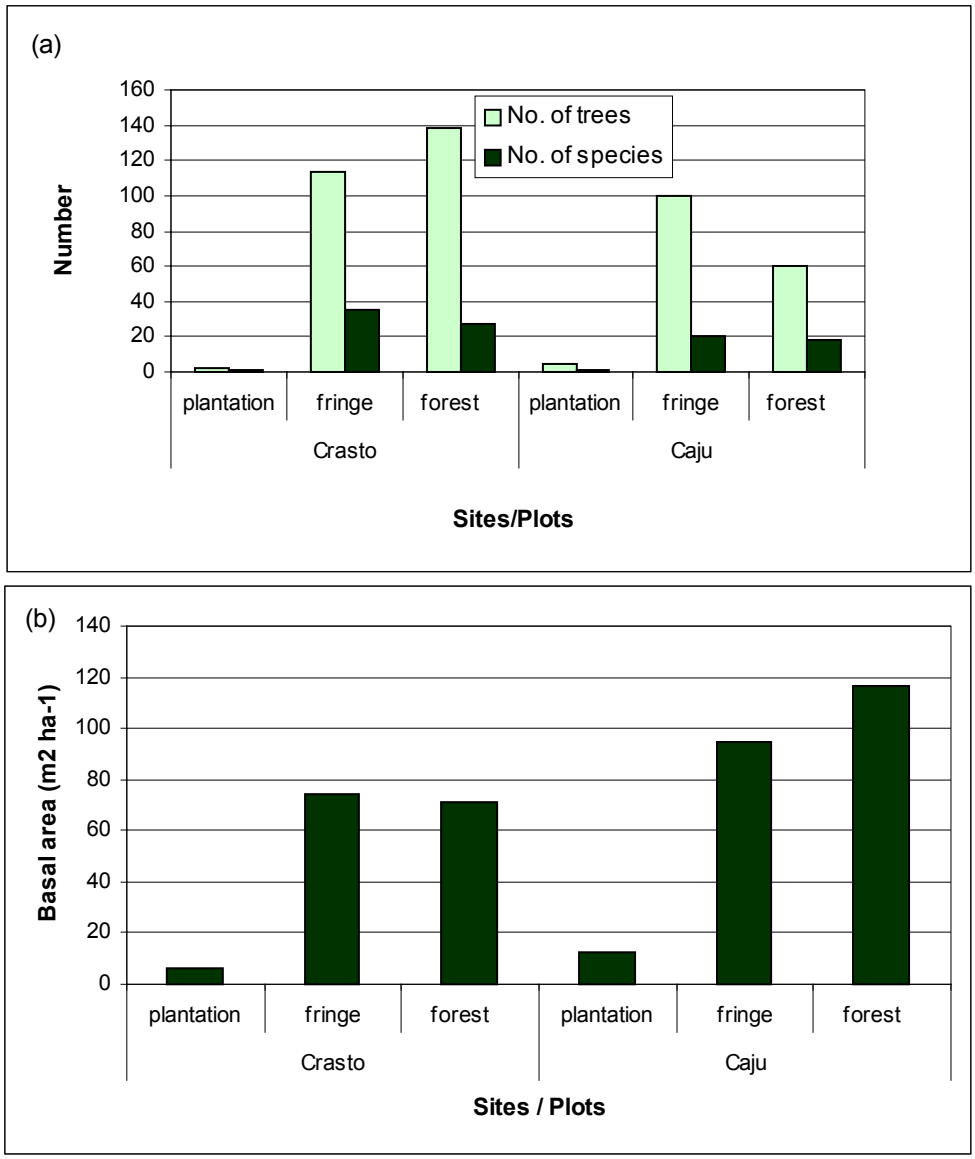

Figure 3.18 Tree and species number (a) and basal area distribution in the studied plots in each site.

Both plots in the two sites presented, however, a great number of young trees (Figure 3.19). Fringe plots in both sites and forest plot in the Crasto site showed a similar inverted J pattern, but forest plot in the Caju site presented a more even distribution of D.B.H. values. Trees in bigger D.B.H. classes (> 65 cm) were only found in the Caju site. However, gaps in some D.B.H. classes, sometimes encompassing even six contiguous classes, were also found in both sites.

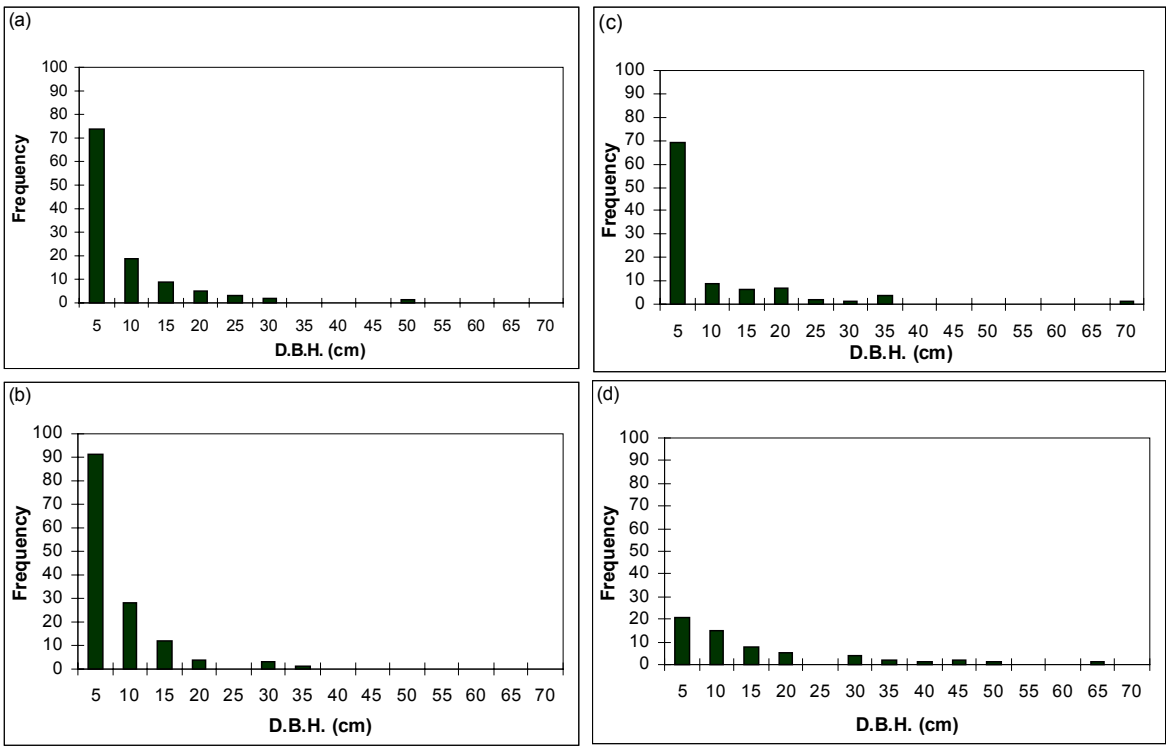

Figure 3.19 Diameter breast height (D.B.H.) histograms in the studied plots (a,c: fringe: b,d: forest) and sites (a,b: Crasto: c,d: Caju).

3.4 Discussion

3.4.1 Climate and microclimate

Seasonality in rainfall within Sergipe's coastal region has been already described (see 2.3 Climate). Actually, spatial and temporal variation in precipitation amount and distribution seems to be frequent in this region, as throughout the 'wet tropics' (Landsberg 1984). The large inter-annual variation in rainfall in the tropics is extremely important, and moisture is considered to limit plant growth in about three-fourths if the arable lands in the tropics (Sanchez 1976).

Distribution of rainfall through the year is regarded to be more important than its total amount, and length of the dry season increases latitudinally (Sanchez 1976). In fact, years with extremely long dry seasons have been found in the analysis performed (see 2.3 Climate), and this characteristic may have determined, at least partly, the structure and composition of the forests growing in this region. Besides influencing forest species composition and growth patterns (Landsberg 1984), length and frequency of the dry season may have some implication in determining success of forest regeneration, specially where soil characteristics have been disturbed after forest removal. Although total rainfall in this area is enough to allow forests to establish, deforestation may not only delay the reforestation due to soil erosion (caused by intense rain during the rainy period), but the relatively long dry season (although with pronounced between-year variation) should hinder the natural and stimulated recruitment. This problem may be worsened by the presence of the hardened horizons, which affect drainage and, consequently, water availability for plant growth, particularly in the dry season.

Distribution of rainfall during the study year (2000) can not be considered typical, with an unusually wet February. However, total rainfall during this year (1485.5 mm) was not markedly different from the mean value reported for Aracaju (1489.965 mm). Nevertheless, results obtained from the two sampling times, must be considered cautiously, as they may not represent the conditions usually prevailing during these months.

With growing rates of deforestation in tropical regions, and increasing concern about it, many studies have been carried to assess effects of forest fragmentation on microclimate (Malcolm 1994, Camargo & Kapos 1995, Malcom 1998, Didham & Lawton 1999), soil nutrients (Didham 1998), animal populations (Didham *et al*. 1998, Stevens & Husband 1998, Silva & Tabarelli 2000), and vegetation (Aizen & Feinsinger 1994, Fox *et al*. 1997, Laurance 1998, Didham & Lawton 1999, Mesquita, Delamônica & Laurance 1999, Restrepo, Gomez & Heredia 1999, Tabarelli, Mantovani & Peres 1999, Oosterhoorn & Kappelle 2000, Silva & Tabarelli 2000). Many of these works have focused on the 'edge effect', changes in the biotic and abiotic environment following forest fragmentation. This can be particularly important in small fragments, and mostly were small fragments are almost the only forested area left, as in the northern Atlantic forest, as a relatively greater area of the fragment would be subjected to this effect. The establishment of a 'fringe' plot in both forest remnants aimed, therefore, to determine possible differences due to this edge effect.

Microclimatic differences between plots were found, but differences were mainly between plantation and fringe/forest, than between forest and fringe plots. Plantation plots showed consistently

higher air and soil temperature values, with higher amplitudes. Soil temperatures were also generally lower than air temperatures, which is expected due to the relatively low heat capacity of soils (Sanchez 1976). In fact, an amplitude of 13°C in air temperature in the February sampling in the Crasto site corresponded to an amplitude of only 2°C in soil temperature. Mean, maximum and minimum values of soil temperatures were, however, greater in the Crasto site. As no broad microclimate sampling was conducted, this may well represent just and artifact caused by insufficient data, as well by the fact that comparison were made between one-week interval samplings. However, as air temperatures do not follow the same pattern, it is probably to correspond to a real difference between both sites. Actually, heat conduction is rapid in soils containing much quartz sand (Kalpagé 1974), and excessively high temperatures are reported for some sandy topsoils, as thermal diffusivity of these horizons is much lower than that of loamy or clayey topsoils, causing them to retain large quantities of heat, particularly when dry (Sanchez 1976).

Thus, soil temperatures amplitude was greater in the plantation plot of the Caju (6°C in February) than in the Crasto site (2°C in the same period), and plantation plots showed higher values than fringe/forest. Plots. Removal of forest cover can lead to increase in the topsoil temperatures by 7 to 11°C (Sanchez 1973 *in* Sanchez 1976) because of the higher solar radiation reaching the soil surface upon exposure. This higher soil temperatures may have also effect on soil microfauna composition, abundance and activity and is supposed to represent another stress factor that forest seedlings must face in order to recolonize these areas. Its effect on the mycorrhizal community is focused in Chapter 4.

Similarly, lower values of air and soil temperature, and relative humidity were found in forest than in the adjacent plantations, in a previous study conducted in the Mata do Crasto (Stevens & Husband 1998). These figures significantly increased with increasing distance from edge into the forest (Stevens & Husband 1998). While soil temperature remained stable after 20m from the edge, a constant increase in air temperature and relative humidity was found, stabilizing just after 60-80 m. As the forest plot was placed 70 m into the forest fragment (See 3.2.2 Sampling design), this area may have been outside the region subjected to this edge effect.

3.4.2 Soil physical and chemical parameters

Although from different geological origins, and presenting different soil types, a general and clear pattern on nutrient distribution between both sites was not found, due to the great spatial (horizontal and vertical) and temporal variability found. In this point, the sampling scheme chosen may have been at least partly responsible for this results, as only compounds samples were taken for the analysis of soil physical parameters, and for chemical parameters in February. Actually, in order to better represent the nutrient spatial variability in forest soil studies, composite samplings should be avoided, and multiple sampling schemes (including different sample numbers) for different depth and parameters are suggested (Sparovek & Camargo 1997), what is in most cases unfeasible. Soil physical properties also vary spatially and sample numbers required for a estimation with 10% error are also found to vary between different properties (Warrick & Nielsen 1980).

Soil structure refers to the arrangement, size and stability of soil aggregates. Texture and structure are important soil physical properties as they influence aggregate stability and infiltration

rate. The latter determine the degree of surface runoff and, consequently, erosion (Kalpagé 1974). For example, sandy topsoils are often more strongly predisposed to erosion than clayey soils (Kalpagé 1974), and this may be important as soils in both sites presented a high to relatively high sand content. While in the Caju site, on sandy soils, it varied between 86-98 and 94-97 %, respectively, in the February and November sampling, these figures were around 59-84 and 60-88 %, in the Crasto site, characterizing loamy sands to sandy loams. Actually, high clay contents are needed for high aggregate stability (Sanchez 1976) and the small amount of clay in quartziferous sands is responsible for their low cohesion (Jacomine 1996). Many sandy Oxisols and Ultisols (this latter, equivalent in the US soil system to the Brazilian Red-Yellow Podzols) are reported to be vulnerable to compaction and erosion, due to its low clay content in the A horizon (Sanchez 1976). Indeed, plasticity and cohesion of kaolinite, the main constituent from the clay fraction of Yellow Podzols (Jacomine 1996), are very low (Brady 1974). Kaolinitic minerals are formed in many areas around the tropics, under acid conditions (Fripiat & Herbillon 1971).

Soil structure, particularly pore size and distribution, is related to the vertical and lateral flow of water and nutrients movement (Sander 2002). In fact, size and distribution of the pores are important physical properties, influencing root growth and determining rainfall infiltration, and, consequently, soil water and air available to roots (Kalpagé 1974). However, available water is not evenly distributed in soils, as roots do not extract it uniformly (Sanchez 1976). Differences in soil structure are thus related to the differences found in their water retention capacities. Ultisols usually present high available water capacities and rates of infiltration, maybe due to lateral water movement (Sanchez 1976). Analyzing soil cores from tropical regions around the world, Sander (2002) did not found the close correlation between texture and porosity, cited for temperate regions. Nevertheless, macroporosity (pores > 10 μm) was considerably high, what would explain rapid drainage and frequently high infiltration capacities after rainfall. Unfortunately, this author do not provide any information on vegetation cover or soil relief, which may also affect water drainage patterns. In fact, sampling in February in the Crasto site was done under heavy rainfall, when significant surface runoff could be observed. However, surface runoff was not found on undisturbed Amazonian forest soils, even after very heavy rain, suggesting a more effective drainage (Hodnett *et al*. 1995). Differences in soil type could explain these discrepant results, but soil in the region is described to be a clayey oxisol "of low water available capacity". The more sandy topsoils in the Crasto site should actually allow a more rapid drainage. Therefore, the reason for these differences may be due to rainfall intensity.

Not only soil texture, but also organic matter content, climate, and vegetation cover are related to soil erosion. Organic matter is probably the most important factor influences aggregate stability (Brady 1974), and decrease in soil humus content is followed by a reduction in the ability of absorbing water (Kalpagé 1974). Soils in regions with alternating wet and dry seasons, as in the coastal area of Sergipe, are usually more prone to suffer erosion than in areas of evenly distributed rainfall (Kalpagé 1974). Soil drainage includes both vertical and lateral flows (Landsberg 1984), but when rainfall volumes exceeds the soil's storage capacity significant amount of soil and nutrient may be lost due to leaching, runoff, and erosion (Sanchez 1976). Forest cover also helps to prevent the impact of water erosion, as tree and shrubs canopies reduce the impact of raindrops on soil, and tree roots build up channels helping infiltration (Kalpagé 1974). Furthermore, soils already prone to face

soil erosion due to their low high aggregate stability and cohesion as the sandy quartziferous sands (Jacomine 1996) will experience increased soil erosion as vegetation is removed (Oliveira, Jacomine & Camargo 1992). Actually, soil cover is the predominant factor affecting erosion (Sanchez 1976) and the general trend of higher nutrient concentrations in the upper layers of soil of fringe and forest plots may reflect not only the greater input of nutrients due to litter fall but also the greater erosion in the plantation site, without a significant tree cover protecting it from weathering.

How the removal of forest cover will affect soil structure in a determined region is related to the soil properties, as well as with the kind of activity after the area is cleared (Sanchez 1976). For example, cultivation was found to reduce the percentage of aggregates larger than 2 mm in a Brazilian Ultisol (Grohman 1960 *in* Sanchez 1976), what is particularly important as these smaller aggregates can reduce water infiltration, as they obstruct the large pores between the larger aggregates (Sanchez 1976). Deforestation and intensive cultivation on African soils also reduced the size of water stable aggregates and aggregate stability (Spaccini *et al*. 2001). Mechanical land clearing resulted in important changes in soil physical properties (Alegre & Cassel 1996) and severe problems of soil compaction have been caused by heavy tractor traffic, specially on sugarcane plantations (Sanchez 1976). Soil disturbance by tractors in the cleared and burned forest areas can also lead to the removal of the original forest topsoil and have a significantly effect on soil hydrological variables (Malmer 1996). Permeability and the water availability were seriously reduced through the use of heavy machinery (Hodnett *et al*. 1995).

Cattle grazing can also affect soil structure, and soil bulk density and water-stable aggregates under pastures were lower than under forests (Krishnaswamy & Richter 2002). As a result, soil compaction, with a correspondingly increase in soil bulk density, mechanical resistance and reduced permeability, is often cited (Hodnett *et al*. 1995, Alegre & Cassel 1996). For instance, a review on forest conversion to pastures in neotropical regions revealed consistent increases in soil bulk density (Fearnside & Barbosa 1998). Overgrazing was also found to reduce earthworms biomass (Alegre & Cassel 1996). The major causes of erosion in tropical soils are thus deforestation, overgrazing of pasture lands, and inadequate cultivation methods (Sanchez 1976).

The two coconut plantation around the forest fragments studied are not subjected to intensive soil mechanical disturbance, but tractors are sometimes used to clear the secondary growth of native vegetation. These areas are also occasionally used as pastures, so soil compaction due to trampling by cattle may occur. However, data on soil bulk density are not available. No clear pattern on particle density was found regarding both sites and plots.

Forest conversion have been found to result in a considerable increase in water drainage (Klinge, Schmidt & Fölster 2001), and both nature of the clearance and relief may affect soil moisture (Nortcliff, Ross & Thornes 1990). In a study in Amazonian region, these authors found an negative exponential relation between soil erosion and cover, with the greater sediment losses being presented by the cleared treatment. Malaysian rainforests also presented lower surface runoff and total sediment outputs than selectively logged or fire-damaged areas, but sites with nutrient-poor sandy soils, were even more susceptible to nutrient losses after forest conversion (Malmer 1996). Although agroforestry systems were found to improve soil physical properties (Alegre & Cassel 1996), nutrient dynamics in agroforestry systems, compared to pastures, were not improved (Tornquist *et al*. 1999). Furthermore,

estimates of nutrient leaching about 10-185 % of that removed in the harvest, poses serious concerns about the long-term sustainability of tropical rainforest conversions to plantation forestry (Malmer 1996).

In regions where a pronounced seasonal variation in rainfall is found, as in Sergipe, deforestation may lead to a particularly low subsequent forest tree establishment. Not only the bare soil will be subjected to greater erosion rates during the rainy season, but drought may be also more intense in the area without forest cover in the dry season. Although mean rainfall and evaporation data do not suggest soil water shortage, significant soil water deficits during dry seasons were observed in Amazonia (Hodnett *et al*. 1995). Forest and pastures did not differed much in soil water storage during the wet season, but in the dry season differences were more pronounced, being found only in deeper soil layers (> 2 m). Forest trees were able to take up water from depths greater than 3.6 m and deep rooting may help these plants to face even unusually severe dry seasons (Hodnett *et al*. 1995). Plant available water in pasture was also found to be significantly different than that of mature and secondary forest in seasonally dry Amazonia (Jipp *et al*. 1998). With deeper roots, forest trees have access to soil moisture even in the dry period. Therefore, in contrast to pastures resulting from forest conversion, these forests appear to be well buffered against the large fluctuations in moisture availability found in this region (Jipp *et al*. 1998). Sergipe's coastal forests may be similarly subjected (as well adapted) to the rainfall seasonality. However, differences in water content in the present study were not so clearly between plots as between sample times, and more accurate studies are yet to be performed in the region, in order to determine the real extension of forest cover removal on soil water dynamics.

Soil fertility is characterized by a complex of physical and chemical properties (Kalpagé 1974). Soil nutrient content is somewhat related to its textural properties (Oliveira, Jacomine & Camargo 1992) and the results in nutrient distribution found in the present study may be, at least partly, explained by them. Quartziferous sands are sand or loamy sands, basically constituted by quartz, are poor and acid soils, usually with high Al saturation. The low content of clay is responsible not only for their low cohesion (Jacomine 1996), but also for the low P fixation in these soils (Camargo *et al*. 1974 in Oliveira, Jacomine & Camargo 1992). These sands posses low cation retention capacity, rarely reaching values up to 2 me 100 g^{-1} soil, with a significant decrease along the soil profile (Oliveira, Jacomine & Camargo 1992). Red-Yellow Podzolic soils are also poor in plant nutrients (Kalpagé 1974). The Yellow Podzols of the Brazilian coastal tablelands (formerly include in the Red-Yellow Podzols) possess chemical and mineralogical properties similar to the ones of the Yellow Latosols (Jacomine 1996), with high bulk density (Souza 1996). Hardened layers are an important feature, and are found just bellow the A horizon, reaching sometimes greater depth of the B horizon.

Soil acidity is a characteristic of regions with high rainfall regimes, where leaching can respond for considerable losses of exchangeable bases in the soil upper layers (Brady 1974). In fact, most of the soils in the humid tropics are acid (Sanchez 1976). Both of the soils considered in the present study, the quartziferous sands and the Yellow Podzols, are acid to strongly acid soils (Jacomine 1996), what is confirmed by the range of variation of pH values found in the present study. This range did no differed much between both sites. Values differed significantly, however, between

plantation plots and fringe/forest plots in both sites. In the Crasto site, plantation plot presented somewhat higher pH values than fringe/forest plots. This agrees with the results of Krishnaswamy & Richter (2002) comparing pastures and forest under Ultisols and Oxisols in Costa Rica. Pastures also presented higher pH than agroforestry treatments in another study in Coast Rica (Tornquist *et al*. 1999). In the Caju site, differences between plots were not so markedly in February. In November, however, the plantation plot showed much lower pH values than fringe/forest plots, what contrasts with the results of Gomes *et al*. (1998), comparing quartziferous sands under native vegetation and pasture, which also found lower pH values under native vegetation. This trend of higher acidity in soils under forest compared to grasslands has been described previously (Jenny *et al*. 1968 *in* Brady 1974). The consistently lower of pH values obtained in the November sampling points out to an apparent seasonal variation. Dryer and warmer conditions are related to higher acidity values (Brady 1974), but temperature do not vary considerably in the region (mean annual amplitude of about 2.9^{o}C in Aracaju), and soil water content was significantly higher in November than in February, as a result of the accumulated water during the rainy season.

Soil acidity is mainly determined by exchangeable aluminum (Sanchez 1976). In neutral and alkaline soils, Al possess low solubility, but under lower pH values, the increased solubility of Al may present toxic effects to plants (Mengel & Kirkby 2001). In fact, aluminum toxicity is reported frequently in tropical soils, and may hinder root development of some crops, as well as increase Ca and P deficiencies (Sanchez 1976). In the present study, soil pH values was always below 5.2 in November, and mostly bellow 5.6, in February. However, only one sample (the 5-10 cm depth in the Caju plantation plot, in November) presented pH values lower than 4.2, which is the point under which AL^{3+} become the dominant cation in the soil solution (Ulrich 1991), and toxic effects can take place (De Leo, Del Furia & Gatto 1993, Elstner & Hippeli 1995, Kabata-Pendias & Pendias 1992). However, plant species from acid soils can present adaptations to these stressed conditions, some of them accumulating Al in their tissues (see references in Davies 1997).

In the Crasto site, under forest, Al concentration was considerably higher in the lower soil layers, while in the plantation plots higher values were found in the upper layers.
However, in the Caju site the plantation plot showed higher values than fringe/forest plots, without any markedly variation along the soil profile. The soil depth studied (0-30 cm) probably was not enough to show this spatial variation of Al in these soils, as an increase in Al concentration in the B horizons (> 80 cm) is described for other quartziferous sands (Gomes *et al*. 1998).

The higher soil Al values in the present study were found in the deeper layers of the Crasto site (about 1.8 me 100 g^{-1} at 10-30 cm), and are higher than the values presented as typical for Yellow Podzols of tablelands (Jacomine 1996). As discussed above, these values refer to soils under cultivation or pastures (Jacomine 1996). The figures obtained in the present study from the plantation upper layers (0-20 cm) in the Crasto site are, with some small seasonal variation, more similar to them.

Soil Al concentration in the Caju site was also higher than the values provided in the literature about quartziferous sands (Gomes *et al*. 1998). Although in the present study slightly higher Al values were present in the plantation plot, these authors found somewhat higher figures in soil under native vegetation. In absolute numbers, these were lower (0.23 and 0.30 me 100 g^{-1}, respectively, for pasture and forest) than the ones found in Caju site (0.6 in all plots in February. No variation was found in

November in the plantation plot, but fringe/forest plots showed more similar figures – 0.15 and 0.3 me 100 g^{-1}). However, a great vertical variation along an apparently homogenous soil horizon may also be observed for the Al concentration, as well as for organic matter, extractable P and CEC by (Sparovek & Camargo 1997). If this is true, comparisons between the present study, when thinner soil layers were analyzed, with the data provided by Gomes *et al*. (1998) could lead to misleading interpretations.

Red-Yellow Podzolic soils are considered to present larger amounts of exchangeable aluminum than latosols of the same texture (Kalpagé 1974), and high levels of aluminum saturation, specially in the subsoil, are reported for some Ultisols (Sanchez 1976). Generally, Ca/Al ratios < 1 in the soil solution are considered perilous for roots in temperate forest soils (Meiwes et al. 1984 *in* Fölster, Dezzeo & Priess 2001). Comparison of this ratio between both sites and the two sample times provided no clear pattern. The Crasto site presented greater number of soil samples with Ca:Al ratio below 1, but while in February higher figures (> 1) were found in the upper soil layers from fringe and forest plots, the plantation plot showed a somewhat inverted pattern. In November, however, samples from all depths and plots revealed values below 1. In the Caju site, a somewhat similar pattern, although inverted, was found. Higher values were also found in the soil upper layers, but most of the soil depths presented values higher than 1. The same is true for both sampling times, although figures were consistently higher (reaching 8.54 in the 0-5 layer of the fringe plot). The plants growing in the Crasto site, therefore, seem to be more subjected to suffer from Al toxicity than the ones growing in the Caju site.

When analyzing soil nutrients under native forests, spatial variability must not be forgotten. The number of samples necessary to estimate mean of soil P within 10 % error was estimated to be as much as 812, as its variance was found to be completely random (Sparovek & Camargo 1997). Results from the five randomly taken samples revealed no significant difference in soil P between both sites, despite differences in soil type. Red-Yellow Podzolic soils are poor in plant nutrients, holding phosphate ions more weakly than latosols (Kalpagé 1974). However, extremely sandy soils can also present substantial phosphorus losses (Sanchez 1976). In soils of tropical regions, usually highly weathered, much of the P can be unavailable to plants, as its become tightly fixed onto aluminum and iron oxides in clay (Lodge, McDowell & McSwiney 1994). This highlights the importance of assessing, when studying nutrient relations in tropical soils, besides total P, also the plant available P.

In fact, soil profiles in southeastern Brazilian coastal quartziferous sands revealed an increase in soil P with depth, reaching up to 24.97 mg kg^{-1} in the B2 horizon (150-180 cm) under native forest and 9.24 mg kg^{-1} in the same horizon (123-182 cm), under pasture (Gomes *et al*. 1998). Therefore, a vertical migration and accumulation of P seems to take place in these soils (Gomes *et al*. 1998), although it is not possible to confirm if the same happens in the Caju site, as only the superficial soil was sampled. In contrast, in the upper layers (0-22 cm) of the restingas studied by Gomes *et al*. (1998), values of soil P were consistently lower compared with the values obtained from the Caju site. In the former, figures ranged from 1.49 and 1.75 mg kg^{-1}, respectively, for pasture and native forest. In the Caju site, P content in plantation plot (3.35 and 5.52 mg kg^{-1}, in February and November) did not differed significantly from that in the fringe plot (3.24 and 6.84 mg kg^{-1}), but from the forest plot (5.03 and 6.07 mg kg^{-1}).

Comparison between Atlantic forest on southeastern tablelands and on quartziferous sands (Kindel & Garay 2002) revealed a range of variation of P concentration in the A horizon similar to the one obtained in the present study (respectively, 2.80-5.61 and 3.30-6.80 ppm). Soil pH values in these soils are also within the range of acidity found in the studied soils (4.5-5.6 and 4.6-5.2). However the soil P values in the upper layers are considerably higher than the values presented (about 1 ppm in the upper 0-20 cm) for a typical Yellow Podzol of the Brazilian coastal tablelands (Jacomine 1996). Even though not mentioned by this author, these data are probably derived from soils under cultivation or pastures, due to the great discrepancy between the soil P in the upper layers (0-20 cm) of fringe and forest plots. Values from the plantation upper layers, although still somewhat higher, are more close to them. The impact of deforestation in soil nutrient pools are, therefore, clearly evident. In fact, many small settlers complain about not being allowed to use the more fertile soils from the forest fragments in the region, not attempting to the fact that this fertility was lost with unsuitable cultivation practices. Consequently, conservation of these forest remnants must include also technical support to farmers aiming to a better management of their soils.

The highest soil P concentrations were found in the November sampling, both in the upper layers (0-5 cm) of fringe plots (9.18 and 6.84 mg kg^{-1}, respectively, in the Crasto and Caju sites). Figures for soil deeper layers were however, similar in fringe/forest and plantation plots, but soil P in soil upper layers were greater in fringe/forest plots than in the plantation plot in the Crasto site. Higher extractable P values in the upper 25 cm soil were also found under agroforestry than under pastures (respectively, 7.24 and 6.19 mg kg^{-1}) in the Atlantic region of Costa Rica (Tornquist *et al*. 1999). However, this difference was not found in the Caju site.

Distribution of other nutrients failed to fit in a definite and clear pattern, the only exception being the expected trend to decrease of nutrient concentration along the soil profile. Exchangeable cations concentration is usually higher in the soil surface layers (Jacomine 1996, Sparovek & Camargo 1997, Gomes *et al*. 1998). Actually, reported differences of nutrient concentration, including organic matter and CEC, even between the upper layers of forest soils, where higher values are commonly found in the first 0-5 cm layer, reinforces the importance of analyzing separately thin soil layers, instead of the usual first 0-20 cm, in soil fertility studies, or the upper horizon, in pedological surveys (Sparovek & Camargo 1997).

Soil Na was significantly higher in the Caju site, while this was not so markedly relating Ca concentration. In each site, plantation values were usually lower than fringe/forest plots. However, soil Na content in the plantation plot of the Caju site was considerably higher than in the Crasto site. This is probably due to the greater proximity to the sea of the Caju site, as NaCl ions may be carried with water particles. This higher soil salinity values may hinder growth of crop cultures unfamiliar to this region, but not of the many plant species adapted to the prevailing conditions of the *restinga*. A seasonal variation was found, but it also did not presented any clear trend. Reasons for this behavior are not clear and must be further studied. Soil K concentration was higher in the February sampling in the Crasto site, but lower in the Caju site. Soil Mg values were higher in February in the Caju site, but did not showed any consistence difference in the Crasto site. It is possible that a high spatial variability in nutrient distribution (Sparovek & Camargo 1997) within these soils plays a role in determining the results found. For instance, data for the November sampling presented sometimes high standard

deviations, mostly in fringe and forest plots. Furthermore, the composite sampling in February excludes more accurate statistical analysis of the data. Therefore, further sampling, with greater sample sizes and, possibly, with more sampling times, in both sites is still necessary and may give more accurate data on spatial and temporal variation on nutrient dynamics in these forests.

Data provided by Jacomine (1996) for K and Na in upper layers (0-10 cm) of a typical Yellow Podzolic soil are lower than the values obtained for soil upper layers in plantation and fringe/forest plots in the Crasto site in February In November, however, these figures were similar, even to fringe and plantation plots, reflecting a strong seasonal variation in the soil nutrient content. This author presented values for Mg and Ca summed (1 $cmol_c dm^{-3}$), and the values of plantation plots fits well to them (0.83 and 1.0 me 100 g^{-1}, respectively, in February and November). However, fringe and forest plots showed values that greatly exceeds these (respectively, 3.88-2.65 and 2.23-2.61 me 100 g^{-1}, for February and November), what may illustrate how removal of forest cover contributes to the nutrient impoverishment of these soils.

Values for Ca + Mg, K and Na in southeastern Brazilian quartziferous sands (Gomes *et al*. 1998) were consistently lower than the results found for the Caju site. In this site, Ca and Mg were significantly lower in plantation than in fringe plots and no difference was found between plots regarding Na and K. However, statistical analysis was made comparing variation in distribution along the soil profile and not only the soil upper layer, what may have obscured these results.

Range of variation for soil Ca in the A horizon was similar between southern tableland and restinga Atlantic forests (Kindel & Garay 2002), respectively, 0.30-1.73 and 0.69-1.69 me 100 g^{-1}. In the present study, however, these lower limits were found consistently in the upper layers of the plantation plots (0.47-0.46 and 0.81-0.62 me 100 g^{-1}, for Crasto and Caju sites, respectively, February-November). In contrast, figures in the fringe/forest plots were similar or even higher. Values for the February sampling in the Caju site, were particularly high (2.09 in the fringe and 2.56 me 100 g^{-1} in the forest plot).

Soil Ca and Mg concentration was consistently higher in the soil upper layer (0-5 cm) in fringe/forest plots than in plantation plots, in both sites. This difference was not so clear regarding Na and K values. Leaching is usually responsible for major losses of Ca, Mg, K and N (Sanchez 1976) and the higher figures in the fringe/forest plots upper layers may represent continuous new inputs due to litterfall. Contrary to this results, agroforestry treatments presented usually lower exchangeable bases concentrations than pastures in the Atlantic region of Costa Rica (Tornquist *et al*. 1999). These agroforestry systems were approximately five years old and may not have reached a structure and dynamics similar to mature forest under the same soil and climatic conditions. However, figures for Ca, K and Na under pasture and forestry were consistently higher than the ones obtained in the present study for both plantation and fringe/forest plots. The only exception was Mg, which value in the agroforestry system was lower than fringe/forest plots in both sites.

No strikingly differences were found between quartziferous sands under pasture and native vegetation (Gomes *et al*. 1998), with the exception of Ca + Mg, which were lower in the latter. This result do not agree with the pattern found for both Ca and Mg in the Caju site, where both nutrients were higher in fringe/forest plots. Na and K did not showed significantly difference between plots in this site, but absolute values were higher in the present study.

Positive significant ($p < 0.01$) correlations were found between soil pH and Ca and Mg. Actually, correlation was expected also for pH and N, P and K, as the soil pH ranges in both sites comprises the region of low pH along which differences in the availability of these nutrients take place (Sanchez 1976). Conversely, a slightly significant negative correlation ($p > 0.05$) between soil pH and Al was also found.

Nutrient pulses may be extremely important in seasonal tropical environments (Lodge, McDowell & McSwiney 1994). Indeed, most of the differences found between the two sampling times may be due not to a random variation but to a seasonal pattern of nutrients availability related to rainfall. This seasonality in rainfall, leading to periodic nutrient release pulses, can cause productivity of forests under these climates to be even higher than that of forests under non-seasonal conditions (Lodge, McDowell & McSwiney 1994).

3.4.3 Litter biomass

The great spatial variation in litter production, may be due to the inhomogeneous tree cover, in the plantation plots, and to local differences in canopy species composition and/or microtopography, in the forest plots. A temporal variability was also found, and seasonality in litter production seems to be important in both forests studied. No accurate information on the deciduous status of the tree species present in these forest remnants is available yet. Although most of them are evergreen species, it is possible that some of them shed some or most of their leaves in the dry season. However, the greater amounts of litter biomass were found in November, end of the rainy season. As no direct measure of litterfall was carried out, the results found on litter biomass may be also partially due to the decomposition processes, which rates may not be, necessarily, constant.

Comparing total amounts collected in both sampling times, the Caju site, on Quaternary sediments, presented the greater values, even if the exceptional high values of the plantation plot (due to fallen coconut leaves) are excluded. Litter biomass was also generally lower under tableland forests (5.89-6.28 t ha^{-1}) than under *restinga* forests on quartziferous sands (9.71-26.3 t ha^{-1}) (Kindel & Garay 2002), and the same pattern was found when two Atlantic forest types in the Brazilian state Espírito Santo were compared (Garay *et al*. 1995). 'Mussununga' forests on with Hydromorphic Podzols (quaternary sediments) presented also more litter than upper forests with dystrophic Red-Yellow Podzolic soils, and litter turnover rates appear to be slower in the former. However, a comparison between Atlantic and *restinga* forest in São Paulo, Southeast Brazil, revealed an inverse pattern, with higher annual litter production in the former (Moraes, Delitti & Struffaldi-De Vuono 1999).

Litterfall is higher in tropical than temperate forests, and within the former, positively related to precipitation (Lonsdale 1988). In the studied sites, litter biomass was significantly positively correlated to soil P, but not to the other soil nutrients analyzed and, interestingly, also not to vegetation structure. Decrease in leaf litter production has been found to be correlated with soil pH and available soil phosphorus, but not with total P content (Van Noordwijk & Hairiah 1986). Vitousek (1984) also found a positive relationship between P content in litterfall and litterfall biomass, probably because productivity in a considerable number of tropical forests is often P limited. However, Vogt, Grier &

Vogt (1986), reviewing litter production and turnover in forests along different climatic conditions found out that nitrogen availability, more than phosphorus availability, would have greater effect on litter production. Nevertheless, the amount of litter biomass present in both studied sites may not necessarily indicate higher production, but also slower decomposition rates. Litter decomposition is affected by litter quality and nutrient content (Vogt, Grier & Vogt 1986). High lignin content and high lignin : N ratios (Cuevas & Medina 1988) as well as low nutrient content, especially N and P, may decrease rate of litter decomposition and, consequently, cause an accumulation of organic matter (Swift *et al.* 1979, Vogt, Grier & Vogt 1986). In fact, lower decomposition rates are found in forests with low nutrient availability in soil and litter, as this reduces microbial activity (Vogt, Grier & Vogt 1986). This seems to explain the differences between restinga and tableland forests found by Kindel & Garay (2002). Litter from the former presented generally higher C : N (32.5-61.4) ratios than the latter (26.7-37.4). Litter from *restinga* forest was also found to be nutrient poorer (in macro and micronutrients) than the litter from an Atlantic forest (Moraes, Delitti & Struffaldi-De Vuono 1999). It seems that restingas forests present, despite the lower litter production (Moraes, Delitti & Struffaldi-De Vuono 1999), comparatively lower decomposition rates than Atlantic forests on similar climatic conditions.

Another important consequence of forest conversion to agricultural land uses is the removal of the litter layer itself (Krishnaswamy & Richter 2002). Litter quality also influences diversity and abundance of different faunal groups, which exert influence on its decomposition rates, as well as seasonality. Wet periods present greater activity of decomposer organisms than dry periods (Hunt, Elliott & Walter 1989). Seasonal variation in soil moisture affects microbial biomass (Van Veen, Ladd & Frissel 1984, Luizão, Bonde & Rosswall 1992) and the activity of soil fungi and bacteria (Cornejo, Varela & Wright 1994). For instance, soil and litter moisture and organic matter were also found to be strongly positively correlated with some Collembola and other microarthropods (Betsch & Cancela da Fonseca 1995).

Deforestation may have also negative effects on these groups, with implications for litter decomposition and nutrient dynamics. Cultivation has been found to reduce biomass of some decomposer organisms, like fungi, nematodes and microarthropods, but not others, as bacteria or protozoans (Hunt, Elliott & Walter 1989). In fact, forest recovery period of 16 years was found to be sufficient to allow a diversity of litter fauna similar to forest sites, but not of the soil fauna (Betsch & Cancela da Fonseca 1995). But not only forest removal, but also forest fragmentation, and the resulting edge effects, can also affect litter dynamics. Leaf litter in open (without secondary vegetation around the border) and closed forest edges presented marked differences in moisture (Didham & Lawton 1999). Edges presented also higher litter decomposition rates, and turnover rates were higher in the edge of smaller fragments (Didham 1998). Although this author failed to find any significant correlation between litter decomposition rates and microclimatic variable or litter invertebrate densities, he found significant effects on forest fragmentation and litter structure and nutrient cycling, with higher variability und unpredictability of litter decomposition rates near forest edges.

Data on nutrient content of litter is not available yet, and it is expected to help to understand differences in nutrient pools between both sites. For example, concentration of C, N, Ca + Mg, K, Na and S were markedly higher in the organic layer on a typical Yellow Podzol in coastal tablelands than

in the subjacent mineral soil (Jacomine 1996), and thus litter may represent the greater pool of these nutrients. As deforestation may not only reduce litter production, but also increase surface runoff and, consequently, removal of this layer, roots may be deprived from this source of nutrients, therefore affecting plant growth.

3.4.4 Root biomass

The presence of a superficial root mat in fringe and forest plots of both sites may imply a an active role of these roots in nutrient acquisition as suggested by Medina & Cuevas (1989). Total root biomass in the soil upper 30 cm was significantly higher in the Caju site than in the Crasto site, being values fairly constant within the three plots. In a study comparing *restinga* and tableland Atlantic forests (Kindel & Garay 2002), the greater root biomass figure (almost 10 t ha^{-1}) was presented by a restinga forest. Within restinga forests, although these figures were somewhat higher than on tableland forts, a higher variation in root biomass was found, even between forests on similar soil types. In contrast, between tableland forests, values were more similar. However, the cumulative root biomass values found, even in the Caju site, where strikingly smaller than the figure (4.9 kg m^{-2}) considered characteristic of tropical forests (Jackson *et al.* 1996). This difference may be due to the longer dry season prevailing in the studied region. Water limitation can reduce root activity in tropical regions and this may be the reason why higher total fine root biomass was found in evergreen than in semideciduous forests (Vogt, Grier & Vogt 1986). However, figures found are also lower than the ones reported (10.3 $t.ha^{-1}$) for a semideciduous forest in Panama (Cavelier 1992).

Differences between the two sampling times, most pronounced in the Caju site, may reflect a seasonal effect on root production following rainfall variability. In the Caju site, on sandy soil, water retention may not be enough to support plant activity during the dry season. But no clear seems to emerge from the results, as higher root biomass values were found in February, what should normally be the end of the dry season. In this year, exceptional strong rainfall occurred in this month, but it is not clear if root growth could be so fast in order to better uptake this resource.

Some nutrients (Ca, Mg, P and Na) were positively correlated with root biomass distribution. Vertical root distribution in tropical forests seems to be positively related to N but inversely to Ca content (Cavelier 1992). Soil pH showed also some significant relationship with root biomass but Al not, although a slightly negative r_s was found, what is expected, as Al can inhibit root growth (see Mengel & Kirkby 2001). This may be due to the smaller sample size available to the statistical analyses for this element, as some soil samples did not have enough material to perform all chemical analysis, or because soil Al levels were not always high enough to cause toxic effects.

Although highly variable, as revealed by the high standard deviations, root distribution along the soil profile was markedly different in both sites. In tropical evergreen forests, about 69% of the root biomass is concentrated in the upper 30 cm of forest soils (Jackson *et al*. 1996). But, while in the Crasto site a more evenly distribution was shown, in the Caju site root biomass was concentrated in the soil upper layers. The exponential reduction in root biomass cited by Cavelier (1992) is therefore found in the Caju, but not in the Crasto site. A greater root biomass in the upper soil layer could also use more efficiently the nutrients released by the slowly decomposing litter (see above 3.4.3. Litter).

The importance of root turnover to nutrient dynamics in forest ecosystems may be underestimated, probably due to the complexity of the methods of study needed. Actually, Vogt, Grier & Vogt (1986) estimate that root turnover may contribute 18-58 % more than litterfall to N addition in soils. Further research, including long-term observations, is still needed in order to assess how the extended dry season present in the coastal region of Sergipe affects root production, activity, and nutrient dynamics in the two forest types studied.

3.4.5 Floristic composition and vegetation structure

The area sampled in this study is certainly insufficient to be representative of the vegetation of both fragments. However, more than to describe this flora, the main objective here was to characterize the environment in a relatively small region (400 m^2), immediately around the areas where soil cores were sampled. If sample area may have been insufficient to assess the floristic composition of each site and plot, some of the results found, regarding forest composition and structure, represent however interesting patterns which deserve further studies.

Fringe and forest plots from the Crasto site, on Tertiary sediments, presented higher or similar values of richness and diversity than the plots in the Caju site, on Quaternary sediments. Species diversity was found to be higher on tertiary sedimentary than on Quaternary sedimentary rocks in Bornean forests (Takyu, Aiba & Kitayama 2002).

Topographical changes in forest structure and species composition were found to correspond to changes in soil nutrients, particularly in pool size and net mineralization of N and P, and soluble P level is suggested to control the magnitude of these topographical changes in vegetation (Takyu, Aiba & Kitayama 2002). Sampling size in the present study was insufficient to test if the same correlation between soil nutrient, topography and vegetation is found in the studied forests in Sergipe. This is going to be performed in the 1-ha where the phytosociological survey of the Mata do Crasto was carried out.

Although the two remnants are not very far from each other (about 50 km), it seems that the soil type may have been determinant in selecting species occurrence, and may be responsible for the low species similarity between them. However, in both sites Myrtaceae is the most important family (in abundance, species number, and I.V.). This is an important family within Brazilian coastal forests (Silva & Leitão Filho 1982, de Jesus 1987, Peixoto & Gentry 1990, Tabarelli & Mantovani 1999, Pereira & Araújo 2000), and was the second most important family in number of trees in the phytosociological survey carried in the Mata do Crasto (Landim *et al*., unpublished), with 218 trees in one hectare (10,3% of the total number of trees).

Unfortunately, no comprehensive floristic or phytosociological inventory of *restingas* forests in Sergipe has been published, and therefore, no comparison can be made with the data here presented. Most studies in *restingas* are concentrated in the Brazilian Southeast region (de Lacerda, de Araújo & Maciel 1993, Assumpção & Nascimento 2000, Pereira & Araújo 2000, Pereira, Araújo & Pereira 2001, Kelecom *et al*. 2002). There is still insufficient knowledge about the *restingas* of the Northeast region, what is more urgent due to the great anthropogenic pressure on these area. This gap should be filled not only in order to provide a better understand of the biogeography of plant families,

genus and species along the Brazilian coast, but also to assure that conservation policies are successful.

Some of the species with higher I.V. in these phytosociological survey (Landim *et al*., unpublished) were also found in this new sampling in the Crasto site, like *Maytenus obtusifolia*, *Manilkara salzmanii*, *Lecythis* cf. *pisonis*, and *Eschweilera ovata*, although some were not, like the emergent tree, *Sclerolobium densiflorum*. *M. obtusifolia* and *E. ovata* showed also high numbers of trees in the present sampling, but some, like *M. salzmani and L* . cf. *pisonis*, showed a much lower abundance. This may reflect not only the spatial variability in tree distribution through the forest remnant, probably related to local differences in relief, but also differences between the species composition in the core central area of the fragment (were the above mentioned phytosociological survey was carried) and its borders. Stevens & Husband (1998) found a transition zone of 60 m in the forest margin for microclimate parameters in the Mata do Crasto. Although the "forest" plot (distant 70 meters from the forest edge) should represent a more similar composition and structure to this core area, it is also possible that a greater zone could be subjected to the border effect regarding plant species establishment. Camargo & Kapos (1995) found that edge effects on forest structure can reach up to 100m into the forest. However, assessment of edge effects were complicated by the 'sealing' of the forest border by the vegetation regrowing in this area. Therefore, care must be taken when comparing these effects between edges of different ages, as this could lead to confusing results. Furthermore, anthropogenic pressure in the studied remnants may not be overlooked, and this can go further deep in the fragment. For example, woody debris and rests of lianas barks were observed on the floor of this plot in the Crasto site, and signals of human activity where also found in the forest plot of the Caju site.

The observed gaps in some D.B.H. classes may indicate past wood extraction. However, further analysis should be done considering each species individually, along a greater sampling area, to confirm this. Nevertheless, this hypothesis is very probable as selective logging, although illegal, has been previously registered (and denounced) in the Crasto (pers. obs.). *Lecythis lurida* and *Manilkara salzmannii* are both species commonly explored for their wood. Human impact by the neighboring communities, although not quantified, may be less selective. Although protected as a experimental research farm from the EMBRAPA, the remnant in the Caju site showed also signs of human interference.

A great number of species presented just one or a few trees, and these species are in fact the responsible ones for the diversity of these systems. Although the sampling scale used in this study do not allow to characterize them necessarily as real rare species, more attention should be laid on this subject. Rare species represent also a problem for the conservation of the diversity, being more prone to be (at least) locally extinguished with forest disturbance. Furthermore, they may also need a greater forest area to support a minimal population size needed to maintain its genetic diversity. How the deforestation and the anthropogenic pressure over these fragments are affecting the reproduction and subsequent recruitment of these species is not yet known. Also important is the fact that standing dead trees were found only in the Crasto site, most of them in the forest plot.

Although only trees of *Cocos nucifera* were found in the plantation plots in both sites, this does not mean a complete absence of a woody layer in this region. A woody shrub, *Vismia guianensis*

(Guttiferae), is abundant and other smaller woody legume species (e.g. *Crotalaria* and *Desmodium*) are also present. However none of these species were tall or thick enough to be included in the sampling criteria (D.B.H. equal or greater than 5 cm). The presence of this woody layer indicates the possibility of natural regeneration around these forest fragments. However, the owners of these regions, most of which occupied by coconut plantations or kept as unmanaged cattle pastures, do not allow process to go further, by cutting them periodically.

3.5 Conclusions

Both sites, although protected by law, are subjected to anthropic influence, and studies enlarging the knowledge about its structure and function, aiming more effective conservation plans are a primarily need.

Strong climatic seasonality prevails in this region and the occurrence of a clear extended dry season seems to determine soil nutrient dynamics, root biomass distribution along the soil profile and litter biomass dynamics. Microclimatic changes, mostly between plantation and fringe/forest plots were also shown. These may be import in limiting tree seedling recruitment in deforested sites. Microclimate measures did no differed much between fringe and forest plots, although other results found in the literature seems to indicate that an area between 60-70 m from the forest border may be subjected to edge effects. Possible differences in species composition between the two areas may be further studied, with more appropriate sample schemes.

Soils of both sites are moderately to strongly acidic, with relatively high Al levels and show oligotrophic characteristics, with pools for at least some nutrient that may somewhat limit plant growth. Differences on soil types, derived from different geological origin (see 2.1 Geology and Geomorphology and 2.2 Soil types) are partially responsible for the differences observed in soil nutrient contents in both forests studied. Different proximity to the sea of both sites may also have influence these results. They may be also, at least partially, explain floristic composition, but further studies on species distribution within this region and physiological are still needed. Selection of species adapted to these nutrient-poor conditions as well as mechanisms of efficiently cycling nutrients may play a role in maintaining productivity in these forest ecosystems.

Although the restinga forest fragment studied presented generally lower soil nutrient levels than the tableland forest, this pattern was somewhat inverted, as in the case of Na, Ca and Mg. This preliminary characterization of the soil nutrient temporal and spatial variation of both studied forest fragments reveal, as expected, a general trend to diminishing nutrient pools in the deforested areas. Both sites showed, generally, a strong trend of nutrient concentration in the soil upper layers. Conversion of these forests to agricultural land use may disrupt the nutrient balance and alter significantly nutrient pools and dynamics. Soil erosion, usually high in tropical regions, may be the main responsible for soil impoverishment after removal of native vegetation.

Aiming the restoration of forest remnants present in these coastal regions, the role of mycorrhizas on plant nutrition (see 1. Introduction) should not be forgotten. Therefore, different aspects of the mycorrhizal symbiosis in both sites will be focused in the next chapters of this work.

4 Spatial and temporal distribution of arbuscular mycorrhizal fungi in soils of forest remnants and adjacent degraded areas

4.1 Introduction

In order to make more efficient reforestation initiatives it is important to evaluate the mycorrhizal function in these remnants, where this symbiosis may posses a significant role, influencing the regeneration and biodiversity maintenance (Smith & Read 1997). The arbuscular mycorrhizal fungi (AMF) are supposed to be always present in undisturbed tropical forests (Janos 1992), and its role may be essential to plant growth on the usually low-phosphorus tropical soils (Janos 1980a).

The AMF has been found to affect positively the plant fitness in the establishment phase, and in doing so, play an important role in influencing community species composition (Francis & Read 1994). These positive effects of the mycorrhizal symbiosis on tree seedlings survival and growth make it particularly an important factor influencing the tropical rainforest regeneration after disturbance (Alexander *et al.* 1992). Actually, different grades and types of disturbance may have different effects on soil mycorrhizal inoculum composition and abundance. Indigenous AMF species or strains should present a better performance in their native environment and must be considered when searching effective VAM fungi not only for use in agricultural ecosystems, but also in the regeneration of tropical rain forests (Louis & Lim 1987).

Laboratory and field approaches must be used to understand the influence of the different mycorrhizal types on the success of individuals and, consequently, on the biome dynamics (Read 1991). The present chapter introduce results of field sampling in the two forest fragments studied. Soil and roots were analyzed for spores and root colonization assessment. Possible differences in spore composition, as well as intensity of root colonization, between and within sites were searched. Plantation plots were supposed to posses less mycorrhizal inoculum, what would represent a hindrance to native forest tree seedling establishment in these areas.

The objective of this chapter was to compare forested and deforested areas and to contribute to the understanding of the role of the arbuscular mycorrhizal interactions in this ecosystem aiming to subside plans of forest restoration.

4.2 Material and Methods

4.2.1 Study sites

Sampling was performed in the two forest fragments already described (see 3.2.1 Study sites), the "Mata do Crasto" (Crasto, hereafter) and the "Fazenda Caju" (Caju).

4.2.2 Sampling design

Sampling was done in the dry and rainy season in three plots (cocoas plantations, forest border and forest) in both sites (see 3.2.2 Sampling design). Similarly to the sampling for soil analysis, five random soil cores were taken in each plot, being subdivided in five samples along the soil profile: 0-5, 5-10, 10-15, 15-20 and 20-30 cm.

4.2.3 Data analysis

Distribution of the occurrence of spores of AMF and of the mycorrhizal colonization intensity was assessed in each sample separately. Roots present in soil were washed and prepared for clearing and staining with trypan blue (Koske & Gemma 1989). The soil was subsequently used to perform wet sieving (Gendermann & Nicolson 1963). Permanent slides with spores were prepared with polyvinyl alcohol lacto glycerol (PVLG) as mounting medium (Koske & Tessier 1983, Omar, Bolland & Heather 1979).

Assessment of arbuscular mycorrhizal (AM) colonization was carried out under the dissecting microscope, examining them at 500x and sorting them out in five classes of percent root colonization: 0-5%, 6-25%, 26-50%, 51-75% and 76-100% (Kormanik & McGraw 1982). Root slides were prepared with some of the samples and analyzed under an Olympus BX60 compound microscope (with Normarski optics), with a video-camera (Sony DXC-9100P) attached. All photographs and measurements were performed with the Optimas image analysis program (version 6.2, 1987-1997 Optimas Corporation).

Statistical analysis were performed using the software SPSS (SPSS Inc., 1989-2001). Non-parametrical variance analysis (Kruskall-Wallis) were used to test differences in root colonization intensity between sites, plots and seasons. Correlation between spore richness and root colonization values between and within sites were carried out using Spearman correlation index (r_s).

4.3 Results

4.3.1 Spore distribution

No attempt was made to quantify AMF spores found in the soil samples. The results presented consist of presence-absence data of species, or, in most instances, morpho-species, as an accurate species identification from field collected spores cannot always be easily achieved.

No spore was recovered from most of the samples (61%). Although both sites presented a similar pattern of general gradual decrease of frequency of spore species richness in the analyzed samples, some differences were found (Figure 4.1). The higher richness found in one single sample (7) was found only in the Crasto site, in the plantation plot. In the Caju site higher richness figures were showed consistently by samples from the plantation plots.

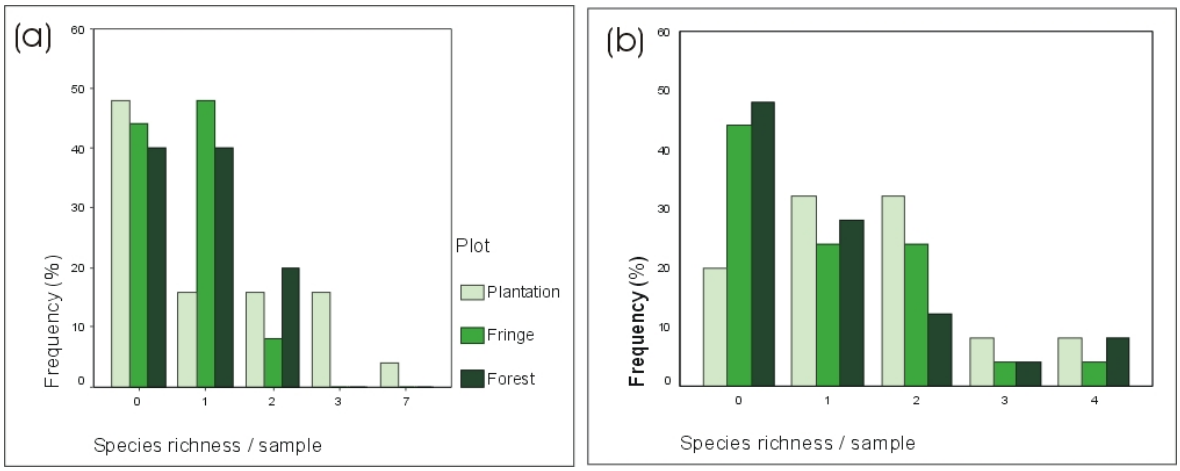

Figure 4.1 Frequency distribution of spore species richness in soil samples between different plots from both sites: (a) Crasto; (b) Caju).

As discussed above, analysis of spore diversity from field collected spores provide limited results. Nonetheless, species and morpho-species of *Gigaspora*, *Scutellospora*, *Acaulospora*, *Glomus*, and *Archaeospora* could be identified in the soil samples analyzed. *Gigaspora* and *Acaulospora* species dominated. A high similarity between both sites (Crasto and Caju) was found, being 12 morpho-species (or 60% of the total) common to both sites. These comprise three *Gigaspora*, two *Glomus*, one *Acaulospora* and other morpho-species from non-identified genera.

Both sites presented the same spore species richness (Table 4.1), although a somewhat high number of rare species contributed for these figures in both sites (6, in each). Plantation plots in both sites presented the higher species richness figures, although not significantly different ($p > 0.05$), neither considering both sites together nor separately. Despite the high similarity between both sites, plantation plots presently markedly lower similarity to spore species colleted in fringe and forest plots in both Crasto (5 species, or 25%) and Caju (6 species, or 30%).

Table 4.1 Spore species richness in each plot of both studied sites.

Site	***Total***	***Plantation***	***Fringe***	***Forest***
Crasto	20	15	7	6
Caju	20	14	10	10

A somewhat general and significant ($H = 11.422$, $p < 0.05$) trend to decrease of spore species richness along the soil profile was found (Figure 4.2). In fact, spore species richness and soil depth were positively correlated ($r_s = - 0.257$, $p < 0.01$). In the Crasto site the decrease in spore species numbers along the soil profile was more marked in the plantation plot, while the border and forest plots presented little variation.

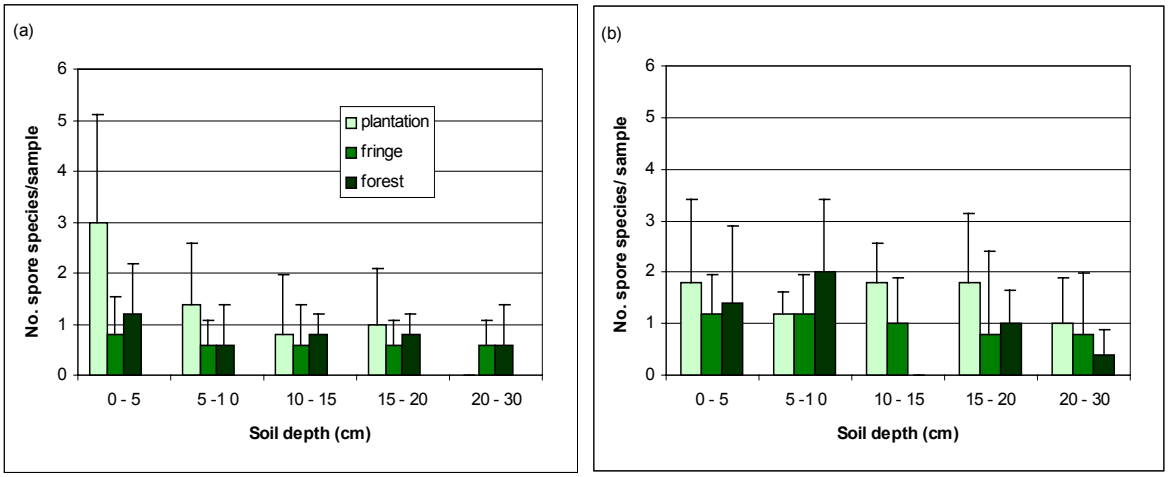

Figure 4.2 Spore species richness distribution along the soil profiles studied in the three plots from both sites: (a) Crasto; (b) Caju.

An attempt to correlate spore richness to root biomass was performed (Figure 4.3), but, as both parameters were determined in different samples, correlation analysis was performed with mean values of the five samples collected per plot. Nevertheless, a significant positive correlation was found between mean spore richness and mean root biomass ($r_s = 0.524$, $P < 0.005$).

Gigaspora cf. *gigantea* was chosen as an example of a species pattern distribution (Figure 4.4) due to its conspicuity. It was found in both sites, being much more frequent in the Caju site. However found in all soil depths in this site, it tended to be concentrated in the superficial layers.

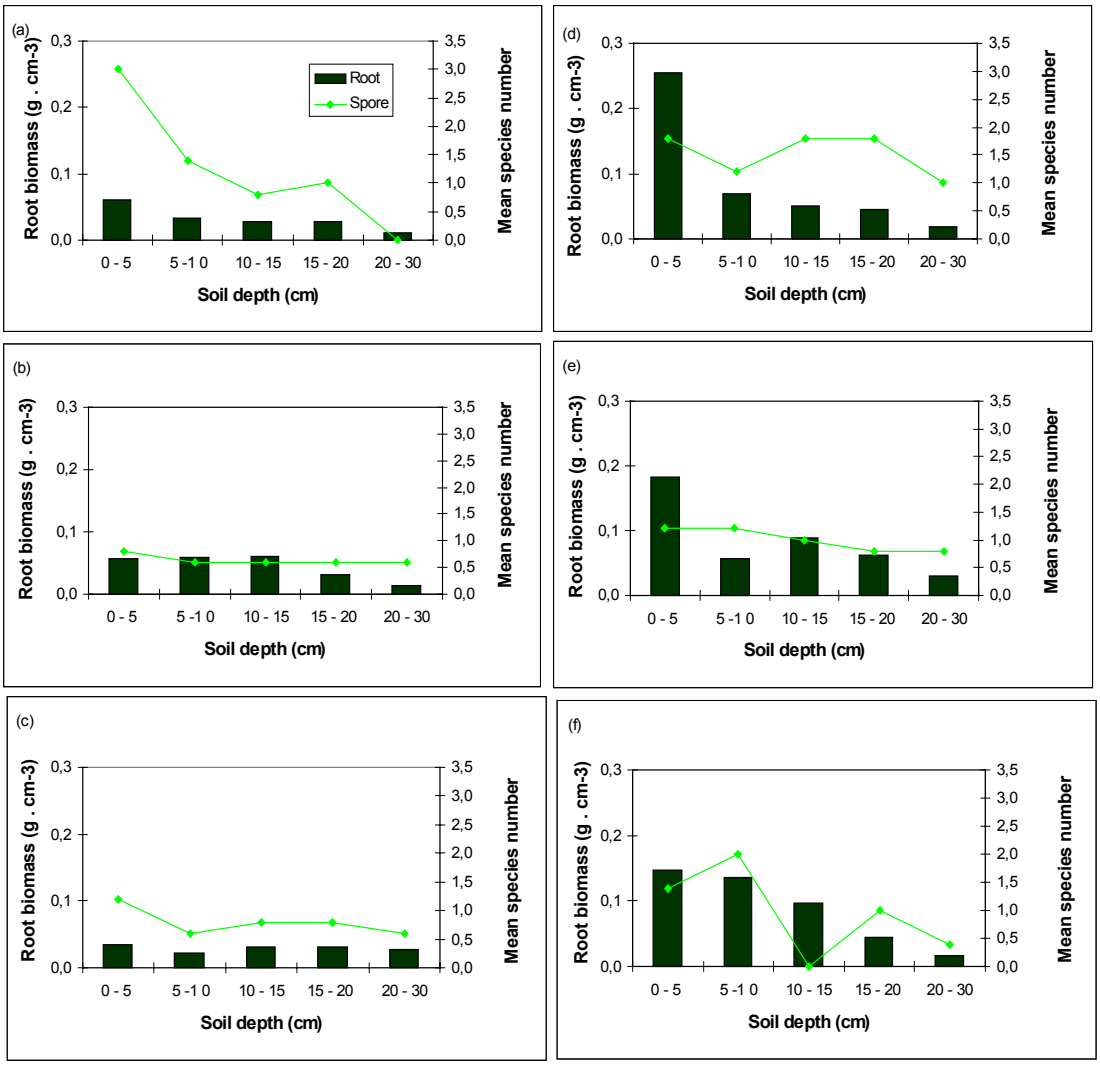

Figure 4.3 Root biomass and mean species number of spores found along the soil profiles studied (a-c) Crasto; (d-f) Caju site; (a, d) plantation; (b, e) fringe; (c- f) forest plots.

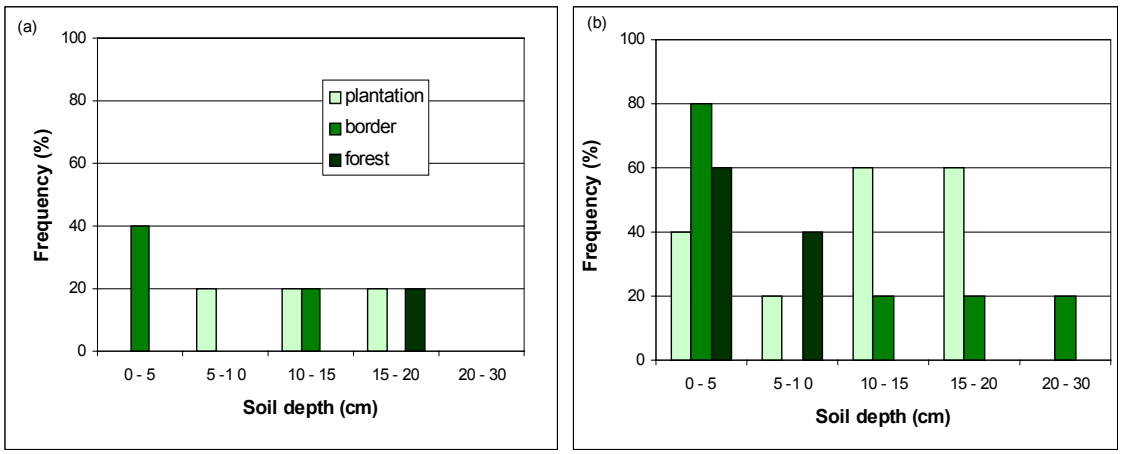

Figure 4.4 Frequency distribution of *Gigaspora* cf. *gigantea* spores within samples collected along the soil profiles studied in the three plots from both sites: (a) Crasto; (b) Caju.

4.3.2 Root colonization distribution

Both sites present distinct root colonization patterns (Figure 4.5). AM colonization differed significantly throughout sites ($H = 47.892$, $p < 0.001$), plots ($H = 22.896$, $p < 0.001$), and depth ($H = 25.625$, $p < 0.001$), but not between the two different sampling seasons ($H = 0.316$, $p > 0.05$). Although root colonization in plantation and fringe plots differed significantly in the Crasto ($U = 194.5$; $p < 0.001$) and Caju sites ($U = 610.5$, $p < 0.05$), it did not differed between plantation and forest plots in none of these sites ($U = 441.5$, $p > 0.05$; $U = 829$, $p > 0.05$, respectively). Interestingly, mycorrhizal colonization intensity in roots of fringe differed significantly from forest plots, this happening in both the Crasto and Caju sites (respectively , $U = 141.5$, $p < 0.005$; $U = 688$, $p < 0.01$).

The Caju site, and the fringe plots in both sites, showed higher levels of root colonization. Root colonization showed also significant negative correlation with soil depth ($r_s = -0.326$, $P < 0.01$).

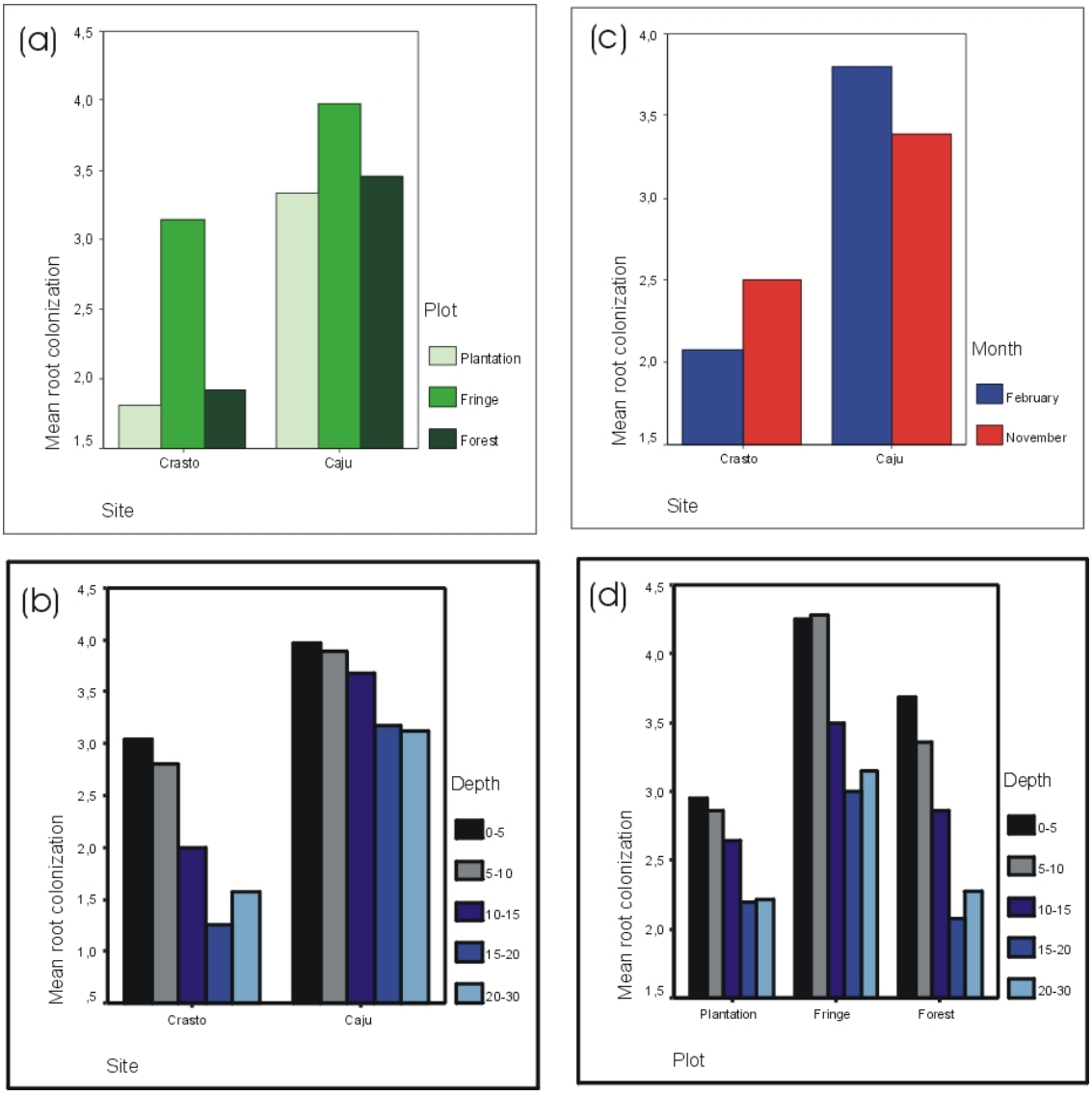

Figure 4.5 Patterns of AM root colonization beneath and within sites, seasons, plots and depths (bars refer to mean root colonization, assessed through percentage classes, see 4.2.3 Data analysis).

4.4 Discussion

4.4.1 Spore distribution

Species identification of AMF is based on characteristics of spores, some of which may be reversible due to local selection pressures (Morton, Franke & Cloud 1992) and is by no way a clear-cut process. Actually, some poor or insufficient descriptions of AMF are available (Abbott & Robson 1991, Smith & Read 1997). Even when clear descriptions exist, some species may be particularly difficult to distinguish, specially from field collected spores. Spores extracted from field soil may be dead, damaged or parasitized, many presenting their outer layer removed or altered, obstructing the analysis of the AMF spore community. In fact, a previous considerable knowledge of these fungi is necessary for the identification of AMF from field samples (Abbott & Robson 1991). A further problem derived from the analysis of field collected spores is when samples possess few spores and/or spores in different developmental stages (Abbott & Robson 1991). In the present study, whenever no clear association to a described taxa could be made, it was considered as a morpho-species. These isolates can be considered as an ecological individual (Morton, Franke & Cloud 1992). However, not much is known on the real diversity of AMF taxa in natural, tropical ecosystems, and at least some of these morpho-species may turn out to be new species. Results here presented represent an tentative comparative analysis between and within the two studied sites, and will be complemented by further successive spore extraction from trap cultures in the greenhouse.

Although most of the soil samples possessed no spore at all, results reveal some interesting patterns. In fact, AMF spore abundance and diversity in soil are very variable and is the result of all factors acting on sporulation, dormancy and infectivity (Smith & Read 1997). Differences are expected to occur between different plant-host combinations and type of ecosystem. Environmental factors as seasonality and disturbance are also probably important (Smith & Read 1997). In fact, reports of spore abundance and richness in natural ecosystem vary from low (Allen *et al*. 1998, Alexander, Ahmad & See 1992, Janos 1992, McGee 1989, Read, Koucheki & Hodgson 1976) to high figures (Johnson & Wedin 1997, Trufem 1995). When high spore abundance is reported, it is associated also high variances (Johnson & Wedin 1997), suggesting a highly spatially variable distribution. Therefore, results obtained may have been somewhat influenced by the sampling design, what is discussed below.

Both sites presented the same number of species/morpho-species, and, despite soil and vegetation differences between both sites, a high similarity in spore species and morpho-species composition was found between them. Although spores have not been counted in the present study, no markedly dominance in abundance of one or more species have been noticed. A distinct result has been found in a Singaporean lowland tropical rainforest, where despite the many different spore types found, one single species predominate in each studied site (Louis & Lim 1987).

Being dispersed by animals (Janos, Sahley & Emmons 1995, Mangan e Adler 1999), AMF abundance and diversity may be diminished where disturbance reduces natural populations of potential dispersers. Contrary to the expectation, forest conversion to coconut plantation did not reduce AMF spore species richness. In fact, plantation plots possessed higher species richness than fringe/forest plots. Plantation plots presented also the higher species richness per soil sample, at least in the Crasto site. Forest conversion is frequently associated with reduction of AMF spore diversity. Dominance by only one species of AMF have been reported in a tropical pasture, while 15 species were found in a adjacent deciduous tropical forest (Allen *et al*. 1998). Grassland soils had lower spore species diversity than dry tropical forest plots in Mexico (Johnson & Wedin 1997). However the species richness was not affected, suggesting that forest regeneration from these pastures may not be limited by native mycorrhizal inoculum (Johnson & Wedin 1997). An interesting result is the markedly decrease in spore numbers along successional stages (Zangaro, Bononi & Trufem 2000). Areas at the initial stage of secondary succession presented not only higher spore abundance than forest areas, but also higher inoculum potentials (Zangaro, Bononi & Trufem 2000), indicating that these spores would be able to effectively colonize plant roots.

Sporulation is at least to some extent related to soil type and properties. Sporulation have been reported to be lower in clay soils (Allen & Allen 1980) and may be related to inhibition in soils with small pore spaces (Griffin 1972 *in* Allen & Allen 1980). Although these authors have considered spore counts, and not spore richness, both the Caju site and the plantation plots in the two studied sites presented the higher soil sand contents (see 3.3.3.1 Texture). In fact, some studies in sand dunes have revealed high spore abundance and diversity (Trufem 1995). Correlation of spore abundances with soil parameters was also found to be variable between AMF species (Johnson & Wedin 1997). Most of their species were positively correlated with soil N and C, and negative correlated with soil C:N ratio, Ca, and Mg. Spore species richness was negatively correlated with soil Ca and pH (Johnson & Wedin 1997). Soil spore numbers and soil NO_3 content have been reported to be negatively correlated, at least for some species of AMF (Burrows & Pfleger 2002). Different AMF species extracted from temperate sand dunes also differed in pH tolerance (Gemma 1987).

On the other side, greater sporulation may not necessarily mean higher diversity or more available AM inoculum. Adverse conditions may stimulate spore production, and so the higher species richness found in the plantation plots may be just an artifact, derived from the harsh environmental conditions present in these areas. Indeed, temperate arable sites presented markedly lower AMF diversity when compared to a woodland (Helgason *et al*. 1998). The molecular methods used by these authors allowed them to find out that these diversity impoverishment from woodlands to arable sites lead to an almost complete dominance of *Glomus mosseae*, or closely related taxa, in the latter.

Similarly, in the present study, disturbance, or removal of the forest cover, has lead to a change in species composition. While both sites presented 60% similarity, this figure is markedly reduced when comparing plantation to fringe/forest plots, in both sites. The most obvious effect of forest conversion to plantations is the reduction in tree species. Higher plant diversity have been related to higher sporulation rates, affecting also AMF species composition (Burrows & Pfleger 2002).

In fact, these authors report that changes in plant diversity affected AMF species composition, but responses differed with spore size, being more consistent with species with larger spores.

Disturbance has also effects on soil physical, chemical, and biological environment. It has been reported to reduce or eliminate native AMF inoculum (Allen & Allen 1980, Perry, Molina & Amaranthus 1987, Powell 1980). For instance, loss of the topsoil in many eroded soils have lead to very low mycorrhizal infectivity (Powell 1980) and tropical forest management has lead to diminishing in spore numbers and mycorrhizal infectivity (Alexander, Ahmad & See 1992). Being soil erosion higher in deforested sites, loss of infective propagules in these areas may be a factor impeding or delaying reforestation. However, the plantation plots in this study could not be considered disturbed sites in the strict sense, as the native forest cover was removed for so long that a system where new relationships between plants and fungi, among all other components of the community, already exists. Moreover, these areas are not intensively managed, with none or low fertilization inputs. Further analysis will show how the removal of forest cover affect the mycorrhizal community and which species could promote better growth and greater survival for forest seedlings in this altered conditions (See Chapter 5, Seedling dynamics and mycorrhizal colonization in roots of some native tree species seedlings).

Spatial and temporal variation in sporulation and spore distribution in soil cores have been reported in different natural and agricultural ecosystems. Distribution of AMF spores in soils was found to be highly uneven, deviating significantly from a normal distribution (Anderson *et al.* 1983, Walker, Mize & McNabb Jr. 1982). This has important implications for designing of field sampling of AMF spores. Large numbers of sample replications may be needed to achieve a better description of the population composition (Walker, Mize & McNabb Jr. 1982), and it is possible that the five randomly taken soil samples may not have been enough to describe it accurately.

Spore species richness showed also a trend to decrease along the soil profile in both temperate (Sutton & Barron 1972), and tropical ecosystems (Ingleby *et al*. 1997, Louis & Lim 1987). However, the relatively high standard deviations found in the present study suggest the existence of a great spatial heterogeneity. The positive correlation between mean spore richness and mean root biomass was expected, as greater root biomass implies more root surface potentially available to be colonized by AMF, possibly by a higher number of different species of fungi. This may be particularly true, if more root density also means higher host diversity. However, seasonal trends in fine root production can affect patterns of spore production along the year. A decrease in fine root production during the dry season was reported in a Mexican tropical wet forest, being however, related to an increase in spore abundance (Guadarrama & Álvazer-Sánchez 1999).

Not only the AMF colonization presents a seasonal variation (Helgason *et al*. 1999, Daniell *et al*. 2001), but also the spore production. Spore numbers increase along the growing season in temperate ecosystems (Gemma & Koske 1988, Sutton & Barron 1972), but seasonal effects vary in tropical ecosystems. Spore abundance has been correlated to climate, and lower sporulation occurs in aseasonal climates (Janos 1992). In fact, even in evergreen tropical forests sporulation is seasonal (Janos 1992). However, both spore richness and abundance were higher in the dry season in tropical wet forest (Guadarrama & Álvazer-Sánchez 1999), while higher in the rainy season in a deciduous

tropical forest (Allen *et al*. 1998). These authors suggest that spore production may be rapidly triggered by the onset of the summer rains, as spores were not long lived. Allen & Allen (1980) also suggest a beneficial effect of precipitation on sporulation in semiarid grasslands. However, Johnson & Wedin (1997) found no seasonal difference in spores numbers in Malaysian tropical forests. The stimuli for production of new spores may be different for annual and perennial plants (Gemma & Koske 1988), and comparison between different studies may consider climate, type of ecosystem, level of disturbance and plant habit. For instance, seasonal forests are supposed to have larger spore populations than aseasonal wet forests (Johnson & Wedin 1997). Unfortunately, results here presented refer to the collects made in November, as samples from February could not be processed fast enough after sampling and had to be discarded. Furthermore, it is important to determine the fine root turnover rates in both forests studied, and whether the prolonged dry season affect mycorrhizal establishment and sporulation. The gradual root decay following natural seasonal environmental changes is considered to stimulate AMF spore production (Janos 1992). The markedly, and sometimes prolonged, dry season occurring in the coastal region of Sergipe may be a significant environmental factor affecting sporulation dynamics in both forest fragments studied.

The analysis of the spatial distribution of *Gigaspora* cf. *gigantea* spores can not be considered complete, as absence of spores in some samples do not mean this species is not present there. However, these results reveal that at least some of the species found may possess a wide range of distribution, or that removal of native forest cover did not imply in changes marked enough to affect their ability to establish under these new conditions. It is still not known whether isolates from the same species, derived from forest and plantation plots, would differ in physiological activity and host preference.

Much more effort is still needed towards the identification of fungal species in tropical forests (Allen *et al*. 1998). Although spore distributions results must be cautiously analyzed due to the manifold chances of error, it provides however a chance to assess the potential identity of the symbionts and of (at least part of) the community composition and its spatial variation. However, the use of recently developed molecular methods can provide more quickly and reliable information on AM community diversity in plant roots, unattainable by classical spore extraction methods (Husband, Herre & Young 2002) and its use is being considered for future research in both study sites, in order to check and improve the results achieved until now.

The figures here presented must be carefully considered, due the above mentioned limitations of analysis of field colleted spores. Furthermore, these results do not mean necessarily the actual AMF diversity present in roots from these areas. The spore species found mean only that these species have recently sporulated, but not that these are the only species present. A more complete view of the AMF composition present in the different plots from both sites can be accomplished with successive trap culturing and isolation procedures, which is currently in progress. Indeed, successive pot culturing had revealed a higher AMF species richness than the obtained through the analysis of field soil or even of the first round of trap cultures (Stutz & Morton 1996).

4.4.2 Root colonization distribution

Although both sites did not differ much in the spore species richness in the three plots, root colonization was significantly different. Higher root colonization rates have been found in the Caju site. The soil type could explain these differences, as the clay content in soils from the Crasto site was higher than in the Caju (see 3.3.3.1 Texture). However, despite the few spores found in a clay soil, roots collected presented relatively high colonization rates (Allen & Allen 1980).

Although the plantation plots in both sites presented higher spore species richness, this was not reflected in mycorrhizal colonization in the Crasto site. Grasses can present heavy mycorrhizal colonization (e.g. Read, Koucheki & Hodgson 1976) and this may be responsible for the high root colonization values found in the plantation plot of the Caju site, despite the removal of forest cover. However, it does not explain the low figures obtained in the Crasto site, as in both sites the plantation plots are covered by a more or less continuous grass cover. The extremely low mean root colonization figures in the plantation plot of the Crasto site, despite its high spore species richness, may indicate that the high spore diversity is not really effective in colonizing roots. There may be many AMF species colonizing roots in these areas, but they may have a restricted ability to colonize these roots, being limited to small extensions. Alternatively, the high AMF diversity could be due to some few plant species, with a wide range of fungal partners, while the great majority would present low fungal diversity and/or root colonization intensities, therefore accounting for the general low colonization levels. This point is not clear and further sampling, analyzing separately roots from the different plant species present in the plantation plots, must still be carried out.

If spore population in soils is low or ineffective, mycorrhizal initiation may rely on AMF hyphae (Read, Koucheki & Hodgson 1976). In fact, under undisturbed, aseasonal vegetation, AMF hyphae are supposed to be the most important AMF propagule (Janos 1992). Although AMF hyphae have been found to remain infective after being detached from roots, soil disturbance have caused significant reduction in their infectivity (Jasper, Abbott & Robson 1989a, b). In fact, soil disturbance may eliminate any infectivity of hyphal fragments, as root fragments with attached hyphae were able to readily colonize roots, whereas fragments of hyphae did not produce any root colonization (Bellgard 1982). Other reports of significant reduction of root colonization in roots growing in disturbed soil (McGonigle & Miller 1996) may be similarly related to disruption of soil hyphae. It has been suggested that soils containing higher spore and mycorrhizal roots abundance will be less affected by soil disturbance (Jasper, Abbott & Robson 1991). Therefore, infectivity in pasture soils, with higher proportion of mycorrhizal hosts, were less affected than forest and heathland soils (Jasper, Abbott & Robson 1991). Actually, although plantation plots can not be considered as intensively managed, but the use of machinery for periodical removal of the growing herb and scrub layer may have a deleterious effect in disturbing soil hyphae and diminishing infectivity in these areas. To test this hypothesis, tractor use should be excluded of at least a smaller region of this areas and monitoring of root colonization (as well as measuring soil hyphae density).

However, mean colonization values in plantation plots did not differ from forest plots in both sites. The reason for this remarkably low infectivity in the forest plot from the Crasto site is still not

clear. Successional status and soil nutrient content may be possible explanations. Higher colonization in nutrient stressed environments have been reported (Allsopp & Stock 1994, Read, Koucheki & Hodgson 1976), and the forest plot in Crasto site presented exceptionally great values in the superficial layer. A maximal pant available soil P threshold has been suggested, above which roots should be non-mycorrhizal (Fitter 1991). Soil fertility may also explain the differences found in root colonization along areas in different successional stages (Zangaro, Bononi & Trufem 2000). In their study root colonization was found to be higher in pioneer species than in mature forest trees. As the studied forest soil had higher nutrient contents, mostly in phosphorus, than the successional areas (Zangaro, Bononi & Trufem 2000), dependence on mycorrhizal under these conditions may not be not particularly great.

A significant decrease in root colonization with increasing depth was observed, in all the three plots from both sites. This may be at least in part an effect of soil nutrient content variation along the soil profile. High root colonization levels are related to low soil nutrient content, and P availability has an important effect of establishment and spreading of colonization, but host plant and environmental factors can have also strong influence on it (Smith & Read 1997). For instance, root biomass was significantly correlated to soil P in the Caju, although not in the Crasto site. Van Noordwijk & Hairiah (1986) have also analyzed mixed root samples from topsoil (0-15 cm), finding that mycorrhizal root colonization was negatively correlated with soil pH, P and Al (Van Noordwijk & Hairiah 1986). Soils from both studied sites did not varied much in pH range, and a decrease in soil pH along the soil profile was generally observed (see 3.3.3.4 Soil pH). Similarly, in both sites the phosphorus content tend to diminish along the soil profile (see 3.3.3.5 Phosphorus). Al has been also suggested to inhibit mycorrhizal colonization, but effects of Al and pH seem to differ between different endophytes (Wang et al. 1985). The two sites differed in Al content along the soil profile. In the Crasto site, both fringe and forest plots showed a increase in Al content with increasing depth, whereas in the plantation plot higher Al concentration was found in the two upper soil layers. (see 3.3.2.5 Aluminum).

It should not be forgotten that the analysis of spatial variation in root colonization levels in natural ecosystems may be hindered by the multitude of plant hosts presenting, by their turn, different spatial distribution patterns. Despite the general idea that AMF do not present any or low degree of specificity with plant hosts, some studies have suggested that at least a certain degree of 'ecological specificity' (McGonigle & Fitter 1990). In fact, the identity of the plant hosts have been found to be more important than root density in determining the spread of AMF hyphae in soil (Warner & Mosse 1982). More recently, analysis in temperate ecosystems with molecular methods have revealed not only a wide range of different AMF colonizing roots but also the existence of physical and functional selectivity in AMF (Helgason *et al*. 2002).

Pattern of root colonization along the soil profile was similar to the spore species richness, both being negatively correlated to soil depth. Spore abundance and mycorrhizal roots were also correlated in Australian soils (Jasper, Abbott & Robson 1991) and in Mexican sand dunes (Sigüenza, Espejel & Allen 1996). However, spore production and root colonization may be not necessarily correlated. For instance, seasonal increase in root colonization did not result in increased sporulation in a deciduous tropical forest in Mexico (Allen *et al*. 1998) and spore counts were not found to be highly correlated with increase in root infection in a Brazilian sandy coastal ecosystem (Trufem,

Malatinszky & Otomo 1994). Actually, an inverse relationship between spore density and colonization in four trees from lowland tropical rainforest, with no markedly rainfall seasonality, has been found (Louis & Lim 1987). However, these authors have analyzed spore numbers and not spore species richness, as in the present study.

Different results obtained by different authors may not only reflect local environmental conditions but also plant specific patterns in root growth and development. Root concentration and total spore numbers in soil colleted around four African leguminous tree species were positively correlated (Ingleby *et al*. 1997), but only one of the studied species also presented positive correlation between mycorrhizal colonization and spore abundance and between root concentration and spore species richness.

In spite of reports of seasonal variation in AMF root colonization (Helgason e*t al*. 1999, Daniell *et al*. 2001, Read, Koucheki & Hodgson 1976), no significant seasonal differences in root colonization were found. In fact, AMF are supposed to adjust themselves to gradual environmental changes without abrupt changes in levels of root colonization, although the effect of fluctuating moisture conditions on the survival of propagules is still not clear (Abbott & Robson 1991). It may probably be variable with plant species and community structure. In addition, the presence or absence of seasonal responses in plant colonization to seasonally varying environmental factors may result from the many different fungi colonizing one single plant root. In fact, plant roots in field soils are usually colonized by more than one AMF species (Abbott & Robson 1991). Actually, amplification from total "environmental DNA" present in roots of the grass *Arrhenatherum elatius* revealed a surprisingly rich fungal community, much of it still unknown (Vandenkoornhuyse *et al*. 2002). Root "community" results would be, therefore, the sum of different response patterns of each individual species.

Although seasonal differences in root colonization were not significant, both sites showed different trends in the two sampling times. In the Crasto, mean root colonization was slightly higher in November, while the opposite was found in the Caju site. These results may no mean necessarily different patterns, as the number of sampling times was not enough to assess effect of seasonality.

Furthermore, this was a rather general assessment, without paying detailed attention to the structures present. So the presence of arbuscules, which may indicate physiological activity and may be seasonally variable, especially considered the rainfall seasonality prevailing in the region, were not registered. Further sampling, this time considering more frequent collations, are planned.

Not only plant species may vary in its response to AMF colonization, but also the different AMF groups may have different requirements and abilities to colonize plant roots. Indeed, differences in colonization strategy has been found to be consistent between AMF families (Hart & Reader 2002). Members of the Glomaceae not only colonize roots faster than members of the Gigasporaceae and Acaulosporaceae but also present the higher colonization levels. Furthermore, species from the Gigasporaceae, which less infective external mycelium, and therefore dependent on spore germination for starting colonization in new roots, may be less competitive in comparison to species from the Glomaceae and Acaulosporaceae. Analysis of mycorrhizal colonization in samples of mixed roots do not considers these differences between hosts from different taxonomical groups.

4.5 Conclusions

This chapter aimed to identify differences between the two areas or between the three habitats in AMF species composition. There is indeed native mycorrhizal inocula on the plantation areas, being their diversity apparently even higher than in the fringe/forest plots, but the relatively lower root colonization intensity may imply an inability or incompatibility of these species. Much more is needed in order to understand not only the spatial and temporal variation, but also the role of this native AMF fungi on the structure and dynamics of these forest fragments. This is just a first preliminary attempt to evaluate the possibility of using the mycorrhizal symbiosis for improving reforestation of these disturbed areas. Rather than testing commercial inoculum, with a mix of introduced species with optimal performance, first of all it was tried to asses the native AMF diversity, supposed to be more adapted to these environmental conditions. In fact, native populations should be maintained to assure the diversity of seedling responses necessary for success in often unpredictably varying environments (Perry, Molina & Amaranthus 1987), as is the case in the studied region

Not only spore density, but also spore species richness were higher in the soil upper layers, making disturbance that means removal of top soil, or increase in soil erosion, particularly harmful, as it will cause reduction of mycorrhizal inoculum (spore and colonized roots) and hinder subsequent recolonization of these areas. In effect, soil disturbance has been reported to reduce soil AMF inocula (Perry, Molina & Amaranthus 1987). However, disturbance in the plantation plots do not imply significantly soils disruption neither removal of soil top soil. Furthermore, not being an intensively managed plantation, with none or low fertilization inputs, mycorrhizal formation in these areas should not be hindered. Native inoculum (spores and colonized roots) was present in plantation plots, particularly in the Caju site. These species are supposed to be more adapted to the prevailing conditions in these areas and, if compatible with tree species, should be tested in reforestation. Isolation and culturing of the species is currently underway.

Differences in mycorrhizal establishment and dynamics between evergreen and seasonal tropical forests are reported in the literature (Allen *et al*. 1998). Both studied forests (Crasto and Caju) receive an annual amount rainfall enough to characterize evergreen forests, but the presence of a markedly dry season, variable between years (see 2.3 Climate), may put these forests in an intermediate condition, which may have important implications for the establishment and dynamics of mycorrhizal symbiosis in these systems.

The present analysis was based on mixed root samples. More specific analyses, within seedling (Chapter 5) and tree (Chapter 6) roots, will help to understand if, and how, these fungi significantly affect plant establishment in natural and disturbed conditions.

5 Seedling dynamics and mycorrhizal colonization in roots of some native tree species seedlings

5.1 Introduction

Despite the complexity involved in mycorrhizal symbiosis in natural ecosystems, it is clear that this symbiosis has a major, yet sometimes undervalued, role in influencing significantly the structure and functioning of these ecosystems. The extent and nature of mycorrhizal colonization might affect survival and growth of tropical tree seedlings, having important implications for the regeneration of tropical rain forest, particularly after disturbance (Alexander, Ahmad & See 1992). A better understanding of the interactions among mycorrhizal fungus species, host species and environment is still needed (Perry, Molina & Amaranthus 1987), and studies focusing the role of AM symbiosis on seedling establishment, particularly, are of great importance (Zobel & Moora 1997). In spite of the low knowledge gathered in natural ecosystems, and the difficulty in proving the beneficial effects of AM under these conditions (Fitter 1991, but see Carey, Fitter & Watkinson 1992, Newsham, Fitter & Watkinson 1994, 1995), the mycorrhizal symbiosis should not be left out of mind when considering reforestation with native species. Influencing tree seedlings survival and growth, mycorrhizas are important factors affecting tropical rainforest regeneration after disturbance (Alexander *et al.* 1992). Indeed, a key step in determining reforestation success is assuring that enough water and nutrients are available to tree seedlings (Perry, Molina & Amaranthus 1987). If suitably managed, and using the most suitable fungal partners, it may enhance seedling nutrient uptake, as well as provide other non-nutritional effects, as increasing the resistance to pathogens (Carey, Fitter & Watkinson 1992, Newsham, Fitter & Watkinson 1994, 1995, Yao, Tweddell & Désilets 2002).

Studies aiming forest restoration should pay special attention to forest remnants left in fragmented landscapes as a source genetic material. Managing and optimizing seed dispersal from these remnants to abandoned plantations or pastures sites may be a economically viable alternative to reforestation and ensuring the establishment of forests with gene pools adapted to the prevailing local conditions. Natural recolonization is also dependent on dispersal efficiency of species with different dispersal syndromes. Therefore, zoochorous species may be dependent of availability of dispersers populations, which, by its turn, may be also dependent on anthropogenic influence.

However, for successful forest restoration, not only efficient dispersal is needed but also favorable conditions for seedling establishment. The seedling stage is a very important phase in plant recruitment. After germination, seedlings must acquire enough water an nutrients to ensure growth until the cotiledonary reserves are consumed. Environmental spatial variability makes this a somewhat random process. This may be particularly true in tropical forests, with high species diversity and irregular distribution pattern of species composition and structure. Nevertheless, deforestation represents the disruption of this equilibrium, causing environmental changes that will hinder or slower subsequent forest growth. Nature of damage and management after native forest removal may

determine magnitude of changes. In the two sites studied in the present study, deforested area (plantation plots), although not subjected to intensive mechanical soil disruption, present lower nutrient and harsher microclimate conditions (Chapter 3), that may prevent successful tree seedlings establishment.

These plots, however, still present native mycorrhizal inoculum (Chapter 4) that can help seedling establishment ensuring them an extra nutrient supply. But how fast infection takes out may depend not only on inoculum availability but also on plant patterns of root development. Specificity was not thought to play an important role in arbuscular mycorrhizas (Smith & Read 1997), but more recent studies have revealed the existence of some selectivity in host plant-fungus associations in temperate natural ecosystems (Helgason *et al*. 2002). Anyway, information is still scarce on tropical species, and host preferences may be indeed an important factor in determining the structure of plant communities. Mycorrhizal inoculum, if available in enough quantities, may therefore help tree seedlings to face these harsh environmental conditions. This may be particularly important when seasonality presents an extra difficulty for seedling reestablishment.

This chapter aims to identify spatial and temporal differences in the seedling establishment in the two studied forest sites, as well as the role of mycorrhizas in the seedling phase. Thus mycorrhizal colonization in forest tree seedlings was compared between sites, plots and season, and the differences found were discussed in view of the implications for supporting an ecologically based reforestation of these remnants.

5.2 Material and methods

Sampling was done as for the litter biomass (Chapter 3), in $1m^2$ quadrats randomly distributed in each plot (in February) and in five $1m^2$ quadrats per plot (in November). Seedlings were sorted out in morpho-species, having then their shoot and root length measured. Shoot biomass were assessed after oven dried (60^oC for at least 48h) and expressed in dry weight m^{-2}. Roots were at first analyzed regarding the presence of nodules or ectomycorrhizal tips.

All root samples where first carefully washed from the soil, and then cleared and stained with Trypan blue (Koske & Gemma 1989). Bleaching with alkaline hydrogen peroxide was necessary in some of the samples. The assessment of arbuscular mycorrhizal colonization of roots was done by examining them under dissecting microscope at 500x and categorizing estimates in five classes of percentage of colonization: class 1, 0-5%, class 2, 6-25%, class 3, 26-50%, class 4, 51-75% and class 5, 76-100% (Kormanik & McGraw 1982)

Furthermore, in both sites (Crasto and Caju) seedlings from two tree species were collected in February aiming to describe patterns of root colonization. Biometric measures and root preparation were done as described above. All of the species are frequent tree species in the forests of the region and were sampled in the fringe plots of both sites: *Maytenus* sp (Celastraceae) and *Annona* sp (Annonaceae) in the Crasto site, and *Tapirira guianensis* (Anacardiaceae) and one unidentified Myrtaceae species, in the Caju site. For each species 45 seedlings were collected, except for *Annona*

sp, with 30 seedlings sampled. For root colonization assessment, 25 from the collected plants were randomly chosen.

Statistical analysis were performed using the software SPSS (SPSS Inc., 1989-2001). Non-parametrical variance analysis (Kruskall-Wallis) were used to test differences in seedling numbers, richness and biomass between sites, plots and season. Spearman correlation analysis were performed between seedling data and plot vegetation structure and seedling data and root colonization. Litter biomass data (Chapter 3) was tested against seedling data using Spearman correlation. Variance analysis were also used to test differences between in root colonization between sites, plots and season.

5.3. Results

5.3.1. Seedlings dynamics

There were not found seedlings of forest species in the plantation quadrats of both sites in the beginning of the rainy season and almost none in the beginning of the dry season (Figure 1a), reflecting the diminished establishment success of these seedlings in the highly modified conditions of the plantation.

In general, fringe plots presented smaller seedling abundance (Figure 5.1a, d), biomass (Figure 5.1b, e) and species richness than forest plots (Figure 5.1c, f). All these parameters were generally higher in the sampling done in November.

Both sites were not significantly different in seedling richness, abundance and biomass distributions, nor the two sampling times differed significantly. But the three plots differed regarding seedling richness, abundance and or biomass ($H = 30.907$, 27.693, and 23.201, $p < 0.001$). Mann-Whitney pairwise tests detected significant differences between plantation and fringe plots in seedling richness, abundance and biomass patterns ($U = 3.500$, 15.500 and 21.000, respectively; $p < 0.01$). Differences were also detected between fringe and forest plots in seedling richness, abundance and biomass distributions ($U = 0.50$, 3.500 and 5.00 respectively; $p < 0.01$). Seedling richness, abundance and biomass were also significantly different between plantation and forest plots ($U = 0$, 0 and 3,00, respectively; $p < 0,01$).

The seedling abundance was significantly correlated to the plot basal area ($p < 0.01$), tree abundance ($p < 0,05$), and tree species number ($p < 0.05$) (Table 5.1). The seedling species number was positively correlated to the plot basal area ($p < 0.01$) and tree abundance ($p < 0.05$). Seedling biomass showed significant positive correlation only with the plot basal area ($p < 0.05$). However, no significant correlation was found between seedling abundance, richness or biomass and litter biomass.

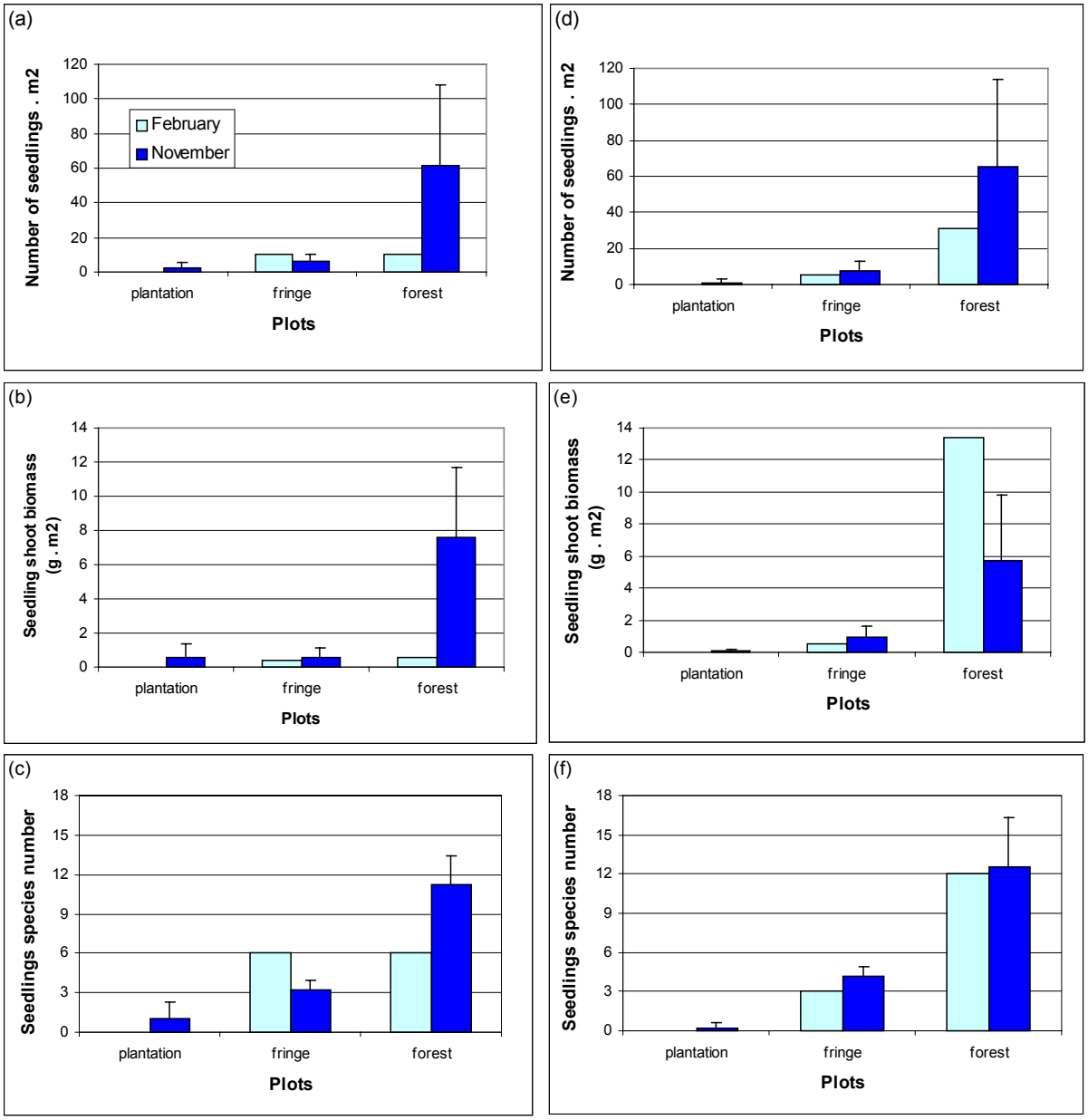

Figure 5.1 Seedling temporal and spatial variation in the Crasto (a-c) and Caju (d-f) sites: seedling abundance (a, d), shoot biomass (b, e), and richness (c, f).

Table 5.1 Spearman Rank Order Correlations between seedling data and plot vegetation structure (N = 12; ** p < 0,01; * p < 0,05)

		Seedling	
	Abundance	***Richness***	***Biomass***
Basal area (m^2. ha^{-1})	0.709**	0.752**	0.667**
Tree abundance	0.638*	0.596*	0.482
Tree richness	0.583*	0.554	0.410
Litter biomass (g . m^{-2})	0.256	0.214	0.200

5.3.2 Mycorrhizal colonization

The root colonization quantification method chosen (Kormanik & McGraw 1982) allows a great number of samples to be more rapidly analyzed and provides a more representative estimate (Giovanneti & Mosse 1980), as a greater root sample is analyzed under the dissecting microscope as under the compound microscope using the magnified intersections method (McGonigle *et al.* 1990). However, due to the small magnification achieved, it does not give an detailed analysis of fungal structures, reason why slides were prepared with some of the samples, which were then microscopically analyzed.

5.3.2.1 Seedlings in quadrats

Results presented refer to the collects made in February, as samples from November, due the greater sample number, could not be processed yet. Both sites did not differed significantly in the distribution of seedlings in root colonization classes. Root colonization was not significantly correlated with root length, shoot length nor with shoot biomass, considering data from both sites together or separately. Root colonization did not differed significantly between the three plots in both sites (Figure 5.2). In the February sampling, legume seedlings were found to be nodulated in the plantation plots of both sites and in the forest plot of the Crasto site one *Inga* sp seedling also presented root nodules (data not shown). All of them presented lower mycorrhizal colonization, ranging from 1 (0-5) and 2 (6-25 %) colonization classes. In November, the same pattern was found in the Caju site (although root colonization, as explained above, could not be assessed), but in the Crasto, no legume seedlings were found. In the Caju site, the high number of Myrtaceae seedlings is remarkable.

Although no clear pattern emerges from the results, it is interesting to note the relatively high number of samples with low colonization grade, especially in the forest plot from the Crasto site. Fringe and forest plots from both sites showed differed patterns of distribution. Plantation plots of both sites also present a significant number of samples with highly colonized roots.

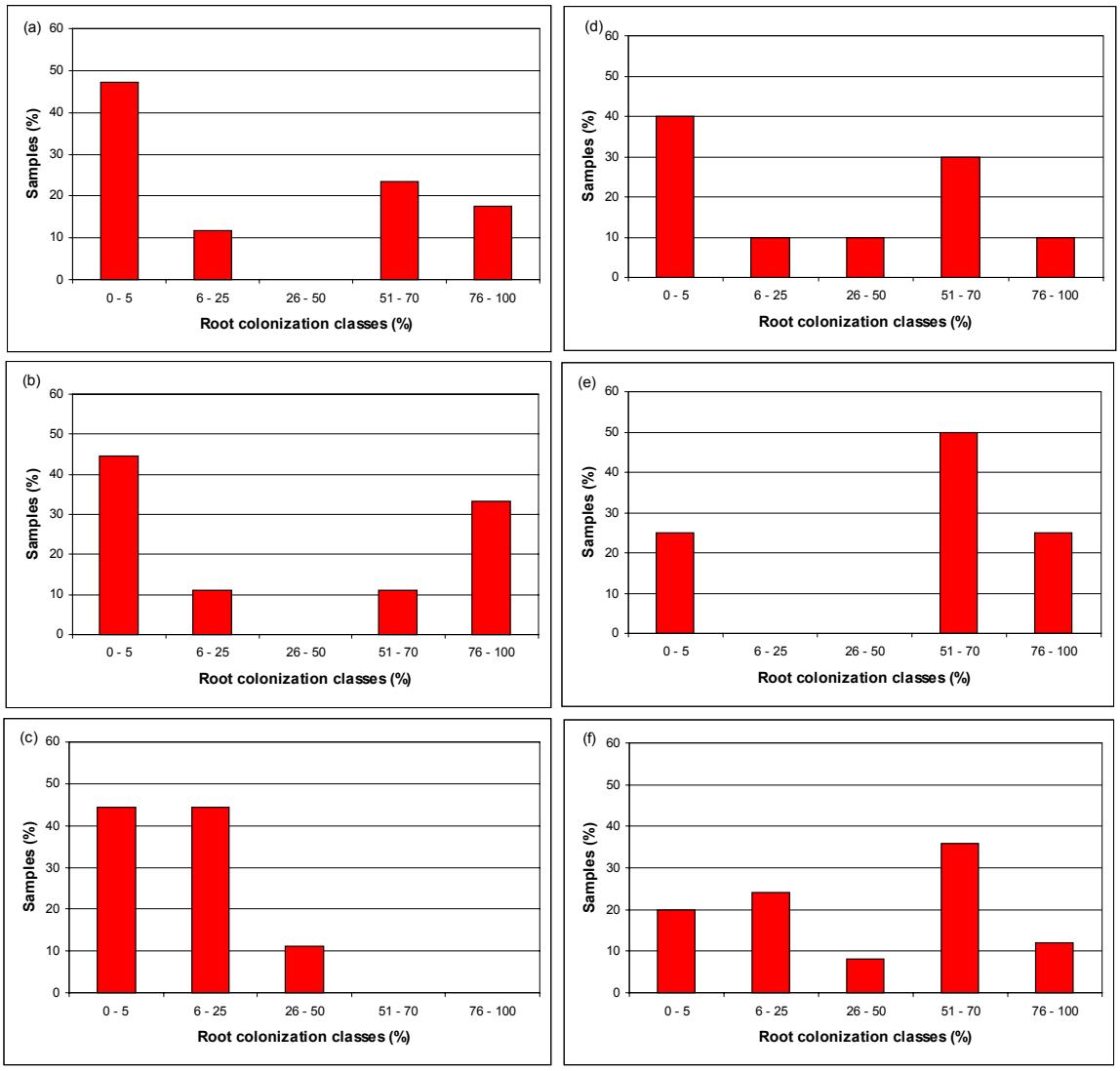

Figure 5.2 Distribution in root colonization classes of root samples from plantation (a, d), fringe (b, e) and forest plots (c, f) from both studied sites: Crasto (a-c) and Caju (d-f).

5.3.2.2 Selected species

The four selected tree species seedling showed great variation in root structure and colonization intensity (Figure 5.3). *Annona* sp showed the lowest values of AM colonization, while *Tapirira* sp the greatest. Seedlings of *Maytenus* sp were most concentrated in the two higher colonization classes and the unidentified Myrtaceae species seedlings were almost equally distributed along four of the classes.

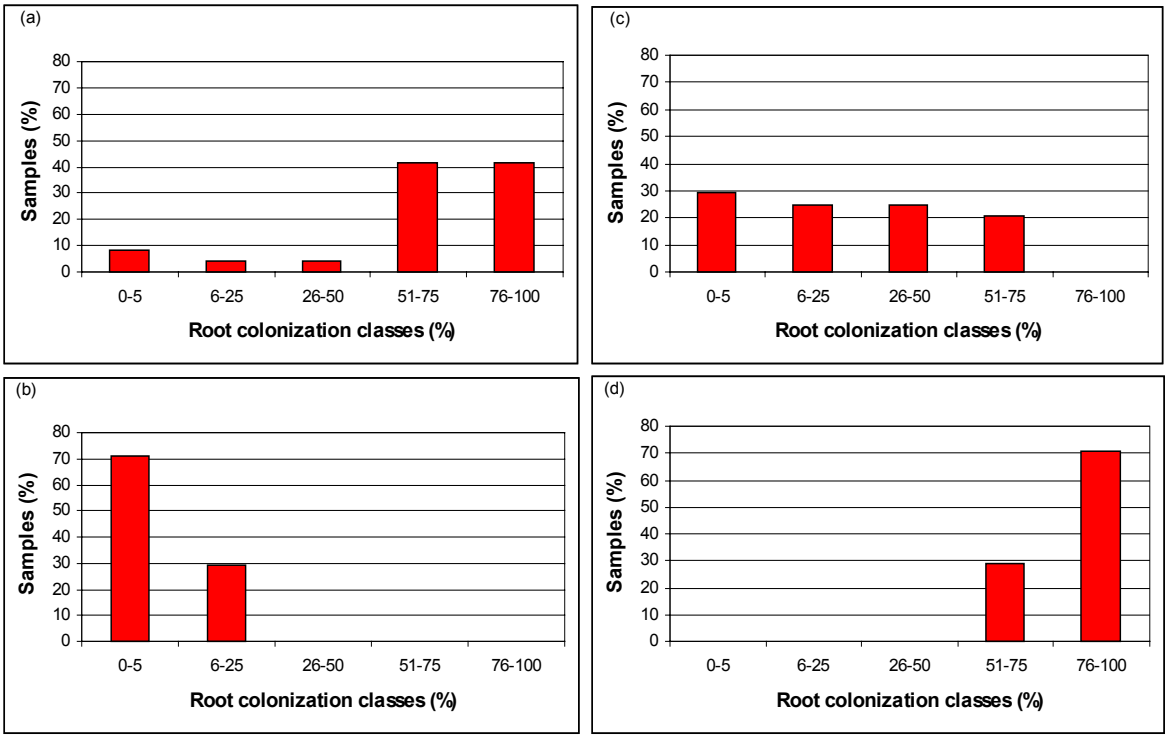

Figure 5.3 Distribution in root colonization classes of root samples from four selected native tree seedling species. (a) *Maytenus* sp, (b) *Annona* sp, (c) Myrtaceae sp, (d) *Tapirira guianensis*.

Seedling biometric measures were tested against root colonization values, but only for *Maytenus* sp positive significant correlation was found (Table 5.2). All species showed also positive significant ($p < 0.01$) correlation between shoot and root length (data not shown).

Table 5.2 Spearman correlation index (r_s) for seedling biometric measures and root colonization values (N = 24; ** p < 0,01; * p < 0,05).

Species	*Root length*	*Shoot length*	*Shoot biomass*	*No. leaves*
Maytenus sp	0.676**	0.463*	0.713**	0.454*
Annona sp	-0.238	-0.073	-0.404	-0.054
Myrtaceae sp	0.201	0.305	0.225	0.136
Tapirira sp	-0.213	-0.292	0.007	-0.135

Analysis of this roots in microscopic slides revealed that AMF were not the only partner. Other non stained fungi, with dark septate hyphae and intracellular microsclerotia (Figure 5.4), were also found, sometimes being even more abundant than the AMF.

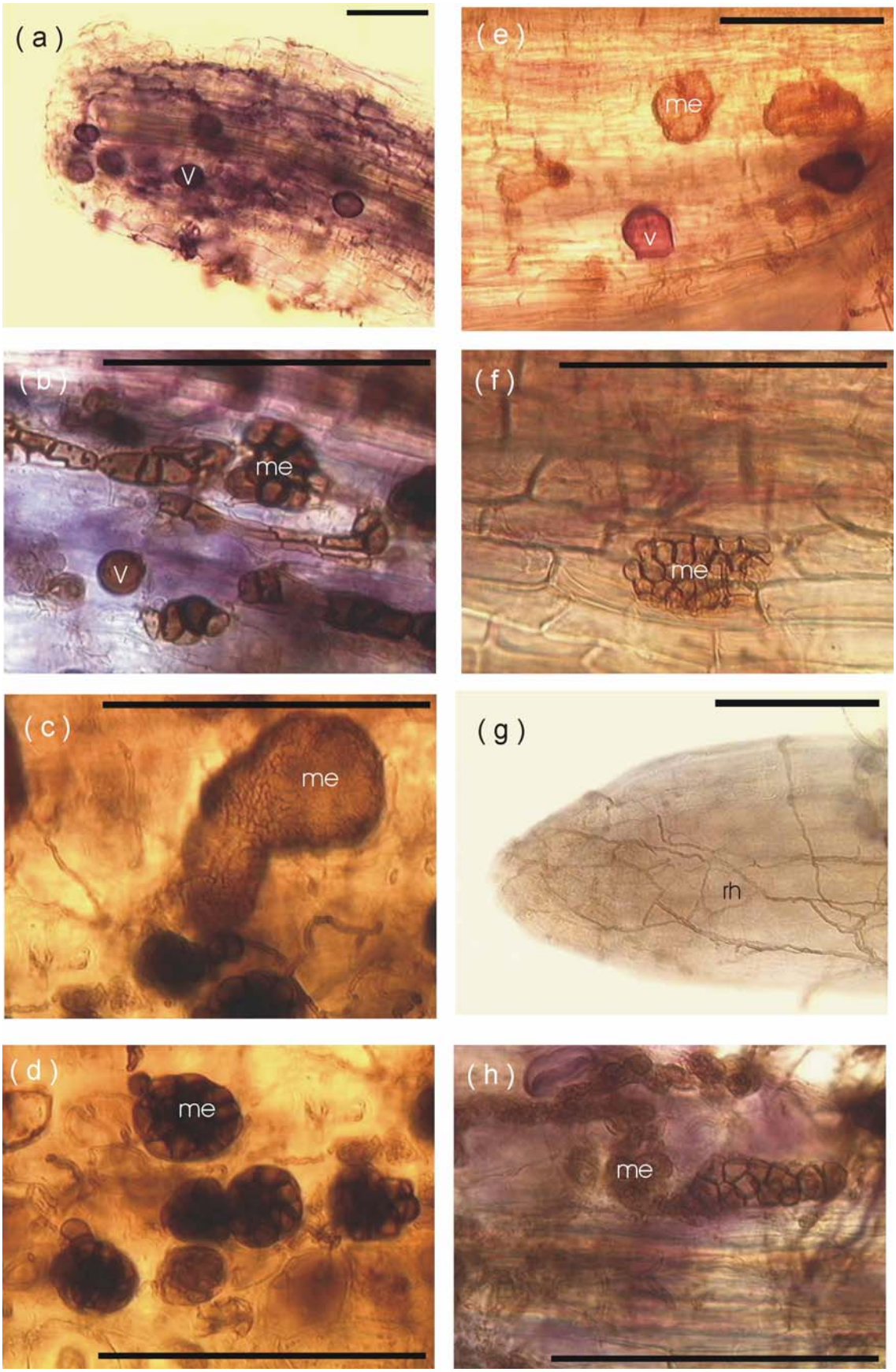

Figure 5.4 Root colonization by AM and "dark septate" fungi in (a-b) *Maytenus* sp, (c-d) *Annona* sp, (e-f) Myrtaceae sp, (g-h) *Tapirira guianensis*. (v = vesicles, me= microsclerotia, rh = superficial hyphae). (Bar = 100 μ)

5.4. Discussion

5.4.1 Seedlings dynamics

The seedling phase is a critical period in plant establishment and density-dependent recruitment has been linked to the high diversity of tropical forests (Harms *et al*. 2000). Herbivory and pathogen pressure is higher in tropical forests than in temperate ones (Coley & Barone 1996) and density-dependent effects of pathogens on forest tree seedlings have been reported (e.g. Augspurger 1984, Gilbert, Hubbell & Foster 1994). Although less studied in tropical forests, pathogens are thought to contribute to the maintenance of tree species diversity in these forests (Coley & Barone 1996). In fact, higher seedling than seed diversity have been found in a tropical forest, these differences being related to interspecific variation in seed and seedling traits (Harms *et al*. 2000). However, differences are found also within the tropical region, with leaves damage being higher in dry tropical forests than in wet forests, and greater in the understorey than in the canopy (Coley & Barone 1996). Herbivory was not quantified in the present study, but leave damage was frequently observed. Whether a species-specific trend exists, and to which point it compromises seedling survival and growth is subject for subsequent studies.

Differences in seedling dynamics between fringe and forest plots seems to indicate distinct recruitment patterns in both areas, probably subjected to microclimatic features (see discussion in 3.4.1 Climate and microclimate). Forest plots presented not only greater seedling abundance and biomass than plantation and fringe plots, but also greater species richness.

As expected, due to the markedly dry season prevailing in the studied region, seasonal differences were also found in these all these parameters. In the November sampling figures were usually higher, probably reflecting the most favorable conditions for the germination and initial establishment during the rainy season, particularly extended this year. A markedly increase in recruitment was also found in response to rainfall in an southeastern Brazilian forest (Santos & Válio 2002), followed by high seedling mortality in the dry season.

It seems that forest structure, or more exactly, plant cover, is an important factor determining the success of seedling establishment. Whether this also reflects in a more effective seedling recruitment in this areas is subject to further study, as subsequent seedling survival rates were not assessed. Nevertheless, plantation plots did not present significant natural forest tree seedling recruitment, what may derive from limited seed dispersal, but also from the unsuitable habitat conditions provided by these areas. The almost constant grass cover may hinder seeds to come in contact to soil surface, making them more prone to predation and diminishing their chances of reaching soil water an nutrient stocks before its own reserves are exhausted. Forest plots, in contrast, presented great spatial variability in seedling abundance, what call attention to the irregular nature of its distribution. Local variation in relief and gaps within the canopy, with correspondent differences in

soil nutrient pools and light intensity, may play a great role in determining the germination and initial establishment success.

Interestingly, seedling abundance, richness and biomass showed no significant correlation with litter biomass. Much has been published recently on the effects of the litter layer on seed germination and seedling establishment. Generalizations are difficult to be made, as many different factors seems to be acting simultaneously (Everham III *et al*. 1996). Litter has been found to reduce seed germination (Vazquez-Yanes & Orozco-Segovia 1992) and seedling emergence (Caccia & Ballaré 1998, Vazquez-Yanes & Orozco-Segovia 1992). But effects may vary between species (Molofski & Augspurger 1992), successional stages (Guzman-Grajales & Walker 1991), and be related to differences in seed size (Kostel-Hughes *et al*. 1998, Vazquez-Yanes & Orozco-Segovia 1992). For instance, in a Puerto Rican montane wet forest (Everham III *et al*. 1996), the litter layer allowed increased seed/seedling survival in all species studied, with the only exception of *Cecropia*, a pioneer species with seeds too small to allow them to go through this layer. Experimental manipulations have shown positive effects of litter removal enhancing seedling emergence (Guzman-Grajales & Walker 1991), but this response was also variable with season (Santos & Válio 2002). However, not only seedling abundance distribution seems to be highly variable (both spatially and temporally) in tropical forests (Santos & Válio 2002), but also the thickness of the litter layer (Molofski & Augspurger 1992). The environmental heterogeneity provided by this litter cover is therefore considered an important factor determining differential seedling establishment, and, consequently, contributing to the maintenance of forest diversity (Molofski & Augspurger 1992). At least in the Caju site, the great standard deviations found (see 3.3.3 Litter biomass) point to the important role of spatial variability in this system.

Seedlings analyzed in the present study were not further subdivided in successional stage. In addition, plots under plantation and fringe/forest conditions may have distinct proportions of shade tolerant/intolerant species. This may be at least partially responsible for the absence of correlation between seedling distribution and litter biomass in the present study. Furthermore, due to the small sampling area, care must be taken in the analysis of the results here presented. Although the forest plots were supposed to represent conditions of the region outside the region subjected to border effects, this region may be indeed subject to some stress, anthropic at least. Human use in these fragments seems to be more intense in the region a little far from the forest edge, maybe in order to avoid conflict with inspectors of the environmental protection agency. If it is difficult, if not impossible, to isolate natural from anthropogenic effects in these fragments, it should be also impossible to plan forest recovery strategies without considering this pressure on the system.

5.4.2 Mycorrhizal colonization

Methods on quantifying AMF root colonization have been recently discussed (Hart & Reader 2002). As different AMF groups present differences in hyphal structure development and root colonization patters, percent root length colonization methods should give somewhat biased estimates for root colonized by fungi of different groups (Hart & Reader 2002). However, as the ergosterol method

suggested by these authors is not specific for AMF, and as the amount of non-mycorrhizal fungal tissue in field-growing tree roots is not know, the traditional assessment within colonization classes were adopted in the present study. This method provides, however, a relatively subjective evaluation of root colonization and results should be taken not as absolute values.

5.4.2.1 Seedlings in quadrats

Differences between both sites in root colonization patterns were found (Chapter 4), the greater root colonization values being found in the Caju site. However, the same does not seem to occur with seedlings, as both high and low colonized seedlings were found in both sites. Subsequent analysis of the material sampled in November may give further insights whether there is a temporal shift in mycorrhizal colonization in the studied communities.

The absence of definite pattern of distribution of root colonization suggests that it may be somewhat randomly determined, possibly related to root, inoculum and nutrient distribution, these also probably related to each other. Variation in colonization grade could be, more than species-specific, spatially determined by differences in inoculum and/or nutrient distribution within soils. However, testing of this hypothesis can not be carried out by the present sampling scheme.

Distribution of root colonization intensities may also be subjected to a temporal variation, but the lost of the samples from February impedes this analysis at least by now. The analysis here performed, however, includes mixed samples with roots from different species. A high, or low colonization value, may therefore result from just one or few species, with abundant roots in these quadrats. Consequently, this analysis is of limited value, and was carried out in order to identify possible differences between natural (fringe/forest) and disturbed (plantation) sites regarding mycorrhizal colonization potential. This difference could not be clearly found, and it seems that mycorrhizal inoculum present in the plantation plots is able to form active symbiosis and may help plants to establish and grow under these conditions.

The presence of nodulated legume seedlings in the plantation site, from both sites, means that soils from this area present not only mycorrhizal (Chapter 4 and 5) but also rhizobia inoculum.

5.4.2.2 Selected species

Seedlings collected in sample quadrats and three of the four selected species did not present positive significant correlation between biometric measures and root colonization values. A general trend toward increasing colonization grade with developing stage has been reported (Herrera *et al*. 1991), and colonization intensity is thought to increase with increase in size class, due to a longer time to allow colonization to take place (Torti & Coley 1999). However, these authors compared seedlings and saplings, while most seedlings analyzed in this study probably belong to the same cohort, at least the seedlings from the four selected species. Size differences between them may not necessarily represent differences in time since germination but genetic differences and/or a patchy distribution of soil nutrients. To plants in the seedling stage, dependent on seed reserves until full photosynthetic activity is achieved, the establishment of mycorrhizas may represent an important nutrient input. On the other side, the mycorrhizal partner may represent a significant carbon cost to small seedlings still

depending on their endosperm reserves. For instance, although high mycorrhizal colonization was found in seedlings in the cotyledonal stage of three tree species, two of these possessed large seed reserves (Herrera *et al*. 1991). Adult plants, with access to the higher light incidence in the canopy have higher photosynthetic rates and have, in contrast to seedlings, more carbon available to fungi (Torti & Coley 1999). Indeed, low colonization infection values were thought to be related to low photosynthetic rates due to the reduced reception of light at the forest floor (Herrera *et al*. 1991). Therefore, comparison between seedlings and adult trees may take into account the environmental and physiological differences between both stages, which by its turn will also influence the mycorrhizal symbiosis (St John & Uhl 1983). Age differences between different developmental stages may also imply more time to roots grow and colonize a greater fraction of soil, increasing therefore the chances of finding (or being found by) suitable mycorrhizal inoculum. Furthermore, root systems and the proportion of it that can be potentially colonized are significantly different between both stages, what makes any comparison more difficult (St John & Uhl 1983). However, these authors reported 'similar levels of infection' in both mature trees and seedlings. The transfer, through the hyphal network interconnecting seedling and mature trees, of organic assimilates would allow the former to overcome the limiting light environment in the forest floor (Francis & Read 1984), and is an important factor affecting nutrient relationships in the early stages of plant establishment.

Colonization patterns between the four selected species also varied. While almost all analyzed seedlings of *Maytenus* sp and *T. guianensis* presented high colonization figures, on the opposite extreme, all *Annona* sp seedlings showed low figures of root colonization. This differences were not related to site, as both *Maytenus* sp and *Annona* sp were collected in the Castro site. The Myrtaceae seedlings showed, however, a more wide distribution within the range of colonization classes. These differences do not seem to be related to amount of seed reserves, as seeds from these species do not vary much in seed size. Variation in specificity levels with AMF inoculum may also play a role in explain some of these differences, but information available is insufficient to test this hypothesis at present.

Despite the variation found between plant species, due to its influence on the fitness of seedlings, AM inoculum is considered to affect the species composition of a community (Francis & Read 1994). Reports on mycorrhizal colonization in tree seedlings in tropical ecosystems are still scarce and provide sometimes contrasting results. Few seedlings were found to be mycorrhizal in natural ecosystems in Australia (Warcup 1980). However, mycorrhizas have been found to have differential effects on tropical seedlings growth (Kiers *et al*. 2000), and mycorrhizal colonization in five tree seedling species in Cuba also differed between species (Herrera *et al*. 1991). With only one exception, with colonization grade about 15.9-30.3%, all the other species analyzed had values higher than 45%, some higher than 90% (Herrera *et al*. 1991). Six of seven Brazilian tree seedlings presented high root colonization, with correspondent positive effects in plant growth and nutrient content after inoculation with AM fungi (Pouyú-Rojas & Siqueira 2000). A more recent study with 29 tree seedling species in Brazil revealed, however, large differences between species in AM colonization, responsiveness, dependency and efficiency in P uptake (Siqueira & Saggin-Júnior 2001). Root colonization was greatly affected by soil P content, but plant characteristics may be the main factor

determining the differences found in plant susceptibility to AMF colonization (Siqueira & Saggin-Júnior 2001). In fact, many of the species studied by these authors were poorly or non-mycorrhizal at all.

A seasonal variation in AM fungal colonization has been already reported (Daniell *et al*. 2001, Helgason *et al*. 1999), and may be responsible for some of the variation found in different surveys. However, in the present study, all the seedlings were collected at the same period, February. This is usually a dry month at the region, but presented exceptionally high precipitation at year the sampling was carried out (see 3.3.1 Climate and microclimate).

Furthermore, not only a seasonal variation in root colonization levels may occur, but also a temporal shift in colonization intensity and identity of the fungal partner may take place during seedling development. For instance, a decrease in root colonization and in diversity of AM fungal types colonizing roots with maturing of seedlings was observed in a Panamanian forest by Husband, Herre & Young (2002). They suggest that AM fungi with superior colonizing abilities may be substituted by other types with superior persistence abilities as seedlings grow older. However, only seedlings of an emergent tree (*Tetragastris panamensis*) were analyzed by these authors, and the high intraspecific variation found in the present study suggests that may not be necessarily a general pattern regarding AM fungal colonization within tropical tree seedlings, and this may be subjected to a multitude of seedling, fungal and environmental features. Nevertheless, an important finding from their study is the shift over time in fungal species composition colonizing seedling roots (Husband, Herre & Young 2002). Moreover, the use of molecular methods by these authors permitted the identification that different fungal partners were found in roots of different ages. A higher diversity of mycorrhizal fungi colonizing seedlings in a tropical forest compared to temperate ecosystems (woodland or agroecosystems) were this analysis has been already carried (Husband, Herre & Young 2002). However, from the 18 AM fungal types found by these authors, 16 belong to the Glomaceae, the two others to the Acaulosporaceae and Gigasporaceae.

Surprisingly, not only AMF were found to colonized abundantly roots of some of the studied species but also another endophyte which septate hyphae did not stained with Trypan blue, being at first thought to be a root pathogenic fungus. Actually, recent uses of molecular methods genetic material of root endophytes in field collected plants have revealed an astonishing high fungal diversity (Kowalchuk, Gerards & Woldendorp 1997, Vandenkoornhuyse *et al*. 2002). 49 different fungal sequences were found in roots of the grass *Arrhenatherum elatius,* being *o*nly seven of them closed to know sequences (Vandenkoornhuyse *et al*. 2002). Genetic analysis of a temperate sand dune grass also revealed a considerable diversity of pathogenic and non-pathogenic fungi within and between plants (Kowalchuk, Gerards & Woldendorp 1997). The pathogenic fungi, however, are considered to surpass in species diversity and economic importance all other groups of root pathogens, like the bacteria, Actinomycetes and viruses (Garrett 1970).

The root-cap mucilage and other root exudates makes the rhizosphere a rich environment for soil microbes, saprophytic and parasitic fungi, besides the mutualistic symbionts (Carlile & Watkinson 1994). These root exudates also seems to be essential for root infection by seedling pathogens and may determine the degree of root infection (Garrett 1970). Invasion of roots by soil fungi are usually done

through younger parts or wounds (Carlile & Watkinson 1994). Seedling roots, with almost all root system being relatively recently formed, do not present adequate barrier against fungi penetration and may be therefore more vulnerable to pathogenic infection than older plants. Seedling of different species, and species in different environmental conditions, may need variable time periods to become resistant to further infection by these pathogens (Garrett 1970).

Although some fungal pathogens (as *Rhizoctonia solani* and *Pythium* species) can extend their mycelia throughout the soil growing from one seedling to another, the majority are usually restricted to spread from infected plant to its neighbors or lie as dormant propagules, waiting for suitable substrates to approach (Garrett 1970). Distribution of inocula of pathogenic fungi in the soil is therefore usually localized around the infection sites, and in this way is the escape of seedlings from disease more due to the local absence of a pathogenic infection focus than to the possession of any significant degree of resistance (Garrett 1970). This may have differential effects on seedlings with different dispersal strategies. The more the seeds are spatially concentrated, the more vulnerable they would be to this infection. All the species analyzed are zoochorous, and although all the seedlings colleted were found more or less restricted to a small region within the forest floor, these seedling do not represent the seeds successfully dispersed to other regions, which should have increased survival chances, at least concerting escaping from pathogens (Augspurger 1984).

In fact, the presence of pathogenic fungi infecting tree seedling roots may have important implications for recruitment and establishment in disturbed areas. Under native forest, the AMF inoculum may not be limiting (with spores and the undisturbed net of hyphae and colonized roots) allowing the early AMF colonization of these seedlings. Furthermore, seedling mortality under forest may be counterbalanced by the abundance of seeds in the seed bank or by the recently produced. However, under disturbed conditions none of these hypothesis are to be fulfilled: inoculum may not be available (at least in enough quantities) and limited seed dispersal may limit recruitment. In addition, seedling in these areas should face greater mortality due to the stress. Therefore, inoculation of seedlings before transplantation may assure greater survival rates. This is particularly true if root pathogenic fungi can infects roots faster than AM fungi, specially where AMF is not available in enough quantities. Actually, one of the many positive effects of AMF cited is the enhanced resistance to pathogens (Newsham, Fitter & Watkinson 1995).

However, although this non-stained fungal partner was at first thought to be a typical pathogenic fungi, no symptoms of root or shoot damage was present on these seedlings. Actually, seedlings seemed healthy, without any noticeable signal of disease or mineral deficiency. Lodge (1996) found several non-mycorrhizal microfungal species, considered as nonpathogenic, growing in plant roots and Carlile & Watkinson (1994) define as ‘fungal endophytes’ these ‘fungal inhabitants of plants (that) do not cause disease or form any close contact with the plant cells, but inhabit the apoplastic spaces of their plant hosts’. However this definition is not very useful as any saprophytic, parasitic or mutualistic fungus growing inside a root is, by definition, an ‘endophyte’. This fungal partner was then associated to the ‘dark septate endophytes’ (DSE), which have been recently subject of a comprehensive review (Jumpponen & Trappe 1998). These fungi are reported for several species of angiospermous families, including mono- and dicotyledons, as well as for members of

gymnosperms and Lycopsida, Polypodiopsida, Equisetopsida and Psilotopsida (Jumpponen & Trappe 1998), and do not seem to have necessarily pathogenic effects (Jumpponen 2001). Although they are more frequently described in artic and alpine ecosystems, they have also been reported in tropical and subtropical regions (see references in Jumpponen & Trappe 1998).

Richard and Fortin (1973) were the first to relate these fungi to the "*Mycelium radicis atrovirens*" described by Melin (1921). Melin (1921) characterized it as a mycelia with thin dark olive green hyphae, not forming ectomycorrhizal nor belonging to mycorrhizal fungi. He also inoculated *Pinus silvestris* and *Picea abies* seedlings with this fungus and found out that the plants were strongly parasited, dying after a few months. After successful sporulation of dark, sterile mycelia of *Mycelium radicis atrovirens* isolates, it was identified as *Phialocephala dimorphospora* (Richard and Fortin 1973), belonging to the Hyphomycetes. Indeed, Jumpponen & Trappe (1998) include DSE in the Deuteromycotina, Fungi Imperfecti. Dark, sterile fungi similar to the Melin's *Mycelium radicis atrovirens* were isolated from roots of conifers and found to be three new species: *P. fortinii* (pseudomycorrhizal), *Phialophora finlandia* and *Chloridium paucisporum* (forming ectendomycorrhizae in conifers) (Wang & Wilcox 1985).

But not all report on DSE presents the identity of the fungi involved, as isolation in agar plates and subsequent sporulation, not always successful (e.g. Johansson 2001, Schadt, Mullen & Schmidt 2001), is needed. Thus, molecular methods are a useful tool to the identification of these endophytes, and will help the comparison from results from studies with different DSE isolates (Schadt, Mullen & Schmidt 2001). Indeed, DSE fungi are considered to have diverse phylogenetic origins (Schadt, Mullen & Schmidt 2001). More interestingly, the sequences obtained by these authors revealed a high phylogenetic similarity to isolates from diverse geographical origins. Furthermore, as this isolate were remarkably successful in colonizing roots from a taxonomically unrelated crop plant (*Zea mays*), it suggests a very broad host range potential (Schadt, Mullen & Schmidt 2001).

If isolation and identification of these fungi is still in a initial phase, their role on root physiology is much more unclear. The usual argument that DSE fungi are found in roots of apparently healthy plants should not be considered necessarily proof of their beneficial status (Newsham 1999). In the same way, the common occurrence of DSE throughout plants within ecosystems or habitats does not necessarily mean that these fungi are not pathogenic, as suggested for arctic and alpine sites (Väre, Vestberg & Eurola 1992). Conversely, a light pathogenicity by unspecialized fungi could also be widespread and stable in a plant community.

Although DSE form interfaces different from the typical mycorrhizal interfaces, they have been found to form root associations functionally similar to mycorrhizas, being suggested that they must be considered mycorrhizal, at least under some conditions (Jumpponen 2001). DSE have a distinct colonization pattern, being mostly characterized by the dark, septate hyphae. Hyphae are described as 'dark', 'dark olive green', 'melanized', usually forming intracellular structures composed of clusters of thick-walled cells, called 'microsclerotia', 'sclerotia', or 'sclerotial bodies', sometimes forming a superficial net of hyphae on the root surface as showed in the figure 5.4g (see references in Jumpponen & Trappe 1998). Although DSE are usually characterized by the presence of these dark hyphae and microsclerotia, recent work using sudan IV, besides the usual trypan blue, revealed also

the presence of vacuolated (vacuoles containing lipids) hyaline hyphae connected to melanized septate hyphae and also forming melanized microsclerotia (Barrow & Aaltonen 2001). Being thinner than dark hyphae, they are supposed to be more permeable and, therefore, to be the active site of carbon exchange with the host (Barrow & Aaltonen 2001). Differently from the AMF, root colonization by DSE have been found to include not only the root cortex, but also phloem's sieve elements (Barrow & Aaltonen 2001). These authors suggest a pattern of variable physiological activity, reflected on the presence of morphological structures formed. Thus, dormant and relatively inactive plants showed a greater abundance of melanized hyphae and microsclerotia, while hyaline hyphae and lipid accumulation prevailed in roots of physiologically active plants (Barrow & Aaltonen 2001). In the present study, hyaline septate hyphae was sometimes distinguished in some of the samples, when roots were analyzed under microscope, but was not considered to be related to the DSE at first. Future analysis of the root material sampled, and new seedling roots sampling, with subsequent staining of the material with Sudan IV are considered, in order to carry out a further additional analysis on the role of these fungal endophytes on seedling survival and growth.

Reports on DSE effects on host plants vary from negative to positive (see references in Jumpponen 2001) and, just like with mycorrhizal studies, they are probably influenced by the experimental conditions (Jumpponen 2001). However, the occurrence of a continuum between mycorrhizal symbiosis and pathogenic infection is suggested, at least in some groups as the dematiaceous fungi (Wilcox & Wang 1987). But, although the host responses obtained with different DSE strains varies, the range of host responses seems similar to those obtained when considering mycorrhizal fungi (Jumpponen 2001). Furthermore, the sometimes inconsistent results on DSE effects on host plants may be partially due to differences between the various fungus taxa and strains involved, being possible that only some of them may be able to form mutualistic associations (Jumpponen 2001). Actually, the absence of a clear taxonomic definition of the fungi encompassed in the DSE designation hinders any conclusive understanding on the functioning of this relationship. This term (or a variation of this, like 'dark septate', 'septate endophytes', 'dark septate fungi') has been indiscriminately used for dark, septate hyphae colonizing roots, either intercellularly or intracellularly (Jumpponen 2001).

Positive effects of DSE infection are not rare in the literature. Indeed, they can be more important than AMF in stressed environments (Read & Haselwandter 1981), being thought to be better adapted to arid ecosystems than aseptate fungi (Barrow & Aaltonen 2001). Alternatively, DSE and AMF can possess distinct ecological roles, for example as suggested for *Vulpia ciliata* populations (Newsham 1999). In this case, the coexistence of two or more fungal types in the same host root system could provide greater variability in the responses to environmental stress (Johansson 2001). Indeed, DSE colonization was found to be positively correlated with root vesicular and hyphal colonization in some cases (Ruotsalainen, Väre & Vestberg 2002).

Much less is know about distribution and nutrient dynamics and requirements of this group. Environmental conditions such as soil pH may affect root colonization by DSE. For instance, colonization by dark septate Hyphomycetes in *Picea abies* seedling roots was found to be negatively correlated with pH, being higher between 3.5 and 4.5 (Ahlich *et al*. 1998). The acid nature of the soils

analyzed in the present study could therefore have affected positively root colonization levels by DSE. There is not much information available about root colonization by DSE and other soil factors yet. No consistent trend in fungal parameters and soil P was found in roots of plants from low-alpine meadows in the Finnish subarctic region (Ruotsalainen, Väre & Vestberg 2002).

The complexity of the mycorrhizal symbiosis, and the different responses of the multitude of host-fungi combinations, under variable environmental conditions, do not allow this relationship to be defined, necessarily, as mutual beneficial (Jumpponen 2001). Though root colonization can take place, effects can be greatly variable (Francis & Read 1995). Thus, AM fungi acts not only improving nutrient relationship on host plants, but are supposed to act negatively on nonhosts, leading to differential impacts on plant fitness (Francis & Read 1995). Therefore, long-term effects should also not be left out of mind (Jumpponen 2001), but any study on the impacts of the mycorrhizal association on individuals and communities should consider firstly the initial phase of plant establishment (Francis & Read 1995), as the seedling phase is of crucial importance for determining community species diversity (Grubb 1977). Whether DSE can also act in such way on plant communities is still questionable, as data on their effects are still insufficient to asses long-term effects on the community level (Jumpponen 2001).

Results from DSE infection experiments in seedling roots suggests that the most frequent DSE species are "harmless if not beneficial" (Ahlich *et al*. 1998), although these authors caution to the observation time (4 months), maybe insufficient to allow the damage to occur. However, beneficial effects of a dark septate fungus, *Phialophora graminicola*, were reported on *Vulpia ciliata* ssp *ambigua*, a temperate grass (Newsham 1999). Reports of DSE infection in seedling roots are common, either in field sampled seedling roots (Ahlich *et al*. 1998, Horton, Cázares & Bruns 1988, Wilcox & Wang 1987) or in laboratory (Wilcox & Wang 1987). Seedlings have also been successfully used as bait plants to dark septate Hyphomycetes (Ahlich *et al*. 1998). However, all the references found until now reports results from studies with herbs, shrubs or trees from the temperate zone. It is of great importance to establish how these fungi affect seedling fitness in tropical forest and whether it can represent, at least under stressful conditions, pathogenic or deleterious effects. Interestingly, similar non mycorrhizal structures were also found in roots of some of the sampled adult trees (see 6.3.1. Root AM colonization – General comments), suggesting that this infection, if not have any positive effects, at least may not hinder seedlings further development.

5.5. Conclusions

The results presented in this chapter are a first attempt to understand some aspects of seedling recruitment in the two studied forest and, in no way, should be considered conclusive. Results points out, however, to important features of the seedling ecology in both forests that should be considered in future efforts of forest restoration. Forest plots had significantly more seedling recruitment than plantation and fringe plots. Some considerable seasonal and spatial variation was also present. Recruitment was significantly positively correlated with basal area.

Root mycorrhizal colonization intensity was highly variable between both plots and sites studied, although no clear pattern could be detected relating differences between forest and non-forest plots. The use of molecular methods in future studies will allow to answer questions on the composition of the fungal community colonizing seedling roots in the studied forest fragments, questions that are only incompletely answered by the classical soil spore extraction (Husband, Herre & Young 2002). The analysis of the roots colonization in different tree seedling species allowed, however, the characterization of different colonization patterns and intensities, reflecting an interesting interspecific variation. Whether it has taxonomic or successional basis is still not clear.

The high root infection by DSE found in seedlings from some selected species raises questions about its effect on the survivorship and growth of these seedlings and the possible interactions with AMF, often found colonizing the same root systems.

In natural ecosystems mycorrhizal diversity, more than one single fungus, may be related to better seedling performance (Perry, Molina & Amaranthus 1987) and, thus, testing with mixed inocula and a native pioneer tree species was performed subsequently (Chapter 7).

6 Analysis of the mycorrhizal status of some selected native species

6.1 Introduction

Tropical forests are important ecosystems, with high species diversity and most of them are still insufficiently studied. However, the need of wood and other products as well as of agricultural land make them vulnerable to human pressure. The Brazilian Atlantic rain forest is a good example of how these factors act on fragmenting and contributing to the lost of its diversity.

Although since Frank's work (1885) it is known that the mycorrhizal association is widely distributed in plant roots, the role of fungi as decomposers and on the mycorrhizal symbiosis in tropical ecosystems have been overlooked until recently (Singer 1984). Information on root symbiosis and fungal flora is still scarce and even absent for many tropical regions.

The mechanisms ruling mycorrhizal symbiosis and its effects on determining and/or maintaining ecological processes in complexes ecosystems as the tropical forests are still relatively not enough studied. Arbuscular mycorrhizas (AM) are the prevailing mycorrhizal type in tropical forests (Alexander, Ahmad & See 1992), being estimated to be ten times more frequent than ectomycorrhizas (ECM) (Riess & Rambelli 1980). In a primary tropical rain forest in French Guyana no ECM were found, being the AM the prevailing symbiosis (Béreau & Garbaye 1994). Almost 94% of the species analyzed in a lowland tropical rain forest in Sri Lanka showed to be mycorrhizal, and most of them presenting AM (de Alwis & Abeynayake 1980). Arbuscular mycorrhizal was dominant in Australian primary tropical rain forest, while one ectomycorrhizal species, *Acacia aulacocarpa*, dominated in secondary forests (Hopkins *et al*. 1996).

However, the role of ECM in tropical forests have been underestimated until recently. Studies have shown that all species of Dipterocarpaceae, most of the Caesalpinioideae from the Amherstieae tribe, and some in the Detarieae and others in the Papilionoideae, among other genus, present ectomycorrhizas, (Alexander 1989). In Africa most ECM species occur in low diversity forests on low fertility soils of under seasonal availability of water and/or nutrients (Alexander 1985). In Asia most of the reports of ectomycorrhizal are of genera from the Dipterocarpaceae (Singh 1966). In the Neotropics, ECM have been cited in the *igapó* e *campinarana* forests in Amazonia (Singer 1984) and in the *cerrado* (Thomazini 1974). Singer (1978) described the *campina* and *campinarana* vegetation types as being dominated by ectotroph plant species, in contrast to the *terra firme* forest, relating the absence of ectotroph species in the later as cause of the increased litter decomposition in the latter, as ectomycorrhizal fungi would be better competitors for nitrogen an phosphorus than other decomposer fungal species (Gadgil & Gadgil 1971). Lodge (1996), however, counter argues that, being formed mostly on poor soils, ectomycorrhizal dominated plant communities would consequently possess a poor quality litter, what would leads to slow decomposition rates.

The importance of understanding the principles controlling of the ectomycorrhizal association is specially relevant for tropical research, mainly when in relation to reforestation of disturbed sites (Redhead 1982). This author refers to *Pine* plantation in the tropics but his plea is equally valid to reforestation with native species. It is important to know what determines the occurrence of the ectomycorrhizal symbiosis in tropical forest ecosystems, what is its role, and how this knowledge can be managed to promote a more ecologically based and successful reforestation.

The objective of this chapter was to investigate the mycorrhizal status of some tropical tree species in the forest fragments studied. In this first phase, species pertaining to families or genus potentially ectomycorrhizal in the tropics and some of ecological interest for reforestation were selected

6.2 Material and Methods

Families, genus and species cited in the literature as ectomycorrhizal in tropical forests where listed (Table 6.1) and compared with the floristic composition of the rainforest remnants studied in the region (Landim, unpublished). Some of the species from these genus occurring in the study area were collected and analyzed (Table 6.2). Some species were also sampled although not necessarily from families or genus formerly cited as ectomycorrhizal, but for being particularly important in these forests, whether possessing high I.V. (*Maytenus* sp), high basal area (*Sclerolobium densiflorum* Benth.), being highly abundant (Myrtaceae species), important pioneer species (*Cecropia pachystachya* Trec.) or abundant in adjacent degraded areas (*Vismia guianensis* (Aubl.) Choisy).

Table 6.1 Taxonomic groups with tropical ectomycorrhizal members (values refer to number of citations; bold values refer to collection in subtropical zones)

Division / Class / Order	*Family*	*Tribe*	*Genus*	*Africa*	*America*	*Asia*	*References*
Coniferophyta							
Coniferopsida							
Coniferales	Pinaceae		*Cedrus*	1			25
Coniferales	Pinaceae		*Pinus*		2	1	14, 20
Gnetophyta							
Gnetopsida							
Gnetales	Gnetaceae		*Gnetum*	1	3		27,32,33,36
Magnoliophyta							
Magnoliopsida							
Casuarinales	Casuarinaceae		*Allocasuarina*			**1**	19
Casuarinales	Casuarinaceae		*Casuarina*			**1**	41

Table 6.1 (Cont.)

Division / Class / Order	Family	Tribe	Genus	Africa	America	Asia	References
Fagales	Fagaceae		*Quercus*			1	35
Fagales	Nothofagaceae		*Lithocarpus*			1	23
Fagales	Nothofagaceae		*Nothofagus*			**1**	41
Urticales	Cecropiaceae		*Cecropia**		1		8
Polygonales	Polygonaceae		*Coccoloba**		6		7,16,17,20,21,28
Caryophyllales	Nyctaginaceae		*Guapira**		1		21
Caryophyllales	Nyctaginaceae		*Neea**		6		2,7,21,31,33,36
Caryophyllales	Nyctaginaceae		*Pisonia**		4	**1**	4,5,17,20,28
Ebenales	Sapotaceae		*Glycoxylum*		3		31,32,33
Ebenales	Sapotaceae		*Manilkara**	1			40
Ericales	Epacridaceae		*Astroloma*			**1**	19
Malvales	Sterculiaceae		*Lasiopetalum*			**2**	18,41
Malvales	Sterculiaceae		*Thomasia*			**1**	41
Theales	Dipterocarpaceae		*Anisoptera*			1	35
Theales	Dipterocarpaceae		*Balanocarpus*			1	35
Theales	Dipterocarpaceae		*Cotylelobium*			1	3
Theales	Dipterocarpaceae		*Dipterocarpus*	1		2	2,3,35
Theales	Dipterocarpaceae		*Dryobalanops*			1	35
Theales	Dipterocarpaceae		*Hopea*			3	2,3,35
Theales	Dipterocarpaceae		*Marquesia*	2			2,13
Theales	Dipterocarpaceae		*Monotes*	3			2,11,13
Theales	Dipterocarpaceae		*Shorea*	4			2,3,18,35
Theales	Dipterocarpaceae		*Vateria*	1			2
Theales	Dipterocarpaceae		*Vatica*	1			35
Apiales	Araliaceae		*Didimopanax**		1		8
Apiales	Apiaceae		*Platysace*			**1**	41
Euphorbiales	Uapacaceae		*Micrandra*		1		37
Euphorbiales	Uapacaceae		*Poranthera*			**1**	19,41
Euphorbiales	Uapacaceae		*Uapaca*	7			2,11,13,22,24,27,38
Fabales	Leg. Caesalpinioideae	Caesalpinieae	Gleditsia		1		10
Fabales	Leg. Caesalpinioideae	Caesalpinieae	*Mora*		1		26
Fabales	Leg. Caesalpinioideae	Cercideae	*Bauhinia**		1		39
Fabales	Leg. Caesalpinioideae	Detarieae	*Anthonotha*	5			1,2,22,24,27
Fabales	Leg. Caesalpinioideae	Detarieae	*Aphanocalyx*	2			22,24
Fabales	Leg. Caesalpinioideae	Detarieae	*Berlinia*	3			22,24,27
Fabales	Leg. Caesalpinioideae	Detarieae	*Brachystegia*	7			2,9,12,11,13,27,30
Fabales	Leg. Caesalpinioideae	Detarieae	*Didelotia*	3			22,24,27
Fabales	Leg. Caesalpinioideae	Detarieae	*Gilbertiodendron*	4			22,24,27,40

Table 6.1 (Cont.)

Division / Class / Order	Family	Tribe	Genus	Africa	America	Asia	References
Fabales	Leg. Caesalpinioideae	Detarieae	*Isoberlinia*	2			2,13
Fabales	Leg. Caesalpinioideae	Detarieae	*Julbernardia*	7			2,9,11,13,24,27,40
Fabales	Leg. Caesalpinioideae	Detarieae	*Microberlinia*	2			22,24
Fabales	Leg. Caesalpinioideae	Detarieae	*Monopetalanthus*	4			9,22,24,27
Fabales	Leg. Caesalpinioideae	Detarieae	*Paraberlinia*[a]	1			27
Fabales	Leg. Caesalpinioideae	Detarieae	*Paramacrolobium*	1			9
Fabales	Leg. Caesalpinioideae	Detarieae	*Tetraberlinia*	3			22,24,27
Fabales	Leg. Caesalpinioideae	Detarieae	*Toubaouate*[b]	1			27
Fabales	Leg. Caesalpinioideae	Detarieae	*Afzelia*	9			1,2,9,12,15,22,24,27,30
Fabales	Leg. Caesalpinioideae	Detarieae	*Eperua*	1	2		26,33,37
Fabales	Leg. Caesalpinioideae	Detarieae	*Intsia*	1			2
Fabales	Leg. Mimosoideae	Acacieae	*Acacia**		**1**	**2**[c]	10,19,41
Fabales	Leg. Mimosoideae	Ingeae	*Calliandra**		**1**		10
Fabales	Leg. Mimosoideae	Ingeae	*Inga**		2		8,28
Fabales	Leg. Papilionoideae	Bossiaeeae	*Platylobium*			**1**	41
Fabales	Leg. Papilionoideae	Millettieae	*Lonchocarpus*		**1**		10
Fabales	Leg. Papilionoideae	Mimoseae	*Prosopis*		**1**		10
Fabales	Leg. Papilionoideae	Mirbelieae	*Brachysema*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Burtonia*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Chorizema*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Daviesia*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Dillwynia*			**2**	19,41
Fabales	Leg. Papilionoideae	Mirbelieae	*Eutaxia*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Gompholobium*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Jacksonia*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Mirbelia*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Oxylobium*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Pultanaea*			**1**	41
Fabales	Leg. Papilionoideae	Mirbelieae	*Viminaria*			**1**	41
Fabales	Leg. Papilionoideae	Phaseoleae	*Hardenbergia*			**1**	41
Fabales	Leg. Papilionoideae	Phaseoleae	*Kennedia*			**1**	41
Fabales	Leg. Papilionoideae	Robinieae	*Robinia*[+]				6
Fabales	Leg. Papilionoideae	Sophoreae	*Ormosia*		1		8
Fabales	Leg. Papilionoideae	Sophoreae	*Pericopsis*	2			2,13
Fabales	Leg. Papilionoideae	Swartzieae	*Aldina*		6		21,31,32,33,34,37
Fabales	Leg. Papilionoideae	Swartzieae	*Swartzia**		3		32,33,34
Myrtales	Myrtaceae		*Backhousia*			**1**	29
Myrtales	Myrtaceae		*Baeckea*			**1**	19
Myrtales	Myrtaceae		*Calistemon*			**1**	41
Myrtales	Myrtaceae		*Calytrix*			**1**	19

Table 6.1 (Cont.)

Division / Class / Order	Family	Tribe	Genus	Africa	America	Asia	References
Myrtales	Myrtaceae		*Campomanesia**			1	39
Myrtales	Myrtaceae		*Eucalyptus*				**2** 19,41
Myrtales	Myrtaceae		*Leptospermum*				**3** 19,22,41
Myrtales	Myrtaceae		*Lophostemon*				1 29
Myrtales	Myrtaceae		*Melaleuca*				3 2,19,41
Myrtales	Myrtaceae		*Syzigium*				**1** 29
Myrtales	Myrtaceae		*Tristania*				2 2,23
Myrtales	Thymelaeaceae		*Pimelea*				**1** 41
Polygalales	Polygalaceae		*Comesperma*				**1** 19
Proteales	Proteaceae		*Faurea*		1		13
Rhamnales	Rhamnaceae		*Cryptandara*				**1** 41
Rhamnales	Rhamnaceae		*Pomaderris*				**2** 19,41
Rhamnales	Rhamnaceae		*Spyridium*				**1** 41
Rhamnales	Rhamnaceae		*Trymalium*				**1** 41
Asterales	Asteraceae		*Helichrysum*				**1** 19
Asterales	Asteraceae		*Podolepis*				**1** 19
Asterales	Asteraceae		*Podotheca*				**1** 19
Asterales	Asteraceae		*Toxanthes*				**1** 19
Campanulales	Goodeniaceae		*Brunonia*				**1** 41
Campanulales	Goodeniaceae		*Dampiera*				**1** 19
Campanulales	Goodeniaceae		*Goodenia*				**2** 19,41
Campanulales	Stylidaceae		*Stylidium*				**1** 41
Rubiales	Rubiaceae		*Ixora*				**1** 29
Rubiales	Rubiaceae		*Opercularia*				**1** 41
Rubiales	Rubiaceae		*Psychotria**			2	31,32
Magnoliophyta	-	-	-				-
Liliopsida							
Arecales	Arecaceae		*Euterpe**			1	8
Poales	Poaceae		*Phyllostachys*+				6

* Genera present in Sergipe; + Collected in temperate regions, but from families or genera with tropical members; [a] Synonymy for *Julbernardia*; [b] Synonymy for *Didelotia*; [c] Citation also in tropical rainforest (Hopkins *et al*. 1996)

References: 1 (Alexander 1985); 2 (Alexander & Högberg 1986); 3 (Alwis & Abeynayake 1980); 4 (Ashford & Allaway 1982); 5 (Ashford & Allaway 1985); 6 (Barsali 1922); 7 (Béreau, Gazel & Garbaye 1997); 8 (Edmisten 1970); 9 (Fassi & Fontana 1961); 10 (Frioni, Minasian & Volfovicz 1999); 11 (Högberg 1982); 12 (Högberg & Nylund 1981); 13 (Högberg & Piearce 1986); 14 (Ivory 1980); 15 (Jeník & Mensah 1967); 16 (Kreisel 1971); 17 (Lodge 1996); 18 (Louis 1988); 19 (McGee 1986); 20 (Miller, Lodge & Baroni 2000); 21 (Moyerson 1993); 22 (Moyerson & Fitter 1999); 23 (Moyerson, Becker & Alexander 2001); 24 (Newbery et al. 1988); 25 (Nezzar-Hocine et al. 1998); 26 (Norris 1969); 27 (Onguene & Kuyper 2001); 28 (Pegler & Fiard 1979); 29 (Reddell, Hopkins & Graham 1996); 30 (Redhead 1968); 31 (Singer 1978); 32 (Singer 1984); 33 (Singer & Araujo 1979); 34 (Singer & Araujo Aguiar 1986) ; 35 (Singh 1966); 36 (St John 1980); 37 (St John & Uhl 1983); 38 (Thoen & Ba 1989); 39 (Thomazini 1974); 40 (Torti & Coley 1999); 41 (Warcup 1980)

Table 6.2 Species sampled in both study sites for analysis of root colonization

Family	*Species*	*Site*	
		Crasto	**Caju**
Cecropiaceae	*Cecropia pachystachya* Tréc.	X	
Celastraceae	*Maytenus* sp	X	
Clusiaceae	*Vismia guianensis* (Aubl.) Choisy		X
Erythroxylaceae	*Erythroxylum* cf. *mikanii* Peyr.		X
Leg. Caesalpinioideae	*Sclerolobium densiflorum* Benth.	X	
Leg. Papilionoideae	*Acosmium bijugum* (Vog.) Yakovl.		X
Leg. Papilionoideae	*Andira nitida* Mart. ex Benth.		X
Leg. Papilionoideae	*Bowdichia virgilioides* H.B.K.	X	
Leg. Papilionoideae	*Crotalaria stipularia* Desv.	X	
Leg. Papilionoideae	*Dioclea violacea* Mart. ex Benth.	X	X
Leg. Papilionoideae	*Swartzia apetala Raddi* var. *subcordata* Cowan	X	
Myrtaceae	Myrtaceae sp 1	X	
Myrtaceae	Myrtaceae sp 2	X	
Polygonaceae	*Coccoloba laevis* Casar		X
Polygonaceae	*Coccoloba rosea* Meisn.	X	
Sapotaceae	*Manilkara* sp	X	

Sampling was done in the two sampling sites, previously described (Chapter 3): Caju and Crasto, from September to November 2000, comprehending the end of the rainy season and beginning of the dry season. Whenever possible, for each species roots were sampled from three different plants, carefully digging along a major root from the base of the trunk following the finer branch until fine roots could be found. The material was placed in plastic bags kept in ice until brought to the laboratory, where it was carefully washed and analyzed at first under dissecting microscope. Ectomycorrhizal tips and/or nodules where registered and collected.

All root samples where prepared for assessing of arbuscular mycorrhizal colonization after carefully washing from the soil, clearing and staining (Koske & Gemma 1989). Bleaching with alkaline hydrogen peroxide was necessary in most of the samples, as the roots were still somewhat dark after the clearing step. The quantification of arbuscular mycorrhizal colonization of roots was done by examining them at x 200 magnification under the compound microscope using the magnified intersections method (McGonigle *et al.* 1990). Analysis of root slides were one using an Olympus BX60 microscope (with Normarski optics) with video-camera attached (Sony DXC-9100P). Photographs and measurements were done using the Optimas image analysis program (version 6.2, 1987-1997 Optimas Corporation).

The ectomycorrhizal tips were prepared for anatomical studies. After washing samples free from soil, they were fixated in F.A.A. (5 ml acetic acid, 5 ml formaldehyde, 90 ml ethanol 50%) overnight. Samples were later washed in running water, dehydrated by changing from 30% to 100% ethanol in 10% steps within the next 24 hours, and prepared for infiltration and embedding with Spurr, through a rising Spurr/ethanol sequence (1:4, 1:2, 4:1 v:v) for 8-12 h each (Spurr 1969).

The material was then sectioned (1µm) on a Reichert-Jung ultramicrotome (Ultracut E), and stained with 0,05% toluidine blue (O'Brien *et al*. 1964). Description of the fungal colonization and the ratio of the fungal sheath area to the total cross sectional area of root was then calculated.

Nodules were fixed in 2,5% glutaraldehyde solution in 50 mM phosphate buffer (pH = 3.0) for anatomical studies. Nodules sections were dehydrated as for light microscopy to 100% ethanol, being then dried using a BAL-TEC critical point dryer (CPD030), coated with gold (200nm) in a sputter coater and examined in a ISI 100B scanning electron microscope. Some nodules samples were prepared for light microscopy as described above.

Isolation of the mycorrhizal partner in ectomycorrhizas was tried, where tips were cultivated in agar medium. Roots were washed free from soil and mycorrhizal tips were selected and cut under dissecting microscope. These were surface sterilized under 30% H_2O_2 for ca. 10 to 20 sec (Molina & Palmer 1982) after what they were rinsed with sterile water and then dried off with sterile paper and transferred to Petri dishes filled with Agar medium (MMN). The inoculated dishes were incubated at room temperature (around 25°C), being periodically monitored.

The identification of the fungal partners in ectomycorrhizas was also attempted, using molecular methods. These were conducted by Dipl.-Biol. Christoph Kulmann, under the supervision of Dr. Carsten Harms, in the Laboratory for Bioanalysis (UFT, Bremen University). For this analysis, the primers ITS1 and ITS7 from the fungal rDNA were chosen. In contrast to the primers ITS1 and ITS4F, until now used for PCR identification of mycorrhizal fungi (Hamelin *et al*. 1996), the use of the primers ITS1 and ITS7 (100 pmol/µl) should improve the specificity of identification of the fungi on root fragments, as a shorter region is amplified. All the primers used were obtained from MWG Biotech AG. The DNEasy® plant kit (Qiagen) was used according to the manufactor's instructions for DNA extraction from ectomycorrhizal tips. 10-25ng DNA was used in a PCR thermocycler (Stratagene Robocycler), using positive (*Amanita muscaria*) and negative (sterile and filtrate water) controls. After electrophoretic separation, the gels were stained with ethidium bromide and visualized under UV light. The resulting ITS-PCR amplicons were then eluted from the gel with the Millipore Ultrafree-DA Kit and used for characterization by DNA sequencing (MWG Biotech AG). Finally, the sequences found were compared using the Basic Local Alignment Search Tool via internet with the sequences already published in the Gen-Bank (NCBI, National Center for Biotechnology Information, www.ncbi.nlm.nih.gov).

6.3 Results

Arbuscular mycorrhizas were found in almost all species analyzed (Table 6.3), with varying grades of colonization. It is possible that seasonality plays a role here, in determining root growth season and/or its activity.

Table 6.3 Root colonization of the studied species by the magnified intersections method (McGonigle *et al.* 1990). "N" = number of intersections analyzed; "np" = not possible to visualize; "+" = present, but not possible to quantify; "-" = absent.

		AMF colonization(%)					ECM	Nodules
Species	**Sample**	**Arbuscules**	**Vesicles**	**Hyphen**	**Total**	**N**		
Cecropia pachystachya	1	np	np	np	np			
	2	-	52.83	42.45	95.29	106		
	3	-	16.33	41.84	58.16	100		
Maytenus sp	1	-	14.89	74.47	86.52	141		
	2	np	np	np	np			
	3	np	np	np	np			
Vismia guianensis	1	-	31.78	76.64	79.44	107		?
	2	-	13.51	78.38	78.38	111		?
	3	-	29.73	99.10	99.10	111		?
Erythroxylum cf. *mikanii*	1	-	+	+	+			
	2	-	+	+	+			
Sclerolobium densiflorum	1	np	+	+	np			
	2	np	np	np	np			
Acosmium bijugum	1	3.7	25.00	93.52	97.22	108		X
	2	np	+	+	np			
Andira nitida	1	8.59	15.62	26.56	53.12	128		X
	2	-	11.94	22.39	34.36	134		X
Bowdichia virgilioides	1	-	+	+	np			
	2	-	+	+	np			
Crotalaria stipularia.	1	6.72	5.22	57.46	69.40	134		
	2	np	np	np	np			
	3	-	8.96	49.25	58.21	134		
Dioclea violacea.	1	-	-	59.85	59.85	132		X
	2	np	np	+	np			
	3	np	+	+	np			
Swartzia apetala var.	1	np	np	np	np			
	2	np	np	np	np			
Myrtaceae 1	1	-	4.38	36.84	36.84	114		-
	2	np	np	np	np			
Myrtaceae 2	1	-	3.64	33.64	33.64	110		-
	2	-	+	+	np			
Coccoloba rosea	1	-	+	+	np		+	
	2	np	np	np	np			
Coccoloba laevis	1	np	np	+	np			
	2	np	+	+	np			
Manilkara sp	1	0.69	10.43	52.41	61.38	145		-
	2	np	np	np	np			
	3	np	np	np	np			

6.3.1 Root AM colonization – General comments

Two AM classes were first described by Gallaud (1905): the *Arum*-type, with "extensive intercellular phase of hyphal growth in the root cortex and development of terminal arbuscules on intracellular hyphal branches", in which "vesicles can be intercellular or intracellular, and are not formed by all VA mycorrhizal fungi". This is the type usually called the "typical" arbuscular mycorrhiza is the Arum-type (Smith & Read 1997). In the *Paris*-type, "defined by the absence of the intercellular phase and presence of extensive intracellular hyphal coils", vesicles, whenever present, are intracellular (Smith & Smith 1997). Both types were found in the plants examined, but the *Paris*-type seemed to be more frequent.

Other non-mycorrhizal symbionts were also found in many roots and were characterized by dark, septate hyphae, not staining in Trypan blue (Figure 6.1), but the identity of these fungal hosts could not be determined. These structures were somewhat similar to the ones found in seedling roots (see 6.3.3.2 Selected species) and were not included in the quantification of the AM colonization. Lodge (1996) found several non-mycorrhizal microfungal species, considered as nonpathogenic, growing in plant roots. However, AMF can present septate hyphae in certain cases, e.g. particular host/ fungus combination, damage, age, and pre-penetration stages of colonization (Smith & Smith 1997).

6.3.2 Root AM colonization – Species description

***Manilkara* sp**

Three samples from this species were collected but only one presented material fine and young enough to be appropriately cleared and stained. Roots were well ramified, without nodules or ectomycorrhizal tips. Abundant young rootlets apex were short, being easily detached from the root. Another sampled tree hat roots somewhat thicker but also well ramified. These were still dark after clearing and staining and could not be considered in the quantification of mycorrhizal colonization.

An intermediate to high root colonization level was found (61%). Vesicles and arbuscules, were present, suggesting it is an *Arum*-type mycorrhizal plant, but the later were much less abundant and probably in senescence stage (Figure 2a). This family was not represented in the revision on VA mycorrhizas types made by Smith & Smith (1997).

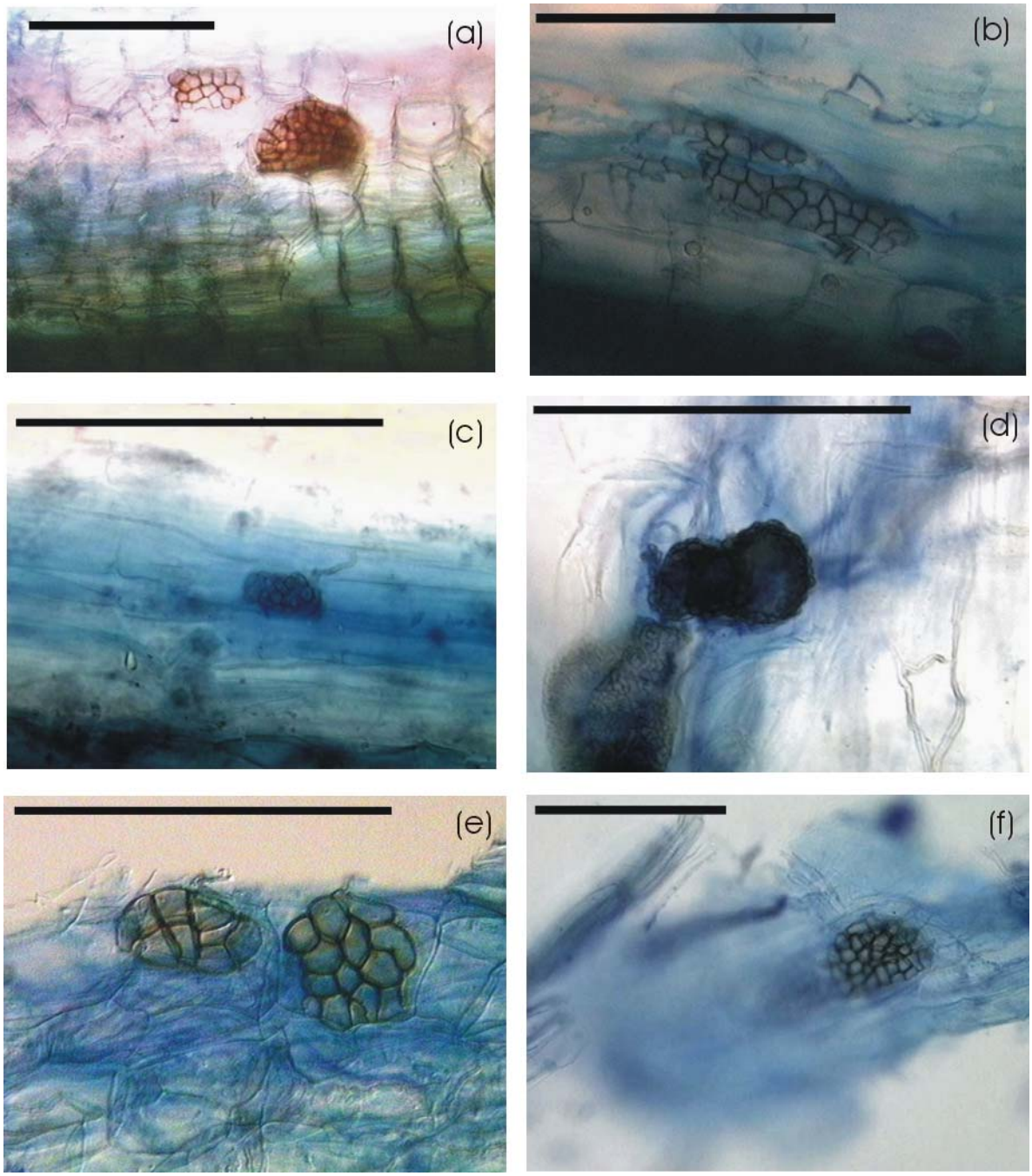

Figure 6.1 Non mycorrhizal fungal structures in roots of some of the sampled trees: (a) *Andira nitida*; (b) *Maytenus* sp; (c) *Crotalaria stipularia*; (d) *Dioclea violaceae*; (e) Myrtaceae "A"; (f) *Coccoloba rosea*. (Bar = 100 µm)

Myrtaceae sp 1

Abundant roots, well ramified, without many root hairs, were found. Some root tips looked like ectomycorrhizas, and were therefore prepared like above described for anatomical studies in order to confirm it.

Arbuscular mycorrhizal colonization was not high (37%). Hyphae and vesicles were found, but no arbuscules or hyphal coils. Hence, the arbuscular mycorrhiza type (whether *Paris*- or *Arum*-type) could not be determined. This family was previously cited as presenting *Paris*-Types (Janse 1897, Smith & Smith 1997).

Myrtaceae sp 2

Roots were fine and well ramified. Some roots seemed somewhat older, maybe senescent. Suspected thicker apexes were, as above, checked for ectomycorrhizal colonization.

Root colonization values were similar to the obtained for the other Myrtaceae species, and arbuscules were also not found. Some hyphal coils (Figure 6.2b), characteristic of *Paris*-type plants, were observed, what agrees with the description of Janse (1897) for *Eugenia* sp. However, the low contrast achieved by these material prevent a more accurate description and analysis.

***Maytenus* sp**

Although three samples were collected, just one presented young roots. These were abundant and well ramified, with many fine and dark roots, while some were brownish-red. The main root and their derived were rust color, clearer than the black lateral roots, with an abrupt change in color between both. No nodules or ectomycorrhizal roots were found.

Although three samples were collected, just one was analyzed after clearing and staining. High root colonization rate was found (86,52%), with no arbuscules, but with vesicles and abundant hyphal coils (Figure 6.2c), which characterizes the *Paris*-type of arbuscular mycorrhizas. No reference on this family was present in the revision on VA mycorrhizas types made by Smith & Smith (1997). Colonization was present even very near the root apex (Figure 6.2d).

***Andira nitida* Mart. ex Benth.**

Three root samples were collected but only two could be analyzed, because no enough fine roots were present in one of them. Roots were well ramified, with abundant nodules in secondary rootlets, but no evidence of ectomycorrhizas.

The two plants analyzed showed some variation in the degree of arbuscular mycorrhizal colonization (34,36 to 53,12%). The stained roots did not presented enough contrast, and it was somewhat difficult to identify the fungal structures. Great variation in colonization intensity was observed between root fragments from the same sample. Vesicles were found in both samples. One of the samples presented arbuscules (Figure 6.2e), but hyphal coils were present in the other sample (Figure 6.2f), what makes difficult to assess the AM type present.

Nodule abundance, size and shape differed between samples, varying from a rod-shaped, "Y"-shaped (2-3cm), to smaller (2–177mm) spherical ones. Nodules morphometric values are listed in the Table 6.4. Rod-shaped nodules seemed to be more abundant, although Y-shaped were bigger and heavier. It seems that they represent different nodule development stages (Corby 1981) and that the final stage represent Corby's astragaloid type, a predominant in tropical legume subfamilies, the Caesalpinioid and Mimosoid (Corby 1981).

Table 6.4 Morphometry of *Andira nitida* nodules.

Shape	*Length / s (cm)*	*Width / s (cm)*	*Weight / s (g)*	*n*
Rounded	0,42 / 0,06	0,28 / 0,03	0,0093 / 0,0020	3
Rod-shaped	1,07 / 0,52	0,38 / 0,11	0,0416 / 0,0361	7
Y-shaped	2,22 / 0,24	0,44 / 0,04	0,1821 / 0,0368	5

Swartzia apetala* Raddi var. *subcordata

Only thick roots, very little ramified, were collected in two samples, and where not suitable for the clearing and staining procedures. No nodules or ectomycorrhizal tips were found but, due to the absence of fine roots, this could not be excluded.

***Bowdichia virgilioides* H.B.K.**

The two samples collected for this species presented thick roots, without nodules or ectomycorrhizal tips. Quantification of AM colonization was not attempted because the roots showed already secondary growth. Stained hyphae and vesicles were, however, found in both samples. Neither arbuscules nor hyphal coils were found.

***Acosmium bijugum* (Vog.) Yakovl.**

Three samples were collected for this species. Roots were relatively well ramified but few young roots could be found in one of the samples, so just two were used for clearing and staining. No ectomycorrhizal roots were found.

One of the stained samples could not be used for mycorrhizal colonization assessment, although hyphae and vesicles were present, because of the low contrast achieved. Arbuscular mycorrhizal colonization was high (97,22%), with hyphae, vesicles and arbuscules being present (Figure 6.2g-h), signs of an Arum-type host.

Just one of the root samples presented nodules. Nodules were big (0,5-1,2 cm) irregularly shaped, more or less spherical, in a lateral position on secondary roots.

***Crotalaria stipularia* Desv.**

Three samples were collected but roots were not abundant, not very well ramified and not very young. No ectomycorrhizas or nodules were observed.

Two of the samples showed intermediate colonization rates (58-69%), with hyphae, some vesicles and structures that seemed to be old, disintegrating arbuscules (Figure 6.3a). Hyphal coils were not found, what suggests it is a Arum-type mycorrhizal host.

***Dioclea violacea* Mart. ex Benth.**

D. violacea is a perennial climbing shrub. Two samples were collected, but in both of them roots were not well ramified and few fine roots were present. No ectomycorrhizal tips were found but

some nodules of the astragaloid type (Corby 1981) were present in one of the samples. One of the samples had somewhat older roots, being discarded after clearing and staining procedures did not resulted in suitable material for analysis and quantification. The sample stained showed also intermediate levels of root colonization (60%), with no arbuscules, few vesicles but dense hyphal infection in some of the root segments analyzed. Hyphal coils were also not observed, and the arbuscular mycorrhiza type (whether Paris- or Arum-type) could then not be determined. Again, colonization was found near the root apex (Figure 6.3b).

***Sclerolobium densiflorum* Benth.**

Two samples from this species were collected. Samples although well ramified, did not possess many fine roots. No nodules or ectomycorrhizal roots were found, although sampled roots did not look very healthy, with the young tips being dark, seeming to be in initial phase of decomposition.

S. densiflorum is an important emergent tree in the Sergipe rain forests, presenting the fourth higher I.V.I. (16.55), and the second dominance value (10,9%) in an phytosociological survey in the Crasto forest (Landim, unpublished). Although roots were not very thick, most showed already secondary growth. In some of the segments, hyphae and vesicles could be found. But, due to the bad condition of samples, neither arbuscular mycorrhizal colonization level nor type could not be assessed.

***Erythroxylum* cf. *mikanii* Peyr.**

This species was chosen, although not present in the prepared list (Table 6.1) or not being particularly abundant in the region, mostly because an ectomycorrhizal seedling collected seemed to belong to this genus. However, no ectomycorrhizas were found on the adults trees sampled. No reference on the mycorrhizal status of this genus could be found, what is not surprising considering the diversity of tropical flora, and the difficulties in sampling roots in tropical forests. Roots were young and fine, light brown, well ramified, without attached hyphae or spores and without root hairs.

Two samples could be analyzed regarding arbuscular mycorrhizal colonization. Quantification of root colonization could not be attempted because some root segments lost their cortex during clearing and staining steps. However, in some of the segments, colonization was very intense, reaching almost 100%. Hyphae and vesicles were found, but none arbuscules. Hyphal coils were present, although not very clearly visible due to the low contrast achieved (Figure 6.3c), what points to an Paris-type AM host. Information on mycorrhizal type of this family was not found in the review of Smith & Smith (1997).

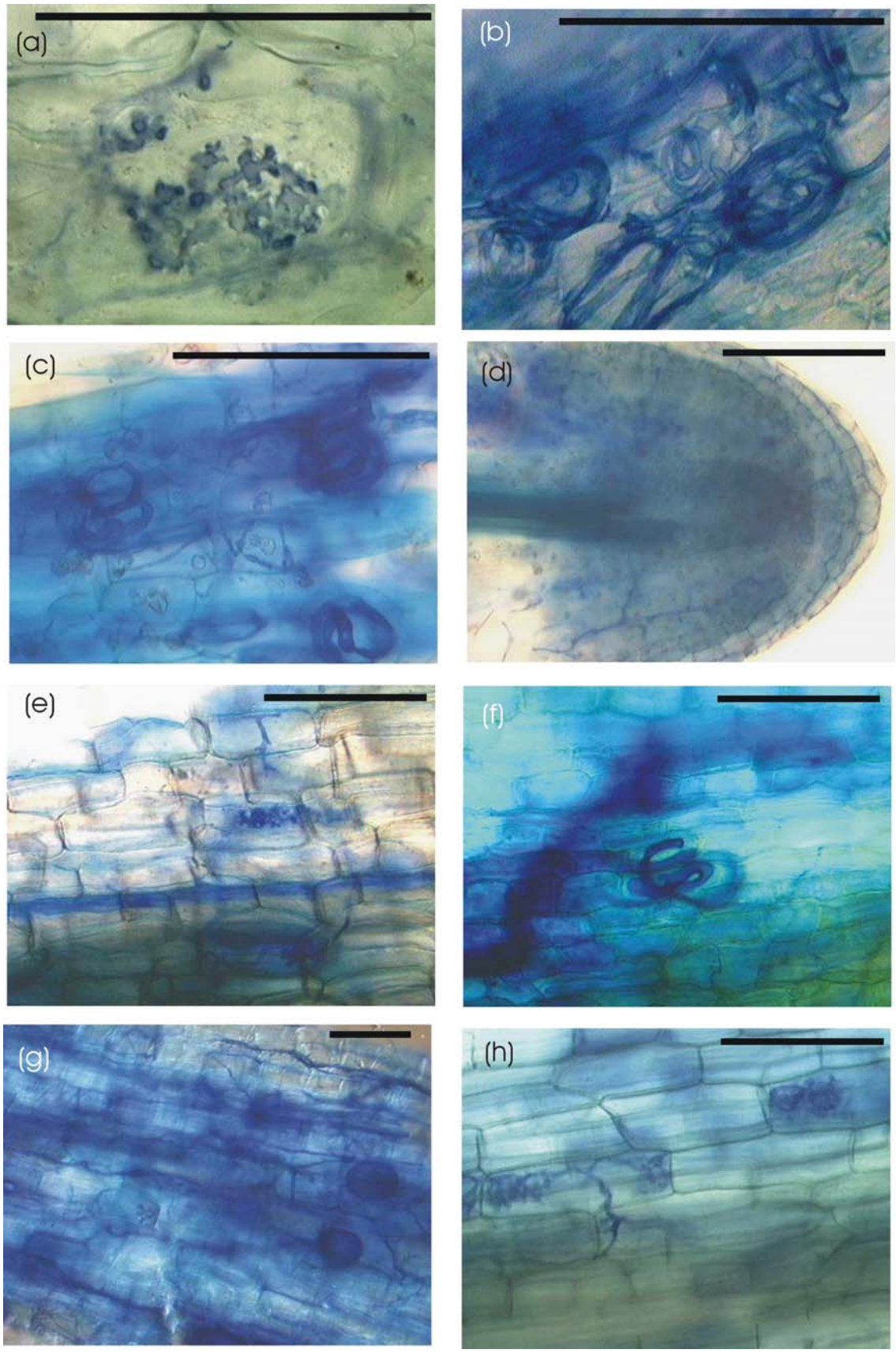

Figure 6.2 Details of the mycorrhizal colonization. (a) *Manilkara* sp; (b) *Myrtaceae* 2; (c-d) *Maytenus* sp; (e-f) *Andira nitida*; (g-h) *Acosmium bijugum*. (Bar = 100 μ)

***Cecropia pachystachya* Trec.**

C. pachystachya roots were reasonably well ramified, without nodules or possible ectomycorrhizal tips. Mycorrhizal colonization was abundant, with many vesicles and two types of hyphae, differing in thickness. Arbuscules could not be found, but hyphal coils were present, usually in the rhizodermis or in the cortex, characterizing an Paris-type mycorrhizal species, in agreement with the review on VA mycorrhizas types made by Smith & Smith (1997).

Few root hairs were present, usually short ones. But even when roots hairs were present, hyphae could be found. Colonization was found even very near to the root apex. (Figure 6.3d). This species showed moderate to intense colonization in two samples (58,16 to 95,22%), but the third sample must be discarded as no sufficient contrast was achieved.

Ecto- and endomycorrhizas were also not found in three other Amazonian *Cecropia* species (Pavlis & Jeník 2000), but Edmisten (1970), sampling in Puerto Rico rain forests, observed ectomycorrhizas in *C. peltata*. However, Lodge (1996), working in the same area years after, found only AM colonization on roots of *C. schreberiana* and did not found ectomycorrhizas in any of the supposed ectomycorrhizal species she had resurveyed. She suggested Edmisten had "mistaken luxuriant growths of extramatrical hyphae of VAM fungi, pathogenic fungi, (…) for ectomycorrhizal fungi".

***Vismia guianensis* (Aubl.) Choisy**

Three samples from this species could be colleted and, due to the good quality of the material, all of them could be cleared and stained with success. Fine roots were abundant, without root hairs. Some unusual "thickenings" in the roots were found (Figure 6.3e-h), some of them presenting short fine lateral roots. These "nodules" or, more exactly, "knobs" or "outgrowths" (Sprent 2002), as they do not seem to be related to N_2 fixation (Thielen-Klinge 1997), were concentrated in the main roots and its laterals. The main roots differed markedly from the lateral ones, the former being brighter than the later. Studies describing AM colonization (Béreau & Garbaye 1994), and the root system of *V. guianensis* (Alexandre 1989-1990), or other *Vismia* species (Pavlis & Jeník 2000) do not cite such "thickenings", nor studies on ecophysiological responses to stress of this species (Dias-Filho 1995a, 1995b, Boehm et al. 2000, Thielen-Klinge 1997).

Ectomycorrhizal tips were not found in this study, but AM colonization was abundant, ranging from 78 to 99%. Hyphal coils were present, what suggests it is an Paris-type AM host. However, two references in Smith & Smith (1997) revision on VA mycorrhizas types place this family (Guttiferae) as presenting Arum-typed mycorrhizas. Seedlings of *V. guianensis* were found to be arbuscular mycorrhizal in a primary rain forest in French Guyana (Béreau & Garbaye 1994) and one unidentified species of *Vismia* in Amazonia also presented AM colonization (St John 1980), while three Amazonian *Vismia* species presented neither ecto- nor endomycorrhizas (Pavlis & Jeník 2000).

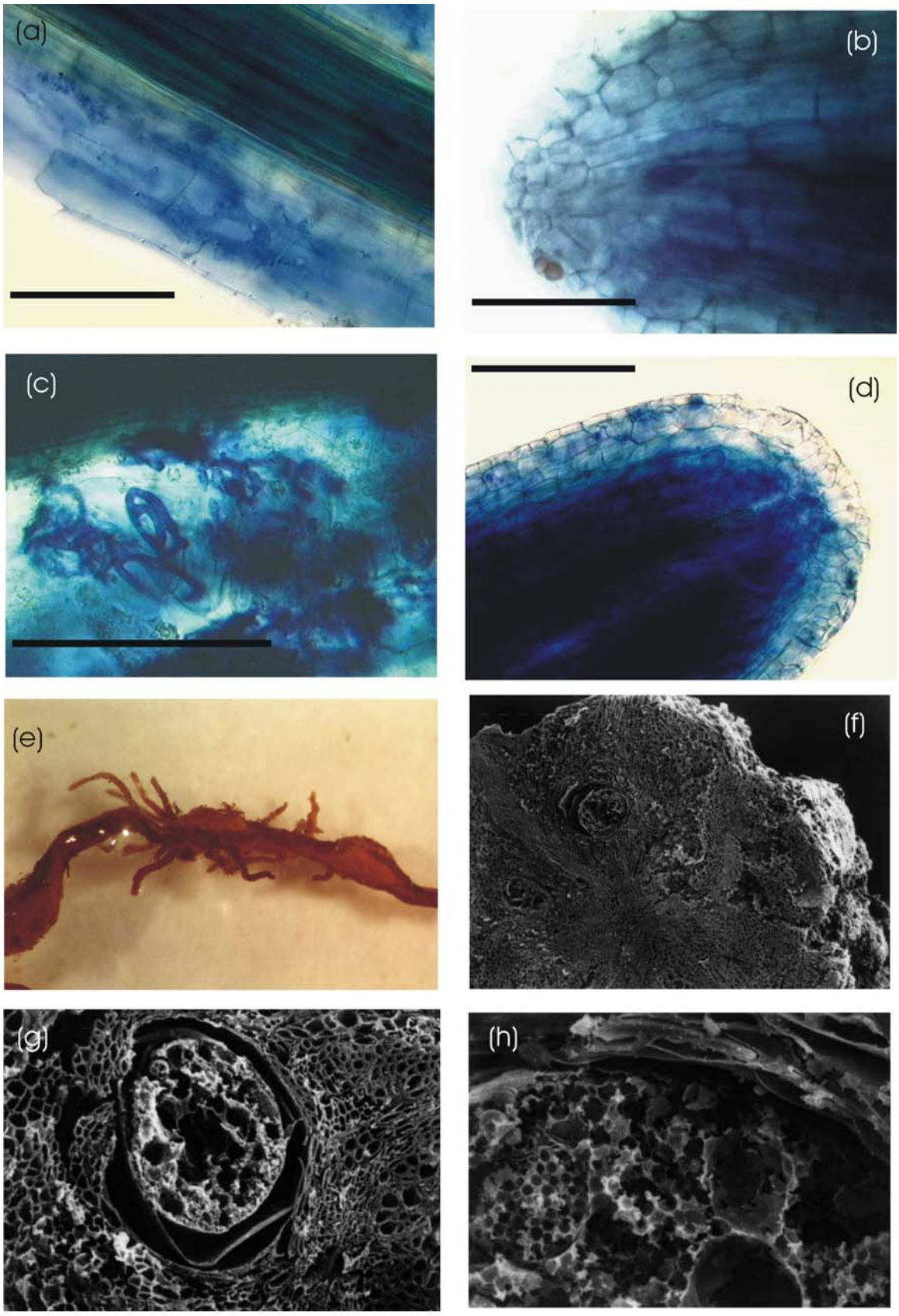

Figure 6.3 Details of mycorrhizal colonization: (a) *Crotalaria stipularia*; (b) *Dioclea violacea*; (c) *Erythroxylum mikanii*; (d) *Cecropia pachystachia* (Bar = 100 μ). (e-h) Outgrowths in *Vismia guianensis* roots: e= 20x, f= 96x, g= 260x, h= 1800x.

***Coccoloba laevis* Casar**

Two samples from *C. laevis* were collected but, being already in the beginning of secondary growth, none could be appropriately cleared and stained, so no information on level of AM colonization can be given. However, both samples presented stained hyphae and, one of them, vesicles. Roots showed not much ramification and some of the short lateral roots looked like ectomycorrhizas but could not be analyzed microscopically. Further sampling is needed in order to assess the relative importance of different mycorrhizal types in this genus.

***Coccoloba rosea* Meisn.**

Three samples were collected and some ectomycorrhizal tips were present in two of them. However, the sample without ectomycorrhizal roots presented somewhat older roots, already in the beginning of secondary growth, and was not considered in this analysis. Although the two cleared and stained samples showed also some secondary development, one of the analyzed samples showed AM colonization, with stained hyphae and few vesicles, but no arbuscules, being present. AM colonization could not be accurately assessed in these samples.

Roots were generally not well ramified, and lateral roots, whenever present, were short (<1cm). However, the sample with two ectomycorrhizal types showed a greater ramification, what means that the two other samplings could not reach really younger roots.

In one of the samples two types of ectomycorrhizal tips were found, differing mainly in color. Most of the tips were dark brown with long "hairs" (Figure 6.4), but some were clearer. Its is not clear whether the two types represent different endophytes or just different developmental stages of the same fungi, as bright hyphae, almost white, could be seen entering younger root tips.

The dark brown mycorrhiza presented monopodial structure, being apparently similar to the "dark brown ECM showing hairy sheath" found by Thoen & Ba (1989) on *Afzelia africana*. The plectenchymatous sheath (Argerer & Rambold 1996) showed, in cross sections, no great differentiation between inner and outer sheath, as found by e.g. Jeník & Mensah (1967), although the outer sheath had somewhat dicker walls compared with the inner sheath. Cell sizes from the inner and outer sheath showed also no great differences, contrary to the observations of Strullu (1979) and Alexander & Högberg (1986), the inner sheath showed sometimes larger cells than the outer. Cells of the sheath were also never "as large or larger than the epidermal cells of the host", as found by Alexander & Högberg (1986). The large epidermal cells showed no clear pattern of elongation, being sometimes circumferentially, sometimes radially elongated, but never as much as in *C. excelsa* (Moyersoen 1993).

A Hartig net was always present, but rarely going further than the host first cell layer (epidermis). Two to three cortical layers were present. No intracellular infection was observed. The mean sheath thickness was 32.55 μm (s = 4.861) .

The identification of the fungal partner in *C. rosea* mycorrhizal tips using molecular methods was unsuccessful. A preliminary collection of fruit bodies in the studied forest fragments was also attempted, in order to identify possible ectomycorrhizal fungal species present in the region.

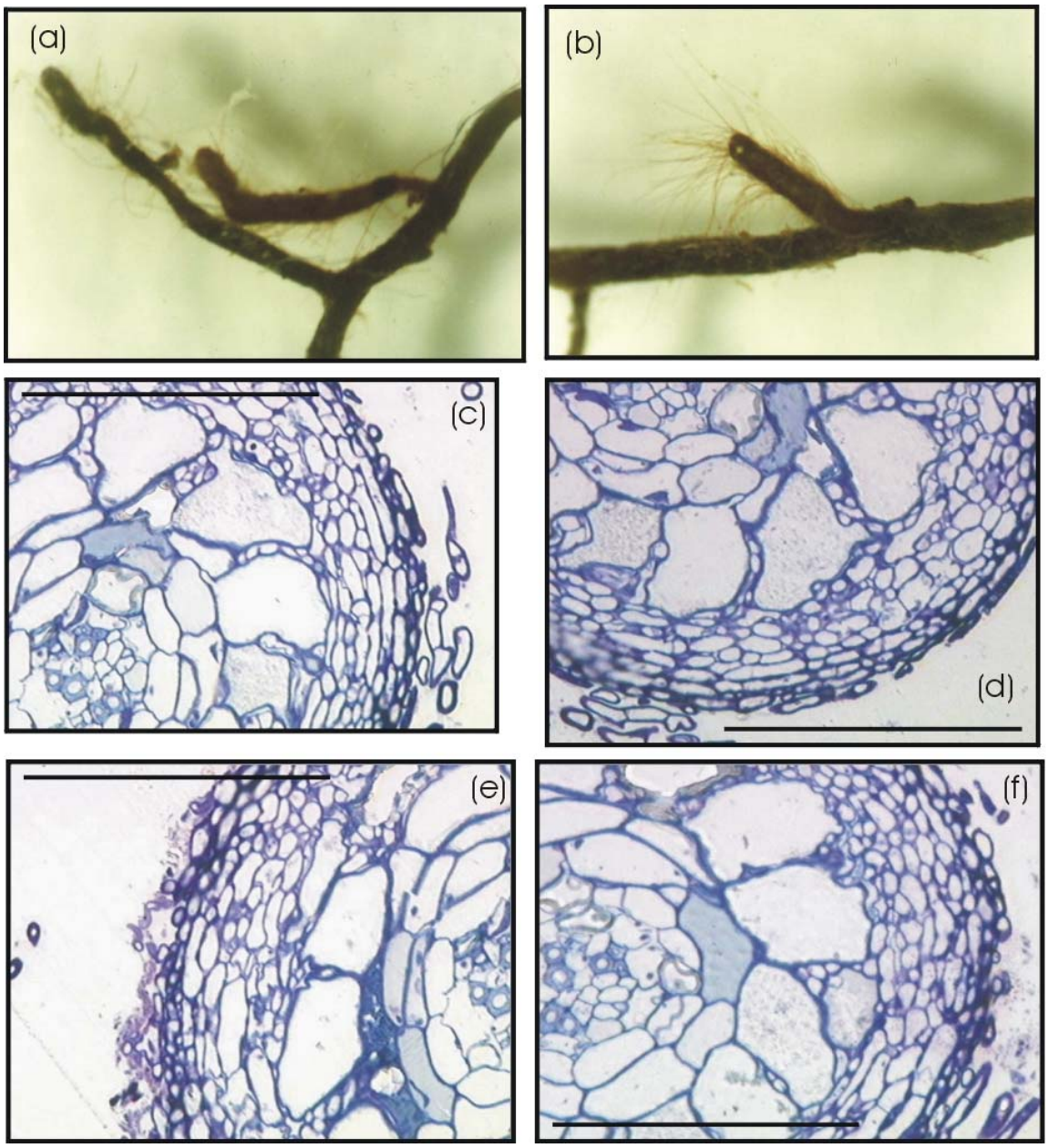

Figure 6.4 Ectomycorrhizas in *Coccoloba rosea*. (a-b) external appearance; (c-f) internal structure. (bar = 100µm)

6.4 Discussion

6.4.1 Sampling and clearing problems

The first problem when dealing with tropical tree forest species is to be sure that one is collecting the roots from the desired plant. As Torti & Coley (1999) describe "it was exceedingly difficult to follow roots on large trees and remain confident that we were harvesting the appropriate fine

roots". As followed by these authors, whenever we could not be sure about root identity, samples were discarded.

Because most of the roots were heavy pigmented, KOH treatment for prolonged times (1.5-2h at 90°C) was used, but nevertheless, sometimes roots must be bleached with alkaline H_2O_2 for 15-20 min (Torti & Coley 1999). Effective post post-clearing bleaching time with alkaline hydrogen peroxide varies between samples and should be carefully used because mycorrhizal hyphae staining can be reduced or even eliminated (Brundrett Melville & Peterson 1994). Bleaching with H_2O_2 has been also found to damage mycorrhizal hyphae and hamper staining (Brundrett, Murase & Kendrick 1990). This may have been a problem in this work, as root from different species, often with tannins and other secondary compounds that may obscure the results, were analyzed together and the time and sample size available were not always enough in order to assess the optimal procedure for each species. Another potential problem in analyzing these results is the possibility of non-staining hyphae (e.g. Morton 1985).

Furthermore, low contrast was found in roots from some species. Roots were not always young enough, although even in somewhat older roots mycorrhizal colonization could be found. Roots from some Amazonian tree species belonging to different successional stages showed different clearing and staining results, maybe due to differences in root anatomy (St John & Uhl 1983), making these authors more confident in quantifying root colonization in successional species. No such difference could be clearly defined between the species sampled in this study, although pioneer species like *C. pachystachya* and *V. guianensis* showed roots with good contrast and *S. densiflorum*, an late secondary or climax species in Sergipe forests, could not have mycorrhizal colonization assessed, as bad the root material was after clearing and staining was.

Due to the several differences in root development, anatomy, and secondary compounds, protocols specifics for each specie should be established, in order to achieve the better staining results possible. It is also needed to continue this analysis with a greater sample size, more species, and seasonal sampling. The great species richness from these forests make difficult a complete assessment from the mycorrhizal colonization, but key species, representing different strategies and successional stages, for example, should be prioritized.

6.4.2 AM colonization types

The *Paris*-type is more frequently found in the plant kingdom than *Arum*-types predominating "in ferns, gymnosperms and many wild angiosperms, herbs and woody species" (Smith & Smith 1997). Morphology of AM colonization is affected not only by the fungus species, but also by the host plant, and both AM types have been produced by the same fungal isolate in different hosts (Gerdermann 1965). The greatest structural diversity was found in AM Angiospermous trees (Brundrett, Murase & Kendrick 1990).

In the species analyzed in the present work, vesicles were more abundant than arbuscules, these being almost absent, what agrees with the results of St John (1980), studying Amazonian tree roots, and Torti, Coley and Janos (1997), analyzing two monodominant tree species in Trinidad and

Panama. Arbuscules may be absent or present only in small numbers in *Paris*-type arbuscular mycorrhizas (Brundrett 1990a e b) and it seems that not all AM form these structures (Smith & Smith 1997). Arbuscule senescence seems to be a gradual and seasonal process probably subjected to environmental stress (Brundrett & Kendrick 1990, Smith & Smith 1997).

Although arbuscules are widely accepted as a transfer region between plant and fungus (Gianinazzi *et al*. 1983), evidence about it is not enough to exclude other interfaces (Smith & Smith 1997). Intracellular hyphal coils, frequent in the *Paris*-type mycorrhizas, could be extensive as arbuscules and it is possible that these structures also take part on nutrient transfer processes (Reinsvold & Reeves 1986, Louis 1990, Smith & Smith 1997). Hyphal coils were, in contrast to vesicles, more abundant in the present study, although not quantified, and it seems its role in tropical tree roots have been undervalued.

No clear pattern relating AM type and plant habit or successional status could be found. Two of the pioneer tree species (*V. guianensis* and *C. pachystachya*) showed Paris-types AM, but the shrub *C. stipularia* presented Arum-type mycorrhizas. Two late secondary species differed in mycorrhizal type: *Manilkara* sp presented an *Arum*-type and *Maytenus* sp, a *Paris*-type. Within the Leguminosae, only two species could have the type defined (*C. stipularia* and *A. bijugum*), both of them presenting the *Arum*-type. Both AM types have been found in the subfamily Papilionoideae of the Leguminosae (Smith & Smith 1997), but Alexander (1989) states that arbuscules are often absent in roots of legume trees, and that infection is characterized by distinctive hyphal coils.

A problem in interpreting the results of this (and similar) work is that roots in different development stages are being compared. But colonization levels may be less a result of the effective importance of the fungi for one species than of the age of the root, and the time it had to establish and grow (this may be true even within a sample of one single species). AM root colonization do not establishes evenly along roots. A preferential colonization of young regions of roots exists (Mosse & Hepper 1975, Smith & Walker 1981) and colony size may be related to distance from the root tip or age (Brundrett & Kendrick 1990).

Differences in root anatomy can be also responsible for different colonization patterns (Brundrett, Murase & Kendrick 1990), and may affect hyphal penetration, e.g. when an exodermis is present (Brundrett & Kendrick 1990). In *Arum*-type species, development of fungal infection was faster and colonies were larger, as roots containing air channels were found not to constrain hyphal growth Brundrett & Kendrick (1990). In contrast, rate of growth of infection in the Paris-type plants seems to be slower than the ones in Arum-type plants because of the chiefly intercellular hyphal growth (Smith & Read 1997). However, root anatomy alone seems not enough to determine mycorrhizal colonization. For example, Janse (1897) did not observed intercellular hyphae in roots of *Eugenia* (Myrtaceae), although the parenchyma in this species showed large spaces.

6.4.3 AM colonization and successional stage

The analysis of mycorrhizal colonization through different seral stages has been already attempted (e.g. Janos 1980, St John & Uhl 1983) in order to search for a pattern on mycorrhizal dependency and plant strategy in relation to habitat.

Pioneer and early secondary species present wide distribution including natural or anthropic disturbed sites, like forest gaps and river banks, with production of abundant small seeds, which are efficiently dispersed and can remain dormant until the light environment is suitable for germination (Budowski 1965). Late secondary species are usually deciduous trees, being also found in dry forests, while climax communities are characterized by a greater diversity of species, frequently endemic ones, with large and short-lived seeds (Budowski 1965).

The strong AM colonization levels in roots of *V. guianensis* and *C. pachystachya*, two pioneer species, differ markedly from the non-pioneer species in this study. This contrasts with the absence of differences between plants from various successional stages found by St John & Uhl (1983) in an Amazonian rainforest. The statement that pioneer species are often non-mycorrhizal (Janos 1980b) relates to habitats on poor soils in which mycorrhizas do not occur, but that is not always the case on deforested sites, as it may depend on the management history of this area after disturbance. Low nutrient levels, resulting from removal of plant cover, with persistence of mycorrhizal inoculum may represent a pressure, on the opposite side, for the establishment of mycorrhizal pioneer species. These were found to be more responsive to mycorrhizal inoculation and phosphorus level than climax species, and more responsive to mycorrhizal inoculation than to phosphorus addition (Siqueira *et al.* 1998).

Cecropia species, although mycotrophic, were thought not to need mycorrhizas when growing in natural habitats (Janos 1980b). Though, *C. pachystachya* was found to be a "very highly dependent" species (Siqueira & Saggin-Júnior 2001), showing a 100% responsiveness index under 0.02, but none under 0.002 mg P l^{-1}. Pioneer species seem to be more dependent, or at least more responsive, on mycorrhizal inoculum under nutrient stressed conditions (Siqueira *et al.* 1998), and these high levels of mycorrhizal colonization may make them able to colonize successfully disturbed habitats.

Janos (1980a) also stated that species with light seeds colonizing disturbed habitats would be least dependent on mycorrhizas, what do not fit to the results obtained in this and other studies for *C. pachystachya* (Siqueira & Saggin-Júnior 2001). *Cecropia* species are important tropical pioneer trees, with its small zoochorous seeds being dispersed by birds, bats, or mammals (e.g. Charles-Dominique 1986, Martínez-Ramos & Alvarez-Buylla 1986, Vázquez-Yanes & Orozco-Segovia 1986, Alejandro Estrada & Coates-Estrada 1986, Alvarez-Buylla & Martínez-Ramos 1990, Garcia, Rezende & Aguiar 2000). The efficiency of *Cecropia* species as colonizers of disturbed habitats may thus be due not only to a suitable dispersal strategy, but also to the enhanced nutrient absorption due to the AM fungal partner.

C. stipularia, a small annual herb present in degraded areas, can also be considered a pioneer species, at least in a broad sense. However, its AM colonization levels were not very much higher than that of other non-pioneer tree species (e.g. *Manilkara* sp or *Maytenus* sp), what is

consistent with the results of St John & Uhl (1983), that did not found difference from infection rates between plants of different life forms or from distinct successional stages.

Maytenus sp, probably *M. obtusifolia* Mart, is an important species in the forests in Sergipe, presenting the higher I.V.I. (35.02), abundance, dominance and frequency values in an phytosociological inventory conducted in the Crasto forest (Landim, unpublished). Its roots were strong colonized by AMF, with almost 90% of colonization. *Manilkara* sp, another late secondary species, presented also significant AM colonization (around 60%), what do not agrees with the findings of Siqueira *et al*. (1998), that found that many secondary and climax species did not presented AM root colonization and that colonization levels diminished with successional stage. These authors, however, studied roots of native woody species seedlings and it is possible that in the initial phases of seedling establishment, different patterns of root colonization occur, irrespective of successional stage, although *Maytenus* sp seedlings showed also high AM root colonization (Chapter 5).

Janos (1980) considered that lowland tropical forest trees should tend to be obligate mycotrophic species, not being able to survive to reproductive maturity without a mycorrhizal partner, because of the low fertility soils found in their natural habitats. This would, therefore, lead them to dominate communities on poor soils. Unfortunately other late secondary or climax species could not have their roots properly analyzed in this survey, which would have given a better picture of the AM root colonization through species of different successional stages. However, spatial variation in mycorrhizal inoculum may be in some way determining some of the results here found while it is also possible that species within a same successional stage presents different strategies related to differences in root anatomy and physiology, and this should bring out different patterns of AM colonization.

6.4.4 Legumes, mycorrhizas and nodulation

All species with roots which could be appropriately cleared and stained presented arbuscular mycorrhizal colonization, which ranged from moderated (*A. nitida*, *C. stipularia*, *D. violaceae*) to high (*A. bijugum*).

No Leguminosae species collected were found to be ectomycorrhizal, although one *Andira* species (*A. inermis*) collected in Puerto Rico, was thought to be a "putative host" for ectomycorrhizal fungi (Miller, Lodge & Baroni 2000). *Swartzia apetala* was also not ectomycorrhizal, though ectomycorrhizas has been found in *Swartzia* species in Amazonian *campinarana* (Singer & Araujo 1979, Singer 1984) and *igapó* forests (Singer & Araujo Aguiar 1986).

Nodules were found on *A. nitida*, *D. violacea* and *A. bijugum* roots, all of them species belonging to the Papilionoideae. Nodulation is cited as most frequent in the Papilionoideae and Mimosoideae subfamilies of Leguminosae (Allen & Allen 1947; Barrios & Gonzales 1971, Allen & Allen 1981, Norani 1983, Faria *et al*. 1989). The coevolution between leguminous species and the broadly classified "*Rhizobium*" microorganisms allowed legume plants to colonize nitrogen-

poor soils (Verma & Stanley 1989). Much of the research on legume nodules has been done with herbaceous species (Sprent 1994) and data on nodulation of native tree species is needed if they are going to be used in reforestation and restoration of degraded soils. Unfortunately, this information is absent for over 40% of legume genera, most of them tropical ones (Sprent 1994).

A. nitida has been cited previously as a nodulating species (Faria *et al.* 1987b), but the records on nodulation is this genus show both nodulating (DeSouza 1966, Norris 1969, Faria *et al.* 1984, Moreira, Silva & Faria 1992, Souza, Silva & Faria 1994) as well as not nodulating species (Norris 1969, Sylvester-Bradley et al. 1980, Moreira, Silva & Faria 1992). Some *Andira* species are cited in both categories in the same (e.g. *A. micrantha*, in Moreira, Silva & Faria 1992) or different works (e.g. *A. parviflora* in Souza, Silva & Moreira 1994, Moreira, Silva & Faria 1992; *A. surinamensis* in Faria & Lima 1998, Moreira, Silva & Faria 1992). The nodulating status of this genus is not yet clear, as other species, e.g. *A. fraxinifolia*, are consistently cited as nodulating in different papers (Faria *et al.* 1984, Faria *et al*. 1987b, while other, e.g. *A. unifoliolata,* are reported as non-nodulating (Moreira, Silva & Faria 1992, Sylvester-Bradley *et al.* 1980). Seasonal and geographical variation, related to soil properties and plant cover, as well sampling efficiency, are not to be excluded from the explanation from this discrepant results, and further research is thus still needed.

Nodules of *Andira* species have been already cited as having a shape corresponding to the astragaloid type of Corby (1981) and indeterminate growth, with a long-lived apical meristem (Faria, Sutherland & Sprent 1986, Faria, McInroy & Sprent 1987). In contrast to the usual form of infection, where N-fixing Rhizobium cells (called "bacteroids") are found within plant membranes (Sprent 1981), rhizobia in this genus was found to be retained within "persistent infection threads" (Faria, McInroy & Sprent 1987), which consist of cell wall material surrounding the *Rhizobium* cells. This is a characteristic found in non-legume nitrogen-fixing species, as *Paraponia* species (Ulmaceae) (Lancelle & Torrey 1984, Lancelle 1985, Trinick 1979). Sprent (2001) has called these structures "persistent **fixation** threads", instead, as they present active nitrogenase. They seems to be a primitive form, being present in all the caesalpinioid legumes examined so far (Sprent *et al*. 1989).

Nodulation has been already reported for *D. violacea* in Trinidad (deSouza 1966) and nodules were found on seedlings growing in pots, but not on field sampled roots (Faria & Lima 1998). Nodulation seems to be frequent throughout this genus as all the surveyed species from this genus were found to be nodulated (DeSouza 1966, Norris 1969, Moreira, Silva & Faria 1992, Souza, Silva & Moreira 1994). Abundant nodulation on a *Dioclea* species roots were found in different sites in the Amazonian rainforest (Norris 1969).

Previous information on nodules on *A. bijugum* was not found, so it seems this is the firs report on nodulation on this species. The genus was not even cited in Allen & Allen (1981) revision. Since then, nodules have been reported for *A. nitens* (Souza, Silva & Moreira 1994, Faria & Lima 1998), although not for *A. nitens* and two other *Acosmium* species in different studies (Faria et al. 1984, Moreira, Silva & Faria, Faria & Lima 1998).

No nodules on *B. virgilioides* roots were found in this study. Absence of nodulation on *B. virgilioides* is also reported in a Venezuelan savanna (Barrios & Gonzales 1971) and in the Brazilian Amazonia (Moreira, Silva & Faria 1992) although *B. nitida* was found to nodulate in this region (Faria & Lima 1998) and nodulating *B. virgilioides* plants were found in Southeast Brazil (Faria *et al*. 1984).

Although nodulation was also not found on roots of *S. densiflorum*, nodules have been already found on roots of this species (Faria *et al.* 1994) and many other *Sclerolobium* species (Norris 1969, Barrios & Gonzales 1971, Faria *et al*. 1987a, Faria *et al.* 1987b, Moreira, Silva & Faria 1992, Souza, Silva & Moreira 1994, Faria & Lima 1998) in contrast to a few reports on non-nodulating *Sclerolobium* species (Moreira, Silva & Faria 1992).

Although no nodules where found on *S. apetala* roots, this species is cited as nodulated (Faria *et al*. 1987a) as well as several other *Swartzia* species (DeSouza 1966, Norris 1969, Faria *et al*. 1984, Moreira, Silva & Faria 1992, Souza, Silva & Faria 1994, Faria & Lima 1998). Some *Swartzia* species did not presented nodules (Norris 1969, Faria *et al*. 1984, Moreira, Silva & Faria 1992), but differences were sometimes found between adult plants in natural habitats and seedlings from the same species, growing in pots (Faria & Lima 1984). These authors did no find nodules on field sampled roots of *S. glazioviana*, but nodules were found on seedlings growing in pot experiments.

Absence of nodulation was registered for most of the legume species analyzed in the present study, what may the considered cautiously, due to the difficulty in obtaining young roots. Furthermore, absence of nodules on a tree roots may not mean necessarily the absence of nodulation on this species (Norris 1969, Allen & Allen 1981). Non-nodulating individuals of species known to present nodules were frequently found in the Amazonian rainforest (Norris 1969). Thus, if the register of nodulation on a given species indicates its ability to establish this symbioses, negative results should not be considered necessarily the absence of this ability. Although differences on soil texture and fertility were not found to affect nodulation establishment in Amazonian soils (Souza, Silva & Moreira 1994), environment may play a role in triggering nodulation (Sprent 1994). Insufficient sampling may also be responsible, at least partially, for negative reports, as nodules can be easily detached in many species, mainly *Acacia* spp, and can be located deep in soil, particularly in arid zones (Sprent 1994).

On the other side, the mere presence of nodules on roots, may not necessarily imply a benefit for the plants bearing them (Allen & Allen 1981). No tests could be performed in order to assure if the nodules found really represent a effective (nitrogen-fixing) symbiosis. Even *Rhizobium* strains able to initiate nodule development on roots, may not be equally capable of establishing effective symbiosis (Corby 1981). Further studies should therefore be carried, as finding nodulating legume tree species in forest ecosystems is only one step towards the understanding of the diversity of nutrient acquisition strategies in natural plant communities.

As a consequence of the *Rhizobium*-plant symbiosis, nitrogen-fixing legumes are thought to have a higher N content than non-legumes. Although no significant differences between nitrogen content in leaves from fixing- and non-fixing legumes species from the Brazilian *Cerrado* were

found, leaves from both legume groups had higher N content than leaves from non-legumes (Sprent *et al.* 1996). Legume species can therefore represent an improvement in soil nitrogen. Greater levels of available nitrate were found in soils around legume than around non-legume forest tree species (Edmisten 1970b) and the same is supposed to occur in deforested sites. Legume species can contribution to increasing soil N content through N-enhanced litter in early successional stages (Vitousek & Walker 1989, Erickson, Davidson & Keller 2002).

Costa Rican native legume tree species have showed results as good as the ones obtained from exotic legume species in reforestation studies on acid soils, with high levels of aluminum (Tilki & Fisher 1998), conditions similar to the ones of the soils in Sergipe. *A. bijugum* is not a typical tree in Sergipe forests, being more frequently found on open, natural or disturbed, areas. Presence of nodulation and high levels of AM colonization may increase the ability of this species to colonize areas with infertile soils. Therefore, this species should also be considered in further studies aiming to determinate how early in plant establishment infection with both partners occurs, how this affects plant growth rates and survival under deforested sites and how this knowledge can be managed to improve soil conditions and support subsequent forest tree seedling recruitment in these sites.

Herbaceous legume species should also be considered in restoration of deforested sites. An herbaceous species of *Desmodium* is cited as important in the succession in cleared sites, having well nodulated roots (Edmisten 1970c). Another legume shrub, *Carmichaelia odorata*, was found to contribute to the build of the soil organic horizons and of nitrogen content in them during primary succession (Bellingham, Walker & Wardle 2001). While the responses to the development of *C. odorata* along succession differed between seedlings from dominant forest tree species, its nitrogen-enriched litter showed positive effects on shoot biomass and foliar nitrogen concentration in all species studied (Bellingham, Walker & Wardle 2001). Although nodulation was not observed on *C. stipularia* roots, nodules have been reported previously for this (Gibson, Dreyfus & Dommergues 1982) and other *Crotalaria* species (DeSouza 1966, Barrios & Gonzales 1971, Faria *et al*. 1989). Nodulation was also found in roots of legume seedlings growing in the plantation site (Chapter 5), what means these soils present not only mycorrhizal (Chapter 4) but also *Rhizobium* inoculum, an important information when considering forest regeneration based on ecological processes with native rather than introduced partners.

6.4.5 Ectomycorrhizas and *Coccoloba* species

Ectomycorrhizal tips in the present work were only found in *C. rosea*, but interestingly not in *C. laevis*. Kreisel (1971) first cited ectomycorrhizal colonization in *C. uvifera* (L.) L., a species occurring in monospecific coastal forests in Cuba (Table 6.5). However, he found no evidence of ectomycorrhizas in the many other *Coccoloba* species present in other mixed stands.

Lodge (1996), analyzing roots in cross- and longitudinal sections in *Coccoloba* species, found no evidence of true ectomycorrhizal infection on any of the species examined. However, 50 to 82% of the fine roots of *C. swartzii* and *C. pyrifolia* was “covered by thick mantles of

basidiomycetous hyphae with clamp connections", what the author describes as "peritrophic mycorrhizal infections". This would differ from the true ectomycorrhizas because of Hartig net absence and presence of transfer cells like the ones described in *Pisonia grandis* by Ashford & Allaway (1982, 1985). All other references on mycorrhizas on *Coccoloba* species seems to fit in the concept of true ectomycorrhiza, and "peritrophic mycorrhizae are formed with the same genera of basidiomycetes as those typically forming ectomycorrhizae with other hosts" (Lodge 1996). Furthermore, Smith & Read (1997) considers *P. grandis* mycorrhizas as true ectomycorrhizas. It is possible that the roots of *C. swartzii* and *C. pyrifolia* presented an initial development phase of ectomycorrhizas, as seems to be the case of the studied roots of *C. warmingii* in rainforests in Southern Brazil (Andrade *et al.* 2000). Although no fungal sheath or Hartig net was present, septate hyphae with clamp connections were found and, as young plants (50 to 70cm) were analyzed, it is probable that it represents an early stage of colonization. A possible temporal succession between mycorrhizal partners should also not be excluded, as arbuscular mycorrhizas were found colonizing their roots (Andrade *et al.* 2000).

Table 6.5 Ectomycorrhizas in *Coccoloba* species cited in the literature

Species	***AM*[1]**	***Vegetation form***	***Country***	***Reference***
C. uvifera (L.) L.	No inf.[2]	Coastal vegetation	Cuba	Kreisel 1971
C. uvifera (L.) L.	No inf.[2]	"Xerophytic" forest	Lesser Antilles	Pegler & Fiard 1979
C. diversifolia Jacq.	No inf.[2]	"Xerophytic" forest	Lesser Antilles	Pegler & Fiard 1979
C. pubescens L.	No inf.[2]	"Xerophytic" forest	Lesser Antilles	Pegler & Fiard 1979
C. excelsa Benth.	+[3]	Amazonian *caatinga*	Venezuela	Moyerson 1993
C. pyrifolia Desf.	+[3]	Lowland rain forest	Puerto Rico	Lodge 1996
C. diversifolia Jacq.[4]	+[3]	Lowland rain forest	Puerto Rico	Lodge 1996
C. latifolia Lamark	3%	Primary rain forest	French Guiana	Béreau, Gazel & Garbaye 1997
C. mollis Casar.	0 - 15%	Primary rain forest	French Guiana	Béreau, Gazel & Garbaye 1997
Coccoloba sp	30%	Primary rain forest	French Guiana	Béreau, Gazel & Garbaye 1997
C. warmingii Meisn.[5]	No inf.[2]	Atlantic rain forest	Brazil	Andrade *et al*. 2000

[1] Information on AM colonization; [2] No information available; [3] Presence, without information on colonization level; [4] *C. swartzii* is cited instead in the text by this author; [5] **No mantle or Hartig net were found.**

Although not quantified, both *Coccoloba* species analyzed in this study, *C. laevis* and *C. rosea*, showed some AM colonization. Other records on mycorrhizas in *Coccoloba* species register not only ectomycorrhizal colonization but also AM. Studies showed no or low to medium low (3 to 30%) AM colonization in *C. latifolia*, *C. mollis* and a *Coccoloba* sp in the French Guiana (Béreau, Gazel & Garbaye 1997). Colonization rates for *C. mollis*, however, varied from 0 to 15%, suggesting an important spatial (or temporal) variation. *C. excelsa* was also showed to be arbuscular mycorrhizal in an Amazonian *caatinga* (Moyerson 1993). However, tropical trees presenting both mycorrhizal types are thought to be rare (Brundrett, Murase & Kendrick 1990) and

the symbiotic nature of the AM association is questioned by these authors, partly due to the absence in many reports of arbuscules what occurs even in heavily colonized AM tree species (see above). Nevertheless, despite the small number of *Coccoloba* species in which colonization has been already quantified (Béreau, Gazel & Garbaye 1997), the usually low levels reported suggest it may not be the principal mechanism of nutrient acquisition, or at least that these endophytes may be present but not much active. This genus is worthy of further study in order to understand the role of ectomycorrhizas in the mineral nutrition of *Coccoloba* species along its distribution area in the Neotropics.

Ectomycorrhizas, although not mentioned in Trappe's (1962) revision, have been already cited in the Polygonaceae (Hesselman 1900; Kelley 1950). Hesselman (1900), studying material from the Artic, described the anatomy of ectomycorrhizas in *Polygonum viviparum* L.

The Polygonaceae is reported to be mainly distributed in North Temperate regions (Conquist 1988), with 15 genera distributed throughout the Neotropics (Maas & Whestra 1998), seven of them occurring in Brazil. The genus from this family cited until now as ectomycorrhizal, *Polygonum* and *Coccoloba,* possess 200 and 125 species, respectively, what may difficult any analysis of the distribution of ectomycorrhizas within this group. *Coccoloba*, a genus of tropical and woody species (Conquist 1988), occurs predominantly in South America (Howard 1961), the largest number being found in Brazil (Howard 1961). Besides being of some economical importance, the genus is also of phytogeographical interest, being found in the main vegetation types of Brazil (Rizzini 1978). A maybe more feasible, and important, task would then be to analyze ectomycorrhizal occurrence in different *Coccoloba* species through its distribution area along different ecosystems, and different soil and climates properties.

From the 44 *Coccoloba* species cited for Brazil (Howard 1961), 24 species are listed in the "Eastern Brazil Database" from The New York Botanical Garden (http://www.nybg.org/bsci/hcol/sebc/Polygonaceae.html). *C. laevis* is cited for restingas in the Espírito Santo states, *C. rigida* in restingas from Rio de Janeiro state, and some other species (*C. alnifolia*, *C. arborescens* and *C. confusa*) occur in restingas from both states (Pereira & Araujo 2000). *Coccoloba* species (*C. confusa*) are also found in Atlantic rainforests (25°17'S 47°00' W) in São Paulo state, (Oliveira, Mantovani & Melo 2001) and in *restinga* forests (21°44'S 41°02"W) of Rio de Janeiro state (*C. alnifolia*), the latter being the fourth species in I.V.I. (22,86) and basal area (0,25 m^2 ha^{-1}) in this study (Assumpção & Nascimento 2000).

A survey of this family in the "Cadeia do Espinhaço" (Espinhaço Range), a phytogeographic province of mountains in Bahia and Minas Gerais states (between 10°-20°35'S and 40°10'-44°30'W), do not lists neither *C. laevis* nor *C. rosea* (Melo 2000). However, *C. brasiliensis* Nees & Mart. and *C. ochreolata* Wedd, other *Coccoloba* species found in Sergipe (Landim, unpublished) were cited.

A part from *C. laevis* and *C. rosea*, two other *Coccoloba* species have been collected until now in Sergipe, namely, *C. ochreolata* Wedd and *C. brasiliensis* Nees ex. Mart. (Landim, unpublished). However, information about *Coccoloba* species in Sergipe is absent or incomplete and, due to the still insufficient floristic sampling, the same probably happens in other regions and

countries. Howard (1960) and Rizzini (1978) cites only collections of *C. rosea* for Bahia. The above mentioned "Eastern Brazil Database" provides also information about collections of *C. rosea*, which includes only one reference for the state Sergipe (Mata do Crasto) and for its Southern neighbor state, Bahia (several collection in the south region), what do not means necessarily a disjoint distribution, due to the relatively greater sample efforts in the South region of Bahia.

Although far from being an complete assessment of *Coccoloba* species distribution in Brazilian ecosystems, this brief review is enough to draw attention to its importance and wide distribution in different ecosystems along East Brazil. If new sampling of this genus throughout its distribution range proves that ectomycorrhizal symbiosis is, at it seems, a constant feature, that will prove that the distribution of ECM species in the Neotropics has also "marked systematic basis", as in the African Leguminosae groups (Alexander 1989).

And if ectomycorrhizal status is a stable characteristic of this genus, and not only defined by determined soil characteristics, compatible ectomycorrhizal fungal inoculum may be, of course, present. In natural forest ecosystems in the Neotropics, native ectomycorrhizal species (see discussion bellow: "Endophyte identification") have been found in monospecific costal stands of *Coccoloba uvifera* in Cuba (Kreisel 1971), in Amazonian forests (Singer & Araujo 1979, Singer & Araujo Aguiar 1986), and in rain forest in Puerto Rico (Lodge 1996), although the species found are considered by this author as apparently forming peritrophic mycorrhizas. Ectomycorrhizal fungi has also been found in exotic tree plantations in Brazil (Oliveira, Schmidt & Bellei 1997, Giachini *et al*. 2000, Baseia & Milanez 2002) and it is possible that at least some of these species do invade natural ecosystems. Fruit bodies of *Pisolithus tinctorius* and *Scleroderma* cf. *geaster*, two exotic ectomycorrhizal species introduced with *Pinus caribea*, were found in a Puerto Rican rainforest (Logde 1996). If it is "unknown but unlikely" that the native basidiomycetous species associated with plants of *Pisonia* and *Coccoloba* can form ectomycorrhizas with introduced pine species (Lodge 1996), it is also not know, but not necessarily unlikely, if the introduced fungal species are able to associate with native ectomycorrhizal plants.

However, due to the still insufficient fungal sampling in Neotropical ecosystems, particularly in the Brazilian ones, it is not clear whether fungal inoculum can really be a limiting factor in the establishment of this association in natural, undisturbed, ecosystems. If no clear pattern of dominance in forest ecosystems or of monospecific stands is found here as in Africa, that does not excludes the existence of another strategy, of colonizing a free niche in different ecosystems.

Coccoloba species have also been found to resprout after cutting or fire, cited for some, respectively, *C. spicata* and a unidentified *Coccoloba* species (Negreros-Castillo & Hall 2000), and *C. cereifera* (Ribeiro & Fernandes 2000). This is an important ecological feature, leading to persistence of its populations after natural or human disturbance.

6.4.6 Ectomycorrhizas in *C. rosea*

Ectomycorrhizas in *C. rosea* do not present the radial elongation of epidermal cells described by Moyersoen (1993) in *C. excelsa* (Figure 6.5d) and by Kreisel (1971) in one of the types (Cd) of *C. uvifera* mycorrhizas (Figure 6.5c). Kreisel (1971) gives no detailed descriptions of the ectomycorrhizas in *C. uvifera*, but five different morphological types (after the classification of Dominik 1969) are cited for this species, but only three of them are illustrated by photographs. Although differing in color, the mycorrhizas on *C. uvifera* are, as on *C. rosea*, branched monopodial. The three types represented in the photographs (Figure 6.5a-c) show a well developed fungal sheath and, possibly, as the quality of some photos do not allow a precise identification, a Hartig net. Differently from the present study, where just some mycorrhizal tips were found, regularly numerous mycorrhizas were found by Kreisel (1971). Moyerson (1993) cites also a great colonization value for *C. excelsa* (56%) and it is possible that seasonal variation in root colonization may be responsible for this difference. Although this author also mention brown mycorrhizas on *C. excelsa*, he describes for these mycorrhizas, however, a irregularly pinnate ramification and a smooth texture what suggests a different fungal partner.

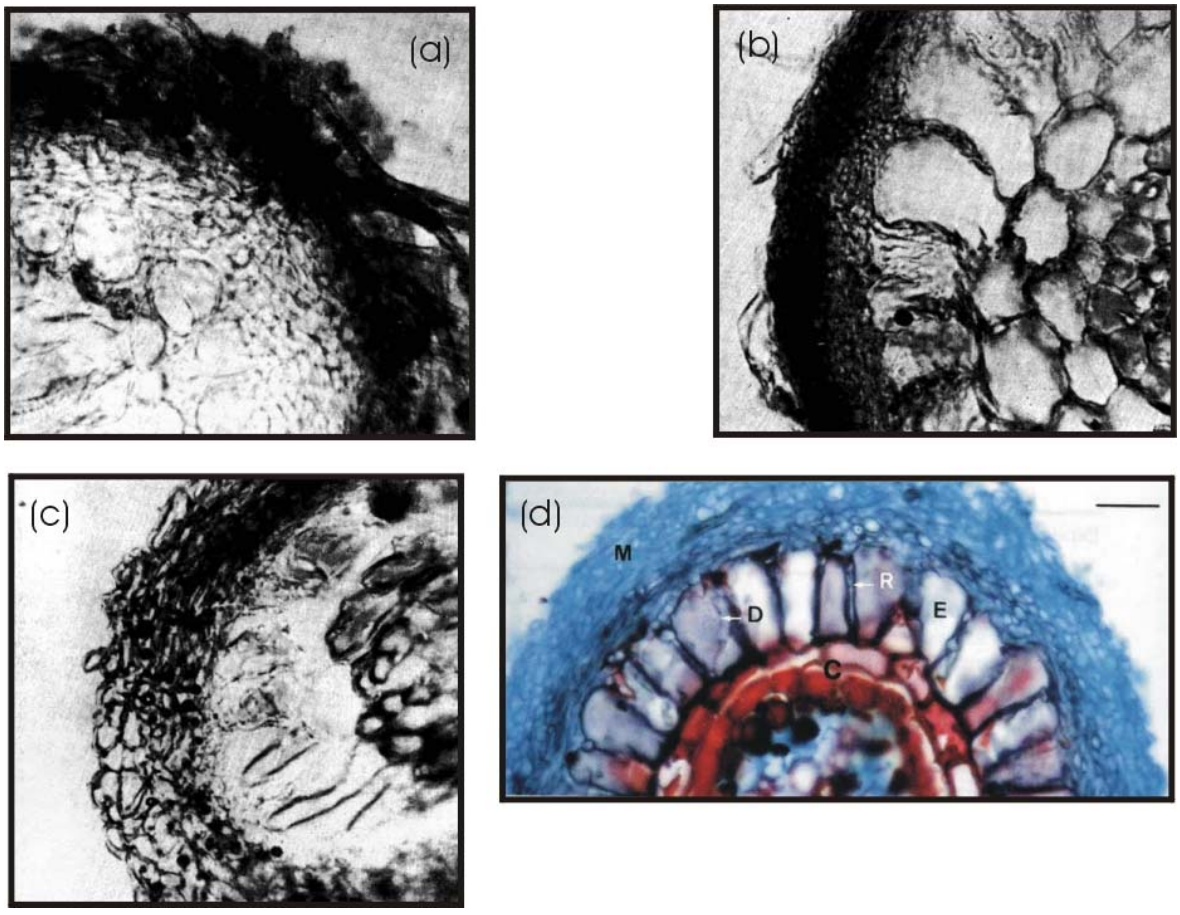

Figure 6.5 Anatomy of ectomycorrhizas in *Coccoloba* species: (a-c) *C. uvifera* (Kreisel 1971); (d) *C. excelsa* (Moyersoen 1993). (bar = 20 µm)

The mean sheath thickness in *C. rosea* mycorrhizas was 32 μm (Table 6.6), well above the values cited by Harley (1969), but within the range of most of the tropical tree species described so far (Alexander & Högberg 1986, Thoen & Ba 1989), and slightly above the values found in *C. excelsa*, in Venezuela (Moyersoen 1993). However, mean sheath thickness alone is of not much use, as root thickness varies considerably within species and tropical ectomycorrhizal species present smaller diameter values than their temperate counterparts (Alexander & Högnerg 1986). A relative measure (sheath area, or the mean ratio of the fungal sheath area to the total cross sectional) is more useful to compare the relative importance of the fungal partner in different plants under different ecosystems, climates and soils.

Table 6.6 Description of ectomycorrhizas morphometry in *Coccoloba* and other selected tropical species (s = standard deviation)

Species	N	*Diameter (μm)* Range	*Diameter (μm)* Mean (s)	*Sheath (μm)* Range	*Sheath (μm)* Mean (s)	*Sheath area (%)* Range	*Sheath area (%)* Mean (s)	*Reference*
Hopea parvifolia[1]			259				13	Alexander & Högberg 1986
Brachystegia bussei[2]			151				64	Alexander & Högberg 1986
Afzelia africana	10	236-466	328 (88*)	14-85	50 (24*)	20-65	50 (14*)	Thoen & Ba 1989
Uapaca guineensis	13	390-640	488 (79*)	18-80	47 (16*)	17-54	35 (9*)	Thoen & Ba 1989
C. excelsa	10	186-276	236 (23)	26-39	28 (4)	40-48	42 (3)	Moyersoen 1993
C. rosea	3	164-253	205 (26)	18-47	32 (7)	40-65	53 (6)	this study

* Standard error values provided; [1] lower % sheath value cited; [2] higher % sheath value cited

From the table comparing mycorrhiza structure from different species of Alexander & Högberg (1986) revision, the species presenting the lower and the higher values of fungal sheath area were chosen, in order to represent the range of variation found by them (Table 6.6). However, *Melaleuca leucodendron*, the species showing the lower values (7%), has a "patchy or absent" sheath, and was therefore, substituted from this comparison by the second species presenting the thinnest sheath, *Hopea parvifolia*. As data on diameter and sheath range are not provided by these authors, no reliable further comparisons could be made.

Mean sheath area in *C. rosea* (53%) was higher than the other values found in the literature (Table 6.6), a result of its smaller mycorrhiza diameter and relatively high sheath thickness. Due to the small number of mycorrhizal tips available for this analysis (3) several different cuts were analyzed and measured, in order to provide a better description of spatial variation in these figures. Although sheath thickness values for *C. rosea* did not differ much from those found for *C. excelsa*, sheath area values were higher, as the mycorrhiza diameter were generally lower. Values from the

two *Coccoloba* species seems to be rather constant, presently smaller standard deviation values in mycorrhizal diameter and sheath area, in comparison with the African species listed.

Alexander & Högberg (1986), considering sheath area as equivalent to percent of mycorrhizal volume, calculated a range from 13-64% (x = 38.4 ± 1.8%) in tropical ectomycorrhizal sheaths, what contrasts with Harley's (1969) estimate of sheaths corresponding to 20-30% of volume, what would represent about 40% of weight. However, Harley & Smith (1997) consider the value of 40% too high for fungal weight and believe values between 20 and 40% to be more common. As these authors seemed to consider only temperate mycorrhizas in this analysis, Alexander & Högberg (1986) statement, of sheaths of tropical ectomycorrhizas encompassing a larger proportion of than in temperate ones, seems to be appropriate. Yet, data on ectomycorrhizal morphology of tropical trees, with detailed measures, are still scarce and most of then concentrated on African tree species (e.g. Alexander & Högberg 1986, Thoen & Ba 1989).

What the causes for this pattern are, if extended sampling in new areas confirms it, it is not clear. In the tropics, being photosynthetically active radiation available during the whole year, a greater carbon input could be achieved all around the year, allowing the development and maintenance of thicker fungal sheaths (Alexander & Högberg 1986). On the other side, if light is not seasonally in short supply, water can be. In this case a thick sheath could be beneficial, storing nutrients in the dry season (e.g. Alexander 1983, Harley & Smith 1983, Read 1983). However, Högberg (1989) did not found abundance of ectomycorrhizal species in dry African savannas, as would then be expected. A thicker sheath may imply also a higher carbon outflow to support it, what may not be desirable under water and nutrient scarcity. Ectomycorrhizal forests were found in the wet savannas, but these were also associated with low P levels in soils (Högberg 1989). The great diversity in tropical forest ecosystems may hinder the analysis of the relationships between climate, soil, fungal and plant species, with the resulting multitude of strategies, these being also responsible for the high diversity in this systems. It seems that much more information should be collected, in a systematic and similar way, and in different geographic regions, until a clear and general pattern, if any, can be determined.

6.4.7 Endophyte identification

Although Singh (1966) suggested that the root association found should be with a Basidiomycetes fungi, as "most roots examined showed clamp connections", the first record of an identified ectomycorrhizal fungi in tropical lowland rain forest was probably made by Redhead (1968). He described the relationship between *Inocybe* sp and *Afzelia bella* in Nigeria, after the development of a sporophore adjacent to one of the inoculated plants, comparing structure of hyphae from the base of the stipe and from the surface of the mycorrhiza.

Redhead (1982) stated that the identity of the fungi-forming ectomycorrhizas on tropical angiosperms was not know, and even twenty years after, efforts in sampling, isolating and identifying these fungi in tropical regions was not enough to present a much better picture. Some of the ectomycorrhizal fungal species already found in the Neotropics are listed in Table 6.7. The

reports available deal with fructifications occurring in monospecific stands (Kreisel 1971), near adult plant trees (Lodge (1996) or collected along forested regions (Singer & Araujo 1979, Singer & Araujo Aguiar 1986). Ivory's (1980) report of macrofungi forming ectomycorrhizal associations were omitted, as dealing with natural tropical pine forests from the Caribic region. Furthermore Miller, Lodge & Baroni (2000) report on putative ectomycorrhizal fungi from Puerto Rican rainforests was also excluded, as no information on mycorrhiza formation was available.

None of the species listed were found within the fruit bodies sampled in the studied area during the sampling period (data not shown), although a unidentified Russulaceae species was collected in a farm areas in the southern region of Sergipe. It is probably an exotic species, as some *Eucalyptus* and *Pinus* plantations are present in this region.

But at least one species of *Ramaria* (Ramariaceae, Phallales, Basidiomycetes), *R.* cf. *toxica* (Basidiomycota) was found in the Crasto site. Trappe (1962) lists six *Ramaria* species as ectomycorrhizal, all of them with temperate host genus (*Picea*, *Fagus*, *Abies*, *Quercus*, *Pinus* and *Larix*), with one exception (*Cedrus atlantica*), which occurs in Algeria and Morocco (Nezzar-Hocine *et al*. 1998). Several *Quercus* species occur in North and Central America but no reports were found for South America (see collections deposited at the New York Botanical Garden at http://www.nybg.org/bsci/hcol/). Thus, other plants should be hosts for *Ramaria* species within south America. However information on *Ramaria* species distribution in this region is also scarce. For instance, only 16 Neotropical deposits of *Ramaria* species are listed at the New York Botanical Garden database, none of them from South America.

Two introduced *Ramaria* species, *R. anziana* and *R. junquilleo-vertex*, were registered in *Eucalyptus* and *Pinus* plantations in Southern Brazil (Giachini *et al*. 2000). However, information on *Ramaria* species in natural neotropical ecosystems, and on their possible mycorrhizal status, could not be found. *Ramaria* is not listed in the survey of basidiomycetous ectomycorrhizal fungi in Amazonia (Singer & Araujo 1979). In fact, according to these authors, the Agaricales is the group better represented. In a comprehensive review of a Puerto Rican rainforest, Lodge (1996) did no report any *Ramaria* species also. Although it is not clear if *Ramaria* could be a fungal partner for the *C. rosea* plants, this possibility should not be excluded by now. Furthermore, the preliminary nature of this survey, and the presence of ectomycorrhizal *Coccoloba* plants points out to the need of increasing sampling in the region.

In the present work, attempts were made to isolate the fungus in mycorrhizal tips of *C. rosea* without success. Different mycorrhizal fungal species may differ in growth ability in culture (Alwis & Abeynayake 1980), due to different nutrient requirements (Molina & Palmer 1982), what can explain this and the other failures reported in the literature (Redhead 1982, Alwis & Abeynayake 1980). Molina & Palmer (1982) suggests that material for 100-200 attempts should be used, while Alwis & Abeynayake (1980) report a 10 per cent successful isolation of ectomycorrhizas in plates. This may be one cause of the failure in this work, as not enough material was available at the time. Furthermore, sterilization of the material may not have been enough. Other methods, including more clearing and sterilizing steps, and other reagents, for example $HgCl_2$ (Zak & Bryan 1963, Zak & Marx 1964), could have provided

better results. This should be tried when new isolation attempts are made. Additionally, the type of substrate may present also an extra difficulty in sterilizing mycorrhizal roots for pure culturing. Mycorrhizas from heavier loams were found to be more difficult to sterilize than the ones present in the litter layer (Zak & Bryan 1963).

Table 6.7 Ectomycorrhizal fungal species already found in the natural neotropical ecosystems

Family / Species	*Vegetation type*	*Country*	*Source*
Amanitaceae			
Amanita campinaranae	*campinarana* [1]	Brazil	Singer & Araujo 1979
A. vaginata	*C. uvifera* stands [3]	Cuba	Kreisel 1971
A. xerocybe	*campinarana* [1]	Brazil	Singer & Araujo 1979
Amanita spp	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
Boletaceae			
Chalciporus trinitensis	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
Xerocomus amazonicus	*campinarana* [1]	Brazil	Singer & Araujo 1979
X. globulifer	*campinarana* [1]	Brazil	Singer & Araujo 1979
X. inundabilis	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
Tylopilus potamogeton var. *aquarius*	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
T. potamogeton var. *mitis*	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
T. potamogeton var. *potamogeton*	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
Xerocomus amazonicus var. *obscuratus*	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
X. chapinii	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
Cantharellaceae			
Cantharellus cinnabarinus	*C. uvifera* stands [3]	Cuba	Kreisel 1971
C. guyanensis	*campinarana* [1]	Brazil	Singer & Araujo 1979
Inocybe squamata	*C. uvifera* stands [3]	Cuba	Kreisel 1971
Hebelomina amazonensis	*campinarana* [1]	Brazil	Singer & Araujo 1979
Russulaceae			
Lactarius gigasporus	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
L. mamorensis	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
L. reticulatus	*campinarana* [1]	Brazil	Singer & Araujo 1979
L. reticulatus	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
Lactarius sp [4]	rain forest	Puerto Rico	Lodge 1996
Russula amnicola	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
R. metachromatica	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
R. nanella	*campinarana* [1]	Brazil	Singer & Araujo 1979
R. pachycystis	*campinarana* [1]	Brazil	Singer & Araujo 1979
R. pluvialis	*igapó* [2]	Brazil	Singer & Araujo Aguiar 1986
Russula sp [4]	rain forest	Puerto Rico	Lodge 1996
Russula sp	*C. uvifera* stands [3]	Cuba	Kreisel 1971
Sclerodermataceae			
Scleroderma stellatum	*C. uvifera* stands [3]	Cuba	Kreisel 1971

[1] Amazonian *campinarana* forests; [2] Amazonian *igapó* forest; [3] monospecific costal stands of *Coccoloba uvifera*; [4] associated with *Coccoloba swartzii*, forming peritrophic mycorrhizas

Identification of the fungal partner in ectomycorrhizas by using molecular methods has been already successfully attempted (Melo *et al*. 1996, Pritsch *et al.* 1997) and is therefore a promising research line. Not only the identity of fungal species can be achieved, but also the importance of specificity in this relationship can, as a result, be confidently evaluated.

6.4.8 ECM in tropical ecosystems

The present review of tropical ectomycorrhizal genera (Table 6.1) is by no means the first, or conclusive. Since the paper of Redhead (1980) new species, genera and families have been found to be ectomycorrhizal in tropical regions. Further sampling in new regions and other taxonomic groups may enlarge this picture. However, any analysis of literature data must be careful in retaining and discarding incomplete or doubtful reports. Data on tropical ectomycorrhizas often lack descriptions or illustrations (Alexander & Högberg 1986). On the other side, discarding such reports may lead to ignoring potential ectomycorrhizal taxonomic groups, that should deserve further sampling and study. When dealing such an literature analysis, it is not always easy to discriminate between "good" and "bad" reports. Therefore, whenever data collected in this assessment could not be accepted in the subsequent analysis, the criteria adopted is explained.

Not all genera listed in the Table 8.1 and present in Sergipe's rain forests could be analyzed in this work and may be subject of subsequent work. At least 10 supposed ectomycorrhizal genera occur in Sergipe (after discarding five doubtful reports: *Cecropia*, *Didimopanax*, *Bauhinia*, *Campomanesia* and *Euterpe*, see discussion below). Moreover, floristic inventories in the region must continue, as a better knowledge of the local flora is still needed.

Most of the records on ectomycorrhizal plants in tropical ecosystems provide from in African studies (Table 6.1). In the Neotropics, reports of ECM species are more scarce and ectomycorrhizal dominated plant communities do not seem to constitute climatic climaxes (Fassi & Moser 1991 *in* Béreau & Garbaye 1994). No ectomycorrhizal species were found among 21 tropical rain forest tree species in French Guyana (Béreau & Garbaye 1994) but ectomycorrhizal species (*Coccoloba* spp and *Neea tristis*) were found in a subsequent sampling of 75 trees rainforest species from French Guyana (Béreau, Gazel & Garbaye 1997). Ectomycorrhizal species (two unidentified Legume species, *Micrandra sprucei* and two *Eperua* spp) were also found in a Venezuelan Amazonian forest (St John & Uhl 1983). Ectomycorrhizas were already found in plants from Amazonian Caatinga forest, in Venezuela, (St John & Uhl 1983, Moyerson 1993), in the rain forest in Guyana (Norris 1969) and French Guyana (Béreau, Gazel & Garbaye 1997), in lowland rainforest (Edmisten 1970, Lodge 1996) and in different ecosystems (Miller, Lodge & Baroni 2000) in Puerto Rico. Alexander & Högberg (1986) gives information on morphology of ectomycorrhizas in *Neea* sp from Peru. Also in Uruguayan subtropical native forests some Legume species were found to be ectomycorrhizal (Frioni, Minasian & Volfovicz 1999).

In Brazil, most information comes from studies done in the Amazonian forest (Singer 1978, St John 1980, Singer 1984, Singer & Araujo 1979, Singer & Araujo Aguiar 1986). In the *cerrado*, a savanna-like vegetation Central Brazil, Thomazini (1974) found ectomycorrhizas in *Bauhina* and *Campomanesia* species, but this information should be taken cautiously, as this author do not provides photographs or descriptions of the material found. This absence in most of the reports has been already criticized by Alexander & Högberg (1988) what would hinder not only the analysis of the nature of the symbiosis found but also comparisons between tropical and temperate ectomycorrhizal plants. Septate hyphae with clamp connections have been recently found on roots from an unidentified *Campomanesia* species in rainforests in Southern Brazil (Andrade *et al.* 2000), what may support Thomazini's (1974) data.

History of land colonization by plants, and their evolution, is linked with the establishment of symbiotic relationships (Pirozynski & Malloch 1975). Among the fungal partners of mycorrhizas, the Glomales are thought to have first originated in the Devonian, while the ectomycorrhizal groups appeared in the Cretaceous, respectively before and after the dislocation of the Pangea (Selosse & Le Tacon 1995). This must have at least some implications on the evolution and biogeography of mycorrhizal plants and communities throughout the globe. In fact, phylogeny, and presence and type of symbiotic partner seems to be somewhat related. For example, Cronquist (1988) describes the trend found in the Ericales on a progressive obligate mycotrophy, which culminates in the Monotropaceae, with achlorophyll members, depending entirely on their mycorrhiza for nutrition. Data of the table 8.1 where therefore analyzed in search for phylogenetic and/or geographic patterns.

Ectomycorrhizas do not seem to be abundant in more primitive groups, although this symbiosis seem to be rather constant through different geographic areas. In the Gymnosperms, like its temperate counterparts (Trappe 1962), tropical members of the Coniferales (*Pinus* and *Cedrus* species) are found presenting ectomycorrhizas along tropical region of the Americas, Africa and Asia. Although *Araucaria* is cited by Trappe as an ectomycorrhizal genus, recent root sampling in *A. angustifolia* in subtropical Brazilian forests only revealed arbuscular mycorrhizas (Breuninger *et al*. 2000). Another ectomycorrhizal genus, not cited in Trappe (1962) revision, *Gnetum* (Gnetophyta), is found in African and American forests.

An analysis of the distribution of number of groups along the different angiospermous subclasses (Figure 6.6) reveals that 78% of the tropical ectomycorrhizal orders, and 64% of the genera, belong to the Rosidae, Dilleniidae and Asteridae, the three most derived, or recent, groups in Cronquist's (1988) classification system, which is adopted in the subsequent analysis. Interestingly, no reports were found on ectomycorrhizal species in the Magnoliidae, a primitive subclass in the Magnoliopsida (dicotyledons), but none of the reports found of ectomycorrhizas in

the Liliopsida (monocotyledons), these being derived from the Magnoliopsida, could be consistently accepted.

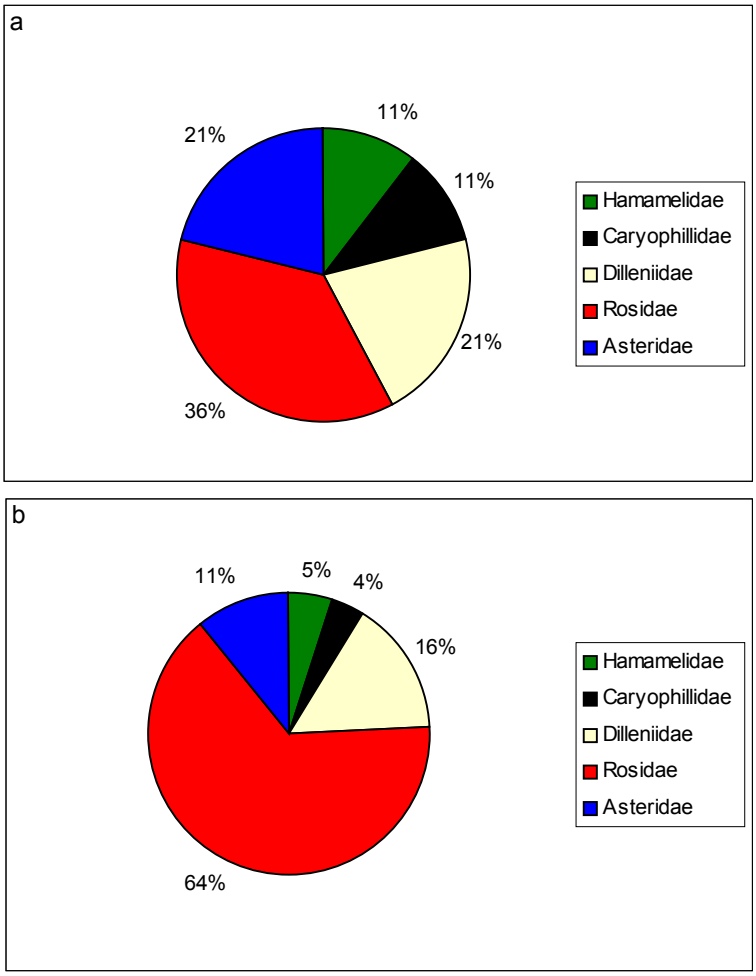

Figure 6.6 Distribution of number of reported tropical ectomycorrhizal orders (a) and genera (b) along angiospermous subclasses

The register of ectomycorrhizas in a member of the Poaceae (*Phyllostachys*) made by Barsali (1922), although not from a tropical region, was included in the Table 6.1 due to the importance of the Poaceae in the tropics. It might then direct new sampling toward insufficiently studied groups. However, Barsali (1922) report an association with a *Clathrus* species, not longer considered a ectomycorrhizal fungal species (Alexander 1989). The other register of ectomycorrhizas in the Liliopsida listed in the Table 6.1 was on Arecaceae (*Euterpe*) by Edmisten (1970). This author also cited ectomycorrhizas on Cecropiaceae and Araliaceae. Because all the supposed ectomycorrhizal species found by Edmisten (1970) were found to present only arbuscular mycorrhizas by Lodge (1996) and no other citation was found to support them, these data were therefore excluded from the analysis. However, much care must be taken when analyzing reports of ectomycorrhizal in the literature. In other instance, a report of Edmisten (1970) on

ectomycorrhizas in *Inga*, although dismissed by Lodge (1996), resampling in the same Puerto Rican forests, was supported by the findings of Pegler & Fiard (1979) sampling in a mesophytic forest in the Lesser Antilles.

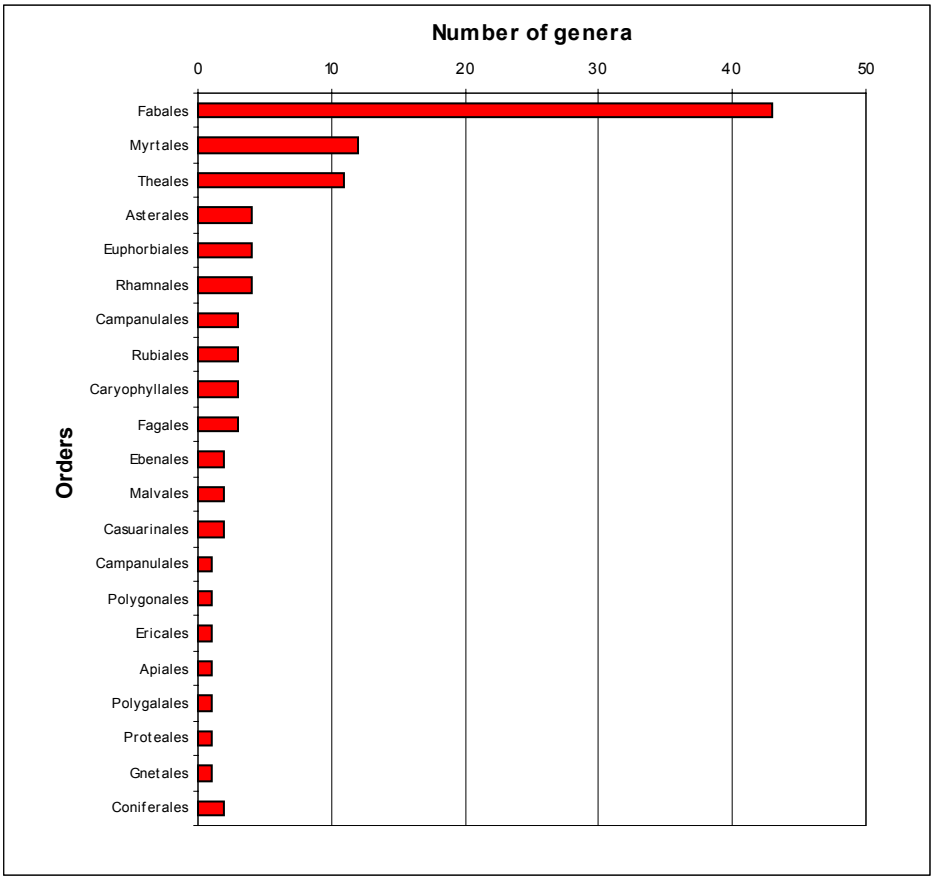

Figure 6.7 Distribution of number of reported tropical ectomycorrhizal genera along different orders.

A concentration of tropical ectomycorrhizal genera was found in the Fabales and Myrtales, both orders from the Rosidae, corresponding to 55% of genera, followed by the Theales (Dilleniidae), with 11% (Figure 6.7). However, the distribution of ectomycorrhizal subclasses throughout the tropics is not uniform (Figure 6.8). In African ecosystems, only members of the Rosidae and Dilleniidae were found, with predominance of the former (72%). All subclasses are represented in American studies, with exception of the Hamamelidae, and Rosidae is also predominant (70%), being followed by the Caryophyllidae (20%). The predominance of the Rosidae is maintained in the studied Asian ecosystems, but substituted by the Dilleniidae, when Australian studies are excluded.

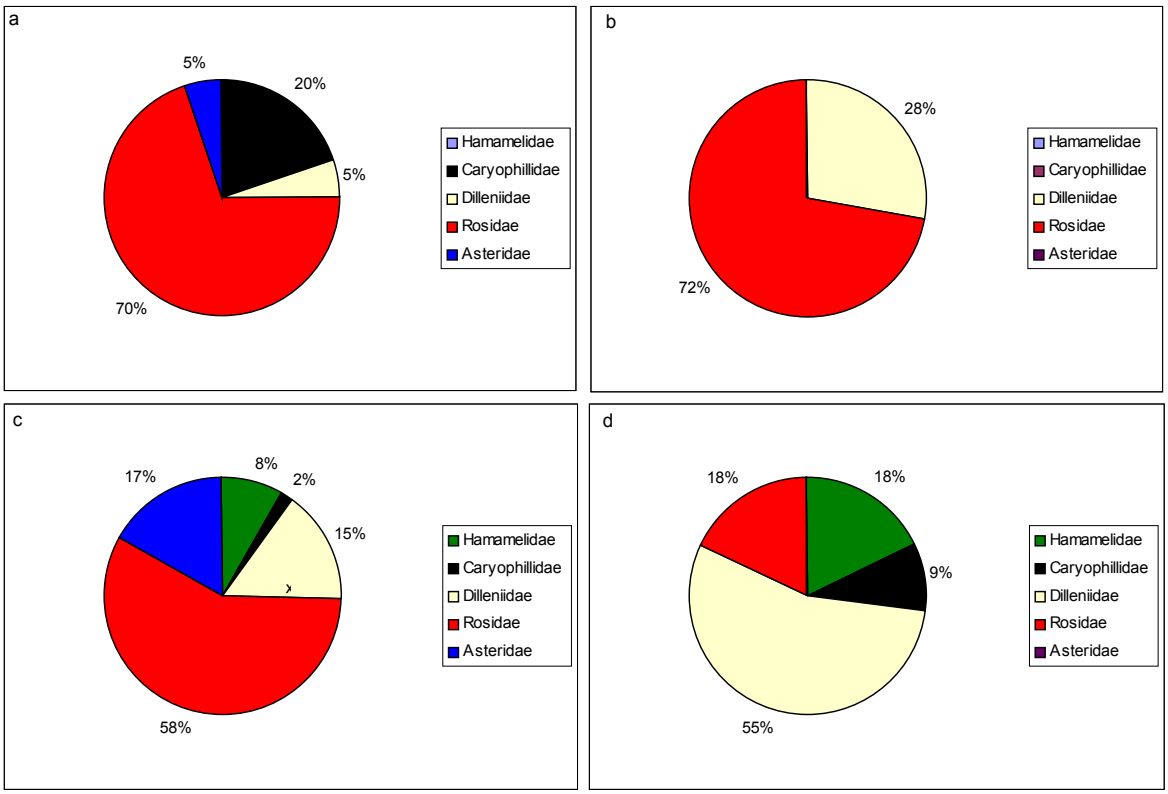

Figure 6.8 Distribution of number of reported tropical ectomycorrhizal genera along different regions. (a) Americas; (b) Africa; (c) Asia; (d) Asia, without Australian reports

Legumes represent an important group of tropical ectomycorrhizal species (Table 6.1). Much of the genera formerly cited as from the Amherstieae tribe are presently considered as belonging to the Detarieae (ILDIS world database of legumes, version 6.05, http://www.ildis.org/LegumeWeb/LegumeWeb6.05.shtml). Two synonyms were found (*Paraberlinia* to *Julbernardia* and *Toubaouate* to *Didelotia*) and excluded from the analysis. Distribution of the legume subfamilies and tribes was also not even through the tropics (Table 6.8, Figure 6.9) The Papilionoideae is the subfamily with the greater number of ectomycorrhizal genera (21), followed by the Caesalpinioideae (18). While the Papilionoideae dominate in Africa, the Caesalpinioideae are more important in Asia, although only found in Australia. Data from Australia used come from studies in subtropical regions (Warcup 1980, McGee 1986) and should be regarded as subject to confirmation. For instance, ectomycorrhizas *in Dillwynia* (McGee 1986) are considered as having atypical Hartig net and sheath development (Harley & Smith 1997), as well as other Myrtaceae species analyzed by McGee (1986). The Papilionoideae is also the most advanced Leguminosae subfamily (Cronquist 1988) and the one with greater incidence of nodulating species (Allen & Allen 1981).

Table 6.8 Distribution of number of ectomycorrhizal genera in leguminous subfamilies and tribes throughout the tropics.

Subfamily	*Tribe*	*Africa*	*America*	*Asia*	*Total*
Mimosoideae	Acacieae		1[a]	1[a]	1
Mimosoideae	Ingeae		2[a]		2
Mimosoideae total		**0**	**3**	**1**	**3**
Caesalpinioideae	Caesalpinieae		2[a]		2
Caesalpinioideae	Cercideae		1		1
Caesalpinioideae	Detarieae	15	1		15
Caesalpinioideae total		**15**	**4**	**0**	**18**
Papilionoideae	Bossiaeeae			1[a]	1
Papilionoideae	Millettieae		1[a]		1
Papilionoideae	Mimoseae		1[a]		1
Papilionoideae	Mirbelieae			12[a]	12
Papilionoideae	Phaseoleae			2[a]	2
Papilionoideae	Sophoreae	1	1		2
Papilionoideae	Swartzieae		2		2
Papilionoideae total		**1**	**5**	**15**	**21**

[a] Reports on subtropical genera.

In the Neotropics a more uniform distribution is found not only among the three subfamilies but also among the different tribes within each subfamily (Figure 6.9b-d). While in Africa the great majority of ectomycorrhizal Caesalpinioideae genera belongs to the Deterieae, being totally absent from Asia, an almost equal number of genera from the Caesalpinieae, Cercideae and Detarieae are found in America. And while Mirbelieae genera seems to dominate in Asia (Australia), four Papilionoideae tribes with ectomycorrhizal members are present in the Neotropics.

Ectomycorrhizal seedlings from four leguminous species, one Mimoisoideae (*Acacia nilotica)*, one Papilionoideae (*Pterocarpus santalinus*), and two Caesalpinioideae (*Tamarindus indica* and *Peltophorum pterocarpum*), all but one not belonging to the genera included in table 1, were produced in pot experiments (Vijaya & Srivasuki 2001). As growth effect of mycorrhizal type varied between species, and no other citation was found on ectomycorrhizal status from field growing *Pterocarpus*, *Tamarindus*, and *Peltophorum* species was found, these genera were not considered in this analysis. However they may deserve further sampling in tropical regions.

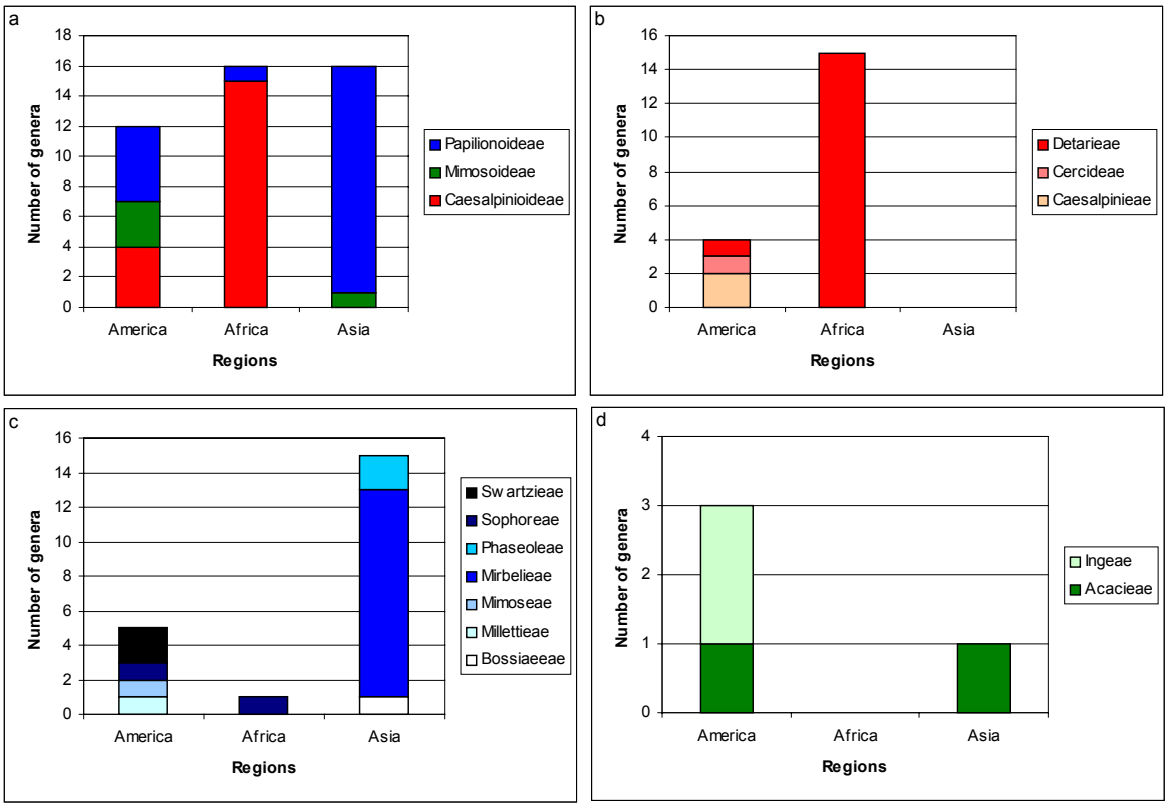

Figure 6.9 Distribution of number of reported tropical ectomycorrhizal leguminous genera along different regions. (a) distribution of leguminous subfamilies; (b) distribution of Caesalpinioideae tribes; (c) distribution of Papilionoideae tribes; (d) distribution of Mimosoideae tribes

Explanation for occurrence of ECM in tropical species was thought to lay on the climatic factors (Redhead 1980), on specific features of its root systems (Jeník & Mensah 1967) or it was thought to an adaptation to the particular biological conditions (Singer & Morello 1960). For example, ectomycorrhizas in the *latosol* forests were thought be restricted to root damaged trees ("cicatrizing mycorrhiza") and the *Gnetum*/*Scleroderma* ectotroph" (Singer 1984).

Being an association where absorption of nutrients plays a main role, poor soil nutrient content may therefore represent a pressure selecting for nutrient uptake strategies that maximize it and would possibly explain cases of dominance in plant communities. Soils from two African vegetation types where ectomycorrhizal species are important components, the "species-rich primary rainforest" from the Korup National Park, and the Miombo, a "single storey, light closed canopy, deciduous woodland", are mostly highly leached with low levels of available N and P (Alexander 1987). These descriptions do not differ greatly from some other neotropical soils. For instance, the Amazonian ectotroph communities, the *campina* and *campinarana* growth also on "white-sand podzol" soils (Singer 1978, Singer & Araujo 1979). Indeed, phosphorus levels in many tropical soils are unlikely to inhibit the development of ectomycorrhizal species (Alexander 1989) and the soils from the two forest fragments studied (Chapter 3) fit also the above description of the

African counterparts. However, arbuscular mycorrhizas where dominant in Amazonian forests on podzols in French Guyana (Béreau, Gazel & Garbaye 1997).

The greater success in acquiring nutrient in such poor soil conditions could lead them to the dominance in numbers and cover of ectomycorrhizal species. In fact, tropical forests do not necessarily mean high tree species diversity systems, being low-diversity forests, whose canopies are often dominated by just one single species, not rare among the tropical region (Connell & Lowman 1989). Redhead (1982) suggested that the presence of ectomycorrhizas would be a factor explaining the dominance of the Caesalpiniaceae and Dipterocarpacae in the miombo woodland of Central Africa and the tropical rain forest of Malaysia and South East Asia, "two of the world's most extensive plant formations in the tropics". This dominance in frequently expressed in high I.V.I. values or percentage of basal area. In Australia, four ectomycorrhizal species presented 25% of the total basal area of a lowland rain forest (Reddell, Hopkins & Graham 1996). In Africa, where most of the important ectomycorrhizal species are legumes, the benefits deriving from both the fungal and the *Rhizobium* partner would then may be one cause of the success of Caesalpiniaceae species in the Miombo vegetation (Högberg & Nylund 1981). Even in tropical America some dominance may be found. *Eperua purpurea* was found to be the dominant species in a 60-year-old forest stand and *E. purpurea* and *Swartzia schomburgkii* were also important in basal area and density in the 80-year-old stands (Saldarriaga & Uhl 1991). Although these authors do not assessed mycorrhizal status of the species studied, both *Eperua* (Norris 1969, Singer & Araujo 1979, St John & Uhl 1983) and *Swartzia* (Singer & Araujo 1979, Singer 1984, Singer & Araujo Aguiar 1986) are supposed to be ectomycorrhizal in Amazonian forests. Nevertheless, more recent studies (Torti & Coley 1999) show that monodominance in tropical ecosystems is "more complex than previously recognized".

Although monodominant forests are also present in the Neotropics, like the *Eperua* and *Mora* communities in the Guyana (Richards 1996) ectomycorrhizal monodominant forests do not seem to be represented in the Neotropics (Torti, Coley & Janos 1997). Here, it seems that some taxonomic groups are relatively constant ectomycorrhizal along different plant communities (e.g. *Coccoloba*, as discussed above). The same appears to be true, for example, for the Nyctaginaceae. *Pisonia grandis* showed the same mycorrhizal structure even in geographically separated regions (Ashford & Allaway 1982, 1985). Although *P. grandis* mycorrhizas were described as an separate category (Ashford & Allaway 1982, 1985), considerable variation in Hartig net, sheath and extraradical mycelium development in ectomycorrhizas occurs (Smith & Read 1997), what make these authors consider *P. grandis* an acceptable true ectomycorrhizal species. Nyctaginaceae is an important family in the coastal areas of Brazil, being species of the genera (*Guapira*, *Pisonia*, *Neea*) also found in Sergipe. Members of the Polygonaceae and the Nyctaginaceae should be therefore analyzed in future studies.

On the other side, factors determining the occurrence in tropical ecosystems of the fungal partner in the ectomycorrhizal association are also important and should not be neglected. Pegler & Fiard (1979) studying associations of *Lactarius* in several plant communities determined by differences in the annual precipitation, hypothesizes that the rainfall seasonality, more than the

temperature variation, is the necessary factor for the growth of ectomycorrhizal fungi in tropical climates. Distribution of *Lactarius* species and individual basidiocarps number, varied greatly throughout the different forest types studied (Pegler & Fiard 1979), being the greatest numbers found in the dry or moderately moist lowland forests. To test this hypothesis, these authors suggest that semi-deciduous continental forests in tropical America should be investigated.

Anyway, by the data available until now, ectomycorrhizal tree species do not seem to be expressively important in number of taxa or dominance (basal area) values in neotropical forests. No ectomycorrhizal roots were found in the soil cores analyzed in the present study (Chapter 4) and ectomycorrhizas, in contrast to arbuscular mycorrhizas, seem to be rare in the seedlings analyzed (Chapter 5). These results seem to reinforce the irregular distribution of ectomycorrhizal species in neotropical forests, in contrast to the clustered pattern of ectomycorrhizal species in African forests. Therefore, much more attention should be given to the role of the arbuscular mycorrhizal symbiosis regarding regeneration of these ecosystems.

However, the present picture of ectomycorrhizal species in tropical ecosystems, mainly in tropical forests, may well represent an under evaluation of its importance, due to the difficulty of studying tree roots in these systems. Less than five percent of African tropical tree species were thought to have been already examined regarding mycorrhizal association (Redhead 1982). Since then this figure may have increased, but it is improbable that it has already reached a significant number of species, representative of this flora. The high diversity of these ecosystems makes this task almost impossible, and it is probable that further sampling may change this picture. For example, ectomycorrhizal species, from genus not previously cited as ectomycorrhizal, have been found lately in Australia (Reddell, Hopkins & Graham 1996). In Neotropical high diversity forests as the Amazonian and Atlantic rainforests, the difficulty in achieving a representative root sampling in these communities and the still insufficient mycological survey still hinder a better understanding of the role of the mycorrhizal symbiosis in these ecosystems.

6.5 Conclusions

This work represents a first contribution to the understanding of mycorrhizal ecology in some rainforest fragments in Northeast Brazil. More species must be further analyzed, preferably including seasonal sampling and a broader geographical area, as this information is basic to the understanding of spatial and temporal variation in root colonization and, consequently, fungal activity.

Although the quality of much of the material collected could not provide a trustful assessment of mycorrhizal colonization, it is clear from these results that arbuscular mycorrhizal colonization are widely distributed among tree species from these forests, in accord with other studies in tropical forests. Quantification could not be done in all cases, some of the quantifications could represent an underestimate of the real fungal colonization, and some variation is thought to occur between seasons and soil types. However, an interesting result is the high colonization found in *Vismia guianensis* and *Cecropia pachystachya*, both pioneer species, rapidly colonizing

disturbed habitats. Thus, these species could act as a source of mycorrhizal inoculum in deforested habitats, being important further studies on the composition and properties of their mycorrhizal partners in order to improve successful establishment of forest tree species in these areas.

Ectomycorrhizal species, in contrast, seem to be a more or less constant and its role in maintaining composition and structure in forest ecosystems is still not clear. The ectomycorrhizas in *Coccoloba rosea* confirms previous studies and shows this character is rather constant within this genera and a wide geographical range. If not yet conclusive, due to the scarcity of data, the analysis of geographical distribution of the several ectomycorrhizal taxa along the tropical regions of the world points to a rather uneven pattern. Particularly, more emphasis should also be given to studies on the evolution of this character in neotropical forests.

Nodules were for the first time reported for *Acosmium bijugum*. Nodules were also found in *Dioclea violacea* and *Andira nitida roots*. Like the mycorrhizal symbiosis, associations with rhizobia represent a extra nutrient input as well, which is particularly important in disturbed habitats and should also be fostered aiming restoration of these forest ecosystems.

7. Effectiveness of native arbuscular mycorrhizal fungi on *Bowdichia virgilioides* H.B.K. (Leguminosae Papilionoideae) seedlings

7.1. Introduction

Native AMF inoculum should be more efficient in promoting seedling growth and survival than introduced inoculum, as the former may be adapted to be environmental local conditions. However, for the isolation of native AMF species, many subsequent trap culture steps are needed, as these fungi do not grow without a plant partner.

Mixed inoculum (soil and roots originating from trap cultures) was therefore used for the testing of its efficiency in promoting plant growth and survival. This inoculum type is produced easily in pots with sterile mixtures of soil-sand and its preparation doesn't consume a long time (Howeler & Sieverding 1983). This is an advantage when considering its use by small farmers and city counts, interested in reforesting Atlantic forest areas in their municipal districts, and who could not afford high-costs procedures. Furthermore, it is more similar to the composition of AMF in nature, where a mixture of different species coexist even in a single root system, than the artificial fungus-host systems. However, results of experiments with these systems often presents higher responsiveness than with mixed inoculum. Pure inoculum can be more efficient in promoting plant growth, and it is necessary to test if the low costs of mixed inoculum would not be compensated by the possible low responsiveness obtained. Furthermore, not only the nature of the fungal partner is important in determining mycorrhizal colonization levels and plant responsiveness, but also soil nutrient content. Trials with different type of inocula and nutrient levels must be carried out before any definitive conclusion on the better approach for using AMF in forest recomposition can be made. This must be performed at a local level, with native plant and fungal species, as results from different regions, varying in climate, soil and vegetation can not be confidently extrapolated for other regions.

An equally important factor when considering the use of mixed inoculum is its origin. It is not known if there is any significant difference between AMF inoculum derived from different sites or habitats. Two hypotheses, each mutually excluding the other, will be tested in these experiments:

- The species of arbuscular mycorrhizal fungi present in the area of the coconut plantation are more adapted to the local conditions. The phosphorus management, for example, can result in a better growth and survival of forest tree seedlings (in this case, the lack of nutrients would hinder the regeneration, and thus the phosphorus management would help the growth of seedlings in these sites).
- The species of arbuscular mycorrhizal fungi of the forest are more adapted to the forest tree species. The absence of that inoculum in the coconut plantation areas would be the cause of the slow natural regeneration.

Two experiments were carried out, one aiming to identify the effect of mixed inoculum under different P levels on plant growth and survival, and the second comparing the effect of mixed inoculum and *Glomus clarum* inoculation under different phosphorous sources on plant growth and survival.

Experiments aiming to test the effectiveness of native mycorrhizal inoculum on plant were therefore carried out with *Bowdichia virgilioides*. *B. virgilioides* (Leguminosae Papilionoideae) is a native pioneer tree species, common in the area of study. It is a secondary initial species (Siqueira & Ribeiro 2001), with potential for use in reforestation programs.

7.2. Material and Methods

7.2.1 Inocula source

In the two sampling times, February and November (see 3.3.1 Climate and microclimate), three intact soil cores were collected in each plot (plantation, fringe and forest) from the two study sites (Crasto and Caju). These were intended for isolating AMF species that were not found in the extraction from field colleted soil (See Chapter 4). Successive subcultures were thereafter tested with these soil cores in plastic pots (16 cm x 18 cm diameter), with different plant hosts (*Petroselinum crispum*, *Brachiaria decumbens*, *Zea mays*, and *Sorghum bicolor)*. Trap cultures using parsley (*Petroselinum crispum*) seedlings were established in intact soil cores, but plant growth was not satisfactory (data not shown) and, therefore, in each subsequent cycle of cultures, a sub sample of the pot soil was used for establishing the next cycle. As a substrate, autoclaved sand (twice for 60 minutes at 121^{o}C) was used. The sand used for the first two experiments had a pH of 5.5, but the third experiment was carried out with a new supply of sand, this time with a pH of 8.5, what was noticed after the experiment had already initiated.

To prevent cross-contamination, pots were placed at least 10 cm from each other, and carefully watered to prevent water drops with soil particles from one pot to reach the neighbor pots. Plants in these trap cultures were watered biweekly with deionized water, and at fortnightly intervals each plants received 250 ml of full-strength Hoagland nutrient solution, without phosphorus. Before initiation a new culture cycle, pots were left to dry and plant shoot was cut out, in order to stimulate sporulation of the symbiotic fungi.

For the comparison of the effectiveness of inoculum of different origins on *B. virgilioides* seedlings, inoculum (soil and roots) of initiated plant cultures with *Zea mays* was used. These cultures consisted of a second cycle of trap culturing, what means these pots received soil collected from a first cycle of trap cultures as inoculum. The first cycle of trap cultures were initiated with field soil colleted in February. Autoclaved sand was always used as substratum. Wet sieving (Gendermann & Nicolson 1963) was performed in samples from these pots in order to select cultures with an adequate mixture of spore species, being these cultures used as inoculum in the subsequent experiments.

7.2.2 Experimental design

7.2.2.1 Experiment 1: Effect of inocula source

A second cycle of trap culturing, deriving also from the February sampling, was initiated with *Z. mays*. Three pots contained soil inoculum from each of the three plots (plantation, fringe and forest) of both sites. However, only two controls (with autoclaved sand and no inoculum) were available, as these were originally intended to check if contamination between pots occurred.

Three seeds of *Z. mays*, where placed to germinate in each plot. After a week, homogenous seedlings were selected in each pot. Plants were wetted weekly or whenever necessary, but each 14 days plants received 250 ml of 100% Hoagland solution. After 3 months, plants were harvested and the length and weight (after drying at 80°C for at least 72 h) of root and shoot was registered. As some of the plants were already in reproductive stage, non-vegetative structures were analyzed separately. A representative fraction of the root system of the plants was prepared for clearing and staining with trypan blue (Koske & Gemma 1989) for the assessment of mycorrhizal colonization.

As a preliminary trial on possible growth differences on *B. virgilioides* seedlings growing with inocula from different origins, after the *Z. mays* plants were harvested, the same pots were used for assessing differential growth of *B. virgilioides* seedlings.

B. virgilioides seeds were provided by the "Centro de Pesquisa Agropecuária dos Tabuleiros Costeiros" (Center of Agricultural Research of the Coastal Tablelands - CPATC), based in Aracaju, Sergipe. Dormancy of seeds of *B. virgilioides* was broken by agitating them in concentrated H_2SO_4 for 10 minutes, washing them afterwards in distilled water several times. Seeds were then surface-sterilized in 5% sodium hypochlorite solution for 10 minutes before placed to germinate in trays with a mixture (50:50) of autoclaved sand and perlite. One recently germinated seedling of *B. virgilioides* was transplanted to each pot (with 16 cm x 18 cm diameter). Seedlings were wetted and fertilized as described above.

As this plants were used for observations on further plant growth and development, they were not harvested. Differential effects on plant growth were assessed, after 7 months, by measuring seedlings shoot length.

7.2.2.2 Experiment 2: Effect of inocula source and P levels

Soil inocula used was as described above. One culture from each of the three plots from both sites was selected as inoculum for this experiment. As control, besides sand with autoclaved inoculum, a second control (control+) with a soil washing was prepared. For each culture used as inoculum, 400 g of soil was mixed with distilled water (1:2) for 3 h, being filtered twice with Whatman No. 1 filter paper afterwards (Hayman & Mosse 1971). Each from these control pots received 50 ml of this soil washing. This should exclude the AMF, but not other soil microorganisms.

Substratum, as described above for the trap cultures, was autoclaved sand (twice for 60 minutes at 121°C). Four phosphorus levels were used and, for each treatment, five replicas were

established. The four P levels were achieved using a progressive increase of the phosphorus solution (K_2HPO_4) of the Hoagland solution: 0, 0.5, 1 and 5ml L^{-1}.

Homogeneous and healthy seedlings were transplanted to the plastic pots (19 cm x 6 cm diameter) were used for this experiment. In each pot, 200 g (approximately) of autoclaved sand were placed under 50 g soil inoculum, which was covered by a layer (50 g) of autoclaved sand.

Care was taken in order to avoid cross-contamination between pots. A completely randomly factorial design, was used. The experiment was carried out in the greenhouse of the Biological Garden of the Bremen University, with extra illumination. Light intensity (measured with a Licor LI-189 Photometer) was 63.55 μmol s^{-1} m^{-2}, air relative humidity was ca. 60% and length of day, 12 h. Day temperature was adjusted for 25°C and night temperature, 21°C, varying, however, about ± 3°C.

One week after seedling transplant pots were soaked with full-strength Hoagland nutrient solution. Seedlings were wetted weekly or whenever necessary, but each 14 days plants received 20 ml of Hoagland solution.

At the end of the experiment, 5 months, seedlings were harvested and the length and weight (after drying at 80°C for at least 72 h) of root and shoot was registered. A sample of the root system was prepared as above described for the assessment of mycorrhizal colonization.

7.2.2.3 Experiment 3: Comparison between mixed and pure inocula with different P sources

For this experiment, mixed inocula was compared with pure inoculum. For this, an AMF starter culture was provided by the European Bank of Glomales (France), *Glomus clarum* (BEG 142). This was first cultured in pots with *Plantago lanceolata* in transparent sunbags (Sigma B-7026) as described by Walker & Vestberg (1994), for attaining sufficient inoculum amount. Soil samples from these cultures were checked for spores through wet sieving (Gendermann & Nicolson 1963), only spores from *G. clarum* being found. *G. clarum* inocula consisted therefore of soil and roots from these cultures. In order to allow comparison between the two trials, mixed inocula (soil and roots) from the same *Z. mays* trap cultures employed in the second experiment were used. However, only inocula from plantation and forest plots were selected for this experiment,.

Substratum was also autoclaved sand. Pots, however, were larger (18 cm of height and 11 cm of diameter, comprising a total volume of 1 L). In each pot, ca. 800 g of autoclaved sand were laid at the pot bottom, followed by 50 g soil inoculum, and a superficial layer (150 g) of autoclaved sand. Only one control, with autoclaved substrate, was used, but 10 replicates were employed. A factorial design, completely random, was also used for this experiment, which was also carried in greenhouse, under the same conditions as above described.

This experiment aimed to compare not only the type of the inocula but also different sources of P. Therefore, half of the pots received 533 mg^{-1} kg^{-2} phytate (inositol hexaphosphoric acid, Sigma), and the other half, 58.52 mg^{-1} kg^{-2} and KH_2PO_4, both being added to the substrate before autoclaving.

Seed germination was performed as described above for the first experiment. Plants were wetted and fertilized as in the first experiment, but no extra source of P was added, therefore full-

strength Hoagland solution (100 ml per pot) was prepared without the K_2HPO_4 solution. After 5 ½ months, seedlings were harvested and analyzed as described above.

7.2.3 Data analysis

For both experiments, analyses considered length and weight of root and shoot, as well as the root/shoot relationship. Testing of differences in biometric measures between inocula source (site and plot), and treatment (P level or P source) were performed with non-parametrical variance analysis (Kruskall-Wallis) and Mann-Whitney U test. All statistical analysis were carried out using the software SPSS (SPSS Inc., 1989-2001).

7.3 Results

7.3.1 Experiment 1: Effect of inocula source

Growth of *Z mays* plants (Figure 7.1) did not differ between the two sites, but differed significantly from the control pots. Plants inoculated differed significantly from the control in dry biomass in both sites ($p < 0.05$, $U = 0$, in the two sites), and in shoot length in the Caju site ($p < 0.05$ $U = 0$).

Plants from all inoculated pots were higher than the control plants. Plant growth was generally higher in pot with inoculum from plantation and fringe plots in the Crasto site (Figure 7.1a), but in the Caju site, plants growing in pots with forest inoculum were higher (Figure 7.1c). Inoculated plants differed significantly in biomass ($p < 0.05$, $U = 8.864$) and shoot length ($p < 0.05$, $U = 8.197$) between inocula source (the three plots) in the Caju site, but difference between plots were not significant in the Crasto site.

Reproductive investment also differed between inoculum source. No control plant developed reproductive tissues. While no clear pattern on plot was found in the Crasto site (Figure 7.1b), in the Caju site plants with forest inoculum had generally higher investment in reproductive tissues (Figure 7.1d).

Similarly, the analysis shoot growth in the from *B. virgilioides* seedlings grown in the *Z. mays* trap cultures pots revealed non significant differences between sites and plots, neither considering pots from the two sites together nor separately. Nevertheless, all inoculated plants presented markedly higher shoot length than the inoculated controls (Figure 7.2, Figure 7.3). However, differences between plots, with data from the two sites analyzed together or separately, were also not significant. The trend of a differential effect between inoculum source in the two sites, as registered in *Z. mays* plants, were not observed in *B. virgilioides* seedlings.

Despite the insufficient number of control plants, effects of inoculation were not observed only on plant growth but also on survival. By the time the photographs were taken, one of the control seedlings had already died (Figure 7.3).

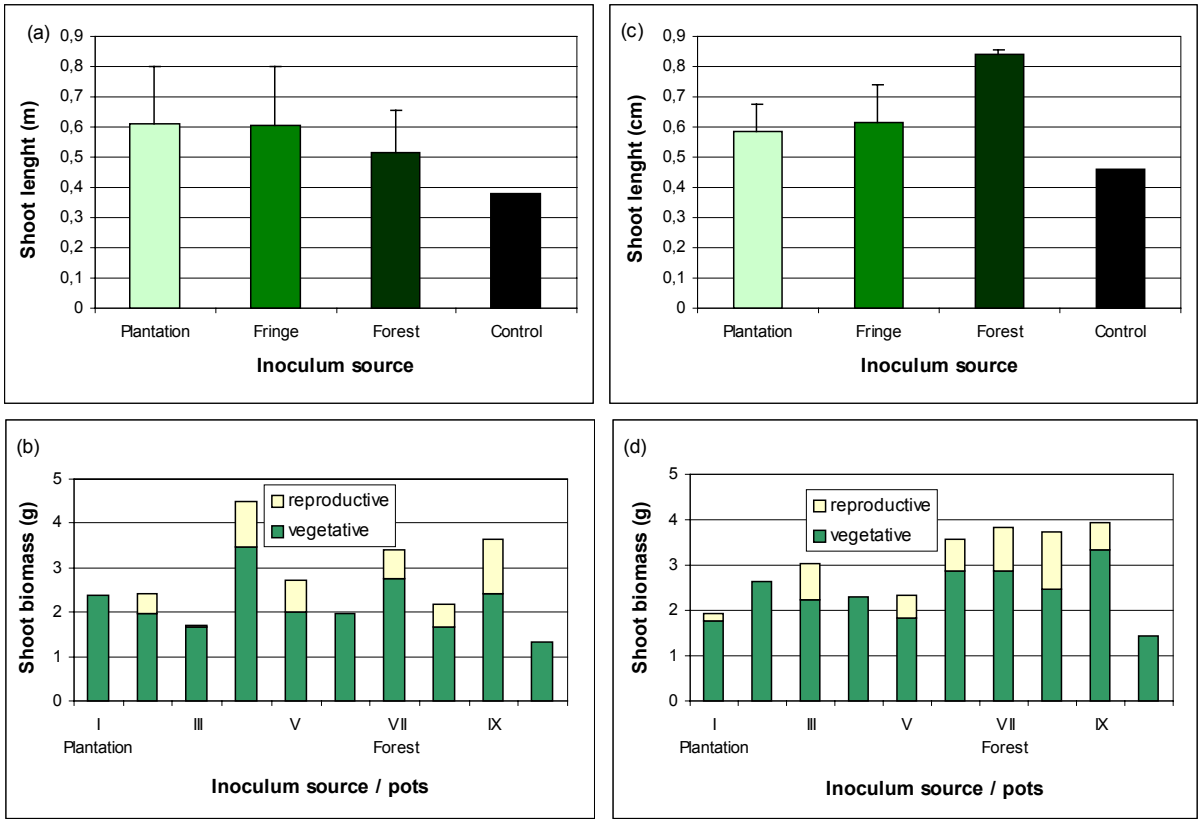

Figure 7.1 Effect of inocula sources on growth of *Zea mays* plants growing in trap cultures for three months: (a-b) Crasto, (c-d) Caju site. (I-X = number of individual plants) (for details, see 7.3.1 Experiment 1)

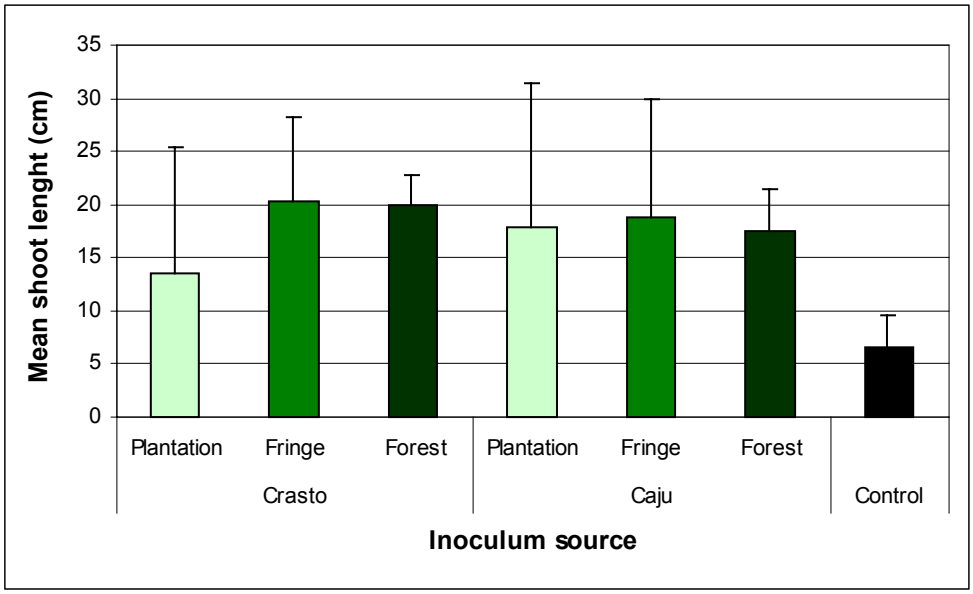

Figure 7.2 Effect of inocula sources on growth of *Zea mays* plants growing in trap cultures for seven months. (N = 3; Control N = 2) (for details, see 7.3.1 Experiment 1)

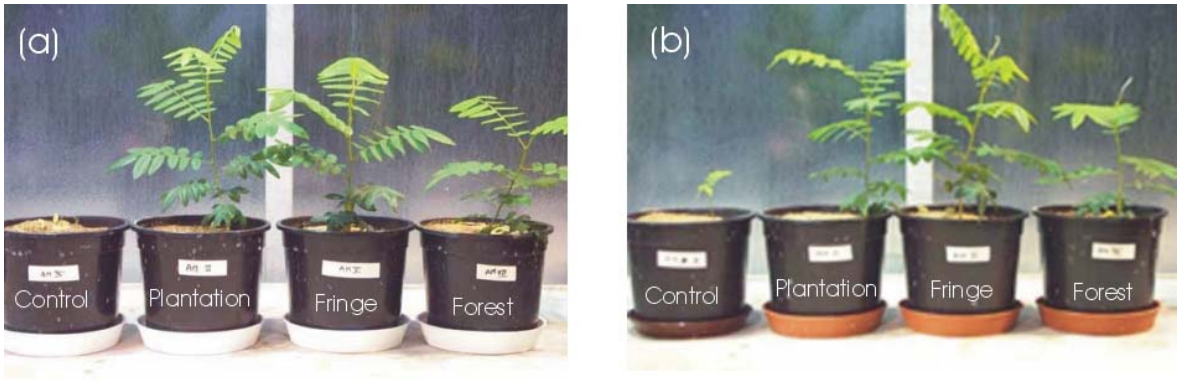

Figure 7.3 Selected seven months old seedlings *B. virgilioides* showing differential growth effects between inoculated and control treatments: (a) Crasto; (b) Caju site. (for details, see 7.3.1 Experiment 1)

7.3.2 Experiment 2: Effect of inocula source and P levels

No significant difference in any of the biometric measures (length and dry weight of shoot, root, root/shoot) between treatments (inoculated and the two controls) or between pots inoculated with soil inoculum derived from the three plots was found. Plants inoculated with diverse P levels, however, differed significantly in most of the biometric measures. Shoot length ($H = 53.537$, $p < 0.001$) and shoot ($H = 70.991$, $p < 0.001$) and root ($H = 9.382$, $p < 0.05$) differed significantly, increasing with increased P levels (Figure 7.4). As roots of some plants were damaged at the extraction, further analysis was carried out only with shoot length and biomass data.

Soil inoculum from the three plots did not result in significantly different results in plant growth. Higher shoot length and biomass were obtained using the higher P level (5ml) (Figure 7.4a,b). Plant dry biomass was, however, markedly improved under higher P levels with inoculation (Figure 7.4c,d).

Interestingly, mortality in this experiment was higher in pots with inoculum from the fringe and forest plots than in the plantation plot (Table 7.1). Mortality was also higher in the inoculated plants and plants with lower P levels. Differences were, however, not significant.

Table 7.1 *B. virgilioides* seedling mortality (%) in pots with inoculum with different origin, P levels, and treatment. (control+ = control plus addition of a soil washing, see text for description)

	Plot:	***Plantation***	***Fringe***	***Forest***	
Mortality (%)		8,3	10,4	12,5	
	Treatment:	***control***	***control+***	***inoculated***	
Mortality (%)		6,3	8,3	16,7	
	P level:	***0***	***40***	***80***	***160***
Mortality (%)		19,4	13,9	2,8	5,6

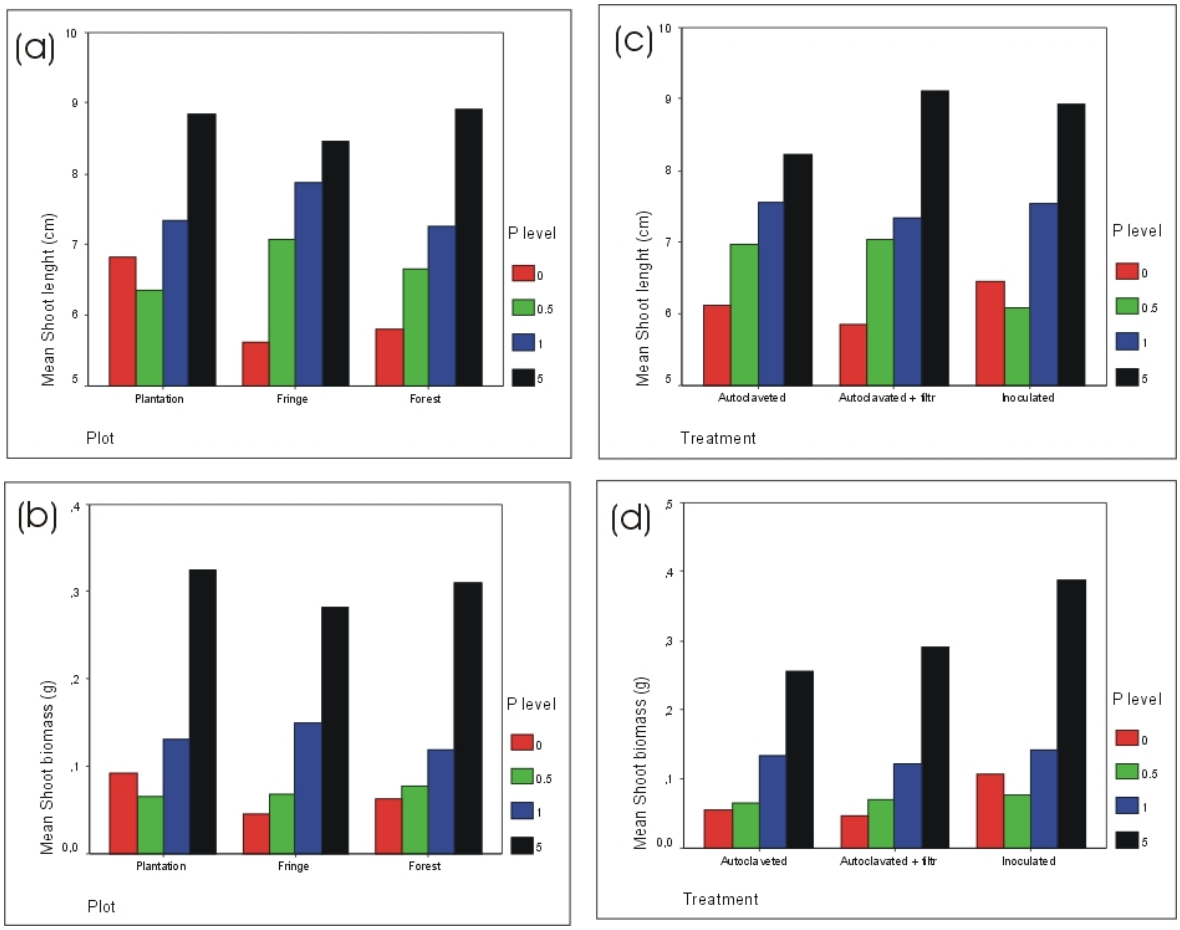

Figure 7.4 Effect of inocula origin and P level on growth of 5 months old *B. virgilioides* seedlings. (for details, see 7.3.2 Experiment 2)

7.3.3 Experiment 3: Comparison between mixed and pure inocula with different P sources

Although some differences between inoculum source were observed, they were not so markedly differences as between P source (Figure 7.5, Figure 7.6). Plants growing with phytate as P source had significantly higher shoot length ($U = 75.5$, $p < 0.05$) and biomass ($U = 23$, $p < 0.001$) than plants growing with K_2HPO_4. Differences in root biomass were also significant ($U = 64$, $p < 0.05$) but not in root length, as plants inoculated with *G. clarum* presented a strikingly higher root length. However this values reflects only results from one single plant, which survived until the end of the experiment.

Surprisingly, plants growing with K_2HPO_4 presented a significantly higher ($U = 180$, $p < 0.001$) mortality figures (Figure 7.6). The inoculum type did not presented any markedly difference on mortality levels, although plants in the treatment growing with forest inoculum and K_2HPO_4 as P source presented lower mortality.

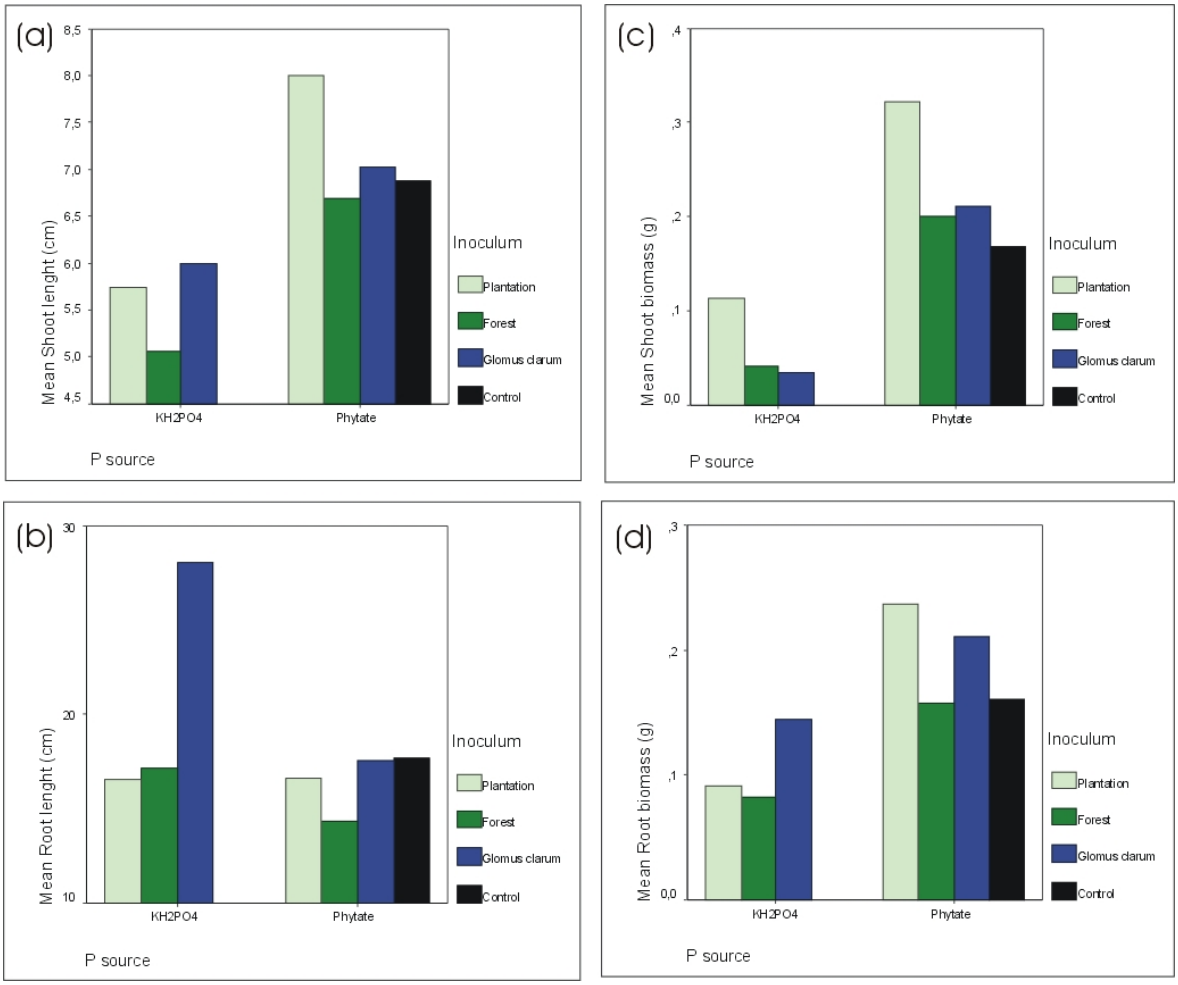

Figure 7.5 Effect of inoculum and P source on growth of 22 weeks old *B. virgilioides* seedlings. (for details, see 7.3.3 Experiment 3)

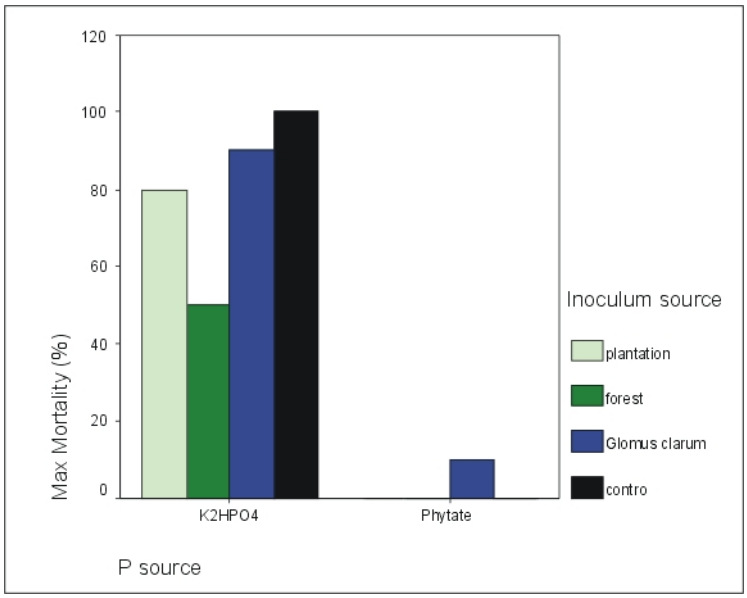

Figure 7.6 Effect of inoculum and P source on mortality of 22 weeks old *B. virgilioides* seedlings. (for details, see 7.3.3 Experiment 3)

Differences were also marked between inoculum type. Mixed inoculum from plantation plots resulted in higher shoot length (Figure 7.5a), biomass (Figure 7.5c), and root biomass (Figure 7.5d). Differences between inoculum sources were significant only with shoot biomass data ($H = 9.596$, $p < 0.05$). However, responses varied between the two P sources used. While comparison between different inoculum sources on *B. virgilioides* biometric data revealed no significant difference in the treatment with K_2HPO_4, significant differences were found between plants growing with phytate as P source in root length ($H = 7.935$, $p < 0.05$) and shoot biomass ($H = 10.14$, $p < 0.05$). Plants inoculated with *G. clarum* did not produce significantly higher shoot and root biometric figures, with the exception, already cited, of higher root length with K_2HPO_4 as P source. However these higher root length were not corresponded to higher root biomass (Figure 7.7). Plants growing with phytate presented higher shoot and root biomass.

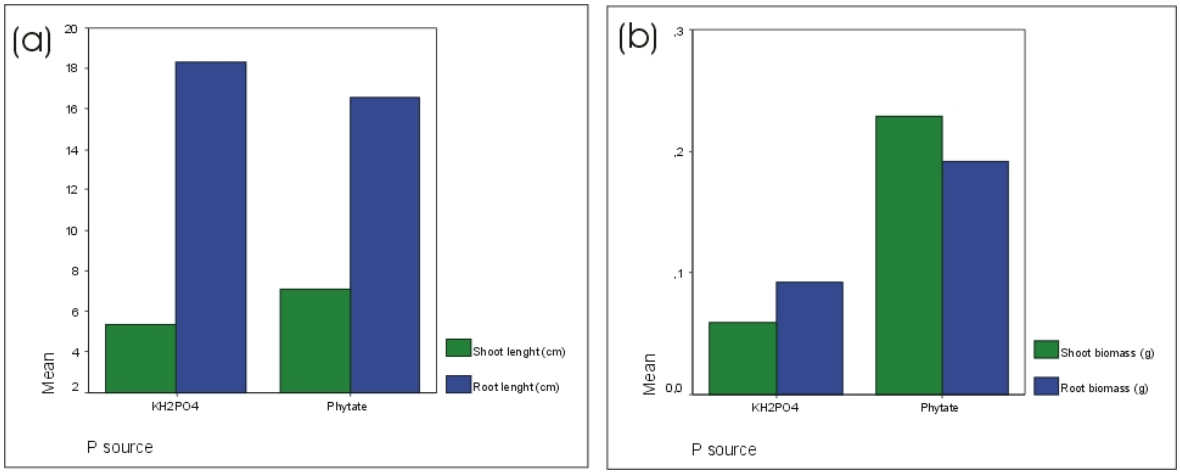

Figure 7.7 Mean shoot and root length and biomass of 22 weeks old *B. virgilioides* seedlings growing with different P sources. (for details, see 7.3.3 Experiment 3)

7.4 Discussion

Mixed inoculum was used in these experiments on the effectiveness of AMF on *B. virgilioides* growth and survival, for two basic reasons. The first one, was because pure inoculum from the study areas was not yet available. Actually, acquiring good quality inoculum of a new isolated AMF fungi can take several years (Brundrett, Melville & Peterson 1994). The first steps, the initiation of successive cycles of trap cultures with different host plants, has already been done, but much more work must be carried out to accomplished this goal.

The second reason, was that this study aims to survey aspects of the AMF ecology in soils from these two forest remnants and apply this knowledge to restoration of these areas. To this, effective as well as low-cost practices are needed. If native AMF inocula, either from forest or deforested areas, are found to be more effective in promoting tree seedling growth and survival, this can represent an improvement in the success of reforestation attempts, reducing the time and costs

involved. Better plant growth has been observed in non-sterilized soil, with native inoculum (Mosse & Hayman 1971) and benefits from inoculation with selected native inoculum has been reported (Hayman 1982). Indeed, native AM fungi were able to colonize extensively roots of clover plants, diminishing the effects on plants from the introduced *Glomus tenuis* (Powell 1980).

Actually, under natural conditions, plant roots present more than one single AM fungus (Clapp *et al.* 1995, Daft & Nicolson 1974, Daniell *et al*. 2001). Roots from forest plants also present a variety of AMF, but effects of different AMF species may vary seasonally (Daft *et al*. 1981). Furthermore, all of requirements for an ideal AMF partner, such as rapid root colonization and growth of hyphae, effective soil exploration, quick transfer of nutrients to the host, wide range of host plants and environmental conditions, among others, can not be fulfilled by a single fungus species (Daft 1983). In fact, different AMF species differ in their effectiveness (Sanders *et al*. 1977). Different host-fungus combinations produce also variable responses (Daft & Hogarth 1982). Different growth rates and P content are produced in response to inoculation with different fungal species (Abbott & Robson 1979). The use of mixed inoculum, composed of species with different strategies, may present better results to host plants and may be, therefore, more adequate than the introduction of one single species (Daft 1983).

In fact, much of the experimental research with AMF has been carried with introduced inocula, and not only more research with native AMF species is needed, but also the development of methods for conveniently measuring their efficiency (Sieverding 1991). Of course, reports of low infectivity of native AMF species also exist (Mosse & Hayman 1971, Fitter 1985) and mixed introduced inoculum has been found to produce better growth responses than native inoculum (Alexander, Ahmad & See 1992). Furthermore, if disturbance has reduced AMF propagule numbers, mycorrhizal colonization on seedling roots may be negligible and inoculation, indispensable (Powell 1980). However, the first step should be the isolation and screening of native AMF species, adapted to the environmental conditions (Hayman 1986). The productivity of some crops could be enhanced by increasing these native AMF populations or by introducing foreign, though well adapted to the local conditions, species (Howeler & Sieverding 1983).

Attempts to inoculate plants in the field may face practical problems such as host selection, and competition with the native AMF species (Daft 1983). The use of native inoculum should minimize both problems. Some indigenous AMF species, although highly infective, may not be equally effective in promoting plant growth (Mosse & Hayman 1971). A solution suggested for minimizing competition between native and introduced effective AMF strains is to pre-inoculate plants or place inoculum near the newly emerging roots (Mosse & Hayman 1971). The latter seems more feasible for use in reforestation programs.

All the experiments here presented had attempted to identify possible increases in plant growth and survival with introduction of AMF native mixed inoculum. The analysis of root colonization is not concluded yet, and will probably explain some of the results here presented. The type of soil inoculum used, containing both spore and colonized root fragments as infective unities, is considered to be highly infective (Sieverding 1991). However, effectiveness of native AMF populations can be subjected to a multitude of factors, like soil fertility, host plant, propagule density, effectiveness of

individual AMF species, and competition between them and other soil microorganisms (Sieverding 1991). Moreover, the efficiency of native AMF populations can vary greatly beneath different areas (Sieverding 1991) and root colonization levels may not be necessarily correlated with plant responsiveness. In fact, significant plant growth was obtained despite low root colonization in three tropical legume species (Ahiabor & Hirata 1994).

Differences between inoculate and control plants were observed in this study using both *Z. mays* and *B. virgilioides* as host plants. Responses to different P levels and sources, however, varied. Although inoculum from both sites (Crasto and Caju) brought about not significant difference in the growth of both host plants, a marked increase in plant growth of *Z. mays* plants was observed with use of inoculum from the forest plot of the Caju site. No clear pattern was found with *B. virgilioides* seedlings. The higher standard deviations obtained, may indicate a higher genetic variability in the seed set used. This may be a problem when analyzing data of responsiveness from experiments with native species, as they are not so homogeneous as some crop seeds, or clonal species.

An interesting result was the increased investment in reproductive tissues, observed in most of the inoculated plants. Control plants did not initiate reproduction, and this is probably due to the stressed nutritional status. AMF species have been found to increase plant fitness (Carey, Fitter & Watkinson 1992). To tree seedlings, increased growth and survival in the establishment phase, even if no effect on later reproduction occurs, is already a great increase in their fitness.

Phosphorus source had markedly different effects on *B. virgilioides* seedlings growth and survival. Plants grown with phytate presented higher root biomass, shoot length and biomass than plants grown with KH_2PO_4 and this effect was clearly higher in some of the inoculated treatments, suggesting some variation between fungal partner. These results are important because P is one of the most critical elements to plant growth in natural field conditions (Brady 1974), particularly, in tropical soils (Sanchez 1976). In fact, not only a small amount of P is usually present in natural soils, but also most of it may be unavailable to plants (Brady 1974). Both inorganic and organic forms are present, being both important as P sources to plants (Brady 1974). The organic P fraction may comprise 20-50% of the total P soil, but in tropical soils this can reach 60-80% (Sanchez 1976). Up to 60% of the soil organic P may be composed by phytates (Halstead & McKercher 1975).

The use of P from less available soil fractions by AMF species is questionable. Bolan (1991), in his review on the role of AMF on P uptake by plants, did not find any evidence that mycorrhizal plants are more able to access less available soil P fractions. AMF hyphae differ from the ectomycorrhizal hyphae in their limited ability to use soil organic P sources (George, Marschner & Jakobsen 1995). Moreover, the contribution of extracellular AMF enzymes to P nutrition of inoculated plants was found to be "insignificant" (Joner, van Aarle & Votsatka 2000). However, AMF mycelia has been found to present active foraging, although their strategies may differ form the ectomycorrhizal mycelia (Olsson, Jakobsen & Wallander 2002). These authors suggest that responses may be variable with different resources. Different AMF species may also present variable capacity to utilize organic P sources (Koide & Kabir 2000). In fact, Koide & Kabir (2000) found that extraradical hyphae of *G. intraradices* were able to hydrolyze organic P. They reported, however, similar levels of root biomass and P content for plants growing with phytate and KH_2PO_4. The strikingly difference

between both sources was surprising, and the high mortality of plants with KH_2PO_4, by no way expected.

The differences between the two inoculum sources in this study may be not due to autoclaving. Koide & Kabir (2000) have also added phytate and KH_2PO_4 to the substrate before autoclaving, and found no release of phosphate from the phytate after autoclaving. On the contrary, the higher initial pH of the available substrate (sand) used may have affected at least part of the results obtained in the present experiments. At pH 6, both the more available $H_2PO_4^-$, and HPO_4^{--} ions are present in soils but, with increasing pH values, this moves to a greater proportion of HPO_4^{--} and PO_4^{---} ions (Brady 1974). At the end of the experiment, sand from pots from both treatments presented a neutral pH (around 7). Phytate, in contrast, is more available under neutral conditions (Brady 1974), and may have been a more available P source than the KH_2PO_4. This could explain the results obtained.

Another possible effect of soil pH on the experiments described is related to mycorrhizal efficiency. AMF species have been found to vary in their pH range of occurrence (Sieverding 1991). If the AMF species present in mixed inoculum are adapted to the more acidic soil conditions prevailing in the soils from both sites, sporulation and activity under the less acid soils used as substratum may have been diminished. Therefore, plant responsiveness to inoculation may have been caused not necessarily by the most effective AMF species present in these soils.

The evaluation of pure and native mixed inoculum revealed that the former (*G. clarum*, in the present experiment) was not more efficient in promoting plant growth than mixed inoculum. *G. clarum* was chosen because it was intended that the performed comparisons would use fungal species occurring in somewhat similar climate and soil conditions, and this was the only culture isolated from Brazilian soils available at the time the experiment was planned. Actually, *G. clarum* may be compatible with legume species, as it has been isolated from *Stylosanthes capitata*, also a Papilionoideae legume. Studies comparing the effectiveness of *G. clarum* on pant growth and nutrient uptake showed favorable results in lentil (*Lens esculenta*), also a legume species (Xavier & Germida 1997), but not in wheat (*Triticum aestivum*) (Xavier & Germida 1997), and apple (*Malus domestica*) (Forge *et al*. 2001). When also rhizobia and native inoculum are present, however, a greater variety of responses are found, depending of optimal combination of rhizobia strain and AMF species (Xavier & Germida 2002).

Differences between inoculum source could only be observe in the treatment with phytate, due to the low number of surviving plants growing with KH_2PO_4. Plants growing with plantation inoculum had higher root and shoot biomass than plants with forest or *G. clarum* inocula, or control. This contrasts with the results obtained in the first experiment, where Z. mays plants grown with forest inoculum presented higher growth, suggesting that inocula source may have diverse responses with different plant hosts.

However, results were not clear enough to allow a definite conclusion, and no significant comparisons between both P sources in the present study could be performed. Further experiments must be carried out, this time with a soil more similar to the soil present in both forests. However, the

high efficiency of *B. virgilioides* seedlings in using phytate as the sole P source, and the enhanced plant growth derived from some of the inocula used, are clearly showed.

B. virgilioides seedlings seem to have high nutrient requirements to grow, at least when rhizobia are not available. Higher biomass was achieved with inoculation and higher P level, in the second experiment. Mortality was also related to nutritional deficiency, but intriguingly, was higher within inoculated plants. As the control+ (with soil leaching) presented the second highest mortality, it could also be that some pathogen may have been carried with the inoculum. However, plants, neither *Z. mays* nor *B. virgilioides,* growing in inoculated soil in the first experiment did experience any mortality. The reasons why mortality was higher within plants growing with fringe and forest inocula are not clear, and this must be subject of further research.

Some features of the experiments carried out may be at least partially responsible for the results obtained. Firstly, pot sizes may have limited plant growth. The better relative growth of *B. virgilioides* seedlings was achieved with the larger pots used in the first experiment. Larger growth rates of *B. virgilioides* roots may have made the small soil volume available to grow in the pots from the second experiment inadequate for this species. Effect of AM fungi have been reported to depend on root density, diminishing when roots reach pot walls (Baath & Hayman 1984). This must be considered in further investigations with this species.

The AMF symbiosis is common in legume species (Hayman 1986) and the increased effect on their N status derived from the rhizobia association make legume plants particularly valuable for use in reforestation programs. The effect of AMF on legume species may be even greater than on other plant groups, as the enhanced P assimilation leads to an indirect effect on N nutrition (Crush 1974, Hayman 1986). Indeed, an adequate P supply is essential to the establishment of nodulation in legume plants (Barea e Azcón-Aguilar 1983). Legume species have been suggested to test the effects of AMF inoculation in the field (Mosse, Powell & Hayman 1976). Actually, the study of AMF and rhizobia symbiosis with legume species is particularly important, due to the possible effects on enhancing production of legumes of economic value (Asimi, Gianinazzi-Pearson & Gianinazzi 1980), but should also consider the potential use in ecosystem restoration (Hayman 1986).

Experiments with AMF inoculation with legumes species are abundant in the literature. Significant increased nutrient uptake and enhanced growth of *Vigna unguiculata* plants were obtained by adding P and AMF inoculum in pot experiments with tropical sandy soil (Bagayoko *et al.* 2000). However, although inoculation of three tropical legumes produced positives effects on plant growth, responses of AMF inoculation may vary between host plant and fungal partner, even under the same growth conditions (Ahiabor & Hirata 1994). Inoculation with AMF species stimulated nodulation and growth of four legume species in soils with low P content, although the two tropical species studied presented higher responsiveness than the temperate species (Crush 1974).

However, an extra difficulty in using legume species as AMF host plants in experiments is the three-partner relationship. The lack of adequate control, needed to identify properly causes of the responses obtained, makes experiments with AMF and legumes a rather difficult task. Although a soil filtrate have been prepared as a second control, plants that received it did not show any inoculation,

nor the control and inoculated plants. It is not clear what effect nodulation would have on plants growing under the experimental conditions.

The choose of *B. virgilioides* was determined by the seed availability of native pioneer/secondary tree species common in the region of study, with potential use in forest restoration. *B. virgilioides* is a common legume tree in the Brazilian *cerrados* and in other South American savannas (Ratter *et al*. 1996, Sarmiento 1984). A positive characteristic for use of *B. virgilioides* on the regeneration of deforested areas is its ability to grow under nutrient-poor conditions. In fact, soils from the south American savannas, including the *cerrados*, are reported to present low macro- and micronutrient contents and CEC, besides high exchangeable Al levels (see review by Montgomery and Askew 1983).

Trees were found to have an important role in nutrient dynamics in a Venezuelan savanna, enriching locally soil nitrogen content (Sánchez, García-Miragaya & Chacón 1997). Soil under trees presented greater N mineralization than soil under grasses, and soils from under *B. virgilioides* trees, particularly, presented the highest N rates (78.25 μg g^{-1}) during the study period (15 days). Furthermore, *B. virgilioides* presented a larger recruitment and lower mortality in areas of *campo sujo* (savanna grassland formation in the Brazilian *cerrados*) than in the *cerradão* (forest formation) (Kanegae, Braz & Franco 2000). In fact, these authors found that the dry season did not significantly affected mortality of this species. However, as typical with tree seedlings, the first months were the more critical for its establishment.

Although *B. virgilioides* seeds present only 45% of germination success (Ribeiro & Siqueira 2001), plants growing in experimental mixed stands reached 5.6m height after 48 months, presenting 100% survivorship (Siqueira & Ribeiro 2001). This species presented the highest growth and survival rate among the other ten species planted in the same stand in the costal zone of Sergipe (Siqueira & Ribeiro 2001).

These characteristics and the results obtained in the present study suggests *B. virgilioides* is an important species to be used in reforestation programs. It may have its growth improved if planted with *Vismia guianensis* and *Cecropia pachystachia*, as these both species present high mycorrhizal colonization rates in the field (see Chapter 6). These two species may act increasing AMF soil inocula in disturbed conditions and making them available to other tree seedlings, like *B. virgilioides*. If some host specificity exists in among AMF species, augmenting plant diversity at the beginning of forest restoration plans may increase their success.

AMF inoculation have been suggested to be more important in soils with low P content or with few native AMF species or ineffective strains (Mosse & Hayman 1971). The first condition applies to plantation sites in the vicinity of both fragments. However, the results from the present study suggest that the second is not necessarily true. Results presented are rather preliminary and derived from extremely artificial conditions, by any means similar to the natural environment. But, despite the methodological drawbacks, the positive effects of AMF inoculation, and the efficiency of native inoculum in promoting *B. virgilioides* seedlings observed in the present study, reinforces the possibility of use of native AMF species for reforestation of these areas. Nevertheless, further investigation, with more adequate experimental conditions, and field trials, are still needed.

7.5 Conclusions

Despite some sub optimal experimental conditions, this study on the differential effects of native mixed inocula on *B. virgilioides* seedlings growth provides interesting results and points out to further research lines.

Growth of *B. virgilioides* seedlings was enhanced by mixed native inoculum. Effects were somewhat higher than with the use of pure inoculum from a selected fungus (*G. clarum*). Therefore, it is worth considering the use of this inocula in reforestation of the studied rainforests areas, what would be not only cheaper but also result in more rapid plant cover. Differences in plant responsiveness to inocula from both sites and from plantation or forest sites could not be worked out in this study and must be investigated.

Origin of mixed inocula (plantation or forest plots) provided different results. In the first experiment, inoculum from forest plots presented higher growth effects. In the second, no significant differences were found. In the third, inoculum from the plantation plot resulted in higher plant growth. As the effect in the first experiment was found only with *Z. mays* plants, it is possible that AMF inoculum from plantation areas may be more effective under low nutrient conditions, even with tree species as hosts.

This study clearly shows up that AMF inoculation can have positive effects on *B. virgilioides* seedlings growth. The transplant of inoculated *B. virgilioides* seedlings to mixed stands with *V. guianensis* and *C. pachystachia*, could lead to rapid plant growth and improved soil nutrient status, what should be taken into account for the restoration of these forest areas.

8 General discussion

This work present results from the investigation of several aspects of the ecology of two Brazilian Atlantic rainforest remnants in the northeastern Sergipe state, as well as implications for the reforestation of these areas. A somewhat wide range of subjects was encompassed, sometimes impeding a more deeper analysis. This approach was, however, intentional. There is not much knowledge gathered on the ecology of these areas. Actually, this is the first survey on the mycorrhizal symbiosis in the costal forests of the Sergipe state and, probably, of the whole northeast region. Some research has been already carried out in this region, but it mostly concentrated on agricultural plants (Melo, Maia & Morgado 1997) or agroecosystems (Ezeta & Santos 1980, 1981, Ezeta & Carvalho 1982, Weber & Oliveira 1994). Some survey of the arbuscular mycorrhizal fungi (AMF) occurring in subclasses of Monocotyledonae has also been done in the region (Santos *et al*. 2000, Silva et al. 2001). Data on the mycorrhizal status of native tree species from the northeastern region was found only in a report of AMF growing in a arboretum (Santos & Vinha 1982), but nothing was found about plants occurring in forests or any other natural ecosystems.

The small extent left of Atlantic rainforest, particularly in the northeastern Brazil, is another factor that has pressured for a more wide ranged approach. Mycorrhizal symbiosis have been reported to be successfully managed in disturbed areas and forest restoration (e.g. Allen *et al*. 1998, Asbjornensen & Montagnini 1994, Cuenca & Lovera 1992, Cuenca, de Andrade & Escalante 1998, Herrera, Salamanca & Barea 1993, Miller & Jastrow 1992). Before some basic knowledge is available on the occurrence and distribution of mycorrhizas in these ecosystems, no utilization of commercial AMF inoculum on reforestation should be carried out, unless one chooses to ignore the unknown native AMF diversity, and the manifold interactions occurring with plant hosts and the soil environment.

The region where both forest remnants are situated is characterized by a marked rainfall seasonality (Chapter 2), which is likely to affect soil nutrient dynamics and plant reproduction and recruitment. Sampling was therefore carried in the two periods (February and November), but sampling scheme was somewhat handicapped by an altered rainfall pattern during the sampling year (2000). Seasonal differences between the two sampling periods may not be typical responses to dry and wet season. Nevertheless, the two sampling times would be insufficient for accurately identifying seasonal trends, and more sampling periods should be used in future studies.

Although the two areas present some similarity regarding floristic composition, soil properties in both differ markedly (Chapter 3). One of the forest fragments (Crasto forest) was a coastal tableland forest, on Red-Yellow Podzols, the other, a restinga forest (Caju forest), on quartziferous sands. Soils are, as typical tropical soils, are moderately to strongly acidic, with relatively high Al levels and nutrient-poor. The Crasto site presented generally higher nutrient levels, maybe due to the higher clay content in its soils, but in both, nutrients were concentrated in the soil upper layers. Collections were also carried out in the adjacent region of each fragment, occupied by a coconut plantation in both sites. With some minor variation, these regions presented lower nutrient levels than the forest plots. Soil

properties are, therefore, probable to hinder tree seedling establishment and, consequently, forest recomposition in these areas.

Aiming to investigate the potential of native mycorrhizal species on forest regeneration, the assessment of mycorrhizal symbiosis in seedling roots (Chapter 5) and trees (Chapter 6) was performed, as well as the analysis of the spatial distribution of inoculum (spores and roots) (Chapter 4). Seedlings of forest species were not found in the plantation quadrats in both areas, reflecting the diminished establishment success of these seedlings in the highly modified conditions of the plantation. However, the forest quadrats presented great variation in the number of seedlings, what results from the irregular nature of its spatial distribution. In the beginning of the dry season it was found the greater density and diversity of species, probably reflecting the most favorable conditions for the germination and initial establishment of the rainy season, particularly extended this year.

Arbuscular mycorrhizal fungi was, like usual in tropical ecosystems, dominant. AMF inoculum was not, contrary to the expectation, excluded from the plantation plots. In fact, spore species richness was higher than in the forest plots. Disturbance in these areas was therefore not enough to eliminate the occurrence of the mycorrhizal symbiosis. However, both sites differed in its response. In the Caju forest, roots in plantation sites presented higher colonization levels than in the Crasto site. Difference in soil properties and/or management practices may be responsible for these results and will be further examined. AMF spore species richness and root colonization levels were generally higher in the upper layers of the soil, similarly to the nutrient and root biomass. Disturbance that involve active removal of the top soil or increasing of soil erosion will therefore remove also the native AMF inoculum important for plant growth and survival, particularly under stressed conditions. Seedling recruitment was lower in the plantation plots, although forest fringe plots presented also similarly low values. Seedling root mycorrhizal colonization was spatially uneven. Analysis of seedling roots from four different native tree species revealed a very variable picture of colonization type and intensity between species. An important finding was the high colonization levels of roots of some of these seedling species by some dark septate endophytes (DSE). Both pathogenic and positive effects have been reported for plants in culture with these fungi, and it is not clear whether the analyzed seedlings benefit from them. As all the seedlings seemed to be healthy, without any noticeable signal of disease or nutrient deficiency, and as this root endophyte was also found in roots of some tree species analyzed, it is probable that these unexpected root partner may have a positive effect in enhancing plant growth and survival. Due to this possible significant effect on tree seedling recruitment, it is an important topic for subsequent studies.

The analysis of some native tree roots revealed also that ectomycorrhizal inoculum, although rare, is present in these forests, as one of the selected species, *Cocoloba rosea* (Polygonaceae), presented ectomycorrhizal roots. The family is already described in the literature as presenting ectomycorrhizal tropical species. In fact, a survey of the data published on tropical ectomycorrhizal plant species suggests an important taxonomic (far wider than within their temperate counterparts), more than biogeographical, component determining the distribution and occurrence of ectomycorrhizas within this region. Species from the genus *Cocoloba*, for example, are reported to form ectomycorrhizas in ecosystems as distinct as coastal vegetation on sandy soils in Cuba (Kreisel

1971), coastal flooded savannas in the French Guyana (Béreau, Gazel & Garbaye 1997), "xerophytic" forest in the Lesses Antilles (Pegler & Fiard 1979), Amazonian *caatinga* in Venezuela (Moyerson 1993) and lowland rain forest in Puerto Rico (Lodge 1996).

For the recomposition of these tropical forests, however, arbuscular mycorrhizas have been found to be far more important. Contrary to some studies relating mycorrhizal colonization and host successional status, roots from pioneer species, *Cecropia pachystachya* and *Vismia guianensis*, were strongly colonized. The high AM colonization found in the roots of these important pioneer species points to the worth of understanding at least some of the ecological relationships of these species in these disturbed habitats. A successful management of these species could then lead to more efficient (i.e. with lower seedling mortality and faster growth and soil cover) reforestation.

Several *Cecropia* species are listed throughout the Neotropics in the "Herbarium Specimen Catalog" from the New York Botanical Garden (http://www.nybg.org). Collections of *C. pachystachya in this* site, however, are restricted to the Brazilian states of Sergipe and Bahia, although Araujo *et al.* (2001) describe this species in some successional forests in the Brazilian state of Pará, in the Amazonian region.

Although no revision of the distribution of *V. guianensis* was found, this native successional species is widely distributed throughout the Neotropics, being frequently found colonizing abandoned or active agricultural areas in Brazilian Amazonia" (Albuquerque 1980) and establishing a woody layer around border of forest fragments in Sergipe, if this regions are allowed to recover without cut (pers. obs.). The "Herbarium Specimen Catalog" from the New York Botanical Garden (http://www.nybg.org) included collections of *V. guianensis* for the Brazilian sates of Acre, Amazonia and Roraima, in the Amazon region, and Bahia, and Pernambuco, in the Northeastern region. Other collections were made in Honduras, Trinidad and Tobago, Colombia, French Guiana, and Venezuela.

Disturbance in natural ecosystems varies in type, size, intensity, and frequency, and may explain the coexistence of a large number of species, with different life-history traits (Loehle 2000). Resprouting after damage is influenced by disturbance intensity and frequency and represent a compromise between resource allocation to vegetative growth or seeding, or in other words, to the current or the future generation (Bellingham & Sparrow 2000). This 'persistence niche', a strategy allowing damaged plants to quickly recolonize its habitat, is thought to be so important to plant demography as recruitment (Bond & Midgley 2001) and is also a feature of interest when considering ecologically based reforestation practices.

Resprouting was found to perform an important role in initial successional stages of forest regeneration (Kammesheidt 1998). Sprouts are able to grow faster than seedlings, at least at the initial phase (Putz & Brokaw 1989), what may confer these species an advantage under unfavorable and/or unpredictable conditions. Deforested areas present not only less nutrients, but also bigger variations in temperature, air vapor pressure deficit and soil moisture stress (Nepstad *et al.* 1996), as well as, of course, higher light incidence (Dias-Filho 1995a). Shading can affect *V. guianensis* seedlings development although even plants growing under shade maintains their full photosynthetic capacity (Dias-Filho 1995a).

A already developed root system may give these plants access to soil water and nutrients not yet available to the young undeveloped root system of seedlings. Resprouting after death of above ground tissues, which may be frequent in habitats such as deforested areas, abandoned agricultural land and pastures by "fire, mowing, or livestock trampling" (Dias-Filho 1995a), is a characteristic of many pioneer trees, among them *Cecropia* spp (Putz & Brokaw 1989). *C. pachystachya* is also reported to resprout in secondary stands in Paraguay (Kammesheidt 1998). This is an important strategy in disturbed habitats, allowing the plant survival after damage that could possibly kill the seedlings and seeds in the soil bank. Furthermore it may also compensate the short life-spans in the soil due to high seed predation and pathogen attack rates found for *C. obtusifolia* (Alvarez-Buylla & Martínez-Ramos 1990) and possible occurring also in other species. *V. guianensis* presents relatively low growth rate at the early stages of development (Dias-Filho 1995b) and its resprouting ability (Dias-Filho 1995b, Boehm *et al.* 2000) may be an important strategy in maintaining its populations in disturbed sites.

Plants growing in disturbed areas are subject to diurnal and seasonal drought stress (Dias Filho 1995c). Besides coping with soils of low fertility, this represents another factor to be counterbalanced when growing under these circumstances. Although not considered an important stress factor in the humid tropics, seasonal drought in tropical hot areas, specially in pastures and deforested areas (Dias Filho 1995c), can be a limiting factor to the establishment of tree species in these areas.

V. guianensis act as an important pioneer under these conditions being able not only to establish but also to survive during the dry season. Some anatomical and physiological traits of *V. guianensis* make this species able to deal with long term water stress successfully, being considered an 'stress-avoider' species (Dias Filho 1995c). Under experimental soil drying, *V. guianensis* seedlings did not allocated more biomass into root tissue (Dias-Filho 1995b). Its small seedlings with shallow rooting depth were, however, able to survive to water stress until the end of the experimental period (42 days). The author suggests that some efficient traits helping to adjust and counteract in some way soil drying. As in this experiment fritted clay was used as substrate, the possible role of mycorrhizas increasing resistance to drying must be excluded.

Whether this characteristics are in some way related to the outgrowths found on *V. guianensis* roots is not clear. The internal structure of the nodule do not resemble leguminous nodules (Faria, Sutherland & Sprent 1986, Faria, McInroy & Sprent 1987). Only one non-legume species, *Parasponia* (Ulmaceae), is know to present nodulation with these organisms (Sprent 2001), but *V. guianensis* knobs are also not similar to the *Parasponia* nodules (Trinick & Galbraith 1976, Trinick 1979, Lancelle & Torrey 1984, Lancelle & Torrey 1985). A complete description of the nature and function of these structures is beyond the scope of this study and will be carried out later.

Seed dispersal is also of importance when considering forest regeneration. As already discussed (see above 'AM colonization and successional stage'), *Cecropia* spp seeds are mainly dispersed by birds, bats, or mammals. *V. guianensis* is cited as a zoochorous species (Albuquerque 1980), and its fruits, oblong berries carrying several small seeds and the "fleshy and mucilaginous pulp, with red coloration and sweetened scent" (Mourão & Beltrati 2001), suggests ornithochory as dispersal syndrome (van der Pijl 1982). Abundant viable seeds of these two species were found in the

soil seed bank of successional Amazonian forests, their numbers decreasing along seral stages (Araujo *et al.* 2001). Seedlings of *Vismia* and *Cecropia* species were also found growing in large forest gaps in Amazonia (Saldarriaga & Uhl 1991).

The high AM colonization levels in these two pioneer species summed to other important ecological characteristics, points to the possible management of this symbiosis in order to improve reforestation in rain forest fragments in Northeast Brazil. The first condition, existence of inoculum in the altered soil condition of deforested areas is already fulfilled (Chapter 4). The second, existence of pioneer species adapted to the same conditions, and able to associate with these fungal species, seems now to be also accomplished. If one assumes that high colonization levels imply also high activity in metabolic exchange, and consequently, high nutrient inputs to these plants, the efficiency in colonizing disturbed habitats could be considered the proof of the positive effects of this relationship. How to manage this association is thus the next step.

Most of the studies dealing with regeneration in tropical forests focus on seedling recruitment under the forest canopy (Dalling, Swaine & Garwood 1998), in canopy gaps (Dalling *et al.* 2001), or even on seedling or tree resprouting under the canopy, but recently more studies have been done on regeneration within abandoned pastures or other disturbed sites (Vieira, Uhl & Nepstad 1994, Nepstad *et al.* 1996, Kammesheidt 1998, Slocum & Horwitz 2000, Slocum 2001, Holl 2002). Most of these pastures were formerly forested areas and, in many cases, are near enough to the forest edge to profit from the seed sources these forest fragments represent. To preserve these fragments is not enough, as the remnant area may be, in many cases, insufficient to assure the maintenance of genetic diversity of some of their populations, and therefore being fated to disappear (see discussion in Janzen 1986). The increase of the forested area by stimulating forest regeneration around the existing fragments left, and the resulting enhance of the connectivity between neighbor fragments, may augment the movement of pollinators and dispersers and, consequently, gene flow between populations. This may be particularly true in tree species with limited pollination and dispersal abilities.

The principle them is to accelerate the natural process, maintaining the advantage of the natural diversity, with the gain in growth rate and soil cover observed in some plantations with exotic tree species. Thus, the expansion of forest fragments by managing the surrounding areas is a viable and not necessarily expensive action, as much of the seed sources, and seed dispersers, lay nearby. Natural regeneration in forest edges in Sergipe rain forest fragments occurs just as these areas are left protected from cattle and cutting (pers. observation), and may be improved if some key species in these processes are suitable managed. Although early successional shrubs may not only "facilitate" but also "inhibit" woody species seedling establishment (Connell & Slatyer 1997), patches of shrubs have been found to have higher seed rain than adjacent grass areas (Holl 2002). As both *V. guianensis* and *C. pachystachya* are zoochorous species, attraction of dispersers may increase diversity in seed rain bring with them also seeds from other species and, thus accelerating soil cover and restoration of species diversity in these sites. Higher seed predation found within patches of shrubs (Holl 2002), might be compensated by higher germination. Moreover, the soil cover may afterwards improve microclimate and make these sites more favorable to the forest tree seedlings.

The use of *V. guianensis* in agroforestry systems has been already considered (Boehm *et al.* 2000), as plants of this species present rapid growth rates and high accumulation of magnesium and phosphorus, while its root distribution in soil do not prevent growth of primary forest species, which grew well in a *Vismia*-thicket in Central Amazonia. Due to these and the above mentioned characteristics of *V. guianensis*, this species could be regarded a "succession facilitator", in the same way as the shrub *Cordia multispicata* in Amazonian abandoned pastures (Vieira, Uhl & Nepstad 1994). These authors suggest the use also of stem or root stocks of *C. multispicata*, which would increase survival chances. If stocks *of V. guianensis* and *C. pachystachya* can also be produced, and if mycorrhizal inoculation of them is successfully achieved, stocks of these pioneer mycorrhizal species, adapted to the local conditions of these disturbed soils, should be planted in the areas near the forest edge as part of reforestation programs in these regions.

Furthermore, *Vismia* and *Cecropia*-bordered forest edges presented lower tree mortality and than pasture-bordered edges (Mesquita, Delamônica & Laurance 1999) and it seems that *Vismia* stands are more effective in this buffer effect than *Cecropia* stands. But, this buffer-effect was not found for *Vismia*-dominated edge, in contrast to *Cecropia* stands, in another study on Amazonian forest fragments (Didham & Lawton 1999). Anyway, border-effect was found to be greater in open-edged fragments (Didham & Lawton 1999) and successional stands of *Vismia* spp in Amazonia did not hinder subsequent establishment of forest tree species (Saldarriaga & Uhl 1991).

It seems that both species present characteristics that may favor the recruitment of forest tree seedlings in deforested regions and its management should be further studied. Although most of the information available on forest fragments dynamics, border effect and forest regeneration in the Neotropics comes from studies in the Amazon forest, many of the underlying processes may be comparable in both Amazon and Atlantic rainforest and results and strategies suggested for the former may as well be of use in the latter.

Some rather preliminary experiments intending to test the ability of native AMF inoculum in promoting seedling growth and survival were performed with a native legume tree species, *Bowdichia virgilioides* (Chapter 7). *B. virgilioides* is a common species in the area of study, with potential for use in reforestation programs. These experiments aimed to compare native mixed inoculum from plantation and forest plots as well as pure inoculum of a selected mycorrhizal fungus (*Glomus clarum*) under different P levels or sources (organic and inorganic). Growth of *B. virgilioides* seedlings was generally enhanced by mixed native inoculum, particularly under the higher P level. Effects were somewhat higher than with the use of pure inoculum (*G. clarum*). Therefore, it is worth considering the use of this inocula in reforestation of the studied rainforests areas, what would be not only cheaper but also result in more rapid plant cover. Differences in plant responsiveness to inocula from both sites and from plantation or forest sites are, however, not yet clear and must be further investigated.

Concluding, conversion of the two forest remnants to coconut plantations do not reduces drastically the AMF inocula from these soils. Although root colonization in these areas seems to be somewhat reduced, diversity of AMF species has been maintained. Soil inoculum from these plots has even proved to be equally effective, if not better than forest and pure inoculum, in promoting growth of a legume tree, *B. virgilioides*. Results may have been fairly biased, due to the inadequate

experimental conditions, and further research must be still carried out. However, the advantages of using a legume species to improve N status of deforested soils would be added to the reported good performance of *B. virgilioides* when growing in experimental stands in the coastal region of Sergipe. It is expected that *B. virgilioides* seedlings should benefit from inoculation with the appropriate AMF inoculum, not necessarily a single species but, more probably, a mix of species adapted to the local conditions. Therefore, the use of *B. virgilioides* together with *Vismia guianensis* and *Cecropia pachystachia*, two pioneer species with high AMF colonization in deforested areas, in restoration programs of these areas should be considered. Thus, ensuring higher seedling growth levels and lower mortality, the management of the native diversity of mycorrhizal species, could lead to more successful reforestation plans.

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Appendix

Results of soil chemical analysis (see Chapter 3). Values for February (Febr.) refers to soil mixed samples (5). Figures for November (Nov.) are mean values for 5 samples, and standard deviation (sd). Values not available refers to insufficient sample size to perform all analyses, or to calculate mean value (N=1).

			pH			Al			P		
Site	**Plot**	**Depth**	**Febr.**	**Nov.**	**sd**	**Febr.**	**Nov.**	**sd**	**Febr.**	**Nov.**	**sd**
Crasto	Plantation	0-5	5,4	4,96	0,17	1,20	0,70		2,56	2,49	0,70
		5-10	5,1	4,72	0,27	0,90	1,10		3,82	2,66	1,61
		10-15	5,4	4,86	0,30	0,70			2,55	2,81	0,31
		15-20	5,4	4,99	0,35	0,50	0,60	0,14	3,09	2,00	0,82
		20-30	5,5	5,21	0,43	0,70	0,6	0,26	3,08	1,11	0,63
	Fringe	0-5	5,2	4,76	0,24	0,60			3,56	9,18	2,41
		5-10	5,1	4,29	0,11	0,60	0,70		2,89	5,96	1,42
		10-15	4,6	4,41	0,12	0,90	0,90		2,73	4,37	2,02
		15-20	4,6	4,32	0,13	0,90	0,90	0	2,28	2,94	0,89
		20-30	5	4,44	0,19	1,10	0,90	0,26	2,39	1,63	0,69
	Forest	0-5	5	4,79	0,42	0,90			3,23	4,69	1,76
		5-10	4,7	4,38	0,06	0,90	1,1	0	1,83	1,99	1,07
		10-15	4,7	4,41	0,07	1,70	1,5		2,44	2,18	0,39
		15-20	4,8	4,32	0,08	1,90	2,20		1,32	1,52	1,22
		20-30	4,8	4,53	0,14	1,80	1,65	0,49	1,32	0,95	0,22
Caju	Plantation	0-5	5,6	4,37	0,22	0,60	0,70	0,1	3,35	5,52	0,20
		5-10	5,2	4,17	0,16	0,80	0,80	0,17	2,91	4,62	0,23
		10-15	5,1	4,26	0,13	0,40	0,83	0,21	1,87	3,56	0,16
		15-20	5	4,25	0,13	0,90	0,83	0,21	1,37	2,72	0,62
		20-30	5,2	4,38	0,23	1,00	0,73	0,23	1,88	1,84	0,52
	Fringe	0-5	5,5	5,21	0,03	0,60	0,15	0,07	3,24	6,84	0,34
		5-10	5,1	4,65	0,31	0,50	0,30	0,1	2,32	5,17	1,36
		10-15	5	4,64	0,46	1,40	0,27	0,12	2,32	3,18	1,56
		15-20	4,9	4,55	0,30	0,70	0,40	0,17	1,34	2,41	0,96
		20-30	5,2	4,38	0,06	0,60	0,37	0,15	1,72	1,80	0,58
	Forest	0-5	5,5	5,11	0,47	0,70	0,30	0,1	5,03	6,07	2,04
		5-10	5,4	4,62	0,38	0,60	0,40	0,1	2,54	2,43	0,64
		10-15	5,4	4,79	0,32	0,50	0,47	0,06	2,3	1,26	0,52
		15-20	4,8	4,84	0,32	0,50	0,40	0	1,98	0,69	0,48
		20-30	4,9	4,63	0,38	0,50	0,37	0,12	1,91	0,52	0,35

			Na			K			Ca			Mg		
Site	***Plot***	***Depth***	***Febr.***	***Nov.***	***sd***	***Febr.***	***Nov.***	***sd***	***Febr.***	***Nov.***	***sd***	***Febr.***	***Nov.***	***sd***
Crasto	Plantation	0-5	0,09	0,05	0,02	0,10	0,04	0,01	0,47	0,46	0,08	0,36	0,54	0,13
		5-10	0,08	0,02	0,02	0,06	0,02	0,00	0,58	0,47	0,27	0,36	0,54	0,22
		10-15	0,09	0,05	0,02	0,04	0,03	0,01	0,70	0,55	0,47	0,47	0,48	0,24
		15-20	0,08	0,05	0,02	0,03	0,03	0,01	0,81	0,40	0,12	0,59	0,44	0,22
		20-30	0,09	0,03	0,02	0,03	0,02	0,02	0,58	0,28	0,05	0,47	0,53	0,15
	Fringe	0-5	0,24	0,05	0,02	0,16	0,06	0,04	1,98	1,95	0,57	1,90	1,20	0,52
		5-10	0,21	0,04	0,00	0,11	0,03	0,01	0,82	0,61	0,04	0,48	0,70	0,20
		10-15	0,07	0,07	0,05	0,09	0,02	0,00	0,93	0,33	0,08	0,59	0,45	0,05
		15-20	0,07	0,02	0,02	0,08	0,02	0,00	0,58	0,47	0,40	0,35	0,45	0,02
		20-30	0,09	0,03	0,02	0,08	0,02	0,00	0,70	0,27	0,02	0,47	0,30	0,07
	Forest	0-5	0,10	0,07	0,02	0,13	0,06	0,03	1,05	1,45	0,35	1,18	1,16	0,36
		5-10	0,11	0,03	0,02	0,10	0,05	0,04	0,70	0,64	0,13	0,48	0,60	0,18
		10-15	0,12	0,09	0,04	0,11	0,10	0,05	0,82	0,41	0,13	0,59	0,42	0,10
		15-20	0,11	0,04	0,01	0,10	0,04	0,01	0,46	0,27	0,07	0,24	0,32	0,08
		20-30	0,11	0,05	0,02	0,06	0,06	0,03	0,58	0,29	0,19	0,48	0,24	0,13
Caju	Plantation	0-5	0,23	0,22	0,02	0,10	0,07	0,00	0,81	0,62	0,17	0,95	0,78	0,42
		5-10	0,26	0,20	0,07	0,07	0,07	0,02	0,35	0,32	0,02	0,36	0,30	0,08
		10-15	0,34	0,14	0,05	0,06	0,05	0,00	0,58	0,31	0,10	0,59	0,14	0,07
		15-20	0,25	0,18	0,06	0,04	0,06	0,01	0,46	0,41	0,16	1,30	0,20	0,07
		20-30	0,26	0,18	0,02	0,04	0,09	0,03	0,47	0,88	1,06	0,47	0,28	0,27
	Fringe	0-5	0,31	0,22	0,03	0,07	0,15	0,02	2,09	1,28	0,69	2,01	0,97	0,55
		5-10	0,31	0,25	0,06	0,06	0,09	0,05	0,70	1,14	0,85	0,59	0,53	0,38
		10-15	0,18	0,21	0,10	0,04	0,11	0,04	1,40	1,01	0,72	1,19	0,75	0,49
		15-20	0,20	0,16	0,04	0,04	0,06	0,01	0,81	0,78	0,34	0,83	0,66	0,45
		20-30	0,17	0,13	0,02	0,03	0,08	0,05	0,81	0,54	0,13	0,83	0,67	0,65
	Forest	0-5	0,25	0,24	0,07	0,12	0,14	0,07	2,56	1,85	0,52	3,09	1,24	0,13
		5-10	0,26	0,20	0,14	0,12	0,05	0,05	1,05	0,80	0,41	1,30	0,82	0,42
		10-15	0,21	0,12	0,00	0,04	0,12	0,08	0,93	0,24	0,12	0,95	0,30	0,05
		15-20	0,23	0,20	0,10	0,04	0,07	0,05	1,05	0,34	0,16	1,07	0,18	0,06
		20-30	0,21	0,11	0,02	0,04	0,04	0,01	0,58	0,33	0,24	0,59	0,39	0,30