

**University of São Paulo  
“Luiz de Queiroz” College of Agriculture  
Center of Nuclear Energy in Agriculture**

**Nutrients dynamics in tropical forest fragments located in an area of  
Atlantic Forest of the state of Alagoas**

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Thesis presented to obtain the degree of Doctor in Science.  
Area: Applied Ecology

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**Bachelor in Biological Science**

**Nutrients dynamics in tropical forest fragments located in an area of Atlantic  
Forest of the state of Alagoas**

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To

*My beloved homeland, Alagoas.*

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*“It is difficult to say whether it is correct to refer to the Atlantic Forest at the present time. Some extent of forest cover still exists in the region, and although most are identifiable as secondary, some small strips may never have been felled or burned by man. Most, however, perhaps all, have undergone some degree of intervention - selective logging, extractivism, or air pollution - so that, it is impossible to say to what extent they resemble the forest that was there before the arrival of the first men.”*

*Warren Dean*

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## RESUMO

### **Dinâmica de nutrientes em fragmentos de floresta tropical situados em área de Mata Atlântica no estado de Alagoas**

A Floresta Atlântica do Nordeste do Brasil contém milhares de fragmentos de florestas em diferentes estágios de regeneração, alguns se recuperando de campos agrícolas abandonados, enquanto outros se recuperam de distúrbios crônicos como incêndio, extração de madeira e caça furtiva. Alguns desses fragmentos ficaram protegidos por várias razões: responsabilidade corporativa, cumprimento do código florestal, preservação da biodiversidade, entre outros. Embora estes fragmentos estejam longe de ter a mesma riqueza de espécies vegetais e animais, podem desempenhar importantes serviços ecossistêmicos, como proteção de corpos d'água, reservatório de importantes nutrientes e carbono. Isso é especialmente importante se considerarmos que há iniciativas globais como o REDD+ que visam avaliar florestas secundárias e de recuperação como importantes reservatórios de carbono (C). Por outro lado, a gestão e preservação destes fragmentos dependem de um conhecimento sólido sobre a sua estrutura e ciclo de nutrientes. Há uma literatura relativamente rica sobre a perda de biodiversidade nestes fragmentos florestais do Nordeste. No entanto, muito menos está disponível sobre a estrutura e o funcionamento destes fragmentos. Este trabalho pretende contribuir para o preenchimento desta lacuna, fornecendo informações científicas sólidas sobre quatro áreas florestais e uma floresta secundária, onde foi implantado um programa de restauração no Estado de Alagoas, Nordeste do Brasil. Após uma breve introdução, o capítulo um discute a estrutura desses fragmentos através de um inventário florestal completo, onde a densidade indivíduos, sua altura e diâmetro à altura do peito (DAP) foram medidos para cada árvore com  $DAP \geq 10$  cm. Com esses dados alométricos, estimamos a área basal e a biomassa viva acima do solo (AGLB), comparando esses atributos com outras estimativas disponíveis na literatura. No capítulo dois, investigamos a distribuição de nutrientes nos fragmentos florestais, onde medimos a concentração foliar de C, nitrogênio (N) e fósforo (P). Ao mesmo tempo, medimos durante um ano a massa da serapilheira em cada fragmento e seu teor de nutrientes. Com essas informações pudemos estabelecer como essas áreas ciclam seus principais nutrientes limitantes e C. Finalmente, no último capítulo abordamos a partição dos estoques de C e nutrientes, acima e abaixo do solo, com o objetivo de avaliar a importância dos *pools* de C e nutrientes nos fragmentos de floresta e entender qual o papel desses fragmentos em um mundo que necessita urgentemente estocar C em um reservatório sustentável.

Palavras-chave: Fragmentos florestais; Estrutura; Nutrientes; Serapilheira; Mata Atlântica

## ABSTRACT

### **Nutrients dynamics in tropical forest fragments located in an area of Atlantic Forest of the state of Alagoas**

The Northeastern Atlantic Forest of Brazil contains thousands of forest fragments at different stages of regeneration, some recovering from abandoned agricultural fields, while others recovering from chronic disturbances such as fire, logging, and poaching. Some of these fragments kept protected for several reasons: corporate responsibility, compliance with the forest code, preservation of biodiversity, among others. Although these fragments are far from having the same species richness of plant and animal, they can perform important ecosystem services, such as protection of water bodies, reservoir of important nutrients and carbon (C). This is especially important if we consider that there are global initiatives such as REDD + with the objective of assessing secondary forests and forests in recovery process as important C pools. On the other hand, the management and preservation of these fragments depends on a solid knowledge about their structure and nutrient cycle. There is a relatively rich literature on the loss of biodiversity in these Northeastern forest fragments. Much less is available, however, about the structure and function of these fragments. This work intends to contribute to fill this gap, providing solid scientific information on four forest areas and a secondary forest, where a restoration program was implemented in the State of Alagoas, Northeast of Brazil. After a brief introduction, chapter one discusses the structure of these fragments through a complete forest inventory, where individuals density, height and diameter at breast height (DBH) were measured for each tree with  $DBH \geq 10$  cm. With these allometric data, we estimated the basal area and aboveground live biomass (AGLB), comparing these attributes with other estimates available in the literature. In chapter two, we investigated the distribution of nutrients in the forest fragments, where we measured the foliar concentration of C, nitrogen (N) and phosphorus (P). At the same time, we measured the litterfall mass in each fragment and its nutrient content for a one year period of sampling. With this information we were able to establish how these areas cycled their main limiting nutrients and C. Finally, in the last chapter we approached the partition of C and nutrient stocks, above and belowground, in order to evaluate the importance of C pools and nutrients in the forest fragments and understand the role of these fragments in a world that urgently needs to store C in a sustainable reservoir.

**Keywords:** Forest fragments; Structure; Nutrients; Litterfall; Atlantic Forest

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## 1 INTRODUCTION

Landscapes have been modified at an unprecedented rate as the economy and the world population grow, with the environment suffering the consequences of these changes (Wright 2005). Global permanent forests cover an area of approximately 1.5 billion ha of the land surface, with tropical forests representing 52% of this total (FAO 2015). Despite the importance of these forests in regulating the climate, half of tropical forest cover was cleared and lands converted into different uses, with the highest rates of deforestation occurring during the 1980s and 1990s (Ramankutty and Foley 1999; Lewis et al. 2015). These forests also play an important role in the global nitrogen (N) cycle exchanging more N with the atmosphere than any other biome on Earth (Townsend et al. 2008). These systems have high N availability with higher rates of soil nitrification and mineralization (Bernhard-Reversat 1977; Silver et al. 2000, 2005; Pardo et al. 2006), higher rates of biological N fixation (BNF) (Cleveland et al. 1999; Galloway et al. 2004; Robertson and Rosswall 1986), and higher N:P ratio in litterfall (Vitousek 1984; Vitousek and Sanford 1986). The tremendous plant biodiversity in tropical forests results in a diversity of nutrient acquisition strategies, leading in turn to a high biogeochemical heterogeneity, such as variations in N and phosphorus (P) distributions, and their stoichiometric ratios (Townsend et al. 2007), making the nutrients dynamics extremely complex in these forests.

Disturbances of all extensions and frequencies, human or natural, have always occurred in all ecosystems of the world, and it is not different for tropical forests (Chazdon 2003). Currently, it is well established that tropical forests are in different stages of recovery (Chazdon 2003). According to Denevan (1992), there is a myth that the tropical forests have always been pristine prior to the Europeans arrival in the Americas in 1492, an invention of those writers who believe in the superiority of primitive tribes, creating the idea of an “heroic pioneer past in need of preservation” (Pyne 1982). When the Europeans first came to the New World, the native populations were large, forest had been modified, erosion could be observed, wildlife was disrupted (Denevan 1992). Although the changes made by early human occupation did not reach the extent of post Europeans changes, it happened in a way that deserves attention as disturbances were nearly everywhere (Denevan 1992). As Whitmore, a tropical botanist whose interests covered all aspects of tropical rain forests, stated in 1991:

*“Primeval tropical rain forest, undisturbed and stable, ‘since the dawn of time’ is a myth. Instability of varying extents occurs on several time-scales. The recovery to a steady-state is likely to take several centuries and is perhaps never achieved in many places.”*

In the modern era, the main reason for deforestation in the tropical and subtropical countries has been the commercial agriculture, which represents 70% of the land use change in tropical regions (FAO 2016). Deforestation leads to a fragmented landscape, where abrupt edges separate forests from different land uses or where forests form a mosaic with towns, pastures or fields (Wright 2005). This highly fragmented mosaic type is already a prevalent landscape in various tropical regions (Turner and Corlett 1996; Ranta et al. 1998; Gascon et al. 2000; Corlett 2000; Sodhi et al. 2004; Mendoza et al. 2005; Harvey et al. 2008).

In 2000, 60% of tropical forests around the world was classified as degraded forest, which include secondary forests, degraded primary forests and degraded forest land (ITTO 2002). According to Faber-Langendoen (1992), secondary forest succession is defined as the vegetation that regrows after clear-cut of primary forests. The presence of secondary forest succession is a common reality in the tropics, however, clear-cut is not the only reason for degradation of tropical forests. As cited previously, ‘since the dawn of time’ tropical forests faced disturbances by native populations and they continue until the present days (Denevan 1992; Chazdon 2003). Poaching, extraction of firewood and non-timber forest products are examples of the called ‘chronic’ disturbances that frequently remove small portions of forest biomass (Martorell and Peters 2005; May-Tobin 2011; Leal et al. 2014a; Ribeiro et al. 2015), with cumulative effects on biodiversity in landscapes, as the fragmented forests (Sodhi et al. 2011; Ellis 2013; Melo et al. 2013; Leal et al. 2015; Ribeiro et al. 2015). Researches tend to overlook the effects of chronic disturbances operating in a small scale, which are not detectable by satellite images (Laurance and Peres 2006), occur in a diffuse way and virtually impossible to cease (Ribeiro et al. 2015).

To evaluate the forest succession ongoing in the tropics, it is necessary to understand to what extent people use land and natural resources (Gardner et al. 2013). The frequency and intensity of these disturbances modify the successional pathways of the fragment remnants (Arroyo-Rodríguez et al. 2017) because some changes lead to losses of nutrients, reducing their stocks in the ecosystems, changing the biogeochemical cycling, thus resulting in a nutritional limitation for vegetation (Jordan 1985; Brown and Lugo 1994 ). Consequently, it is expected loss of biodiversity, loss of soil fertility and changes in local physical, chemical and biological

characteristics (Reiners et al. 1994; Neill et al. 2006). When considering fragmentation effects, there is also a number of changes in the structure and population dynamics of plant species, mainly due to the so-called edge effect (Bierrgaard et al. 2001). For example, a common effect is the loss of large trees (diameter at breast height - DBH  $\geq$  70 cm) on the edges of these fragments, with consequent decrease in forest biomass (Laurance and Bierregaard 1997; Oliveira et al. 2008). These changes also cause drastic changes in the abundance and distribution of several groups of organisms in the fragmented tropical forests (Turner and Corlett 1996; Bierrgaard et al. 2001). Therefore, in order to understand how the succession of the secondary forests occurs and affect the ecosystem, studies have mostly focused on the structural description (e.g., basal area, species richness and composition and biomass), whereas functional characteristics, as nutrient cycling or net primary productivity, have received less attention (Guariguata 2001). Some of these studies have shown that the forest structure, soil nutrients stocks and richness of the species can rapidly recover (Chazdon 2003).

Currently, fragmentation has reached unprecedented levels, especially in areas such as the Atlantic Forest of the Brazilian Northeast (Silva and Tabarelli 2000). The Atlantic Forest *stricto sensu* is located along the Brazilian coast and characterized by high diversity and endemism (Morellato and Haddad 2000; Talora and Morellato 2000). Changes on climate and soil conditions along the Brazilian coast, coupled with altitudinal variations, determined a great heterogeneity in the structure and floristic composition of the Atlantic Forest (Joly et al. 2012). Today it is considered one of the hotspots for biodiversity conservation (Myers et al. 2000), due to significant destruction of this biome since the Portuguese colonization to the present day. Currently, 12 to 15 % of the Atlantic Forest remains from its 1.5 million km<sup>2</sup> original cover (Ribeiro et al. 2009), with most of the fragments with areas smaller than 50 ha and only a few are larger than 100 ha (Ranta et al. 1998).

The Northeastern Atlantic Forest used to cover an area of 56,400 km<sup>2</sup> of the original Atlantic Forest biome cover (IBGE 1985), extending from the state of Rio Grande do Norte to the state of Sergipe. This part of the Atlantic Forest has a biogeographical origin highly influenced by Amazonian elements and known as one of the most unique centers of endemism in South America, the Pernambuco Endemism Center (Prance 1982; Oliveira et al. 2004; Santos et al. 2007; Lôbo et al. 2011). However, according to Silva and Tabarelli (2000), the Northeastern Atlantic Forest is the most threatened South American Atlantic Forest, with a high degree of fragmentation, and considering 300 m away from the edge to the forest interior, only 6 % of the total area have old-growth forest characteristics. Ribeiro et al. (2009) suggest that

preserving the remaining forests is a priority and that the maintenance of small fragments is necessary for the functionality through the connections between them.

Based on the discussion above, with this study we attempted to understand how the severe fragmentation of the Brazilian Northeastern Coastal Atlantic Forest affects the dynamics of nutrients, such as N and P. To achieve this goal, we worked in forest fragments of the State of Alagoas, Brazil, that have been protected against fire and selective logging (Cícero Bastos de Almeida, personal communication; Santos et al. 2008). In the first chapter, we describe the forest structure and biomass distribution in the studied fragments. In the second chapter, we describe the foliar nutrients concentrations and their fluxes through litterfall. Finally, in the third chapter, we investigate the nutrients dynamics in the soil, describing their distribution and stocks in the different forest fragments and their variations with depth.

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## 2 FOREST STRUCTURE AND ABOVEGROUND LIVE BIOMASS ESTIMATION IN FRAGMENTS OF THE NORTHEASTERN COASTAL ATLANTIC FOREST, BRAZIL

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### Abstract

Aboveground live biomass (AGLB) is responsible for about 60 % of carbon (C) stocks in terrestrial ecosystems. The balance of this element in tropical regions is still uncertain due to the lack of complete and reliable AGLB estimates in tropical biomes. With the increasing number of abandoned forest areas across tropical countries and the increasing CO<sub>2</sub> fluxes to the atmosphere in the modern era, it is of high priority to estimate C stocks to evaluate the importance of the ecosystem as a sink of CO<sub>2</sub>. In Brazil, studies on forest structure and biomass stocks are focused on well preserved forest areas as the Amazon and Southeastern Atlantic Forest, with only a few data on fragments of the Northeastern Atlantic Forest along the Brazilian coast. In addition, it is known that throughout the Brazilian Northeast history, the Atlantic Forest fragments have suffered different degrees of disturbance, which gives an opportunity to understand how and in which pace the forests have been recovering. In order to evaluate how forest structure and AGLB varies in this ecosystem, we performed trees DBH ( $\geq 10$  cm diameter at breast height) and height measurements in 16 0.1-ha old-growth forest area plots established in the state of Alagoas, in addition to four 0.1-ha plots in a restored area. The stems density of all fragments was higher (888 stems ha<sup>-1</sup>) than lowland and montane physiognomies of the Southeastern Atlantic Forest and than the Amazon forest, while the basal area was similar when compared to these ecosystems and other tropical forests. Regarding the old-growth forest areas AGLB, which ranged from 175 to 231 Mg ha<sup>-1</sup>, there were no differences between the four areas. Even though lower averages of AGLB stocks were found in the Northeastern fragments, the basal area was similar to those found in old-growth tropical forests, concluding that these ecosystems can rapidly recover the basal area but not the AGLB. This fact highlights the importance of the preservation of these ecosystem patches for stocking C and maintenance of environment equilibrium.

Keywords: Basal area, Carbon pools, Stems density, Tropical forest

## 2.1 Introduction

Global forests cover ~ 1.5 billion ha of the Earth surface (FAO 2015), with its largest area located in the tropics and half of it in the tropical America (Dixon et al. 1994). Tropical forests account for, approximately, 35% of the net primary productivity (NPP) in terrestrial ecosystems (Melillo et al. 1993). In 1984, most of the net CO<sub>2</sub> fluxes (~ 80%) were due to changes in the land use in the tropics (Brown and Lugo 1984). Since that time, studies to understand the global C cycle try to estimate aboveground live biomass (AGLB) and rates of deforestation due to their importance to the cycle of this element (Brown and Lugo 1984). The magnitude of CO<sub>2</sub> fluxes related to deforestation rates were uncertain and continues to be because there is a scarcity of reliable estimates of live AGLB and its different distribution across landscapes (Brown and Lugo 1984; Houghton 2007; Saatchi et al. 2007; Houghton et al. 2009).

Studies have demonstrated that fragmentation of forests leads to changes in tree species, where pioneers species replace late successional species, a feature that seems to be increasingly observed in tropical forest fragments (Tabarelli et al. 2010). Other studies have shown that edges will have lower species richness (Oliveira et al. 2004; Santos et al. 2008), fewer emerging trees (Oliveira et al. 2008) and trees with large seeds (Santos et al. 2008), and less diversity of pollinating vertebrate species are observed compared to the forest interior (Lopes et al. 2009). These changes in fragmented landscapes may lead to changes in the forest structure (Laurance and Bierregaard 1997; Bierregaard et al. 2001).

In Brazil, most of the studies describing forest structure and AGLB have focused on the Amazon forest and the Southeastern Atlantic Forest (e.g. Alves et al. 2010; Vieira et al. 2004; Vieira et al. 2008). The Northeastern Atlantic Forest represents 3% of the Atlantic Forest original cover because most of the forest was converted into agricultural lands (Viana et al. 1997; Ranta et al. 1998; Ribeiro et al. 2009). This portion of the Atlantic Forest biome has been recognized as an important endemism center in South America (Müller 1973; Prance 1982). However, currently it is dispersed as small patches inserted mainly in sugar cane fields (Ranta et al. 1998), constantly suffering from chronic disturbances (Arroyo-Rodríguez 2017), which is defined as the disturbances that do not result in forest loss or fragmentation, but cause impacts on the biological integrity of the biota, for instance, poaching, extraction of firewood or non-timber forest products (Arroyo-Rodríguez et al. 2017).

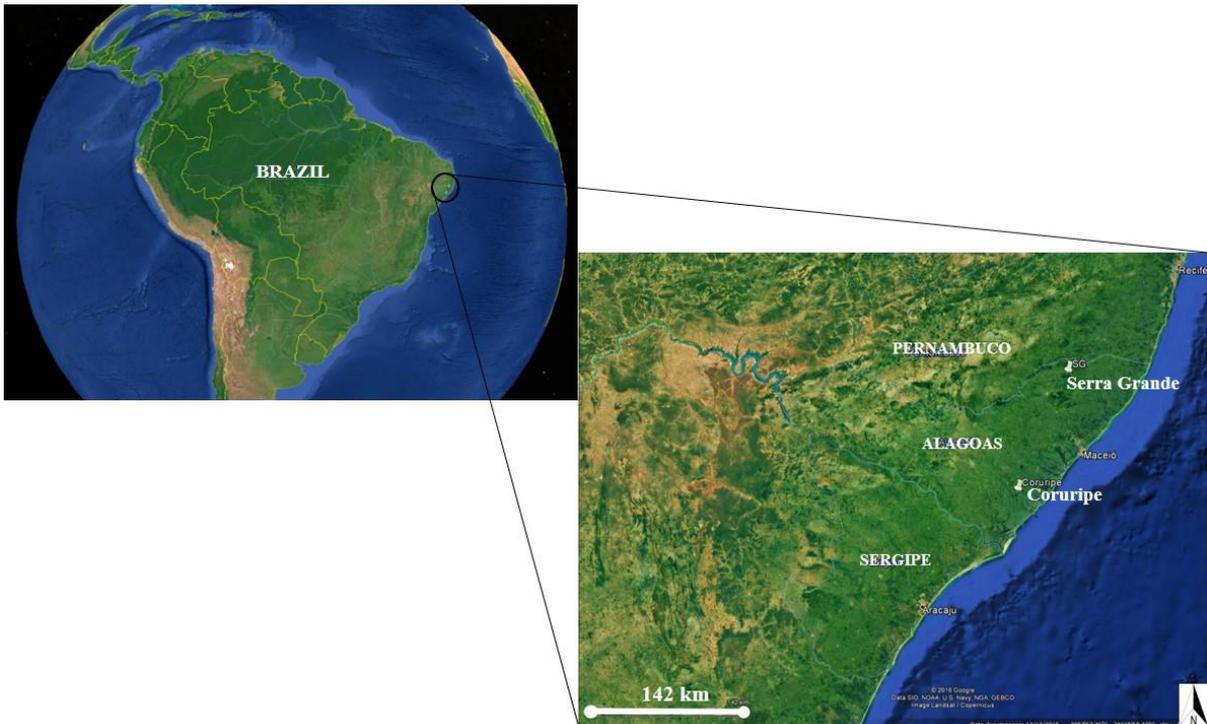
Despite its importance, C pools and estimates of AGLB stocks in the Northeastern Atlantic Forest have not been studied. Therefore, our study focused on fragments known to be the most well preserved remnants of the Brazilian Northeastern Atlantic Forest. Our main

objectives were: (i) to quantify the live AGLB of the studied forest fragments under chronic disturbances; (ii) to compare forest structure and AGLB results from this survey to the other Neotropical forests. According to our knowledge, studies describing the Northeastern Atlantic Forest fragments can be found, however, AGLB measurements have not been carried out in this region. Therefore, since these forests are the most threatened of the South American Atlantic Forest (Silva and Tabarelli 2000), understanding the forest structure in these areas is the first step to describe the nutrients distribution and C stocks of this important ecosystem.

## **2.2 Methods**

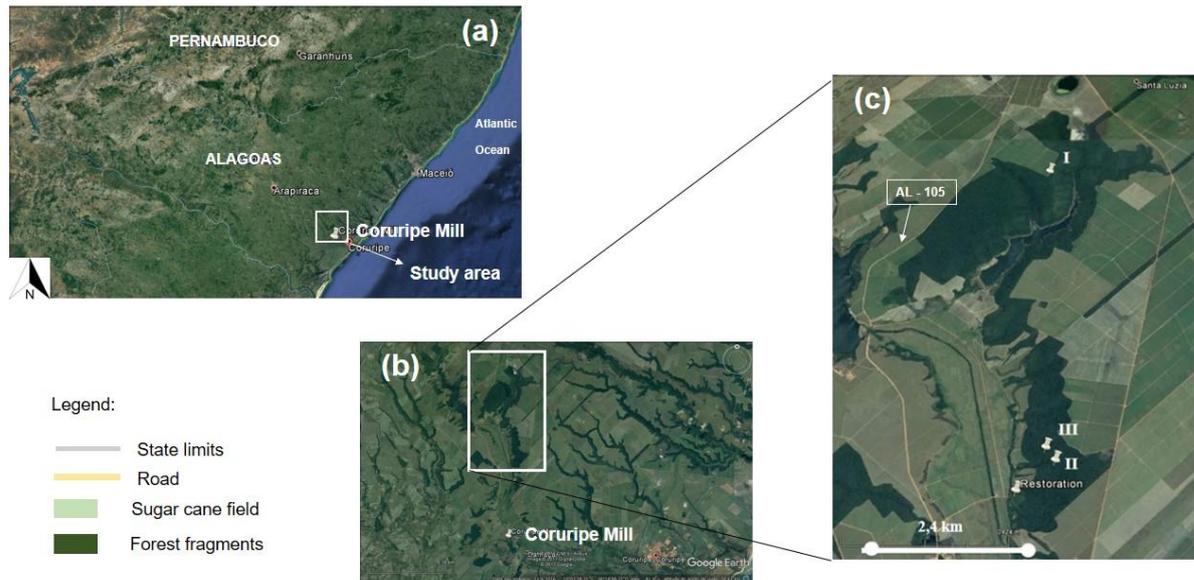
### **2.2.1 Study Area**

The study was conducted in Open Ombrophyllus Forest areas. This physiognomy occurs in the Amazon, scattered areas in the northeast of Brazil, and in the state of Espírito Santo (IBGE 1993), being characterized by the occurrence of spaced emergent trees with heights up to 35 m and open canopy (Veloso et al. 1991). Ombrophyllus Forest is a Greek term created by Ellenberg and Mueller-Dombois (1965), meaning “rain friend” (Veloso et al. 1991). Therefore, the Ombrophyllus Forests occurring along the Brazilian coast are characterized by marked rainy seasons. The study was conducted between April, 2014 and April, 2016. The studied areas are located in the lands of Coruripe sugar cane mill (hereafter Coruripe), located mainly in Coruripe municipality, and Serra Grande sugar cane mill (hereafter Serra Grande), in the municipality of São José da Laje, both within Alagoas state, Brazil. The city of Coruripe is located on the south coast of Alagoas state, about 90 km south from Maceió, capital of the state (Figure 1), while the city of São José da Laje is far inland, 100 km from the capital (Figure 1). We chose these areas because they well represent the fragmented landscape of the Northeast of Brazil.



**Figure 1:** Above, map of Brazil showing the location of the study area. Below satellite image showing the State of Alagoas borders and the location of Coruripe and Serra Grande sugar cane mills.

In Coruripe, three areas were selected in a fragment of approximately 1000 ha (Figure 2) within an area called Capiatã A. In addition to them, it was also included in this study a restored area (hereafter called restoration). In restoration ( $10^{\circ}03'25.42''\text{S}$  and  $36^{\circ}16'41.92''\text{W}$ ), fifteen native species were planted almost fifteen years ago (see Appendix), where sugar cane crops used to be grown. According to a 30 years monitoring dataset from Coruripe weather station, mean annual precipitation is  $1500 \text{ mm yr}^{-1}$ , including four drier months, with averages  $< 60 \text{ mm mo}^{-1}$  (November to February), with more intense rainy season occurring in the second quarter of the year. No historical data were available for temperature at Coruripe Mill dataset. Therefore, according to the National Institute of Meteorology (INMET), the mean annual temperature is  $24.4^{\circ}\text{C}$ . The studied areas were between 60 and 130 m above the sea level. The soil, according to surveys by Coruripe is classified as dystrophic Ultisol, and developed on sediments of the Barreiras Formation, mineralogically constituted mainly by kaolinite and quartz (Filizola et al. 2001). Myrtaceae, Fabaceae, Sapindaceae and Annonaceae are the most frequent families in Coruripe forests (Machado 2003).



**Figure 2:** (a) Alagoas state with limits of part of Coruripe Mill lands; (b) Coruripe Mill land with limits of the biggest forest fragment, where the study was conducted; (c) Areas distribution in the biggest forest fragment, Coruripe, State of Alagoas. Area I, where plots A, B, C and D are located; area II, plots E, F, G and H; area III, plots I, J, K and L; Restoration area, plots M, N, O and P. Source: Google Earth (Central area of the biggest fragment to the right of the image:  $10^{\circ}01'48.23''S$  and  $36^{\circ}17'00.87''W$ ).

In highly fragmented landscapes, such as the Brazilian Northeast (Tabarelli et al. 2004), it is extremely difficult to establish the history of land use and, consequently, the recovery time from certain areas. For instance, the initial idea was to establish a chronological sequence of disturbance to classify the areas with a recovery time and investigate the nutrients dynamics in each of them. According to the mill administration, clear-cut in the chosen forest areas had never happened, they guaranteed the forests were old-growth forests. However, as we established the plots and with the first samplings in Coruripe, it became clear that it would not be possible to establish a chronological sequence of disturbance. We could not find a reference area for that. Signals of fire, selective logging and the presence of charcoal in soil samples of some plots could be observed in all areas. This is the reason why it was difficult for us to determine in which stage of forest succession the areas were without a botanist help in the field. Therefore, taking into account the initial information, we considered the studied plots as old-growth forest areas under chronic disturbances.

In order to have more preserved areas included in our experiment, a fragment of 3500 ha, which we called Serra Grande Fragment (hereafter SG), was added to our experiment (Figure 3).



**Figure 3:** (a) Alagoas state with limits of the region where Serra Grande fragment is located; (b) Serra Grande fragment location near the border of Alagoas and Pernambuco states, Brazil; (c) Serra Grande fragment, where the plots were established (plots P1, P2, P3 and P4). Source: Google Earth (Central area of Serra Grande fragment: 9°00'22.17"S and 35°51'36.45"W).

SG is 200 km far from Coruripe and 450 m above the sea level (a.s.l), with the predominance of *Latosolo Amarelo - Vermelho* and *Argissolo Amarelo - Vermelho*, according to the Brazilian Soil Classification (IBGE 1985). We are aware that the difference between altitudes of Coruripe (100 m a.s.l - Lowland) and SG (450 m a.s.l - Submontane) could be not ideal for comparison purposes. However, from the few studies describing the floristic composition in the landscapes of the northeastern Atlantic Forest, they have shown, for instance, that Lowland and Montane (above 600 m) of the northeastern coastal Atlantic Forest are similar in floristic composition (Tavares et al. 2000; Ferraz 2002). Therefore, we expected SG to be similar to the other areas despite the differences in altitude. Additionally, there were no other alternatives to overcome the problem faced in the field in terms of the high degree of chronic disturbances observed in all Coruripe areas, and choosing SG was the best option in

order to have a better preserved northeastern Atlantic Forest fragment included in our survey for comparison.

According to Prof. Marcelo Tabarelli of the Federal University of Pernambuco, SG is known to be one of the best preserved of the Pernambuco Endemism Center, which is a biogeographical area that used to have 56,000 km<sup>2</sup> of the Northeastern Atlantic Forest, but currently has only small fragments. SG is larger than the average of the remnants of this endemism center and presents late successional stage characteristics of the vegetation (Oliveira et al. 2004). As in Coruripe, the mill protects its fragments against selective logging, poaching, and fire, but this protection is not totally effective, since even in smaller scale, selective logging and poaching still happen (Tabarelli et al. 2004). The mean annual precipitation in the region is approximately 2000 mm, with the dry season ranging from November to January, and the mean annual temperature is 23°C (Oliveira et al. 2004). Most of the trees are in the families Fabaceae, Lauraceae, Sapotaceae, Euphorbiaceae, Chrysobalanaceae, and Lecythidaceae (Tabarelli et al. 2010).

Due to the presence of a rugged relief in the areas, we prioritize the establishment of all plots in plateaus. We assessed the AGLB and forest structure in three areas in Coruripe (areas I, II and III), consisting each of them of four plots of 1000 m<sup>2</sup> (0.1 ha), or 0.4 ha in total; additionally, in restoration four plots of 0.1 ha were also established. In area I (280 m far from the edge) the plots were called A, B, C and D; in area II (280 m from the edge), E, F, G and H were established and in area III (260 m from the edge), plots I, J, K and L. Finally, in restoration, plots were called M, N, O and P. And in SG, four plots of 0.1 ha were established, named P1, P2, P3 and P4 (the closest one to the edge was established 190 m far away from it).

### **2.2.2 Forest Structure Measurements**

All trees with diameter at breast height (DBH)  $\geq 10$  cm in areas I, II, III and SG, and all individuals with DBH  $\geq 5$  cm in restoration were tagged, perimeter was measured and overall height of the individuals was taken. The perimeter was measured with a measurement tape at 1.3 m above the ground and the total tree height was measured using a laser range finder (Impulse-200LR, Laser Technology Inc., Englewood, Colorado). The height measurements were not taken at individuals with sharply inclined heights or where it was not possible to target appropriately the top of the trees. DBH was obtained by dividing perimeter by  $\pi$ .

The basal area (BA) was estimated according to the following equation:

$$BA = \pi r^2 \dots\dots\dots(1)$$

where,  $\pi$  is equal to 3.1416 and  $r$  is the radius of each measured tree obtained by dividing DBH by 2.

### **Biomass estimation**

The AGLB was estimated in old-growth forest plots, where we considered  $DBH \geq 10$  cm, by the following pantropical allometric equation (Chave et al. 2015):

$$AGLB_{est} = 0.0673 (\rho D^2 H)^{0.976} \dots\dots\dots(2)$$

where,  $D$  is the DBH,  $H$  is the tree height and  $\rho$  is the average wood density equal to 0.645. As it was not part of this study to identify the plant species of the areas, in addition to the difficulties faced during the field campaigns, identification of species composition of the areas were not performed. Therefore, for mean wood density used (0.645) is based on a study developed by Chave et al. (2006), where they analyzed regional, taxonomic, and phylogenetic variation in wood density of 2456 trees species of eight geographical regions in Central and South America, considering dry and wet forests and also including the Atlantic Forest of Brazil.

### **2.2.3 Statistical Analyses**

The plots of each area were treated as repetitions of a block. To test for differences of basal area and AGLB between the blocks, it was used the general linearized model (GLM). All differences to 5% probability were considered significant. The analyses were performed using the statistical package STATISTICA version 13 for Windows (STATSOFT, Inc. 2013).

## **2.3 Results**

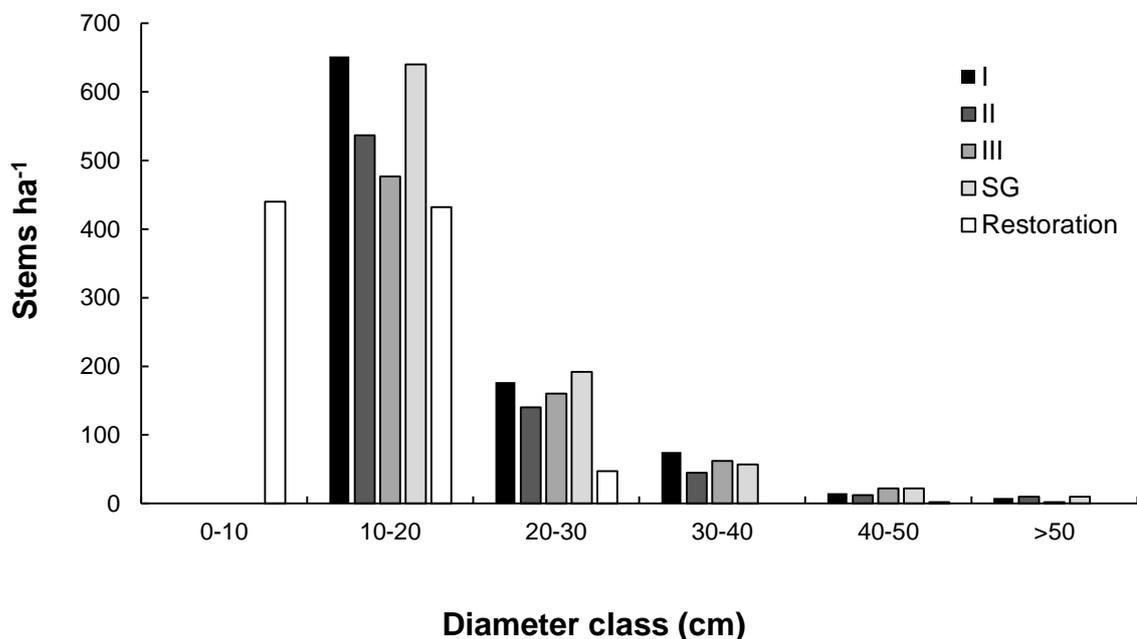
### **2.3.1 Forest Structure**

Considering the number of stems in each studied plot, there were no significant differences ( $p > 0.05$ ) while comparing forest areas I, II, III and SG. Overall, a total of 927

stems  $\text{ha}^{-1}$  in area I were found, 745 stems  $\text{ha}^{-1}$  in area II, 725 stems  $\text{ha}^{-1}$  in III, and 922 stems  $\text{ha}^{-1}$  in SG. The stem density in restoration reached 990 stems  $\text{ha}^{-1}$ .

The stems density distribution within the 10-20 cm DBH in Coruripe ranged from 477 to 652 stems  $\text{ha}^{-1}$  and from 140 to 177 stems  $\text{ha}^{-1}$  within the 20-30 cm diameter class (Figure 4). SG results were similar, where 640 stems  $\text{ha}^{-1}$  were within 10-20 cm. The 20-30 cm DBH class accounted for around 192 stems  $\text{ha}^{-1}$  in SG. Considering the 30-40 cm class, the stems density decreased, varying from 45 to 75 stems  $\text{ha}^{-1}$  in Coruripe and accounting for 22 stems  $\text{ha}^{-1}$  in SG (Figure 4). For the 40-50 cm class, in Coruripe the stems density varied from 12 to 22 stems  $\text{ha}^{-1}$ , and in SG it was 22 stems  $\text{ha}^{-1}$ . In all areas, stems density of trees with diameter  $\geq 50$  cm was lower than 10 stems  $\text{ha}^{-1}$ . Areas I and II had 8 and 10 stems  $\text{ha}^{-1}$  with DBH higher than 50 cm, respectively. While in area III there was only 2 stems  $\text{ha}^{-1}$ . The largest individual was found in area II, measuring 82 cm DBH. In SG, there were 10 stems  $\text{ha}^{-1}$  with DBH  $\geq 50$  cm, representing only 1 % of the total. The largest individual found in SG had a DBH of 60 cm.

For the total of stems sampled in restoration area, 440 stems  $\text{ha}^{-1}$  were within the DBH  $\leq 10$  cm, and 432 stems  $\text{ha}^{-1}$  were within the 10-20 cm diameter class (Figure 4).



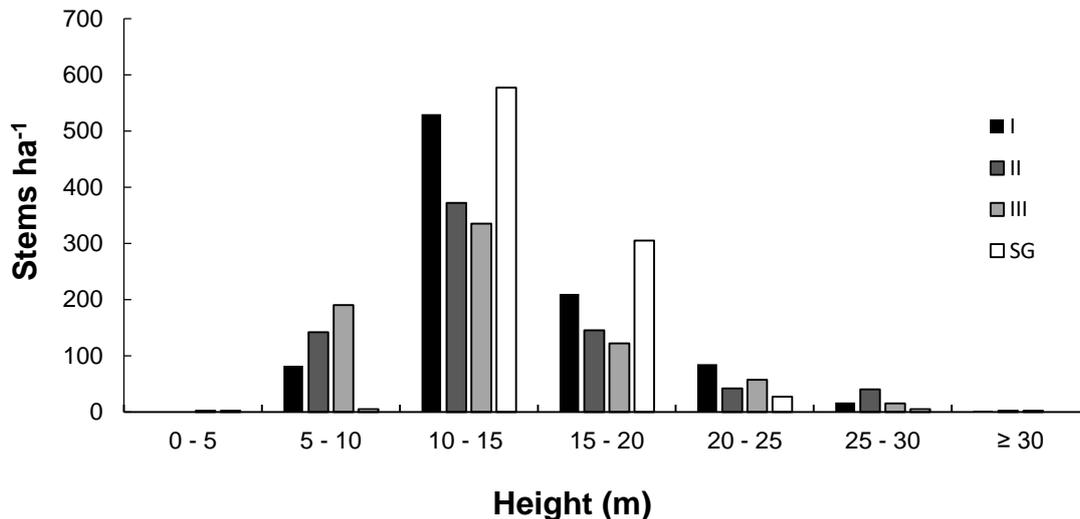
**Figure 4:** Stems  $\text{ha}^{-1}$  considering different DBH classes in areas I, II, III, SG and restoration, established in Coruripe and Serra Grande lands.

In all old-growth forest areas, approximately 68% of trees were shorter than 15 m. Significant shorter stems were found in area III ( $13 \pm 4.7$  m) ( $p < 0.05$ ) when compared to areas

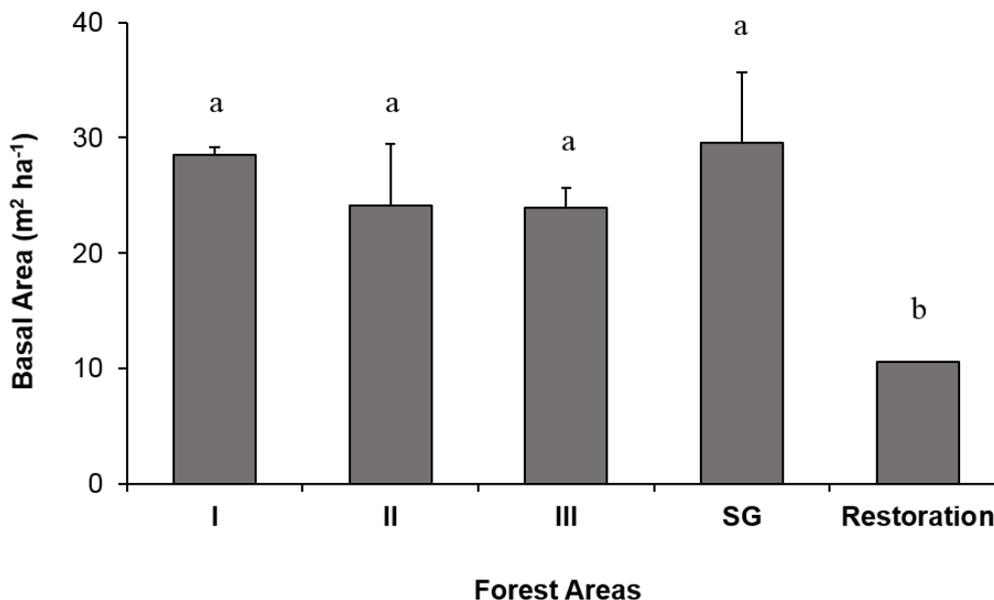
I ( $14 \pm 4.2$  m), II ( $14 \pm 5$  m) and SG ( $14 \pm 2.6$  m) (Figure 5). The tallest individual in SG was 26 m in height. In SG only 3.5% of the individuals were grouped within the 20 – 30 m height interval, and in areas I, II and III this percentage varied between 10 – 11% (Figure 5). The tallest stem in area I measured approximately 31 m, in area III, 38 m and area II held the tallest one, with almost 48 m.

Stems height was shorter in restoration, with half of the individuals reaching only 6 m (50%) and 19% measuring 8 m high. Only 7% of the individuals were between 8 and 12 m, with the tallest stem measuring around 18 m high (Figure 5).

The basal area ranged from 24 to 30  $\text{m}^2 \text{ha}^{-1}$  (Figure 6), with no significant differences ( $p > 0.05$ ) between old-growth forest areas. Restoration had a basal area of 10  $\text{m}^2 \text{ha}^{-1}$ , which was significantly different from the other areas in Coruripe and SG ( $p > 0.05$ ).



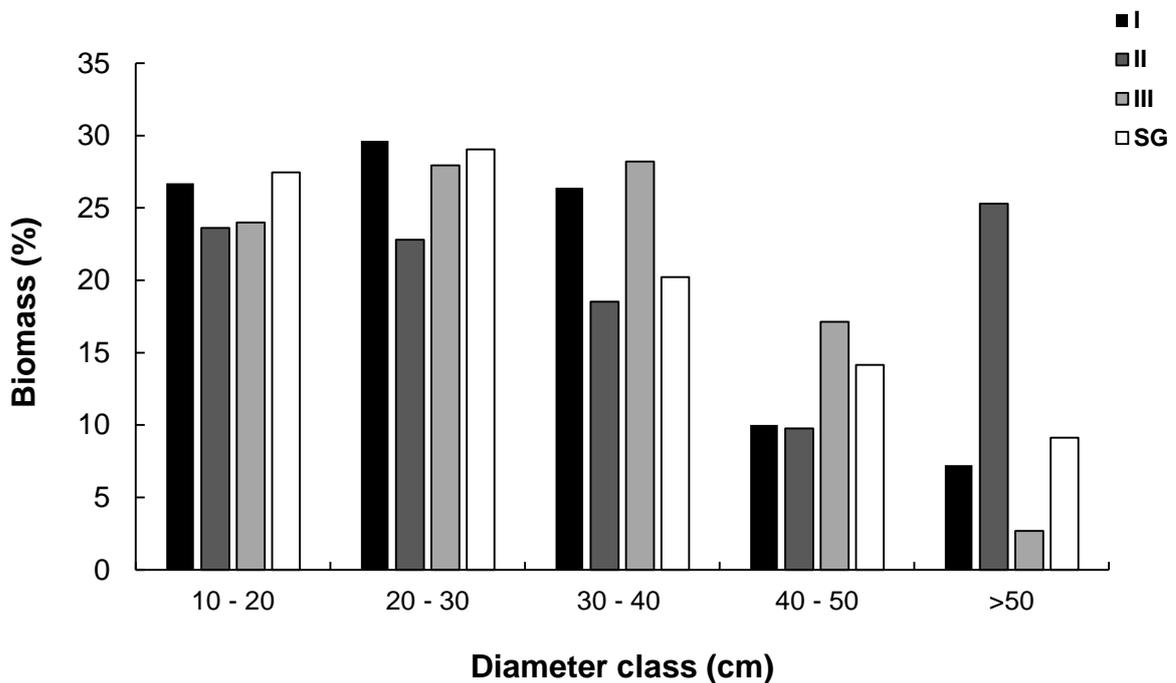
**Figure 5:** Stems number of height classes in areas I, III and II and SG.



**Figure 6:** Basal area in different areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil. Values are means  $\pm$  sd n = 4.

### 2.3.2 Aboveground Live Biomass (AGLB)

AGLB ranged from  $164 \pm 7 \text{ Mg ha}^{-1}$  (area III) to  $222 \pm 47 \text{ Mg ha}^{-1}$  (SG), with no statistical differences ( $p > 0.05$ ) among the old-growth forest areas. In area II only four individuals  $\geq 50 \text{ cm}$  were responsible for  $52 \text{ Mg ha}^{-1}$ , which represents 25 % of the total biomass found in this area (Figure 7).



**Figure 7:** (A) Diameter at breast height (DBH) distribution; and (B) percentage of biomass by diameter class (DBH cm) in different areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil.

## 2.4 Discussion

Stems density, basal area and AGLB are reliable attributes to predict stages of forest succession (Chazdon 2003). If we consider the stems density of our study by DBH class in old-growth forest areas only, it is represented by a reversed J-shaped curve distribution (Figure 5), with large trees often absent. The density of stems ( $\text{DBH} \geq 10 \text{ cm}$ ) per hectare varied from 725 to 928 stems  $\text{ha}^{-1}$ , which are lower than those found in other Northeastern forest fragments (Table 1). However, it is worth saying that most studies related to forest structure in the Northeast include stems with  $\text{DBH} \geq 5 \text{ cm}$  in their inventories (Table 1). Our data were similar

to the stems density reported by Mendes et al. (2016) on the edge of a humid Brazilian Northeastern Coastal Atlantic Forest fragment, in Igarassu, Pernambuco state ( $760 \text{ stems ha}^{-1}$ ) ( $\sim 1690 \text{ mm yr}^{-1}$  and  $25^\circ\text{C}$ ) (Table 1). Rodal and Nascimento (2006) found a total of  $1525 \text{ stems ha}^{-1}$  in a dry Brazilian northeastern Atlantic Forest in Serra Negra Biological Reserve, between Floresta and Inajá, Pernambuco state, with an average precipitation of  $900 \text{ mm yr}^{-1}$  and  $25.3^\circ\text{C}$  average annual temperature. It is important to remember that they included  $\text{DBH} \geq 5 \text{ cm}$ . In addition, comparing our results to the Southeastern Atlantic Forest, we found similar stems density averages in *Restinga* (0 m) and Submontane (400 m), while Lowland (100 m) and Montane (1000 m) physiognomies have higher averages (Alves et al. 2010) (Table 1). Submontane is located on a very steep slope constantly suffering landslides, which frequently exposes young soils, creating gaps and making disturbances in this forest physiognomy (Furian et al. 1999). This characteristic may explain why the stem density is similar to that found in our study. Finally, in the South of Brazil, Paraná state, Viani et al. (2011) found stems density similar to those of the northeast fragments (Table 1). However, it is worth saying that their sampling design also included  $\text{DBH} \geq 5 \text{ cm}$ , which makes the difference because smaller stems usually encompasses the higher number of stems.

In a study conducted in secondary forests in the State of Bahia, it was found that the basal area increased in 30 years from  $11.8 \pm 4 \text{ m}^2 \text{ ha}^{-1}$  to  $29.0 \pm 7.9 \text{ m}^2 \text{ ha}^{-1}$  (Table 1) (Piotto et al. 2009). Despite that, after 30 years, the basal area was still smaller than the old-growth forest area used for comparison ( $45.6 \text{ m}^2 \text{ ha}^{-1}$ ). These results show that it takes more than 30 years for a forest in that region to acquire old-growth forest characteristics after clear-cut (Piotto et al. 2009). In another study that has been conducted in areas of the Atlantic Forest, also located in Bahia, and including different forest cover characteristics, most of the basal areas was within a range of  $10$  to  $15 \text{ m}^2 \text{ ha}^{-1}$ , with only a few values higher than  $20 \text{ m}^2 \text{ ha}^{-1}$  (Moraes 2017). The areas average basal area of our study ranged from  $24 \text{ m}^2 \text{ ha}^{-1}$  to  $30 \text{ m}^2 \text{ ha}^{-1}$ , which are comparable to those results found by Piotto et al. (2009) for 30 years old forest (Table 1). We speculate that our average basal area is similar to forest in late recovery from clear-cut because Coruripe and Serra Grande sugar cane Mills have been protecting the areas for around 40 years, decreasing the amount of wood extraction and poaching by the local population. Our average basal areas are also comparable to those found in the edge and interior forests investigated in Pernambuco (Mendes et al. 2016). These authors also reported that the forest interior had been disturbed at some point, due to the observation of greater trees recruitment and lower mortality rates found in their studied plots. Additionally, our data are comparable to those found in São Lourenço da Mata ( $23.9 \text{ m}^2 \text{ ha}^{-1}$ ), and to those of Mata dos Macacos (varying from  $28$  to  $35 \text{ m}^2 \text{ ha}^{-1}$ ), both in

Pernambuco state (Table 1). The basal area of the Southeastern Atlantic Forest physiognomies along the altitudinal gradient ranged from 24.5 to 37.5 m<sup>2</sup> ha<sup>-1</sup> (unpublished data), where *Restinga* and Lowland had the lowest values, 24.5 m<sup>2</sup> ha<sup>-1</sup> and 26.6 m<sup>2</sup> ha<sup>-1</sup>, respectively, compared to Submontane (37.5 m<sup>2</sup> ha<sup>-1</sup>) and Montane (36.6 m<sup>2</sup> ha<sup>-1</sup>) forests (unpublished data). Therefore, our data are similar to those found in *Restinga* and Lowland forests. Additionally, our data were lower than those found in the further south of the Atlantic Forest, Brazil. The basal area found there varied from 33.2 to 46.2 m<sup>2</sup> ha<sup>-1</sup> (Viani et al. 2011). However, the authors highlight that the higher basal area found in the region may be related to the multistems trait of the individuals in this area due to past antropogenic disturbances. Finally, comparing our average basal area (27 m<sup>2</sup> ha<sup>-1</sup>) to the Amazonian sites, Manaus, Rio Branco and Santarém, we found also that they are in the same range (Vieira et al. 2004). Based on the aforementioned discussion, it is fair to conclude that fragments of Coruripe and Serra Grande are in a late stage of recovery from chronic disturbances suffered in the past, including poaching, fire events, and timber exploitation.

Regarding the AGLB estimative, that basically adds tree heights and wood density to basal area, our results varied from 196 Mg ha<sup>-1</sup> (Coruripe) to 222 Mg ha<sup>-1</sup> (SG), which are similar only to the one found in Lowland forest (198 Mg ha<sup>-1</sup>) of the Southeastern Atlantic Forest by Alves et al. (2010). Higher than that found in *Restinga* (154 Mg ha<sup>-1</sup>), but lower than those found in Submontane and Montane forests (Table 2). Moreover, when compared to the Amazonian sites, Manaus, Santarém and Rio Branco, the average biomass of the present study was smaller than all of them (Table 2). When compared to other Neotropical forests, the average AGLB of the Northeastern Coastal Atlantic Forest areas were between the lowest ones, more comparable to the Southeastern Atlantic Forest results (Table 2). There were no AGLB data available for the Northeastern fragments, where basal area was measured. Therefore, we lack data for a more detailed comparison between our fragments and other fragments of the Northeast region. With the data available, it is clear that the AGLB of Coruripe and Serra Grande fragments are similar to the Southeastern Atlantic Forest physiognomies and lower than the Amazon basin forests. We hypothesized that this different relation to the Amazon is due to the lack of the so called large trees (DBH ≥70 cm). Most of trees biomass (88%) are located in large branches and in trees trunks (Brown et al. 1995), then it is expected that large trees would keep higher amounts of AGLB. Consequently, if large trees are rare in the fragments, the lower AGLB found in the present study, when compared to other Neotropical forests, can be explained by the absence of higher number of these large individuals.

**Table 1.** Basal area and stems density comparison between Northeastern Coastal Atlantic Forests, Southeastern Coastal Atlantic Forests altitudinal gradient, and Amazon forest areas.

	Basal Area (m <sup>2</sup> ha <sup>-1</sup> )	Stems ha <sup>-1</sup>	DBH (cm)	Altitude (m)	References
<b>Northeastern Atlantic Forests</b>					
Mata do Curado 2, Recife - Pernambuco	41.7	970	≥ 5	22	Espig 2003
Mata de Dois Irmãos, Recife - Pernambuco	41.5	549	≥ 5	60	Guedes 1992
Gurjaú Ecological Reserve, Cabo de Santo Agostinho - Pernambuco	32.6	1166	≥ 5	115	Silva Jr. 2004
Mata dos Macacos, Igarassu - Pernambuco	31.5	1482	≥ 5	135	da Silva et al. 2000
<b>Serra Grande - Alagoas</b>	<b>30.0</b>	<b>943</b>	≥ 10	<b>450</b>	<b>This study</b>
Serra do Conduru, Southern Bahia - 40 yr old fragment	29.0	3025	≥ 10	120-300	Piotto et al. 2009
Mata do Zumbi, Cabo de Santo Agostinho - Pernambuco	27.5	1657	≥ 5	70	Siqueira et al. 2001
Mata de BR, Igarassu - Pernambuco - Interior	27.3	1455	≥ 5	20-85	Mendes et al. 2016
Serra do Conduru, Southern Bahia - 25 yr old fragment	27.0	-	≥ 10	120-300	Piotto et al. 2009
Mata de BR, Igarassu - Pernambuco - Edge	26.3	1525	≥ 5	20-85	Mendes et al. 2016
<b>Coruripe - Alagoas</b>	<b>26.0</b>	<b>869</b>	≥ 10	<b>100</b>	<b>This study</b>
Mata do Curado 1, Recife – Pernambuco	24.7	780	≥ 5	26	Lins-e-Silva and Rodal 2004
Mata do Toró, São Lourenço da Mata - Pernambuco	23.9	1145	≥ 5	120	Andrade and Rodal 2004
Mata de BR, Igarassu - Pernambuco - Intermediate	19.6	1460	≥ 5	20-85	Mendes et al. 2016
Serra do Conduru, Southern Bahia - 10 yr old fragment	11.8	1530	≥ 10	120-300	Piotto et al. 2009
<b>Southeastern Atlantic Forest</b>					
Dense Ombrophyllus Forest - Submontane, Ubatuba, Brazil	37.5	721	≥ 10	400	Alves et al. (2010)
Dense Ombrophyllus Forest - Montane, Ubatuba, Brazil	36.6	589	≥ 10	1000	Alves et al. (2010)
Dense Ombrophyllus Forest - Lowland, Ubatuba, Brazil	26.6	543	≥ 10	100	Alves et al. (2010)
<i>Restinga</i> , Ubatuba, Brazil	24.5	763	≥ 10	10	Alves et al. (2010)

### Southern Atlantic Forest

Mata do Palmital, Paraná, Brazil	38.5	1327	$\geq 5$	450	Viani et al. (2011)
Mata do Polonês, Paraná, Brazil	46.2	1557	$\geq 5$	550	Viani et al. (2011)
Mata das Antas, Paraná, Brazil	33.3	1553	$\geq 5$	550	Viani et al. (2011)

### Amazonian Forests

Tropical Forest - Lowland, Manaus, Brazil	30.0	626	$\geq 10$	50 - 100	Vieira et al. (2004)
Tropical Forest - Lowland, Santarém, Brazil	25.0	460	$\geq 10$	175	Vieira et al. (2004)
Tropical Forest - Lowland, Rio Branco, Brazil	23.0	467	$\geq 10$	214	Vieira et al. (2004)

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Considering that 1329 individuals (average of 830 stems  $\text{ha}^{-1}$ ) were inventoried in 1.6 hectares, and taking into account that the average basal area found in our old-growth forest areas were not totally different from those found in mature forests (e.g. Amazonian forests), the presence of only one tree with the  $\text{DBH} \geq 70$  cm and a dozen individuals with  $\text{DBH} \geq 50$  cm suggest a lack of large trees. What are the reasons for such low incidence of these individuals and the presence of only one large individual ( $\text{DBH} \geq 70$  cm, according to Clark and Clark, 1996; Turner 2001; Laurance et al. 2000) in a tropical forest characterized by high heterogeneity and high biodiversity and endemism (Morellato and Haddad 2000; Talora and Morellato 2000; Lacerda 2001)? For individuals of this size, our results do not even represent 1 % of the total inventoried, which is an amount considerably under the percentage usually found for non-disturbed tropical forests, usually between 1 and 6% of trees with  $\geq 10$  cm  $\text{DBH}$  (Clark and Clark 1996). Some studies have been reporting this characteristic of lower large trees incidence in the edges of fragments (e.g. Oliveira et al. 2008; Laurance et al. 2000). For instance, Oliveira et al. (2008) in a study conducted in 109 fragments of Serra Grande, including the fragment where we conducted our experiment, found a considerable impoverishment of large trees on the edges of fragments and in small fragments, compared to the interior of the old-growth forest fragments. Possible explanations rely on: edge effects and chronic disturbances.

Past and present human disturbances are common and important factors interfering in the Atlantic Forest structure and biomass (Denevan 1992; Chazdon 2003; Tabarelli et al. 2004). Santos et al. (2008), studying the fragments of Serra Grande, including the one of the present study, concluded that the presence of large individuals on the edges is rare, compared to the interior of the fragment. The existence of large trees may be affected by edge effects due to several factors: (1) increased wind turbulence near the edges, exposing the roots (D'Angelo et al. 2004) (2) microclimate changes, such as increased light intensity and evaporation and consequent physiological stress, since large trees are vulnerable to drought (Laurance et al. 2000); and (3) the presence of lianas that in abundance on the edges interfere on the survival of these individuals (Laurance et al. 2000). Besides that, Laurance et al. (1998b) demonstrated that edge effects caused by the wind in the Amazon can be seen in the interior of the forest up to 300-400 m from the edges. Therefore, distance to forest edges of the established plots for the present experiment ranged from 190 to 280 m and should be considered as one of the factors that influenced the structure of our areas, where only 12 individuals with  $\text{DBH} \geq 50$  cm were found.

Another possible explanation for the absence of large trees in the studied areas is the past selective logging and fire in the areas, both representing examples of chronic disturbances

(Arroyo-Rodríguez et al. 2017). For instance, charcoal was found in soil samples of area I and a few cut tree trunks could be seen in most areas, as a signal of selective logging. Our field assistant in Coruripe, Nilo Pinto, a former lumberjack, witnessed selective logging for commercial purpose some decades ago, before the establishment of more restricted environmental laws during the 1980's. Additionally, in the middle of the eighteenth century, there was a large naval industry in Salvador, Bahia state, from where merchant vessels and war ships constituted the greater part of the fleet under Portuguese control (Dean 1996). The wood for these ships was obtained in Pernambuco and Alagoas and in the south of Bahia. In these places, large patches of old-growth forest persisted until the end of the eighteenth century (Dean 1996). The wood from Alagoas had a great value for its shapeless, usefull for ribs, bows and sterns (Dean 1996), suggesting that chronic disturbances in the Northeastern Atlantic Forest happen since long time ago until the present days.

According to Oliveira et al. (2008), it can be found complete sets of plants and vertebrates in Serra Grande fragments, present only in well preserved areas of the Atlantic Forest. Despite this finding, Tabarelli et al. (2004) points out that the presence of wood extraction and poaching by the local population, still happens today, even under the protection of Serra Grande Mill. Serra Grande is the best preserved fragment of Atlantic Forest in northeastern Brazil, but it is not intact. The same is true for the forest fragments owned by Coruripe Mill, where there is great care in protecting the fragments; however, ceasing chronic disturbances seems to be virtually impossible (Ribeiro et al. 2015).

**Table 2.** Aboveground Live Biomass (AGLB) of Neotropical Forests. Source: Alves et al. (2010). MAP = Mean Annual Precipitation.

	MAP (mm yr <sup>-1</sup> )	AGLB (Mg ha <sup>-1</sup> )	Stems ha <sup>-1</sup>	References
<b>Neotropical Forests</b>				
Tropical Forest seasonaly dry - Lowland, Mexico	1650	109	-	Vargas et al. (2008)
Tropical Forest - Lowland, La Selva, Costa Rica	4000	149	367	Clark and Clark (2000)
Tropical Forest - Montana, Venezuela	3000	157	-	Delaney et al. (1997)
Tropical Forest - Montana, Venezuela	2400	173	-	Delaney et al. (1997)
Tropical Forest - Lowland, Venezuela	2500	179	-	Delaney et al. (1997)
Tropical Forest - Lowland, BCI, Panama	2600	179	377	DeWalt and Chave (2004)
Tropical Forest - Lowland, La Selva, Costa Rica	4000	203	471	DeWalt and Chave (2004)
Semideciduous Tropical Forest - Submontana, La Chonta, Bolivia	1580	237	129	Broadbent et al. (2008)a
Tropical Forest - Lowland, Manaus, Brazil	2650	240	654	DeWalt and Chave (2004)
Tropical Forest - Lowland, Rio Branco, Brazil	1940	244	467	Vieira et al. (2004)
Tropical Forest - Lowland, BCI, Panama	2600	260	461	Chave et al. (2003)b
Tropical Forest - Lowland, Santarem, Brazil	1909	281	460	Vieira et al. (2004)
Semideciduous Tropical Forest - Lowland, Roraima, Brazil	2083	292	364	Nascimento et al. (2007)
Tropical Forest - Lowland, Santarem, Brazil	1920	295	498	Rice et al. (2004)
Tropical Forest - Lowland, Santarem, Brazil	1909	298	441	Pyle et al. (2008)
Tropical Forest - Lowland, Rondonia, Brazil	2300	307	450	Cummings et al., 2002
Tropical Forest - Lowland, Manaus, Brazil	2650	308	518	Castilho et al. (2006)b
Lowland wet forest, Nouragues, French Guiana	2757	317	534	Chave et al. (2001)
Tropical Forest - Lowland, Manaus, Brazil	2650	326	623	Nascimento and Laurance (2002)
Tropical Forest - Lowland, Cocha Cashu, Peru	2165	333	666	DeWalt and Chave (2004)
Tropical Forest - Lowland, Manaus, Brazil	2650	334	621	Pyle et al. (2008)
Tropical Forest - Lowland, Manaus, Brazil	2650	360	626	Vieira et al. (2004)

### Atlantic Forest, Brazil

Semideciduous Tropical Forest - Lowland, Linhares, Brazil	1200	335	-	Rolim et al. (2005)
<b>Open Ombrophyllus Forest, Coruripe, Brazil</b>	<b>1500</b>	<b>196</b>	<b>869</b>	<b>This study</b>
<b>Open Ombrophyllus Forest, Serra Grande, Brazil</b>	<b>2000</b>	<b>222</b>	<b>943</b>	<b>This study</b>
<i>Restinga</i> , Ubatuba, Brazil	2640	154	763	Alves et al. (2010)
Dense Ombrophyllus Forest - Lowland, Ubatuba, Brazil	2200	198	543	Alves et al. (2010)
Dense Ombrophyllus Forest - Submontane, Ubatuba, Brazil	2640	239	721	Alves et al. (2010)
Dense Ombrophyllus Forest - Montane, Ubatuba, Brazil	3000	263	589	Alves et al. (2010)

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## 2.5 Conclusions

This study provided the first AGLB estimates for the Northeastern Coastal Atlantic Forest fragments under chronic disturbances, improving the AGLB estimative for the whole Atlantic Forest biome.

The presence of only one large tree decreased the AGLB found in our study areas, since most of the forest biomass is stored in large individuals (Brown et al, 1995; Martinelli et al., 2000; Alves et al, 2010). Despite that, our averages were similar to those found in the Southeastern Coastal Atlantic Forest physiognomies. In addition, our results showed that even though a disruption in the forest structure was observed with the almost absence of large trees, the basal areas are still similar to other Northeastern Coastal Atlantic Forest and to the old-growth forests basal areas. Consequently, considering that these parameters are known as good proxies for C stocks estimative and, consequently, for C emissions estimative (Houghton 2007; Saatchi et al. 2007; Houghton et al. 2009), the capacity of these fragments in stocking C cannot be diminished. It is very important to consider that this highly threatened ecosystem can represent an important role to the global C cycle, preventing higher CO<sub>2</sub> emissions and functioning as a sink of C. The understanding the Northeastern Atlantic Forest fragments structure and AGLB distribution is important when we consider that they are the best preserved forest remnants also harboring endemic species of the Pernambuco Endemism Center, besides representing the only areas for C stocks in this highly disturbed landscape. Therefore, we claim that it is of high priority to protect these areas, and make conservation and sustainable management plans.

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### 3 FOLIAR CARBON, NITROGEN AND PHOSPHORUS CONCENTRATIONS AND FLUXES THROUGH LITTERFALL IN FRAGMENTS OF THE BRAZILIAN NORTHEASTERN COASTAL ATLANTIC FOREST

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#### Abstract

The Atlantic Forest is one of the most important biomes of Brazil. Originally covering approximately 1.5 million of km<sup>2</sup>, today it has been reduced to 12 to 15 % of its original cover. The Northeastern Coastal Atlantic Forest is the most threatened portion of the biome, with only a few remnants left. In this study we explore how fragmentation affects the dynamics of carbon (C), nitrogen (N) and phosphorus (P) in forest fragments of Alagoas state. We investigated foliar and litterfall leaves concentrations of these elements in 16 0.1-ha old-growth forest area plots established in the state of Alagoas, Brazil, in addition to four 0.1-ha plots in a restored area. Our main finding in this chapter was that litterfall flux in old-growth forest areas (6 Mg ha<sup>-1</sup> yr<sup>-1</sup>) was lower than the mean litterfall flux found in more than 100 forest plots scattered over the Atlantic Forest biome (7 Mg ha<sup>-1</sup> y<sup>-1</sup>). The nitrogen (N) litter concentration was similar to other tropical forests, and N flux in old-growth forest areas of Alagoas was in the range found in other tropical forests. Accordingly, nutrient use efficiency (NUE) of N was similar to NUE-N found in other areas of the Atlantic Forest. The phosphorus (P) leaves litterfall concentration was lower than in other tropical forests. As a consequence, the P flux via litterfall was significantly lower, leading to much higher NUE-P in the forest fragments of the Alagoas. Therefore, with our findings there were not much change in N cycles, while P seems to be a very limiting nutrient in the fragments.

Keywords: Nutrients, Nutrient use efficiency, Leaves, Tropical forest

### 3.1 Introduction

Terrestrial ecosystems directly affect the climate regimes besides suffering the effects of climate (Cox et al. 2000; Yurova et al. 2010). As one of the most important ecosystems, tropical forests are responsible for stocking large amounts of carbon (C) and nitrogen (N) above and belowground (Melillo et al. 1993; Brown and Lugo 1984; Dixon et al. 1994; Brown et al. 1995; Phillips et al. 1998; Houghton 2005; Houghton et al. 2009). Changes in land use of tropical forests have increased the number of areas exposed to edge effects, posing a threat to the systems, limiting the persistence of late species that are characteristic of primary forests and crucial for stocking and fixing atmospheric carbon C in relation to young species or smaller individuals (Tabarelli et al 2008; Martinelli et al. 2000).

Due to deforestation there is only 15%, 12%, 8% of tropical forests in Asia, America and Africa, respectively (Hansen and Defries 2004), creating forest fragments inserted in different matrixes and degrading the few remaining forests (Wright 2005). According to Laurance et al. (2000), with fragmentation the exposure of large trees at forest edges may cause a 40% increase in the mortality rate and may die three times faster than those located in the interior of the forest (> 300 meters). Most of the changes in fragmented landscapes leads to changes in the long run, the persistence of certain groups of species in these disturbed landscapes, as well as variations in the cycling of nutrients and energy flow (Laurance and Bierregaard 1997; Bierregaard et al. 2001). Disturbance of ecosystems which are in equilibrium causes changes in the environment, as loss of nutrients by reducing their stocks in the ecosystem, altering the biogeochemical cycling and thus resulting in a nutritional limitation of vegetation (Jordan 1985; Brown and Lugo 1994).

Climate, composition of species, successional status and soil fertility are the most important factors influencing the nutrient cycling in forest ecosystems (Vitousek and Reiners 1975, Bormann and Likens 1979, Jordan and Herrera 1981, van Cleve et al. 1983, Vitousek 1982). For instance, higher amounts of N cycle through lowland tropical forests compared to temperate forests (Proctor et al. 1983, Vitousek 1984, Vitousek and Sanford 1986). Tropical ecosystems are very heterogeneous, and Vitousek and Sanford (1986) have shown that soil fertility changes with soil types even within lowland tropical forests. These forests also show higher rates of N cycling and losses in lowland than temperate forests (Vitousek and Denslow 1986, Lewis 1986, Matson et al. 1987, Neill et al. 1995), suggesting that N is in excess in tropical forests, with some exceptions, such as white-sand and montane (Salati et al. 1982; Cuevas and Medina 1988). Regarding the phosphorus (P) availability, the main source of P to

the ecosystem is through the weathering of mineral present in the parent material (Lajtha and Schlesinger 1988). Some studies have shown that P is a limited nutrient in highly weathered soil of the lowland tropical forests (e.g. Vitousek 1984; Vitousek and Sanford 1986; Tiessen et al. 1994; Raaimakers et al. 1995; Sollins 1998). Despite the lack of more data to better understand in which extent tropical ecosystems are limited in P, it is established now that the supplies of N and P to plants present high variation across the terrestrial biomes (Jenny 1950, Walker and Syers 1976, Vitousek and Sanford 1986, Vitousek and Farrington 1997, Sollins 1998, Chadwick et al. 1999, Hedin et al. 2003, McGroddy et al. 2004).

Investigating foliar nutrients concentrations is a way to predict the availability of nutrients in the soil for vegetation, and the cycling of the elements in tropical forests (e.g., Grubb 1977, Aerts and Chapin 2000; Hobbie and Gough 2002; Vitousek 2004). However, these analyses do not account for the reabsorption of nutrient and energy before leaf senescence (Grubb 1977), and besides foliar, leaf litterfall N:P ratios are used to predict the presence of these nutrients in the soil, since litterfall fluxes are one of the major pathways for returning the nutrients from vegetation to soil (Herrera et al. 1978; Montagnini and Jordan 2002). Generalizations for stoichiometry are reliable for mature forests, however, in secondary forests changes on nutrient cycling as a consequence of the rapid changes in the land-use are not well understood (Davidson et al. 2007). The same is true for forests under chronic disturbances, such as poaching, selective logging and extraction of firewood (Arroyo-Rodríguez et al. 2017). These continuous human disturbances cause important biomass removal (Arroyo-Rodríguez et al. 2017, Martorell and Peters 2005, May-Tobin 2011, Leal et al. 2014a, Ribeiro et al. 2015) with almost unknown evidences about the consequences to nutrients cycling. Very few studies have tried to understand the effects of forest fragmentation or chronic disturbances on litterfall dynamics, for example, in spite of its importance for nutrient cycling.

The Brazilian Atlantic Forest is located on the coast and for centuries suffers from intense logging, formation of agricultural fields, the presence of farms, planting of exotic species and hunting being exposed to fragmentation and habitat loss (Tabarelli et al. 2008, Laurance 2015). In the present days, only 12-15% of this biome is left due to urbanization and agriculture (Ribeiro et al. 2009). The Brazilian Northeastern Atlantic Forest portion is the most threatened region of the whole biome, only 6% of its original area is covered by mature forest (Silva and Tabarelli 2000). However, even the mature forest fragments suffer chronic disturbances in this region. For instance, Serra Grande fragments, located in the state of Alagoas, and encompassing the best and bigger preserved fragment of the biome, have been under more strict protection against disturbances caused by human population (Tabarelli et al.

2004). The same authors points out that these activities still happen and the Northeastern Coastal Atlantic Forests are not intact.

In order to better understand how these chronic disturbances and the fragmentation of the Northeastern Atlantic Forest may affect C and nutrient cycles, as N and P, we investigated foliar and litterfall leaves concentrations of these elements. Our study sites (as it was described in chapter one) are located in two important areas of the Northeastern Coastal Atlantic Forest remnants. Therefore, our main objectives are: (1) to describe patterns of foliar and leaves litterfall N and P cycling after long-term chronic disturbances; (2) to quantify fluxes of these elements through litterfall of a one-year study; (3) to compare the nutrients use- efficiencies (NUE) in these forest areas with those of other tropical and temperate forests. We hypothesized: (i) disruptions in the distribution of N and P will be observed, such as lower N concentrations, higher N:P ratios and lower  $\delta^{15}\text{N}$  values (Davidson et al. 2007); (ii) Nitrogen use-efficiency (NUE-N) will be higher than the mature Neotropical forests averages.

## **3.2 Methods**

### **3.2.1. Study area**

The study areas are located in the Northeastern Coastal Atlantic Forest fragments in the municipalities of Coruripe and São José da Lage, both within Alagoas state, Brazil. Sixteen 0.1-ha plots were established in chronically disturbed old-growth forest areas, totalizing four study areas of 0.4-ha each, in addition to a restoration area (0.4-ha) encompassing four plots of the same size (0.1-ha). The studied areas are the same used for the study presented in the first chapter. More details regarding the areas can be found in the subheading 2.2.1.

### **3.2.2 Sampling**

For nutrients and isotopic analyzes, around five to eight healthy and mature leaves were sampled with a telescoping measuring rod (Crain Enterprise, model No. 90182), giving priority to those in the outer portion of the tree canopy receiving direct light incidence. To avoid the variability in nutrient concentration along the canopy, the leaves were randomly collected at the same height, when possible. In total 209 leaf samples were analyzed in areas I, II, III, restoration and SG. Of this total, 38 samples belong to area I, 46 to area II, 32 to area III, 35 to restoration and 58 to SG.

The litterfall sampling and carbon and nutrients fluxes estimatives were done only in areas I, II, II and restoration, within Coruripe area, with prohibitive timing to execute the same experiment in SG by the time we concluded that a more preserved area should be included in this study (see chapter 1). For litterfall sampling in Coruripe plots, five circular litter traps with 0.25 m<sup>2</sup> and a metal screen with a fine mesh of 1 x 1 mm were randomly placed in each plot 1 m above the ground, totalizing 80 traps. Litterfall samples were collected biweekly over a period of 12 months. On the average, leaf litterfall represents 70% of fine litterfall (Bray and Gorham 1964; O'Neill and DeAngelis 1980). Therefore, for the estimatives of litterfall production and carbon and nutrients fluxes, the leaves were separated from the other parts of the collected material, as fruits and branches, and then only them were prepared for chemical analyses.

### 3.2.3 Chemical Analyses

For foliar and leaf litterfall C, N and P concentrations, the sampled materials were air dried, later dried in the oven and crushed to fine powder in a Willye mill (ET - 648). Sub-samples of 2-3 mg of the prepared material were wrapped in tin capsule and total N and C were determined in a Carlo Erba 1110 elemental analyzer (Milan, Italy). The gases generated by the combustion are purified in a chromatography column and directly introduced into a mass spectrometer for the determination of the isotopic ratios (IRMS Delta Plus, Finnigan Matt, San Jose, CA, USA). Sugarcane leaves were used as reference for plant material, when analyzing the isotopes. The natural abundance of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  was expressed as parts per thousand (‰) from a standard internationally recognized by the equation:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000 \quad (1)$$

where, R is the molar ratio  $^{13}\text{C} / ^{12}\text{C}$  or  $^{15}\text{N} / ^{14}\text{N}$  in the sample and standard.

The standard used for C is Peedee Belemnite (PDB; limestone of the Grand Canyon region, USA), while for N it was used the atmospheric air as standard. The acceptable analytical error is  $\pm 0.3\%$ ,  $0.1\%$ ,  $0.3 \text{ ‰}$  and  $0.5 \text{ ‰}$  for C, N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. For the determination of total P concentration in the plant material (leaves and leaf litterfall), the digestion was carried out with perchloric acid + nitric acid (ratio 5:1) (Johnson and Ulrich 1959), using 0.5 g of sample and 6 ml of a mixture of acids. After digestion, the material was

diluted with 50 ml of deionized water. The concentration of P was determined by spectrophotometry using reactive "ammonium metavanadate + ammonium molybdate" (Jackson 1964; Lott et al 1956).

All nutritional and isotopic analyses relative to C and N were executed in Laboratory of Isotopic Ecology of CENA / USP, while total P in leaves and leaves litterfall were determined in the Brazilian Institute of Analyses (IBRA).

### **3.2.4 Litterfall fluxes and carbon and nutrients annual fluxes**

The annual litterfall mass was based on one year of experiment, from April of 2014 to March of 2015. The weighed material was divided by the area of the collector and values were then divided by the number of days of each collection interval, and extrapolated to 1 ha. The results were multiplied by 30 days to estimate the monthly leaf litterfall production for each plot and, then, summed the 12 collected months to obtain the annual leaf litterfall production.

To calculate C and nutrients annual fluxes, annual litterfall mass was multiplied by mean C, N and P concentrations in litterfall leaves. Nutrient use efficiency (NUE) was calculated as the ratios of the annual litterfall mass to its N and P fluxes (Vitousek 1982).

### **3.2.5 Statistical Analyses**

To test the differences between areas, we used the general linear model (GLM) grouping the data by plots of the same areas, followed by an Unequal N HSD posthoc test. Plots were used as repetitions. To test for differences between areas for the dependent variables C, N and P concentrations, ratios, nitrogen and carbon isotopic compositions, we considered the areas as categorical factors. For litterfall fluxes, nutrients fluxes and NUE, the categorical factors did not include SG. All the dependent variables cited were tested between old-growth forest areas and also restoration later on. To test the correlations between seasonal variations and litterfall fluxes, nutrients fluxes and NUE, we also used GLM followed by Unequal N HSD posthoc test, considering mean monthly precipitation (MMP) as categorical factor and the different studied areas as grouping variables. All differences to 5% probability were considered significant. The analyses were performed using the statistical package STATISTICA version 13 for Windows (STATSOFT, Inc. 2013).

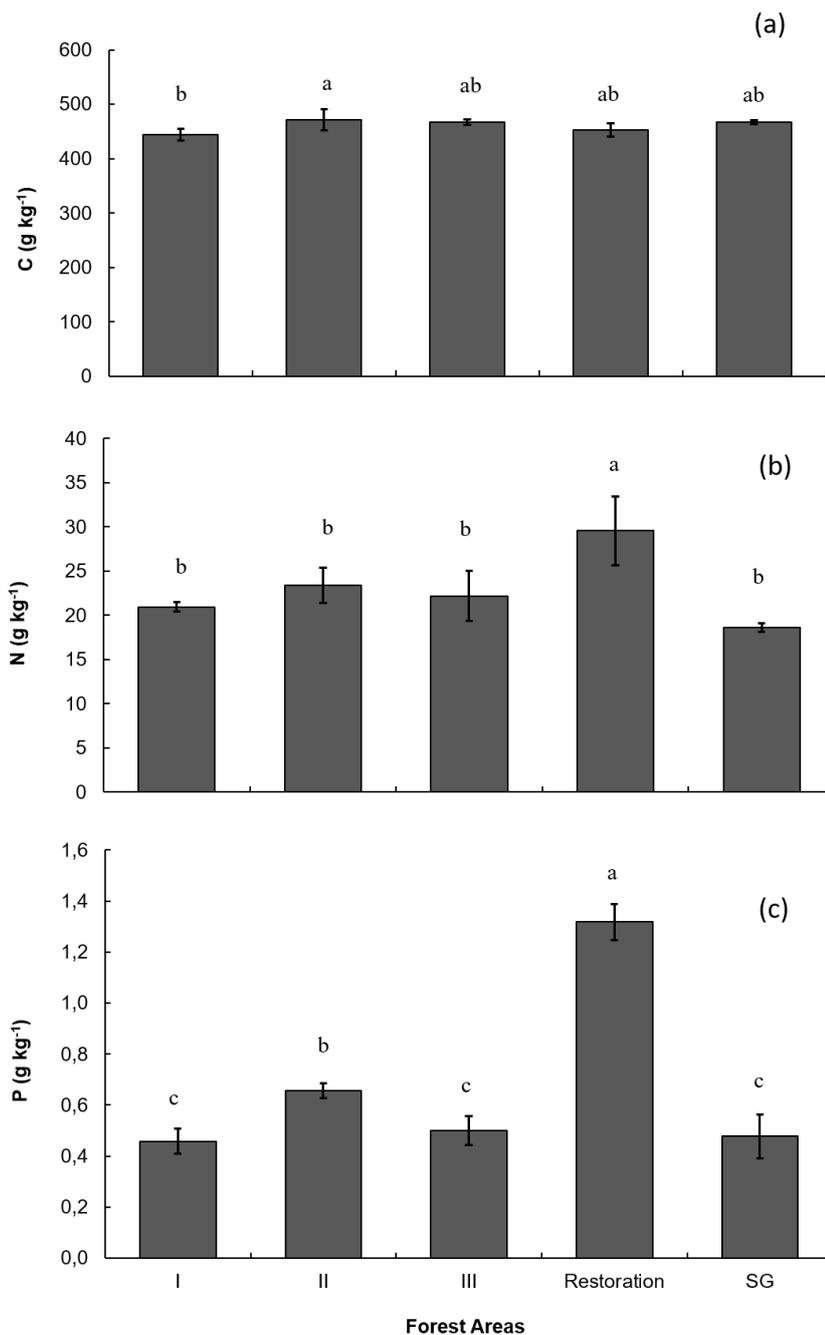
### 3.3 Results

#### 3.3.1 Foliar carbon, nitrogen and phosphorus concentrations; elements ratios and carbon and nitrogen stable isotopes

The overall mean ( $\pm$  standard deviation) foliar C concentrations among forest areas varied from  $444 \pm 10 \text{ g kg}^{-1}$  to  $471 \pm 19 \text{ g kg}^{-1}$  (Figure 8a). The only significant difference was between areas I and II, where the former had lower concentration value than the latter ( $p < 0.05$ ) (Figure 8a). Restoration had a mean foliar C concentration of  $450 \pm 28 \text{ g kg}^{-1}$  and did not differ from the old-growth forest areas (Figure 8a).

The overall mean ( $\pm$  standard deviation) value of foliar N concentration among areas was  $23.0 \pm 4.3 \text{ g kg}^{-1}$ , varying from  $18.6 \pm 0.5 \text{ g kg}^{-1}$  to  $29.5 \pm 4.0 \text{ g kg}^{-1}$  (Figure 8b). There were no significant differences when comparing mean foliar N concentration among old-growth forests areas ( $p > 0.05$ ) (Figure 8b). However, restoration had a significant higher N concentration than old-growth forest areas ( $p < 0.05$ ) (Figure 8b).

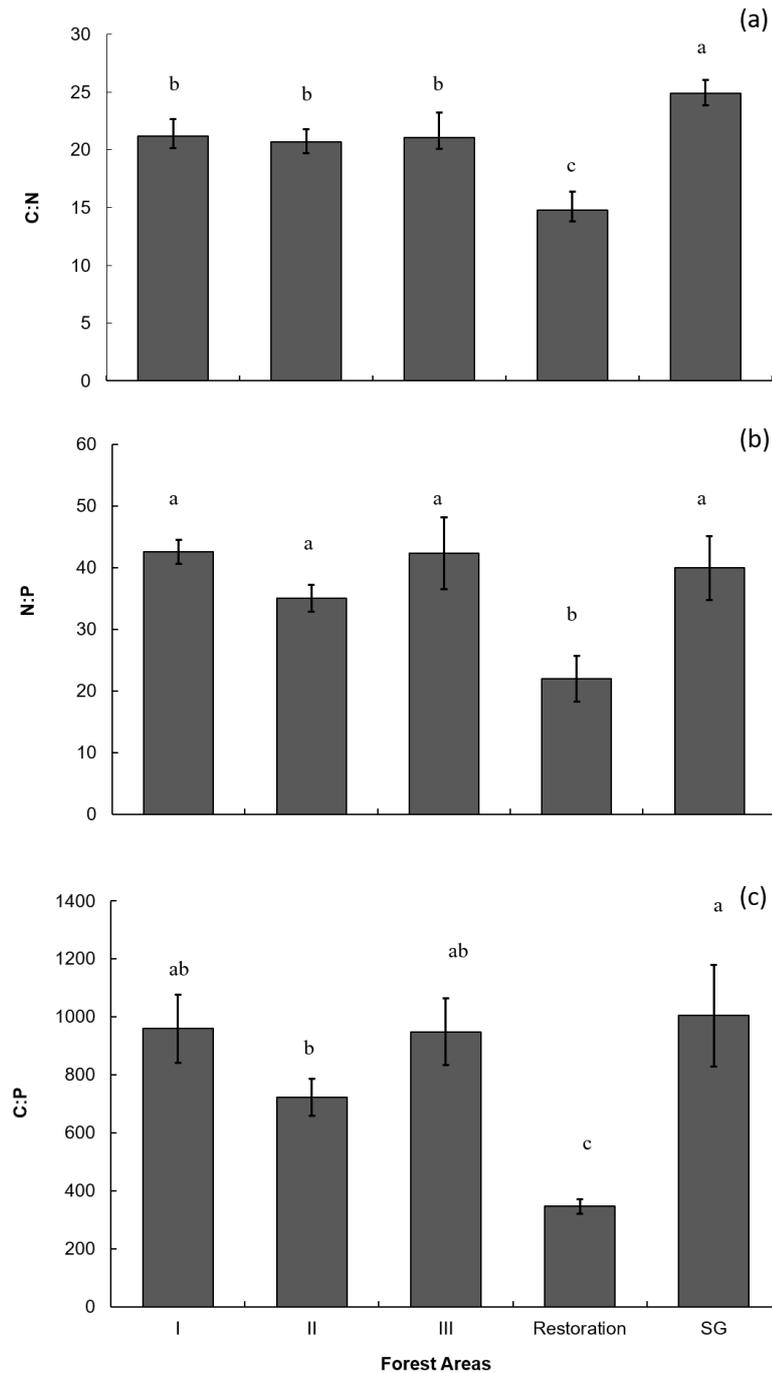
Foliar P concentrations ranged from  $0.45 \pm 0.04 \text{ g kg}^{-1}$  to  $1.31 \pm 0.07 \text{ g kg}^{-1}$  (Figure 8c). The P concentration of area II was significantly higher than the other old-growth forest areas, but lower than restoration, which was significant higher than all the old-growth forest areas ( $p < 0.05$ ) (Figure 8c).



**Figure 8:** Mean foliar C (a), N (b) and P (c) concentrations in different forest areas of the Northeastern Coastal Atlantic Forest in Alagoas state, Brazil. Values are means  $\pm$  sd n = 4.

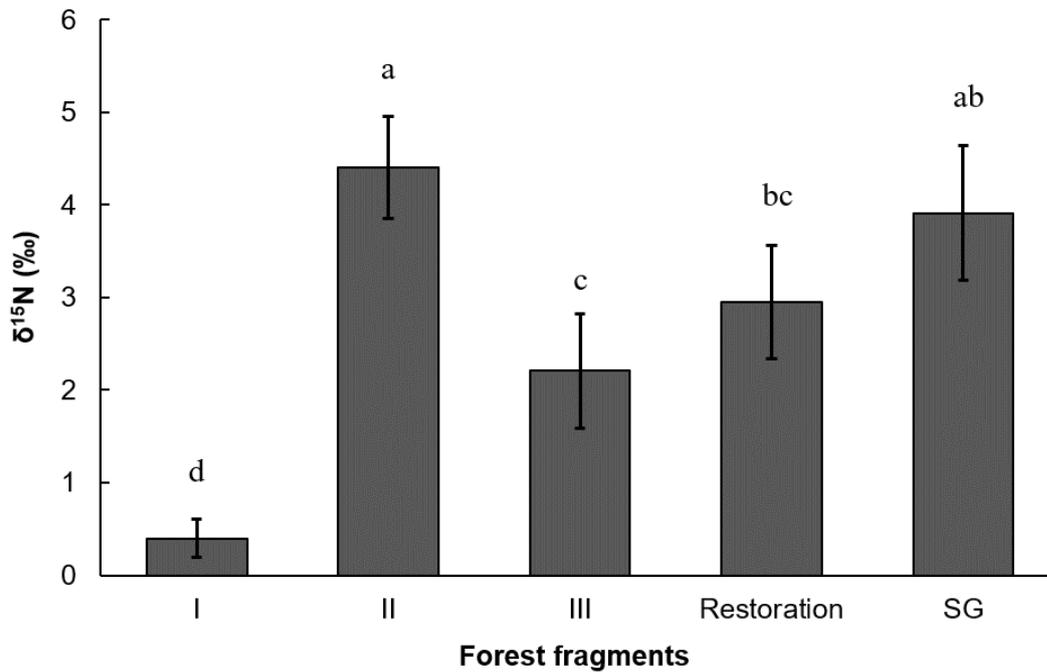
The overall mean ( $\pm$  standard deviation) foliar C:N ratio was  $20.5 \pm 3.6$ , varying from  $14.8 \pm 1.6$  to  $24.8 \pm 1.2$  (Figure 9a). The area of Serra Grande (SG), which had the higher C:N ratio mean value, was significantly different from the other old-growth forest areas and restoration ( $p < 0.05$ ) (Figure 9a). In turn, restoration had a mean value of  $14.8 \pm 1.6$  (Figure 9a), which was significantly lower than all the old-growth forest areas ( $p < 0.05$ ) (Figure 9a). The overall

foliar N:P mean ( $\pm$  standard deviation) ratio was  $36.3 \pm 8.6$ , varying from  $22.0 \pm 3.7$  to  $42.6 \pm 1.9$ . Restoration was significantly lower than old-growth forest areas ( $p < 0.05$ ) (Figure 9b). There were no differences between old-growth forest areas ( $p > 0.05$ ) (Figure 9b). The same trend was found for mean foliar C:P ratio, where restoration was lower than old-growth forest areas (Figure 9c) ( $p < 0.05$ ). Additionally, the C:P ratio of SG area was significantly higher than area II ( $p < 0.05$ ) (Figure 9c).



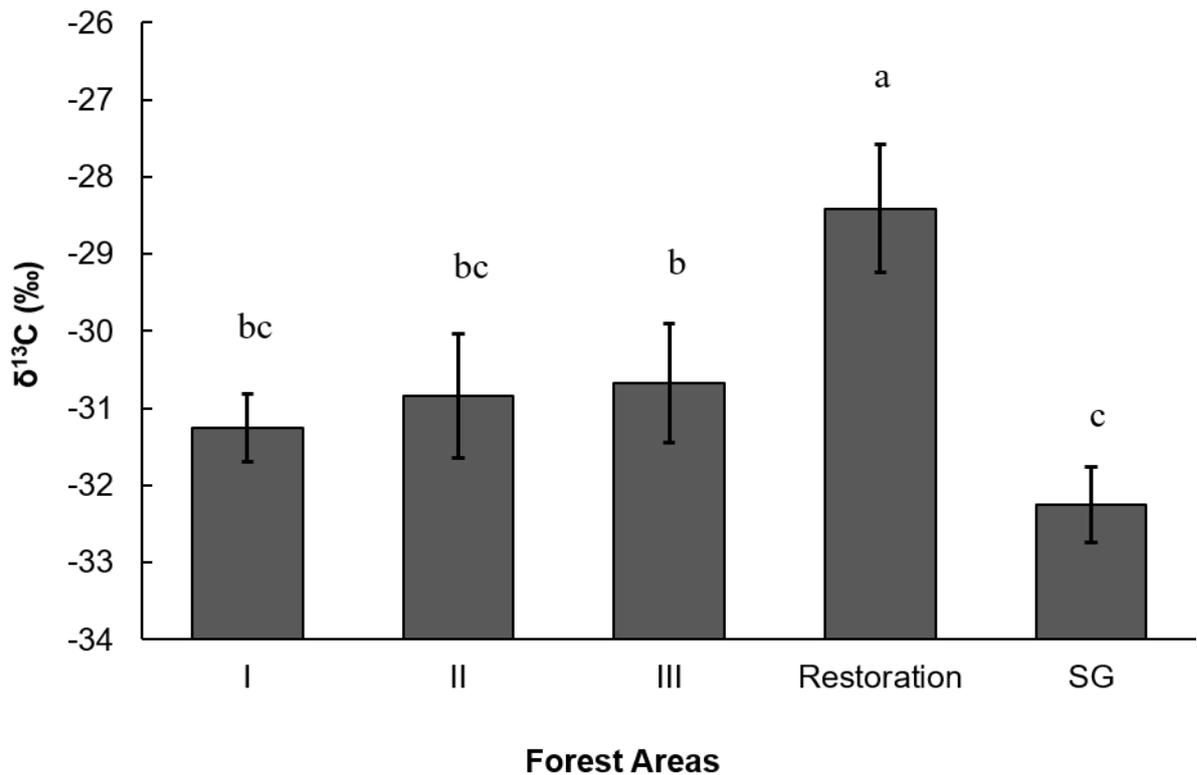
**Figure 9:** Mean foliar C:N (a), N:P (b), C:P (c) ratios in different forest areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil. Values are means  $\pm$  sd n = 4.

The minimum average foliar  $\delta^{15}\text{N}$ , considering all areas, was  $0.4 \pm 0.2$  ‰ (area I) and the maximum  $4.4 \pm 0.5$  ‰ (area II) (Figure 10). There were significant differences between areas I, II, III ( $p < 0.05$ ), where area I had the lowest mean value and II the highest foliar  $\delta^{15}\text{N}$  ( $p < 0.05$ ) (Figure 10). Additionally, area I was the lowest not only comparing to areas II and III, but also to SG and restoration ( $p < 0.05$ ). SG was equal to area II and restoration, but significantly higher than I and III ( $p < 0.05$ ) (Figure 10).



**Figure 10:** Mean foliar  $\delta^{15}\text{N}$  values in different forest areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil. Values are means  $\pm$  sd n = 4.

Foliar  $\delta^{13}\text{C}$  ranged from  $-32.2$  ‰ to  $-28.4 \pm 0.8$  ‰ (Figure 11). SG foliar  $\delta^{13}\text{C}$  was significantly lower ( $p < 0.05$ ) than area III and restoration. Areas I, II and III did not differ from each other ( $p > 0.05$ ) (Figure 11). Restoration was significantly higher than any old-growth forest area ( $p < 0.05$ ).

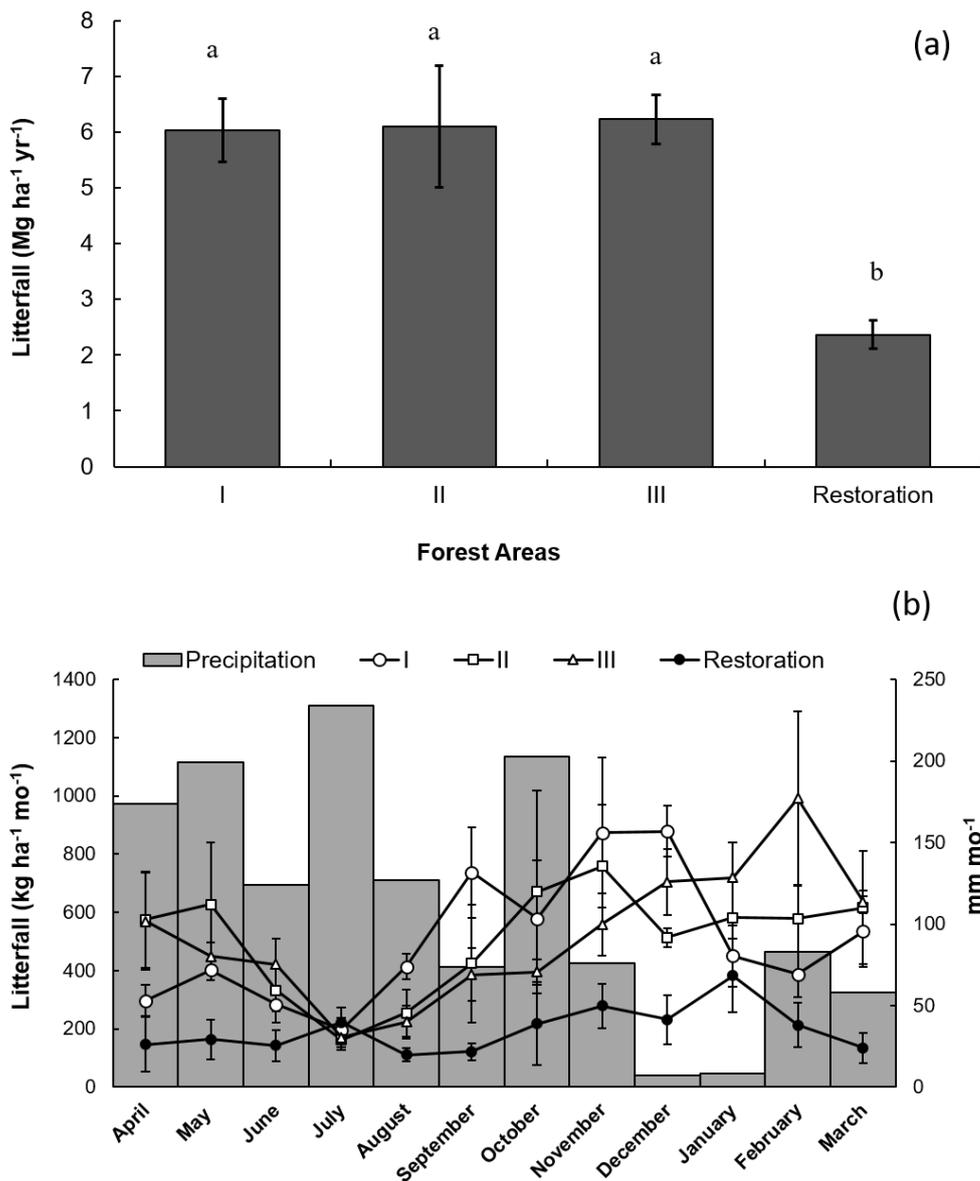


**Figure 11:** Mean foliar  $\delta^{13}\text{C}$  values in different forest areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil. Values are means  $\pm$  sd n = 4.

### 3.3.2 Litterfall

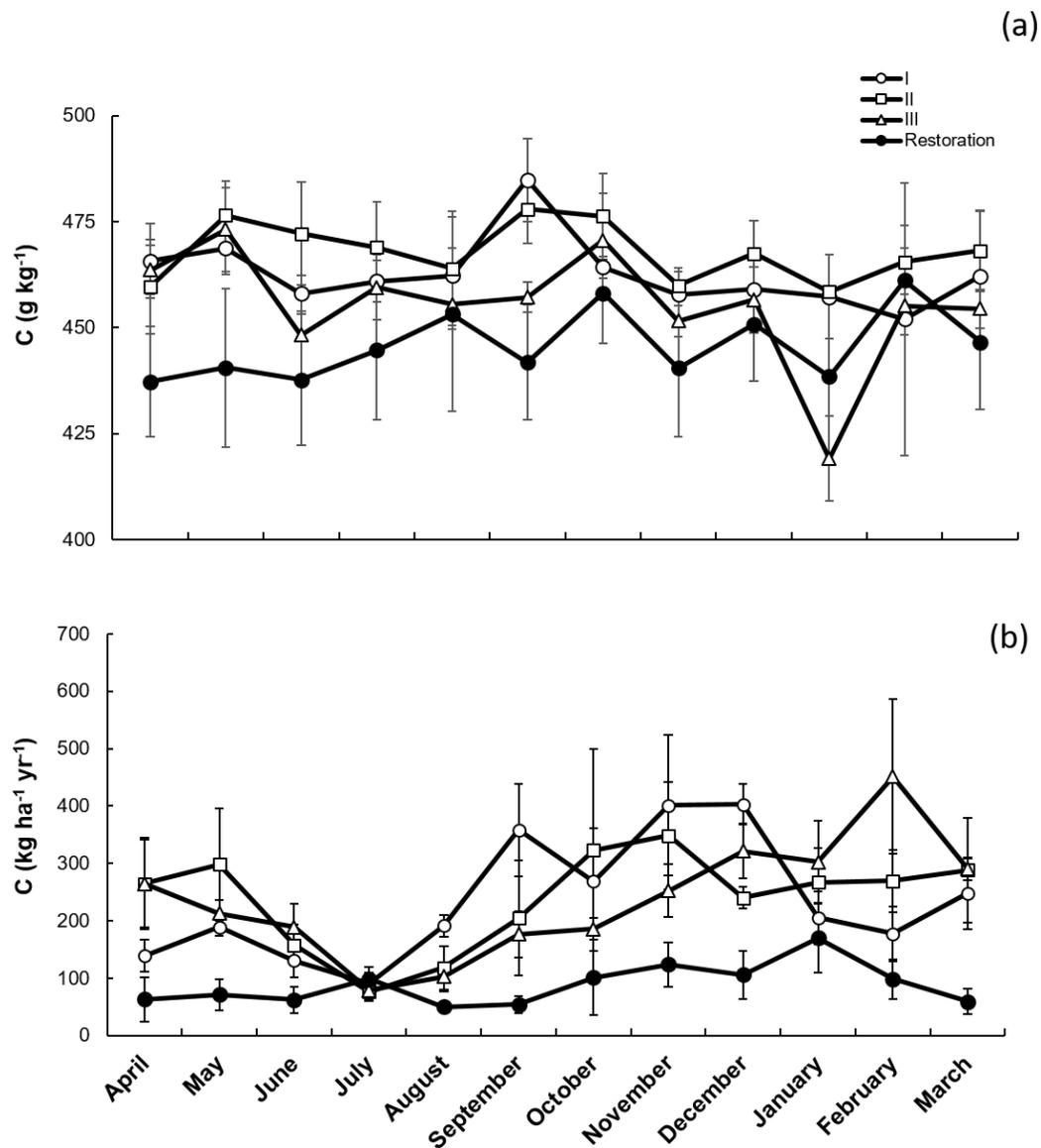
The overall mean annual litterfall flux across old-growth forest areas was  $6.1 \pm 0.7 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ , without any significant difference among them (Figure 12a). The restoration had a mean annual litterfall flux of  $2.4 \pm 0.3 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ , which was significantly lower than all the other three old-growth forest areas of Coruripe ( $p < 0.05$ ) (Figure 12a).

Although the lowest litterfall flux was observed in July, peak of the rainy season, the only significant difference, considering the seasonal variation, was in area I, where the wet season had lower litterfall fluxes compared to the drier season ( $p < 0.05$ ) (Figure 12b).



**Figure 12:** (a) Annual litterfall fluxes and (b) monthly litterfall fluxes and precipitation rates throughout the year in different forest areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil.

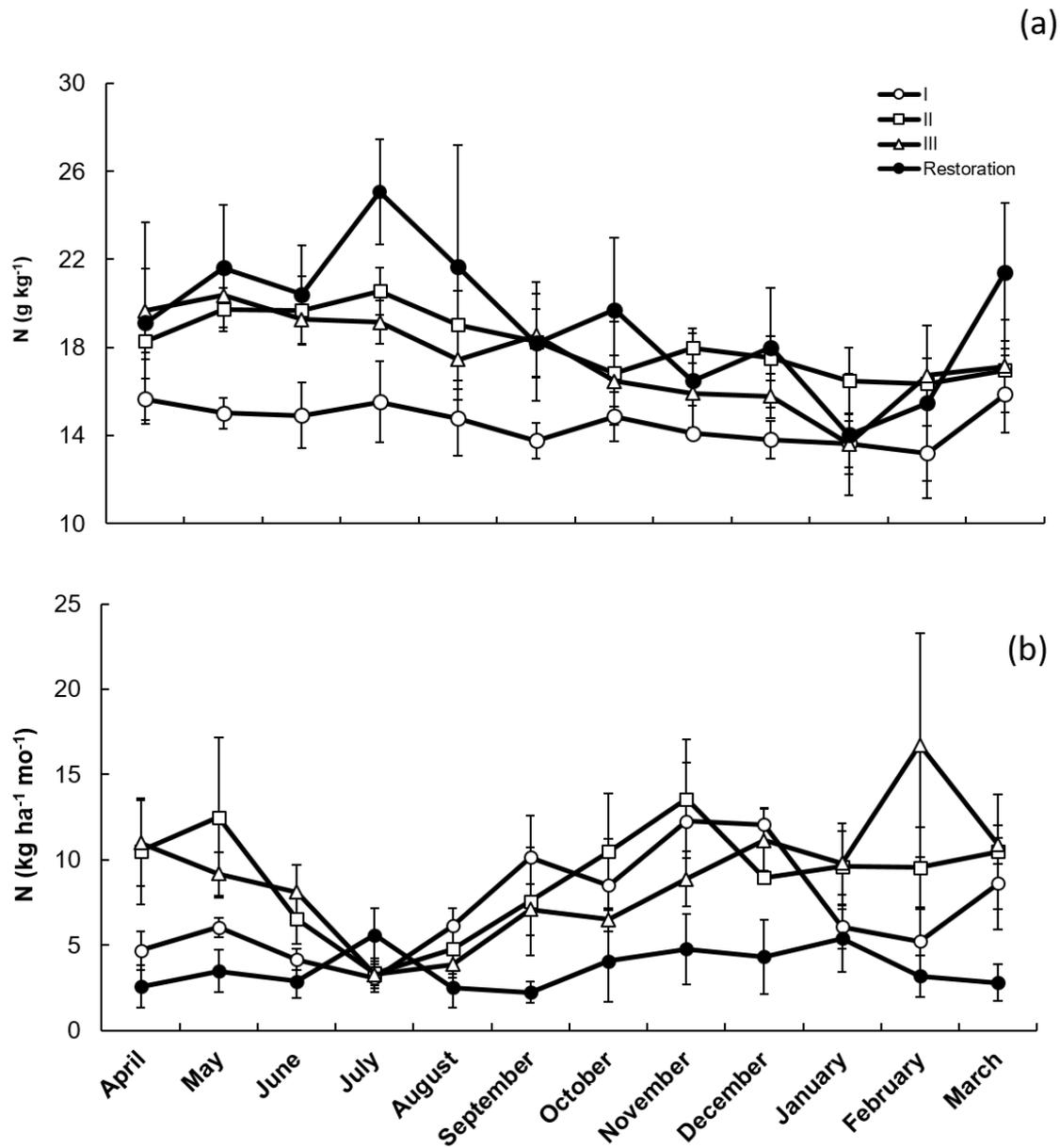
Overall, the C concentration in litterfall showed no significant seasonal variation ( $p > 0.05$ ) (Figure 13a). The minimum mean value for the areas was  $445 \pm 15 \text{ g kg}^{-1}$  (restoration) and the maximum  $467 \pm 7 \text{ g kg}^{-1}$  (area II), where restoration was significantly lower than area II ( $p < 0.05$ ), but equal to I and III (Figure 13a). There were no significant differences between old-growth forest areas ( $p > 0.05$ ) (Figure 13a). However, there were significant differences between the C fluxes in wet (April to August) and dry (September to March) seasons in all the areas, including restoration, with higher C fluxes in the drier season ( $p < 0.05$ ) (Figure 13b).



**Figure 13:** Monthly variations of C concentrations in litterfall leaves (a) and monthly variations of C fluxes through litterfall (b) in different forest areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil.

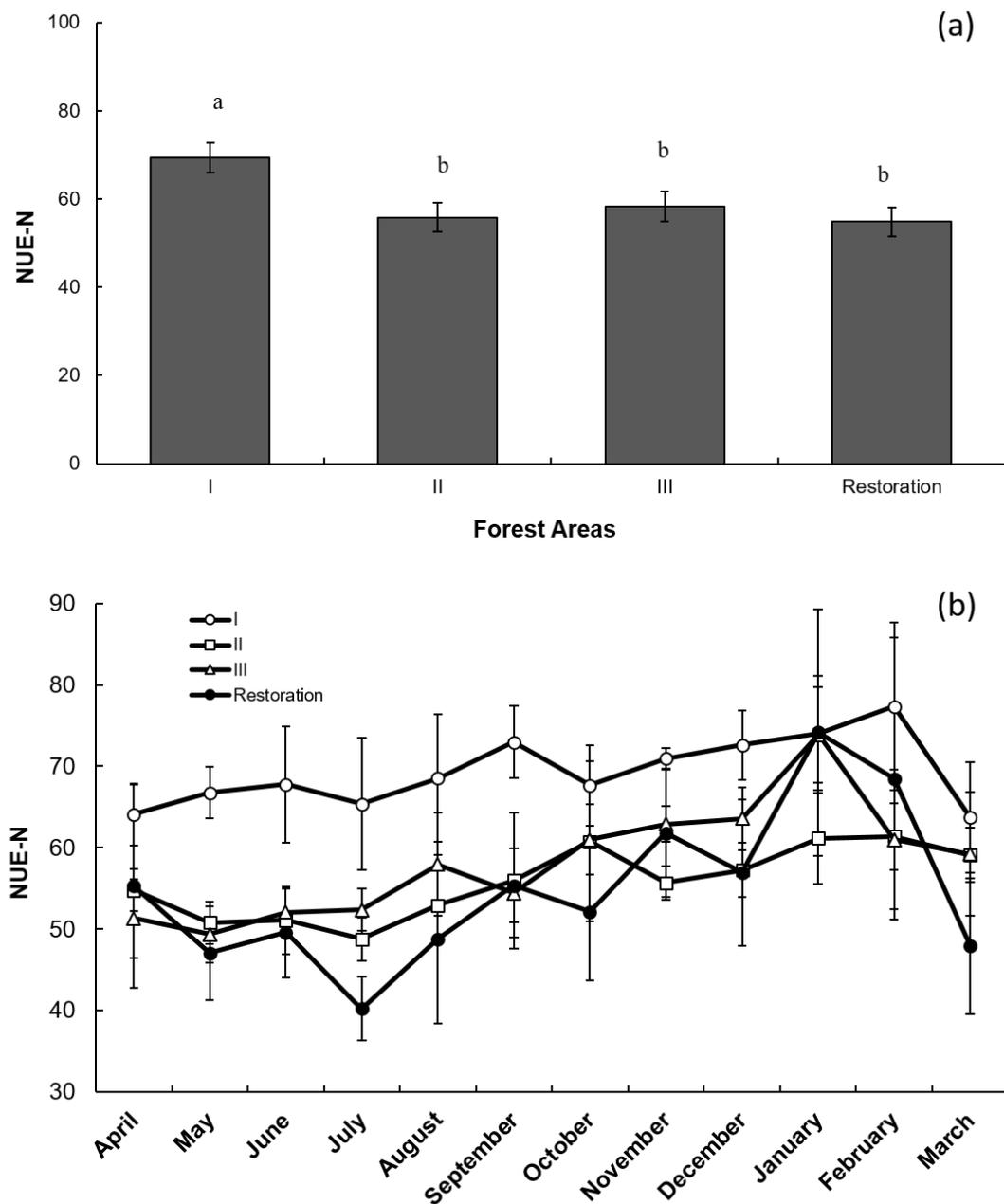
Overall, the N concentrations in litterfall ranged from  $14.6 \pm 1.1 \text{ g kg}^{-1}$  to  $19.3 \pm 2.4 \text{ g kg}^{-1}$ , where area I was significantly lower than II and restoration ( $p < 0.05$ ) (Figure 14a). Only area II data showed a significant variation with season, where wet season had higher mean value than dry season ( $p < 0.05$ ) (Figure 14a). In terms of N flux, restoration had lower N fluxes than old-growth forest areas ( $p < 0.05$ ), with no significant differences among old-growth forest areas ( $p > 0.05$ ) (Figure 14b). There were significant differences between N fluxes in wet and

dry seasons in all old-growth forest areas ( $p < 0.05$ ) (Figure 14b), but this difference was not observed in restoration ( $p > 0.05$ ).



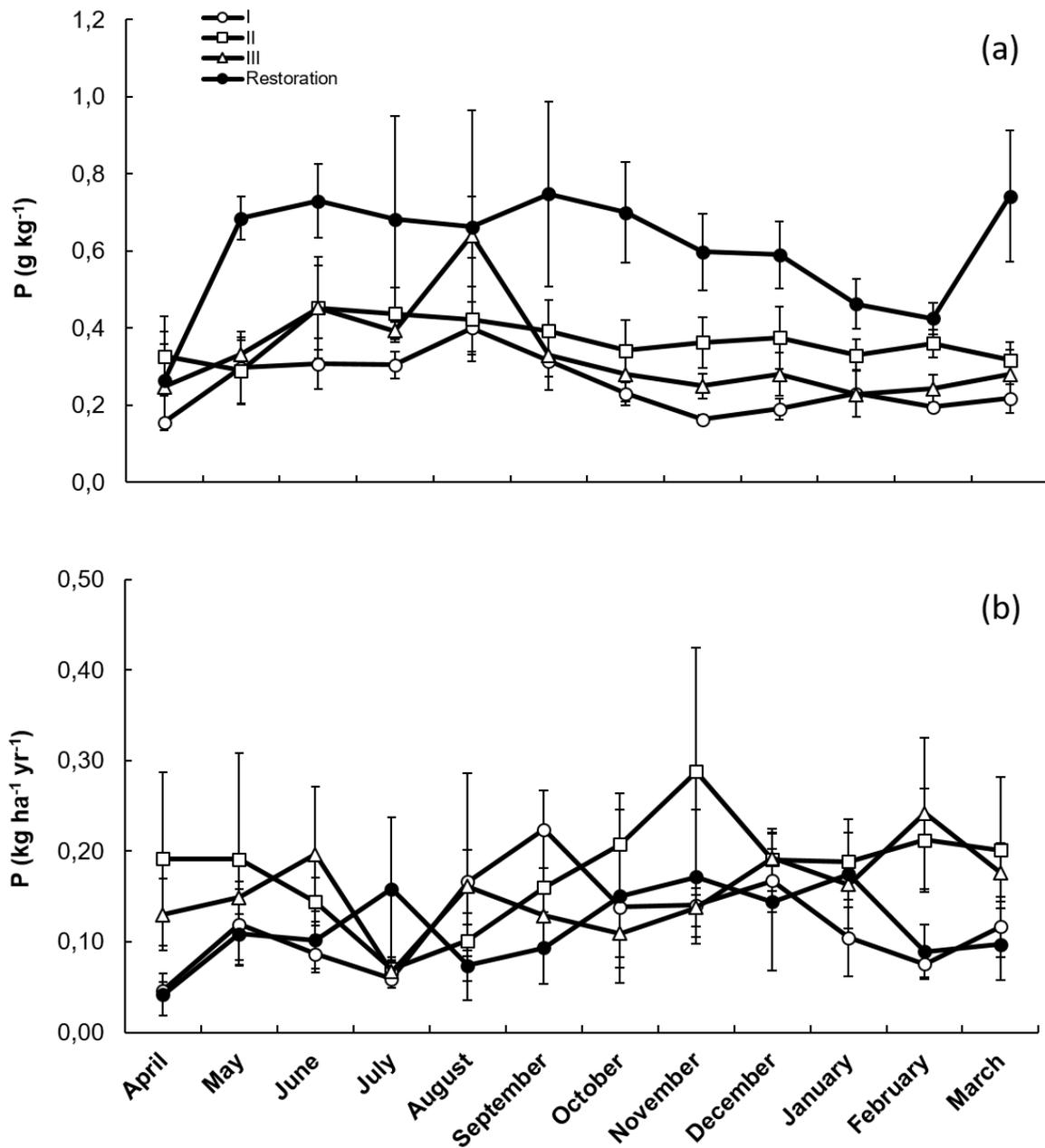
**Figure 14:** Monthly variations of N concentrations in litterfall leaves (a) and monthly variations of N fluxes through litterfall (b) in different forest areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil.

Nitrogen use efficiency (NUE-N) varied from  $55 \pm 8$  to  $69 \pm 5$ , with significantly higher NUE-N ( $p < 0.05$ ) in area I compared to area II and restoration ( $p < 0.05$ ) (Figure 15a). There was a significant seasonal difference when considering all the areas together ( $p < 0.05$ ) (Figure 15b).



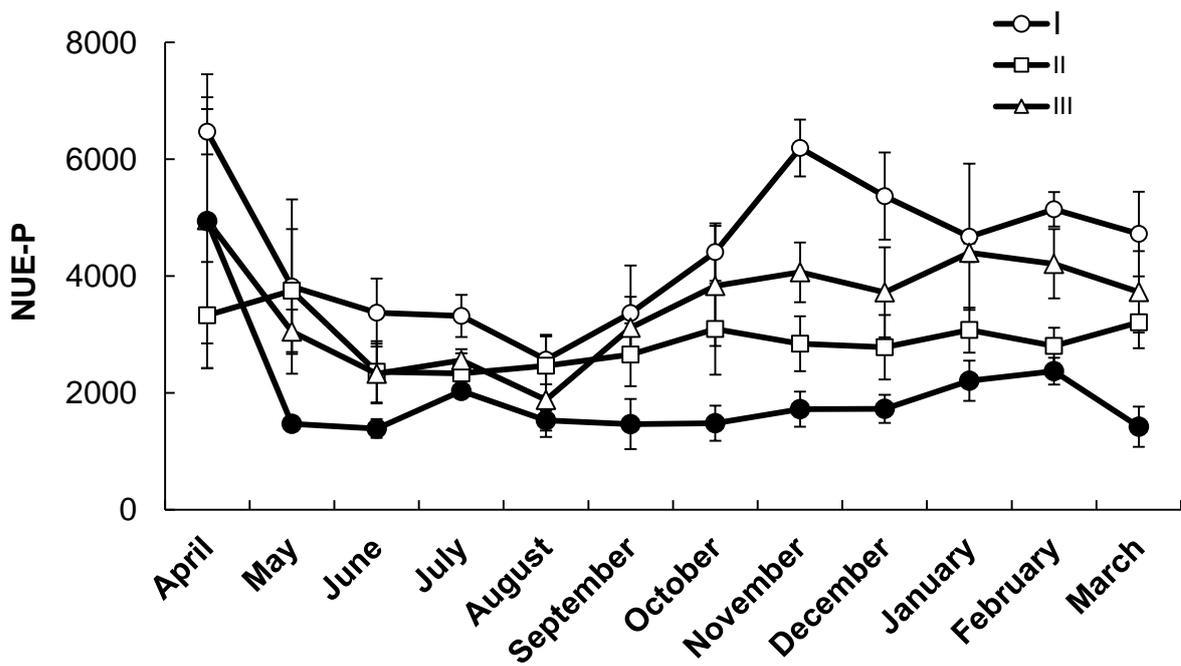
**Figure 15:** Mean NUE-N values (a) and monthly variations of NUE-N through litterfall (b) in different forest areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil. Values are means  $\pm$  sd n = 4.

Overall, P concentrations in litterfall was lower in area I and higher in restoration ( $p < 0.05$ ), with no significant difference between areas II and III ( $p > 0.05$ ) (Figure 16a). There were no seasonal differences ( $p > 0.05$ ) (Figure 16a). Litterfall P fluxes were significantly in the dry season than in the wet season ( $p < 0.05$ ) (Figure 16b). Area II had significantly higher mean P fluxes than area I and restoration ( $p < 0.05$ ) (Figure 16b).



**Figure 16:** Monthly variations of P concentrations (a) and P fluxes through litterfall (b) in different forest areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil.

Additionally, area I had the highest mean value of NUE-P, followed by areas III, II and restoration, respectively ( $p < 0.05$ ) (Figure 17). The whole data did not differ between wet and dry season ( $p > 0.05$ ). (Figure 17).

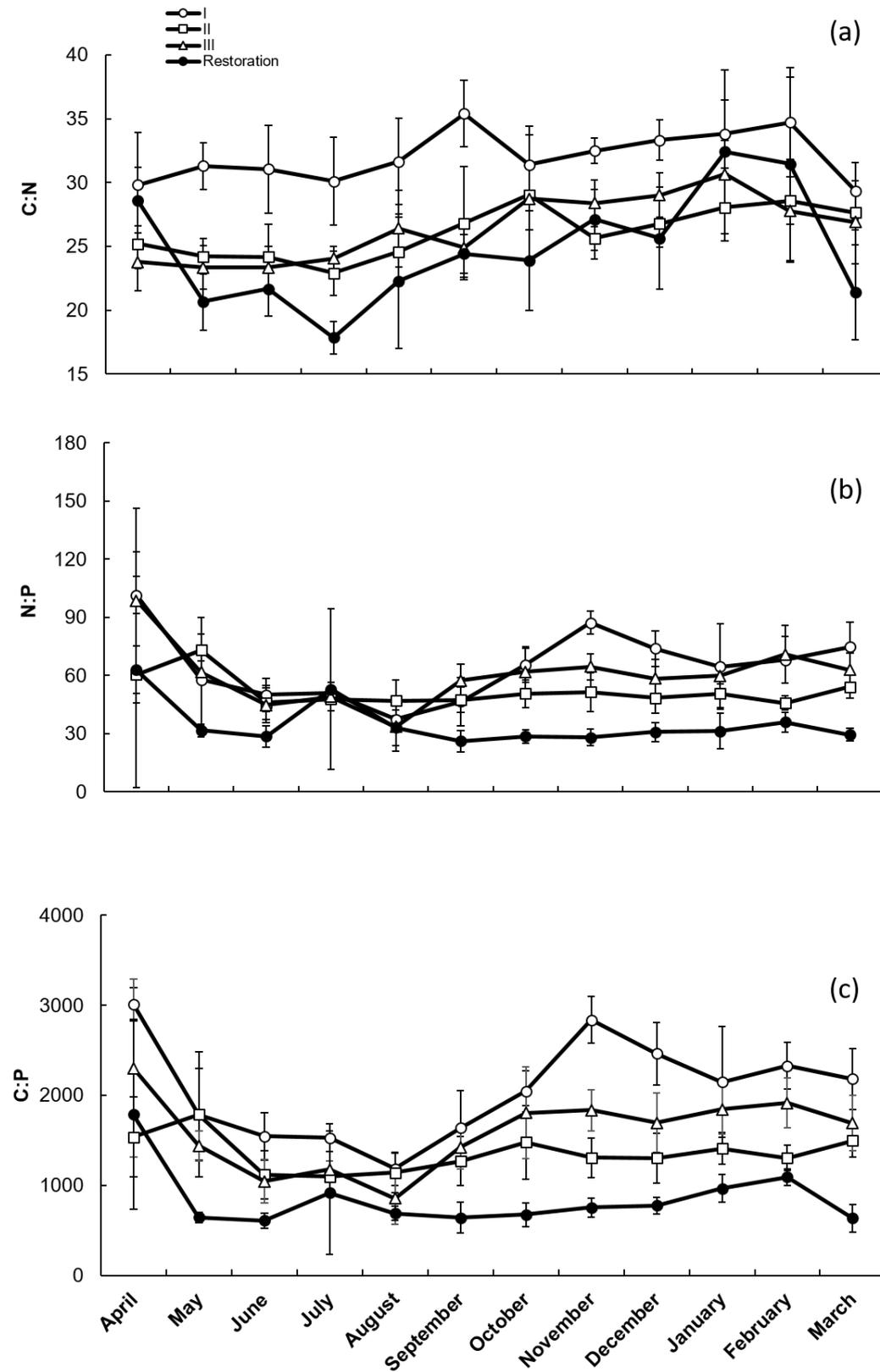


**Figure 17:** Monthly variations of NUE-P through litterfall in different areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil.

Overall, higher mean litterfall C:N ratio ( $32.0 \pm 2.3$ ) in area I was found compared to areas II, III and restoration ( $p < 0.05$ ) (Figure 18a). Considering mean C:N ratios for all areas, there was a significant difference with higher mean values in dry season ( $p < 0.05$ ) than in wet season (Figure 18a).

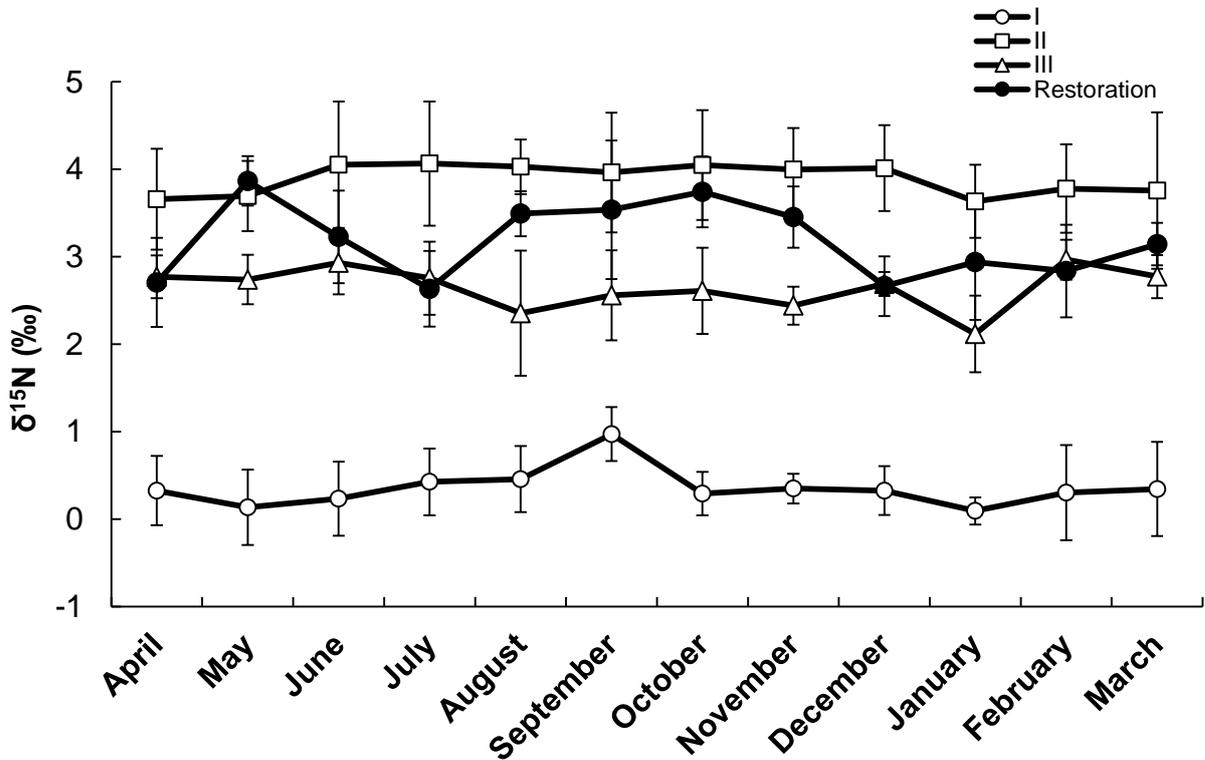
The litterfall N:P ratios varied from  $36.1 \pm 5.7$  to  $64.8 \pm 5.3$ . Restoration had a significant lower ratio than all mature areas ( $p < 0.05$ ) (Figure 18b). Additionally, the litterfall N:P ratio of area I was higher than area II ( $p < 0.05$ ) (Figure 18b). Considering the mean N:P ratio of all the areas together, including restoration, there were no significant differences between wet and dry seasons ( $p > 0.05$ ) (Figure 18b). However, when analyzed individually, the N:P ratio of area III in the wet season was lower than the dry season ( $p < 0.05$ ) (Figure 18b).

The litterfall C:P ratio varied from 726 to 2075. Restoration had the lowest and area I had the highest ratios ( $p < 0.05$ ), with no significant difference between areas II and III ( $p > 0.05$ ) (Figure 18c). Considering the mean C:P ratio of all the areas together, including restoration, there were no significant differences between wet and dry seasons ( $p > 0.05$ ) (Figure 18c).



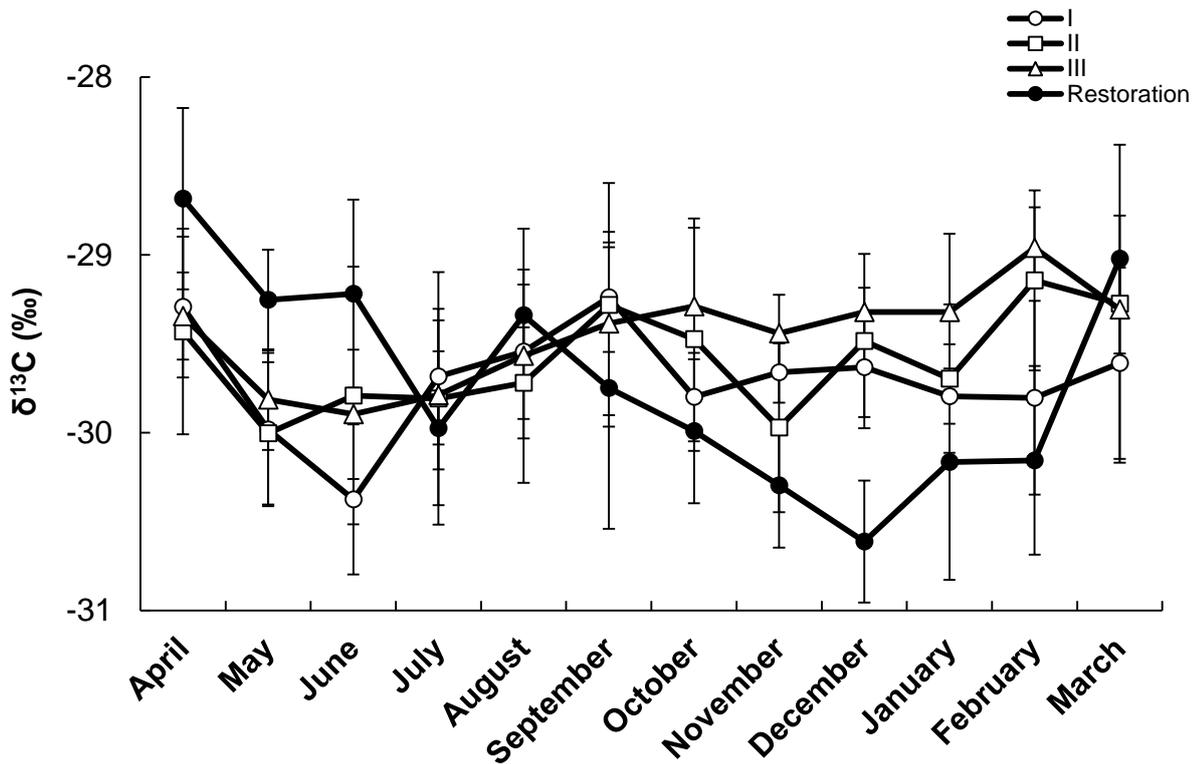
**Figure 18:** Monthly variations of C:N (a), N:P (b) and C:P (c) ratios through litterfall in different areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil.

Finally, the lowest litterfall  $\delta^{15}\text{N}$  mean value was observed in area I ( $0.4 \pm 0.4$  ‰) ( $p < 0.05$ ) and was significantly different from all old-growth forest areas, and restoration (Figure 19). On the other hand, among the old-growth forest areas, the highest  $\delta^{15}\text{N}$  mean value was observed in area II ( $3.9 \pm 0.1$  ‰) ( $p < 0.05$ ) (Figure 19).



**Figure 19:** Monthly variations of  $\delta^{15}\text{N}$  through litterfall in different areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil.

There was no difference in the litterfall  $\delta^{13}\text{C}$  among areas ( $p > 0.05$ ) (Figure 20). There was little variation in  $\delta^{13}\text{C}$  mean values of old-growth forest areas throughout the year, with no significant differences between wet and dry seasons ( $p > 0.05$ ) (Figure 20). Only restoration showed lower  $\delta^{13}\text{C}$  in the wet season than in the dry season ( $p < 0.05$ ) (Figure 20).



**Figure 20:** Monthly variations of  $\delta^{13}\text{C}$  through litterfall in different areas of the Northeastern Coastal Atlantic Forest, Coruripe - Alagoas state, Brazil.

### 3.4 Discussion

Ostertag et al. (2008) suggested that litterfall can rapidly recover in secondary forests. The same conclusion was achieved by Martinelli et al. 2017 (in press) analyzing more than 40 forest areas where litterfall was already measured in the Atlantic Forest. However, the mean litterfall of old-growth forest areas of this study ( $6.1 \pm 0.1 \text{ Mg ha}^{-1}$ ) was lower than the overall litterfall average for Atlantic Forests ( $8.0 \pm 2.5 \text{ Mg ha}^{-1}$ ) (Martinelli et al. 2017, in press) and also lower than old-growth forests of South America ( $8.6 \pm 1.9 \text{ Mg ha}^{-1}$ ) (Chave et al. 2010). One possible reason that may explain the lower litterfall fluxes found in our study is the forest physiognomy, since IBGE (1993) classified this portion of the Atlantic Forest as Open Ombrophyllus Forest, which is characterized by the presence of spaced trees and sparse vegetation, and heights up to 35 m (Veloso et al. 1991).

In general, traits that characterize the nutrient distribution and dynamics in old-growth forest areas of Coruripe and Serra Grande were in the range commonly observed for old-growth tropical forests. Foliar N concentrations of the old-growth forest areas was  $21.3 \text{ g kg}^{-1}$ , which

is lower than the averages found in Lowland ( $31.1 \text{ g kg}^{-1}$ ) and Montane ( $30.1 \text{ g kg}^{-1}$ ) of the Southeastern Atlantic Forest (Lins et al., unpublished data). The old-growth forest areas average was higher than the total average found in tropical forests ( $19.0 \text{ g kg}^{-1}$ ), and than temperate forests ( $16 \text{ g kg}^{-1}$ ) (Martinelli et al. 1999). N-rich ecosystems, with high N contents in the soil, are characterized by losses of isotopic light N during nitrification and denitrification processes, accumulating isotopically enriched N in the system (Amundson et al. 2003). Besides fine roots, leaves are responsible for the largest proportion of the annual nutrient uptake (Pregitzer et al. 2010), and foliar N concentrations and  $\delta^{15}\text{N}$  averages are used as a proxy of N availability in the soil (Amundson et al. 2003; Evans, 2001). Our foliar  $\delta^{15}\text{N}$  values were similar in three of four old-growth forest areas, with the exception of area I, where there was a significant lower  $\delta^{15}\text{N}$  value ( $0.4 \pm 0.2 \text{ ‰}$ ). The higher averages (2.2 to 4.4 ‰) found in our study were a little higher than the averages found in the Southeastern Atlantic Forest, 1.7 ‰ in Lowland and 0.5 ‰ in Montane forests (Lins et al., unpublished data), in line with those usually found in old-growth tropical forests ( $3.7 \pm 3.5 \text{ ‰}$ , Martinelli et al. 1999).

A variety of factors interfere in foliar  $\delta^{15}\text{N}$  values and the stands characteristics are very important in defining the reasons for  $\delta^{15}\text{N}$  values variations. The causes of the more  $^{15}\text{N}$ -depleted average in area I are not well understood, but we speculate some possible explanations for this finding. The different sources of N for plants will define their foliar isotopic composition, for instance, soil biota can define different isotopic compositions due to their N transformations (Nadelhoffer et al. 1996; Högberg 1997). Therefore, depending on the form of N used by plants,  $\text{NO}_3^-$  or  $\text{NH}_4^+$ , their isotopic composition will be different. Averages of  $\delta^{15}\text{N}$  in soil, from the surface to depth, in area I were lower (3.7 to 5.2 ‰ – see chapter 3) and may have influenced the foliar lower average, since plants usually present lower N isotopic composition relative to soil (Nardoto et al. 2013). Additionally, in areas with N limitation, vegetation and mycorrhizal fungi association is found, and these organisms usually deliver isotopically light N to vegetation (Michelsen et al. 1996, 1998, Hobbie et al. 2000). Although there are no information regarding the presence of this organisms in the region and their influence on biogeochemical cycles, Thomazini (1974) and Sprent et al. (1996) points out that the association with these organisms can be an explanation for lower  $\delta^{15}\text{N}$  averages.

Differences between foliar and litterfall leaves C:N:P ratios are usually used to understand the resorption of nutrients before leaves senescence (Aerts 1996). The overall N:P ratio in leaves litterfall of our areas ( $40.0 \pm 3.5$ ) did not differ significantly between old-growth forest areas of Coruripe. Additionally, our average was in the range of other old-growth tropical forests ( $62.7 \pm 4.9$ ,  $n=50$ ) (McGroddy et al. 2004), but higher than the average for broadleaf

and coniferous temperate forests ( $29.1 \pm 2.0$ ,  $n=30$  and  $26.0 \pm 4.6$ ,  $n=20$ ) (McGroddy et al. 2004). Davidson et al. (2007) found the same pattern in remnants of old-growth forests, in a chronosequence study carried out in the state of Pará, eastern Amazônia. However, their young stands had a N:P ratio average closer to the temperate forests results. Our data on N:P suggest that, as in other tropical forests (McGroddy et al. 2004), our old-growth forest areas tend to reabsorb P relative to N before the abscission of the leaves. The NUE-N ranged from 55 to 68 in our old-growth forest areas and were within the range found in the Atlantic Forest (Table 3).

We were expecting to find clear disturbances in nutrients cycling as consequence of the evident chronic disturbances ongoing in the Northeastern Coastal Atlantic Forests (Arroyo-Rodríguez et al. 2017). However, our study did not confirm our initial hypothesis that clear disturbances in the distribution of N and P in the areas would be found, with most of our data ranging between those found in tropical forests. Almost all studies in secondary forests report that the recovery of forest structure, stocks of nutrients in the soil, and species richness happen faster than recovery of species composition (Chazdon 2003). Consequently, despite the chronic disturbances, we speculate that the areas are recovering in a speed that the provision of nutrients to the vegetation has not been totally affected.

**Table 3.** Mean ( $\pm$  standard-deviation) of annual inputs of nitrogen and phosphorus ( $\text{kg ha}^{-1}$ ) via litterfall, and nutrient use efficiency (NUE) in forest types (evergreen vs. seasonal) and forest status (old-growth vs. secondary) of the Brazilian Atlantic Forest, in mature areas of the Brazilian Northeastern Atlantic Forest (this study) and in old growth forests of the Amazon region. *N* is the number of data.

Forest type	Forest status	<i>N</i>	N ( $\text{kg ha}^{-1}$ )	NUE-N	P ( $\text{kg ha}^{-1}$ )	NUE-P
<b>This study</b>	<b>Disturbed</b>	<b>3</b>	<b><math>100 \pm 9.5</math></b>	<b><math>60 \pm 5</math></b>	<b><math>1.8 \pm 0.2</math></b>	<b><math>3612 \pm 1119</math></b>
Evergreen		13	$111 \pm 37$	$68 \pm 24$	$4.0 \pm 1.6$	$2092 \pm 1135$
Seasonal		20	$196 \pm 48$	$48 \pm 10$	$6.0 \pm 2.6$	$1679 \pm 522$
All	Old-Growth	24	$167 \pm 65$	$55 \pm 21$	$5.5 \pm 2.7$	$1838 \pm 901$
All	Secondary	9	$150 \pm 46$	$56 \pm 14$	$4.5 \pm 1.3$	$1851 \pm 644$
All	All	33	$163 \pm 60$	$56 \pm 19$	$5.2 \pm 2.4$	$1842 \pm 829$
Amazon	Old-Growth	13	$98 \pm 31$	$85 \pm 19$	$2.7 \pm 1.1$	$3348 \pm 1441$

### 3.5 Conclusions

The results here presented improve our understanding relative to the Atlantic Forest as a whole, besides providing the first data attempting to describe the nutrients dynamics in the Northeastern Coastal Atlantic Forest areas under chronic disturbances. Neotropical forests are characterized by a relative abundance of N. The forest areas of Alagoas seems to follow this trend. Although the litterfall fluxes were lower than in other sites of the Atlantic Forest, the N fluxes via litterfall, and, consequently, the NUE-N was similar to other forests in the same biome. On the other hand, the foliar and leaves litterfall P concentration was lower than in other forests; consequently, the P flux via litterfall was also lower, leading to a high very high NUE-P. This high efficiency in the use of P suggests that this nutrient is the main limiting nutrient of these areas. Due to constraints in our sampling design, especially the lack of litterfall measurements in Serra Grande, we could not conclude if this P impoverishment in the forest areas of Coruripe was caused by chronic disturbances, or was also present in more preserved forest areas. However, it is clear that a more complete investigation of P cycling is needed in these areas.

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#### 4 CARBON, NITROGEN AND PHOSPHORUS STOCKS IN SOIL AND ABOVEGROUND BIOMASS OF FOREST FRAGMENTS OF THE NORTHEASTERN ATLANTIC FOREST

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##### Abstract

As the secondary forest areas increase worldwide, it also increases the recognition of these forests and all disturbed forests as carbon (C) reservoirs. One of the first steps to evaluate the role of forests as a reservoir is to conduct a forest inventory to determine forests C pools. In order to investigate soil C, nitrogen (N) and phosphorus (P) concentrations and stocks in soil and vegetation in the highly fragmented areas of the Brazilian Northeastern Atlantic Forest, plots were established in four 0.4-ha chronically disturbed old-growth forest areas, and one 0.4-ha restored area. In all areas, soil concentrations and stocks of the elements were determined at the depth intervals of 0-10, 10-20, 20-30 cm, in addition to trunks sampling in the bigger trees. Soil C stocks were similar to those found in other tropical forests of Brazil. The aboveground C stock was comparable with lowland physiognomy of the Brazilian Southeastern Atlantic Forest, and lower than submontane and montane physiognomies, which has a higher aboveground biomass. Above and belowground stocks of N were also similar to other Neotropical forests. Comparing to the physiognomies of the Brazilian Southeastern Atlantic Forest, the nutrient use-efficiency (NUE) for P was very high, the belowground stock of available  $P_{resin}$  was comparable, and aboveground P stocks were lower. The results from this study show that apparently chronic disturbances have not disrupted the organic matter input to the soil in the areas. Concluding that these forest hold large amount of C in the soil. In addition, the areas seems to be N-rich as other tropical forests. P results emphasize the importance of the P in these forests.

Keywords: Belowground, Nutrient, Tropical forest, Vegetation

## 4.1 Introduction

Secondary forests are increasing as well as the protection of chronically disturbed forest fragments, especially in the Neotropics as a result of several factors (Nagy et al. 2017). One of them is the so-called forest transition, which consists in forest recovery of abandoned agricultural land (Molin et al. 2017). A second important factor is the increasing efforts to maintain biodiversity and use forests as a carbon sink. A good example of initiative to maintain biodiversity is the Convention on Biological Diversity programme ([www.cbd.int/forest/](http://www.cbd.int/forest/)), and a bold example of the second is the UN-REDD ([www. http://www.unredd.net/](http://www.unredd.net/)), which is United Nations Collaborative Programme on Reducing Emissions from Deforestation and forest Degradation (REDD+) in developing countries. The UN-REDD has a sub-programme called Forest Carbon Partnership Facility, that demonstrates the importance of forests as living C reservoirs. These are global initiatives supported by local or regional initiative such as the Atlantic Forest Restoration Pact, created in 2009 with the purpose of restoring 15 million ha and protect existent forest fragments (Calmon et al. 2011). Only in the Northeast region of Brazil there are more than 3 million ha that could be recovered, being approximately 10% of that located in the State of Alagoas, where this study was conducted.

One of the first steps to evaluate the role of forests as carbon reservoirs is to conduct a forest inventory to determine forests carbon pools (Silver et al. 2000). This is especially important in secondary forests considering the fast recovery of above and belowground C stocks in these forests (Marín-Spiotta and Sharma 2013; Martin et al. 2013; Holl and Zahawi 2014; Poorter et al. 2016).

The Atlantic Forest of the Northeast region of Brazil is highly fragmented (Silva and Tabarelli et al. 2000). Several of these fragments were subject of several chronic disturbances, like selective logging and poaching (Arroyo-Rodríguez et al. 2017). On the other hand, corporative agroindustries, like sugar cane mills, are slowly understanding that there are several benefits in protecting large forest fragments in their lands. Corporate responsibility, and compliance with environmental laws, with the necessity of being a “green” enterprise are among these benefits. Another benefit that these agroindustries may have by protecting their fragments is to keep in their farms a living carbon reservoir. As mentioned above, the first step toward this initiative is to measure fragments C stocks. Previous studies suggest that the aboveground biomass can take 20 years to 80 years to recover depending on the soil and environmental conditions (Martin et al., 2013). As suggested by Nagy et al. (2017), there are more available data on aboveground recovery in secondary forests than on belowground stocks.

However, it seems that belowground recovery would be slower than aboveground (Martin et al. 2013). As a consequence, there are few studies that have made the partitioning of stocks of nutrients between above and belowground. Specifically in the Atlantic Forest, there is no data about nutrient stocks and partitioning in forest fragments that suffered chronic disturbance in the past.

Therefore, in this chapter, we will discuss the carbon (C), nitrogen (N), and phosphorus (P) stocks above and below ground in Atlantic Forest areas of the State of Alagoas. We first present C and nutrients concentrations in the soil, as well as, their C and N isotopic composition. Secondly, we present the concentrations in tree trunks, and finally we estimate stocks of carbon and nutrients in the above and belowground compartments. Finally, we compared the partitioning between these two compartments in an attempt to understand how these areas cycle carbon and nutrients between soil and vegetation.

## **4.2 Methods**

### **4.2.1 Study Area**

This study was conducted in the Northeastern Coastal Atlantic Forest fragments located in the municipalities of Coruripe and São José da Lage, both within Alagoas state, Brazil. In each area, 20 0.1-ha plots were established, with five main areas of data collection in total. The mentioned areas are the same ones described in the chapter 1 of this thesis. Therefore, more details about the study area can be found in the subheading 2.2.1.

### **4.2.2 Field sampling**

For nutritional and isotopic soil analysis, the O horizon was removed and twelve soil cores per plot were collected with an auger at three depth intervals (0-10, 10-20, 20-30) in three different points. The 60 samples were analysed and results grouped by depth and areas.

To estimate AGLB C, N and P stocks, it was used the data presented in chapter 1 on AGLB and multiplying them by the average concentration of those elements in the trunks. Overall, 227 trunk samples were collected, 129 from Coruripe (I = 46; II = 46; III = 37), 28 samples from Restoration, and 60 from SG. Ideally, each stem within all the areas should have the trunk samples collected. However, the timing for that would be prohibitive. Therefore, samplings of trees trunks (about 20 individuals per plot) were done with a screw auger, selecting

trees with larger diameters, inserting it to approximately half the diameter of the tree. Beeswax was used to fill the hole left in each individual sampled to avoid the proliferation of pathogens.

### 4.2.3 Chemical Analyses

After sampling, soil samples were air dried, homogenized and sieved at 2 mm. To determine the concentration of total N and C sub-samples of 20-25 mg of the prepared material were wrapped in tin capsule and introduced into the elemental analyzer Carlo Erba Model 1110 (Milan, Italy). The analyzer generate gases by combustion that are purified in a chromatography column and directly introduced into the mass spectrometer to determine the isotopic ratios (IRMS Delta Plus, Finnigan Matt, San Jose, CA, USA). Tropical soils were used as standard material for soils samples. The natural abundance of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  was expressed in parts per thousand (‰) from a standard internationally recognized equation:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = (R_{\text{amostra}} / R_{\text{padrão}} - 1) \times 1000 \dots\dots\dots(1)$$

where, R is the molar ratio in the sample and standard between  $^{13}\text{C}$  and  $^{12}\text{C}$  or  $^{15}\text{N}$  and  $^{14}\text{N}$ .

Peedee Belemnite (PDB; limestone of the Grand Canyon region, USA) was the standard used for C, while the atmospheric air the standard for N. The analytical errors accepted were  $\pm$  0.3%, 0.1%, 0.3 ‰ and 0.5 ‰ for C, N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively.

For P in soil samples, the element was extracted using the ion-exchange resin procedure as proposed by Raij et al. (1986), where the extraction is made by a mixture of anion and cation resin saturated with sodium bicarbonate. In this procedure, the extraction of P occurs in an aqueous suspension of soil and the resin, transferring the element to the resin, which adsorbs the phosphate ( $\text{PO}_4^{3-}$ ), after 16 hours shaking period. Then, P is extracted from the resin, followed by the analytical determination.

All nutritional and isotopic analyses related to C and N were done in Laboratory of Isotopic Ecology at CENA / USP.  $P_{\text{resin}}$  was determined at the Department of Soil Science of ESALQ / USP.

#### 4.2.4 Carbon, nitrogen and phosphorus stocks in soil

The stocks of C, N and  $P_{resin}$  in the soil were determined as a function these elements concentrations in the soil ( $mg\ g^{-1}$ ), soil density ( $g\ cm^{-3}$ ) and sampled depth interval thickness (cm).

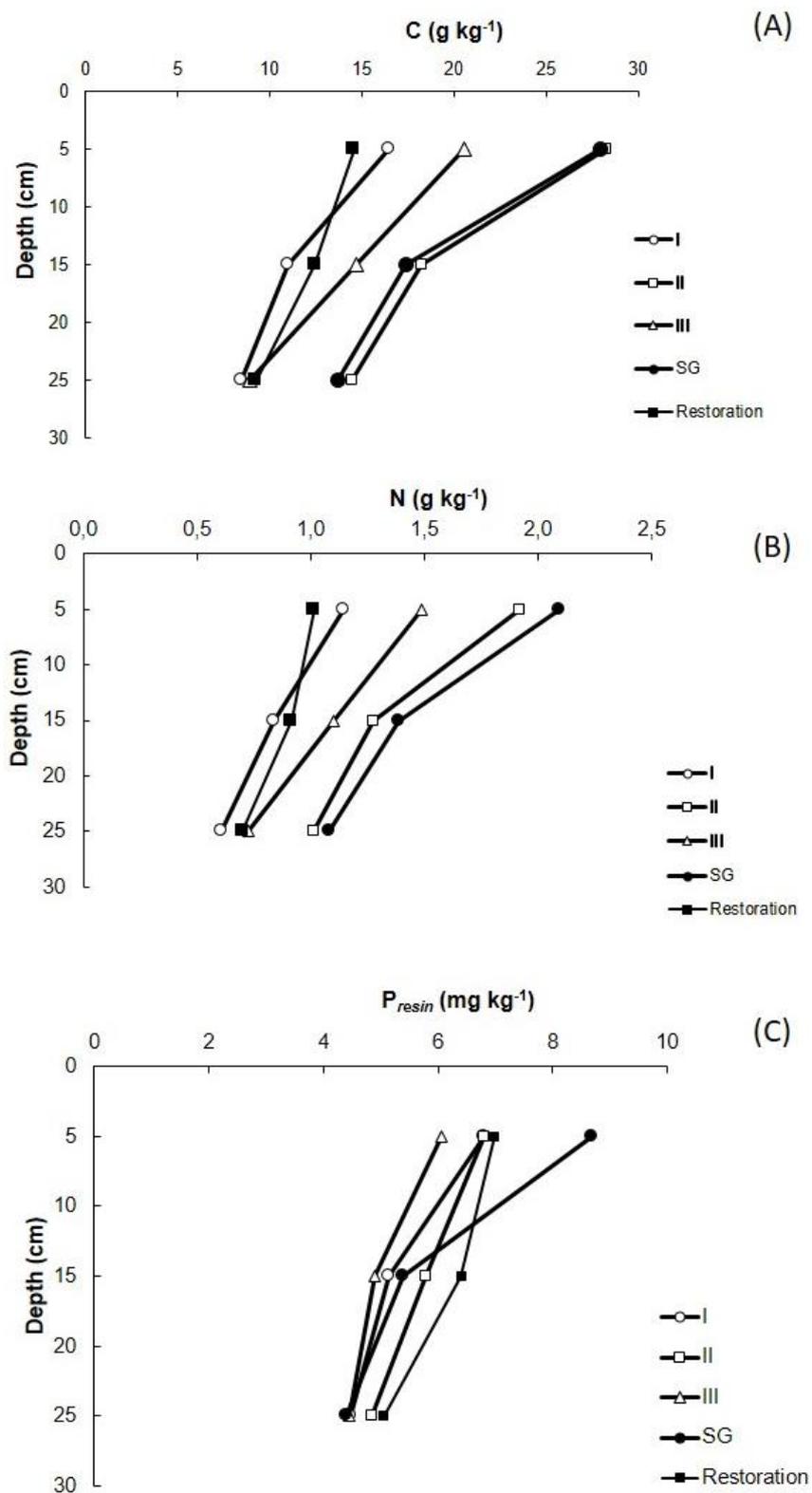
#### 4.2.5 Statistical Analyses

In each area, the plots were treated as repetitions, totalizing four blocks of 0.4-ha. Among the blocks, there are differences in the degree of disturbance and regeneration. To test for differences between the sampling areas, it was used the general linearized model (GLM), followed by a Unequal N HSD test for unequal variances. All differences to 5% probability are considered significant. The analyses were performed using the statistical package STATISTICA version 13 for Windows (STATSOFT, Inc. 2013).

### 4.3 Results

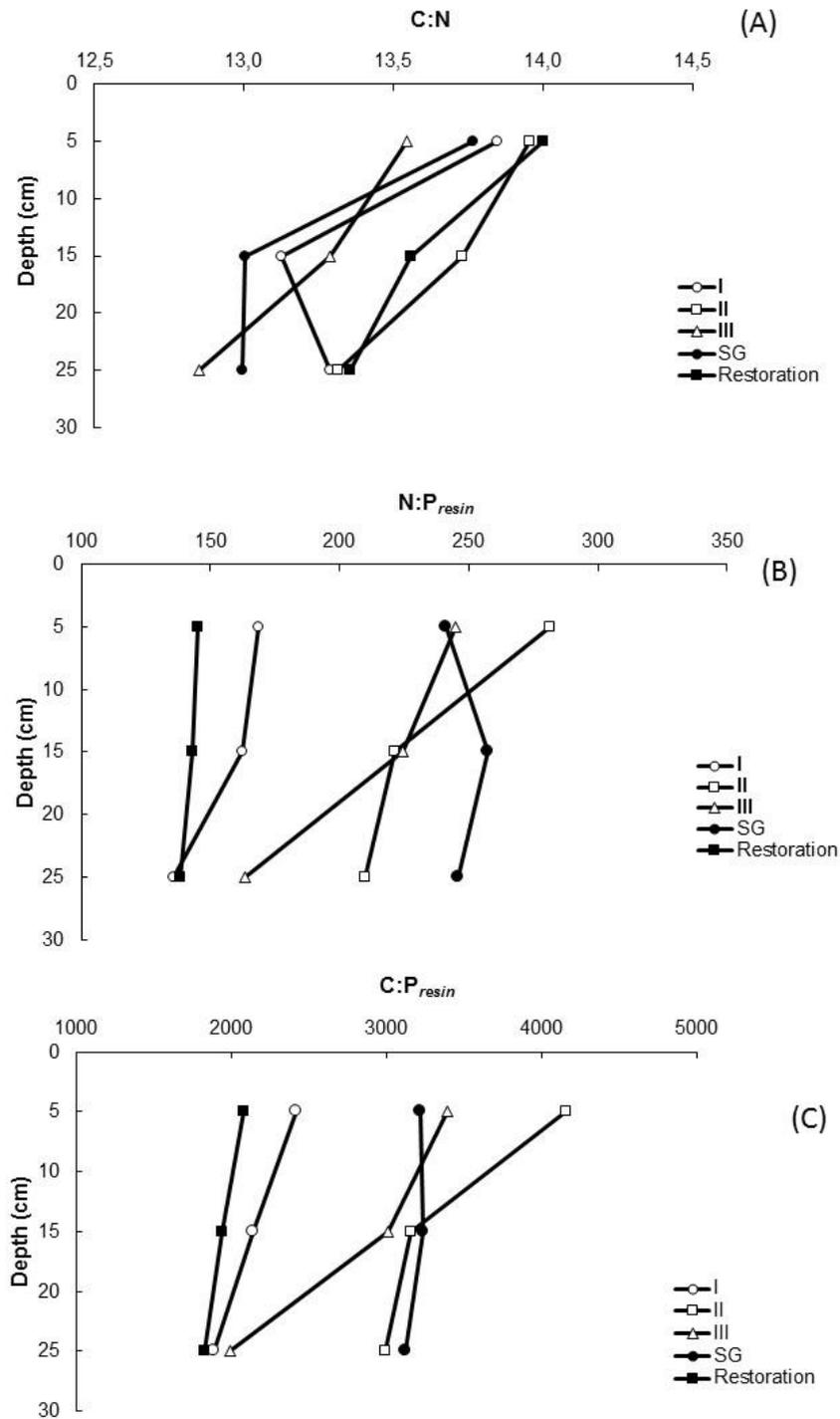
#### 4.3.1 Soil carbon, nitrogen and phosphorus concentrations; ratios and carbon and nitrogen stable isotopes

There was a decrease in C, N and  $P_{resin}$  with soil depth in all areas. Considering the soil depth interval 0-30 cm, no statistical differences were observed between the areas mean soil C concentrations between areas ( $p > 0.05$ ) (Figure 21a). The average soil N concentrations followed the same pattern as C, with no significant differences ( $p > 0.05$ ) between the areas considering the first 30 cm (Figure 21b). For  $P_{resin}$  concentrations, the only significant difference was observed between restoration and area III, where the former had higher mean  $P_{resin}$  concentrations than the latter ( $p < 0.05$ ) (Figure 21c).



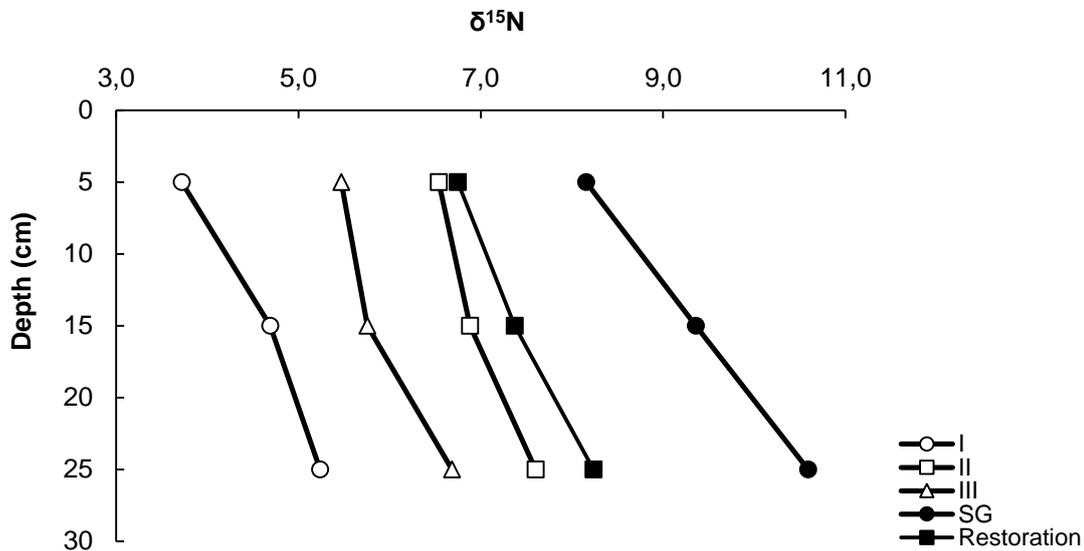
**Figure 21:** Soil depth variability of (A) C, (B) N, and (C)  $P_{resin}$  concentrations in different forest areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil.

Considering the 0-30 cm depth interval, C:N ratio decreased with depth, and no significant differences between old-growth forest areas nor restoration ( $p > 0.05$ ) were found (Figure 22a). For mean  $N:P_{resin}$  and  $C:P_{resin}$  ratios, there were also no significant differences, neither between old-growth forest areas nor restoration ( $p > 0.05$ ) (Figure 22b and 22c).



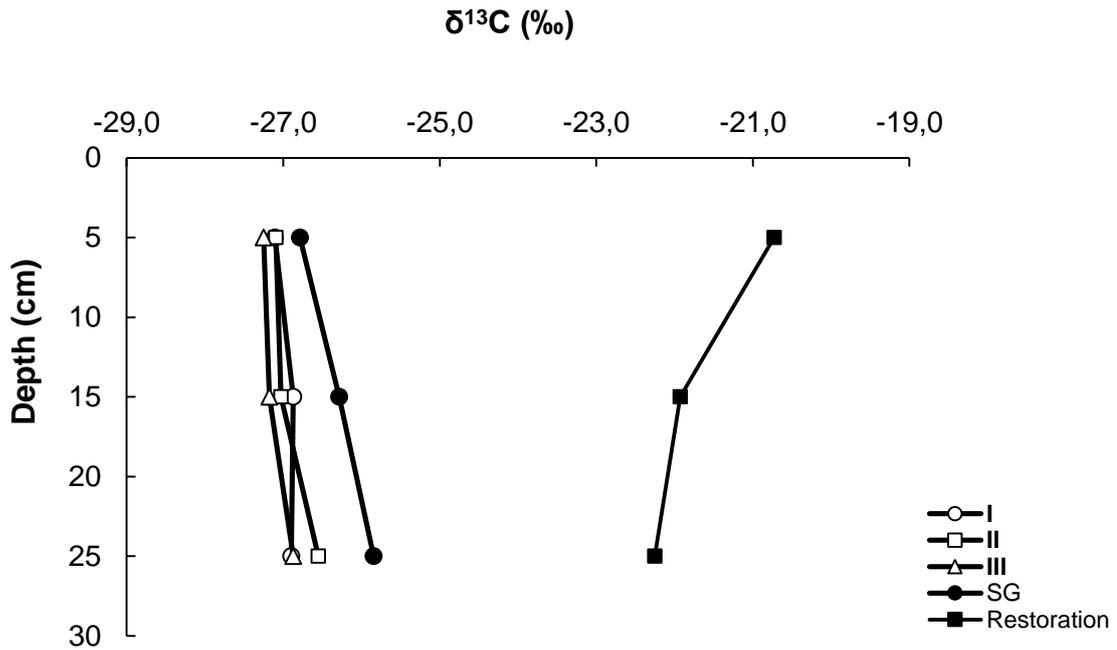
**Figure 22:** Soil depth variability of (A) C:N, (B)  $N:P_{resin}$  and (C)  $C:P_{resin}$  ratios in different forest areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil.

The  $\delta^{15}\text{N}$  mean values ranged from  $4.5 \pm 0.4 \text{ ‰}$  to  $9.4 \pm 0.3 \text{ ‰}$ . Area I had the lowest  $\delta^{15}\text{N}$ , and SG had the highest mean value ( $9.4 \pm 0.4 \text{ ‰}$ ) ( $p < 0.05$ ), compared to all the other areas (Figure 23). However, restoration was not different from II ( $p > 0.05$ ). Additionally, there was a significant  $\delta^{15}\text{N}$  increase with depth in all areas ( $p < 0.05$ ) (Figure 23).



**Figure 23:** Soil  $\delta^{15}\text{N}$  mean values variability with depth in different areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil.

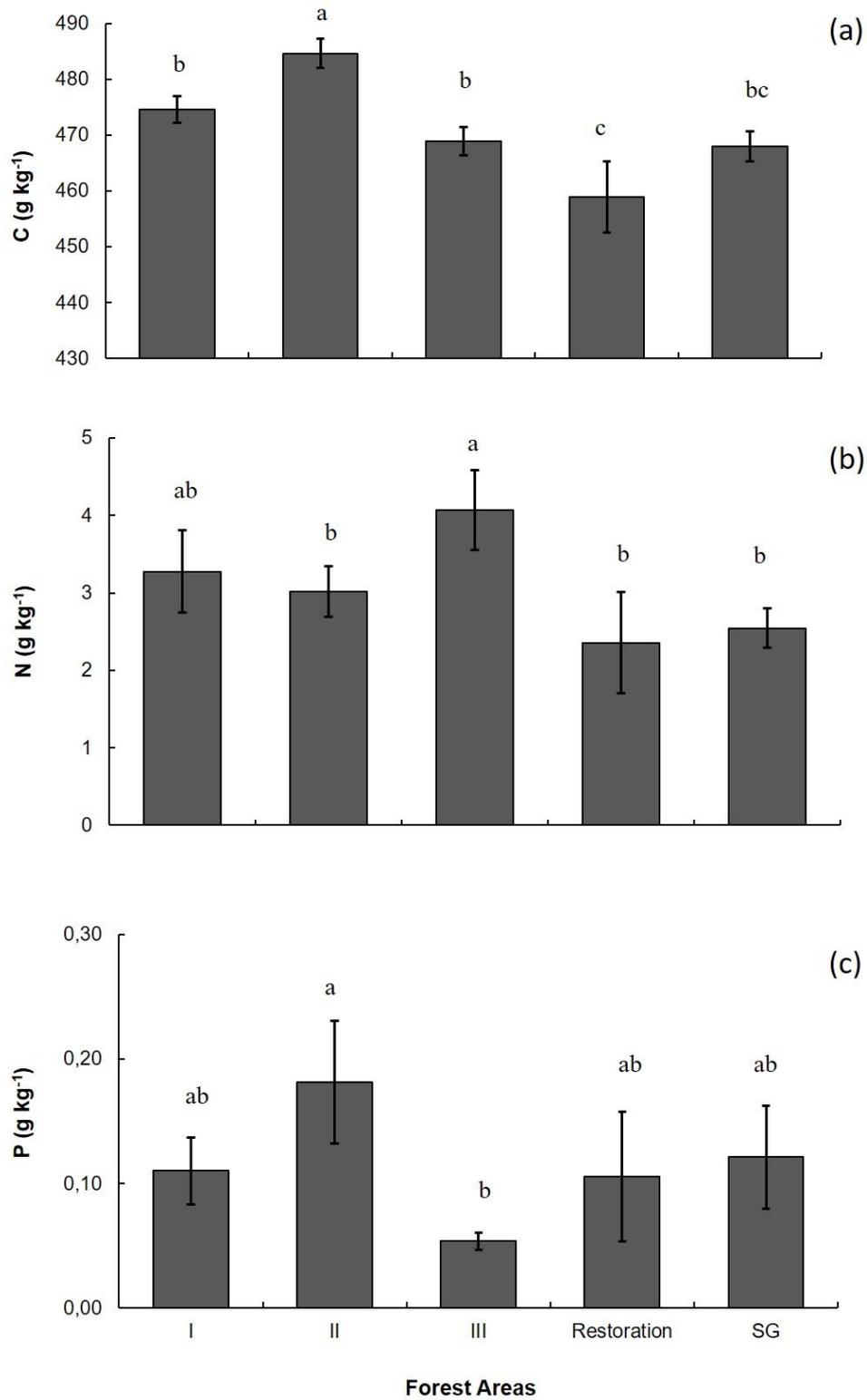
The main purpose of the determination of the  $\delta^{13}\text{C}$  values in the soil was to evaluate past presence of C source  $\text{C}_4$  (sugar cane or forage pastures). We observed a uniform distribution in  $\delta^{13}\text{C}$  values with soil depth in old-growth forest areas (Figure 24), indicating the absence of  $\text{C}_4$  sources. As expected, there is still the presence of  $\text{C}_4$  carbon in the soil profile of restoration plots, a consequence of previous sugar cane crops (Figure 24). There were significant differences between restoration and old-growth forest areas ( $p < 0.05$ ). However, there were no significant differences between old-growth forest areas ( $p > 0.05$ ) (Figure 24).



**Figure 24:** Variability of soil  $\delta^{13}\text{C}$  values with depth in different forest areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil.

Concentrations of C and nutrients were determined in tree trunks samples in order to estimate the stock of these elements in the vegetation. Here we present the concentrations of such elements and variability between areas.

There were significant differences between trunk C concentrations, where area II had higher mean C concentrations than all areas, including restoration ( $p < 0.05$ ) (Figure 25a). Restoration was lower than areas I, II and III ( $p < 0.05$ ), but equal to SG ( $p > 0.05$ ) (Figure 25a). Regarding N concentrations, the difference was between area III and area II, SG and restoration, where area III had a higher mean N concentration ( $p < 0.05$ ) than other old-growth forest areas and restoration, but with no difference when compared to area I ( $p > 0.05$ ) (Figure 25b). There was only a single difference when comparing mean P concentrations of the areas, where area II was significant higher than area III ( $p < 0.05$ ) (Figure 25c).

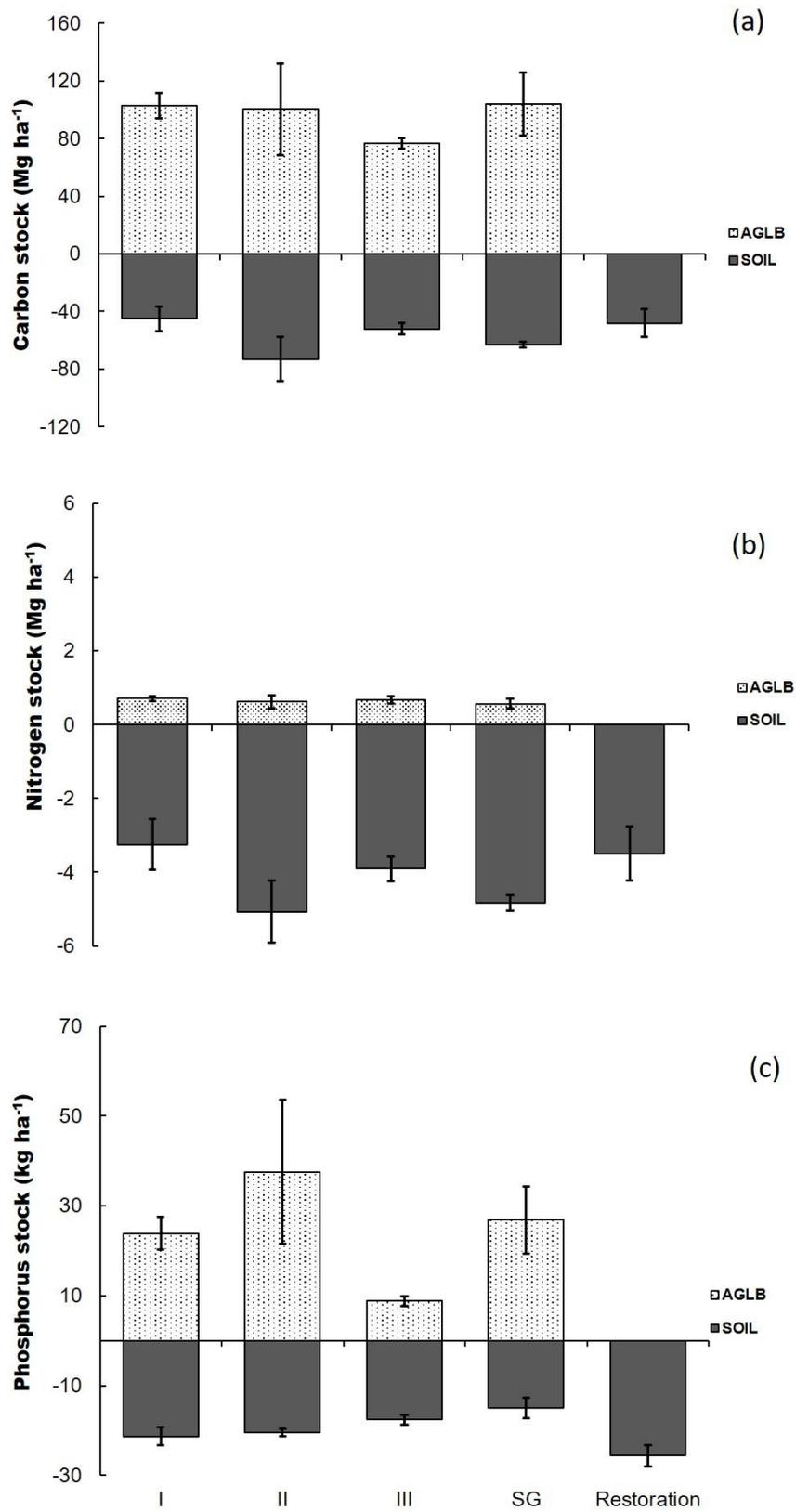


**Figure 25:** Trees trunks concentrations of: (A) C, (B) N and (C) P in different forest areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil. Values are means  $\pm$  sd n=4.

Soil C stocks ranged from  $47 \pm 1 \text{ Mg ha}^{-1}$  to  $68 \pm 17 \text{ Mg ha}^{-1}$  (Figure 26a) and the N stocks from  $3.5 \pm 0.8 \text{ Mg ha}^{-1}$  to  $5.0 \pm 1.0 \text{ Mg ha}^{-1}$  (Figure 26b), with no significant differences between areas ( $p > 0.05$ ). While for soil  $P_{\text{resin}}$  stocks, restoration had higher ( $p < 0.05$ )  $P_{\text{resin}}$  soil stocks than SG and area III (Figure 26c).

The C in the vegetation varied from 77 to  $104 \text{ Mg ha}^{-1}$ , with no difference between areas (Figure 26a). The aboveground N stocks varied from 0.57 to  $0.71 \text{ Mg ha}^{-1}$ , also with no significant differences between areas ( $p > 0.05$ ) (Figure 26b). The significant difference for P stocks was between area II and III, where area II had higher mean value than III ( $p < 0.05$ ) (Figure 26c).

The comparison of C, N and P stocks between soil and vegetation, considering all the areas together, showed significant differences for C and N stocks ( $p < 0.05$ ), but no difference was found for P stocks ( $p > 0.05$ ). When analyzing each area individually, C stocks in vegetation was significantly higher than in soil in all areas ( $p < 0.05$ ), except in area II, where there were no differences ( $p > 0.05$ ). In all mature areas there were higher stocks of N in soil compared to vegetation ( $p < 0.05$ ). While for P stocks the only significant difference between soil and vegetation was observed in area III ( $p < 0.05$ ).



**Figure 26:** Aboveground and soil depth variability of (A) C, (B) N and (C) P stocks in different forest areas of the Northeastern Coastal Atlantic Forest, Alagoas state, Brazil. Values are means  $\pm$  sd n=4.

#### 4.4 Discussion

Soils may effectively be managed in order to increase their carbon content, especially the more labile fraction of organic matter (Lal 2010). The implication of this is that belowground carbon can accumulate in the soil with time. At the same time, that regeneration of aboveground biomass is fast in tropical forests (Guariguata and Ostertag 2001; Bonner et al. 2013; Anderson-Teixeira et al. 2016). In this sense, forest under chronic disturbance that are in a regeneration process could be an important sink of carbon that is currently considered an important ecosystem service (Chazdon 2003). Under this scenario, it is useful to compare below and aboveground carbon stocks with old-growth and secondary neotropical forests to address the status of the areas investigated in this study in comparison with these other forests.

By suggestion of the IPCC's Guidelines for National Greenhouse Gas Inventories (IPCC 2006), soil carbon stock should be considered in the 0 - 30 cm soil layer. In the mature areas of Coruripe the mean soil carbon stock among three areas was 57 Mg ha<sup>-1</sup>, and in Serra Grande the stock was 63 Mg ha<sup>-1</sup>. These stocks are very similar to the mean stock found for low activity clay soils of the Atlantic Forest biome (Bernoux et al. 2002), and about 10 to 15 Mg ha<sup>-1</sup> higher than stocks determined by IPCC (2006) considering soil of low activity clay of tropical moist forests.

The soil carbon stock of the restoration plots was not different from mature old-growth plots. In general, cultivation with time tend to decrease soil carbon stocks, especially in the first 30 cm (Baker et al. 2007; Don et al. 2011; Eclisa et al. 2012). However, several studies have reported non-difference between soils carbon stocks under native vegetation and cultivated soils, or even an increase in soil carbon stocks in cultivated soils (Ogle et al. 2005; Zinn et al. 2005; Braz et al. 2013). Restoration in our plots started more than 10 years ago, and this may explain the lack of difference in soil carbon stocks between these plots and mature areas. However, we cannot exclude the possibility that sugarcane cultivation had no effect on the soil carbon stocks. By analyzing the study conducted by Ivo (2012) in the Coruripe farm, we think that this possibility is rather difficult; especially because this author found that after 35 years of sugarcane cultivation, soils under cultivation have lost half of its carbon stock in comparison with soils of a mature forest located not far from our plots.

The soil  $\delta^{13}\text{C}$  reflects a mixture of the new and old vegetation in the soil, and can be used to estimate the relative proportion of C<sub>3</sub> and C<sub>4</sub> carbon present in the soil according to the following equation:

$$C_{4p} = \frac{\delta^{13}C_{\text{soil}} - \delta^{13}C_{C_3}}{\delta^{13}C_{C_4} - \delta^{13}C_{C_3}} \dots\dots\dots(2)$$

Where,  $C_{4p}$  is the relative proportion of  $C_4$  carbon;  $\delta^{13}C_{\text{soil}}$  is the C isotopic composition of the soil sample;  $\delta^{13}C_{C_3}$  is the C stable isotopic composition of a soil under an old-growth forest; and  $\delta^{13}C_{C_4}$  is the C stable isotopic composition of a soil under a natural  $C_4$  vegetation or that have been cultivated with a  $C_4$  vegetation for a long period of time (> 30 years).  $\delta^{13}C_{C_3}$  was assumed to be the mean surface soil  $\delta^{13}C$  among old-growth forest areas (-27‰), and the  $\delta^{13}C_{C_4}$  was assumed to be the highest  $\delta^{13}C$  among  $C_4$  (sugarcane or pasture) cultivated soils in the country, which was -14‰ (Assad et al. 2013).

Using the equation above, and the  $\delta^{13}C_{\text{soil}} = -21‰$  that correspond to the mean soil C isotopic composition of the 0 -10 cm soil layer of the restoration plots (Figure 24), it was estimated that the proportion of  $C_4$  in the surface soil (0 – 10 cm) of the restoration plots was approximately 45%. The  $\delta^{13}C$  of the 0 -10 cm soil layer cultivated for 35 years with sugar cane was equal to approximately -19‰ (Ivo 2012). Using the same equation above, it was estimated that the proportion of  $C_4$  carbon in this soil was 62%. Therefore, assuming that the restoration soil had a similar  $\delta^{13}C$  of the soil cultivated with sugarcane for 35 years (-19‰), since the beginning of the restoration (15 years ago), there was an increase in the proportion of  $C_3$  carbon from 45% to 62%. Probably, the main source of this new  $C_3$  carbon is the litterfall from the trees used to restore the area. The mean annual litterfall in these plots was 1.2 Mg C ha<sup>-1</sup>, that after more than a decade would represent an input at least of 12 Mg C ha<sup>-1</sup>. Obviously, not all this C will be incorporated into the soil, because part will be lost by decomposition. We have not enough data available to make these estimates, but, certainly a part of this C input via litterfall has been incorporated into the soil.

Soil N stocks are difficult to compare with other sites because of the lack of such estimates (Groppo et al. 2015). We compare the soil stocks found here with the average of 16 forest sites compiled by Groppo et al. (2015), which was equal to 5.1 Mg N ha<sup>-1</sup>. This value although in the range of the values found here, is a bit higher if compared with the mean soil N stock of Coruripe mature areas that was 4.1 Mg N ha<sup>-1</sup>. The soil N stock in Serra Grande was a bit higher (4.8 Mg N ha<sup>-1</sup>) and closer to the mean stock found by Groppo et al. (2015). Once again, there was no difference between Coruripe mature areas and the restoration soil (Figure 35). This lack of difference also contradicts the lower soil N stock observed in cultivated soils when compared with old-growth soils compiled by Groppo et al. (2015). As for C, we believe

that the N input via litterfall could contribute to compensate for any previous difference between restoration soil and old-growth forest areas. Clearly, there is not a way to prove that with the available data, and this subject indeed needs further investigation.

Finally, the mean  $P_{\text{resin}}$  soil stocks found in this study were very similar to the mean soil  $P_{\text{resin}}$  stocks of old-growth forests compiled by Groppo et al. (2015). But, again, these authors found that cultivated soils, even low-input pastures, had the double of  $P_{\text{resin}}$  stocks compared with forests. In that sense, we could not detect any statistical difference between the  $P_{\text{resin}}$  stocks of restoration soils and old-growth forest areas. It is important to remember that for years the restoration soils received approximately  $15 \text{ Mg P yr}^{-1}$  (Cícero Bastos de Almeida, personal communication). Soils of Coruripe have a low pH that contribute to the adsorption of P by the clay mineral fraction, and Al and Fe hydroxides (Clinebell et al. 1985). This could explain the lack of increase in  $P_{\text{resin}}$  stock of the restoration soils. Alternatively, it can also be hypothesized that the extra P provided by mineral fertilizers was used by trees planted in the restoration area, since, the growth of tropical trees is especially limited by this nutrient (Mercado et al., 2011).

As already seen in Chapter 1, the aboveground live biomass (AGLB) of Coruripe and Serra Grande areas is in the lower end of biomass found in other neotropical areas (Table 2). Aboveground C stocks in the mature areas followed a similar tendency in relation to the AGLB, since woody vegetal tissues has low variability in their C concentration (Vieira et al., 2010). Anderson-Teixeira et al. (2016) compiling a high number of C stock in old-growth forests of the tropics found a mean stock of approximately  $135 \text{ Mg C ha}^{-1}$ , which is higher than the stocks found here that varied from approximately 80 to  $100 \text{ Mg C ha}^{-1}$  (Figure 26). In contrast, these stocks found in Coruripe and Serra Grande are higher than secondary forest older than 20 years and younger than 100 years, also summarized by Anderson-Teixeira et al. (2016). These authors found for these secondary forest a C stock that is approximately half of the stock found in the areas of the Brazilian Northeast. This comparison highlights the importance of these areas in stocking C, although these stocks are not quite equal to old-growth intact forests, they are large enough to consider the conservation of these areas for this purpose.

There is much less data available on N stocks in the aboveground vegetation, especially because, there are very few determinations of N concentrations in tree trunks (Vieira et al. 2011). One of the few studies that have such stocks available was conducted in the coastal Atlantic Forest of the State of São Paulo (Vieira et al. 2011). The N stocks in aboveground areas of Coruripe and Serra Grande are similar to the stocks found in the old-growth lowland forests of the State of São Paulo. Other similarity between these two areas is the much higher

belowground N stock in comparison with the aboveground stock (Figure 26). Finally, there was no difference between the above and belowground  $P_{\text{resin}}$  stocks in Coruripe and Serra Grande Fragments (Figure 26). This was a surprising because one of the paradigms of tropical forest ecology is that most of P is locked in the vegetation, not in the soil, especially P readily available for plants, which is the case of  $P_{\text{resin}}$ . However, this paradigm was built more on the general idea that in order to weathered tropical soils support a luxuriant vegetation should rely in a closed nutrient cycle, with minimum losses (Milne, 1937). Real comparisons between soil available P and aboveground P stocks were made available only recently. Although still very few of these comparisons were made, they have been showing that available P is frequently higher in the soil than in the vegetation (e.g. Dalling et al., 2016; Denise Bizutti, personal communication). Therefore, our findings are in line with these more recent studies.

#### 4.5 Conclusions

The forest fragments of Alagoas state, despite missing the large trees (DBH > 70 cm) still holds a significant amount of C above and belowground. This is very important, because, if other forest fragments of the Northeast region have similar C stocks, they are providing an important ecosystem service that only recently has been appreciated. This could be an important incentive to preserve these fragments, and also would serve as an incentive to buster restoration programs. Although we could not measure above ground C stock yet, it is clear that in 10 to 15 year the amount of C in the aboveground is significant, which suggest a fast growth of the tree species in the restoration area. Following the findings in other Neotropical areas, N is also abundant in the forest fragments of the Alagoas. It would be interesting in the future to investigate the role of trees of the Fabaceae family as a N source, since species of this family in symbiosis with rhizobia can fix N from the atmosphere. The aboveground of P was lower than forests of the State of São Paulo, this result could be a consequence of the low P inputs via litterfall described in Chapter 2. Maybe this is the reason for the comparable stocks of P between above and belowground. This is interesting because highlights the fact that available P ( $P_{\text{resin}}$ ) is not very low and it is comparable with the concentration in other tropical soils. However, the dynamics of P in these fragments deserve futher more detailed investigation, including the role of mycorrhiza populations in these forest fragments.

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## APPENDIXES

### APPENDIX A.

Foliar nutrient concentrations and  $^{15}\text{N}$  and  $^{13}\text{C}$  stable isotopes compositions. Samples number (n), mean  $\pm$  standard deviation of the dependent variables C, N, P,  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and C:N ratio in relation to the independent variables I, II, III, Restoration, SG, and their plots.

Area	Plot	n	C (g kg <sup>-1</sup> )	N (g kg <sup>-1</sup> )	P (g kg <sup>-1</sup> )	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	C:N
I	A	10	446.7 $\pm$ 25.3	20.9 $\pm$ 5.6	0.48 $\pm$ 0.15	0.4 $\pm$ 0.7	-30.7 $\pm$ 1.6	22.8 $\pm$ 6.7
	B	8	432.2 $\pm$ 25.4	22.7 $\pm$ 8.7	0.46 $\pm$ 0.15	0.6 $\pm$ 1.2	-31.9 $\pm$ 0.8	21.6 $\pm$ 7.9
	C	10	453.5 $\pm$ 20.8	20.4 $\pm$ 5.1	0.51 $\pm$ 0.25	0.7 $\pm$ 1.1	-31.2 $\pm$ 0.7	23.5 $\pm$ 5.8
	D	10	430.2 $\pm$ 14.7	19.7 $\pm$ 4.6	0.53 $\pm$ 0.06	0.1 $\pm$ 1.3	-30.8 $\pm$ 1.0	23.1 $\pm$ 6.5
	Mean		4	444.6 $\pm$ 10.8	21.0 $\pm$ 0.5	0.46 $\pm$ 0.04	0.4 $\pm$ 0.2	-31.2 $\pm$ 0.4
II	E	9	452.0 $\pm$ 18.6	22.4 $\pm$ 3.6	0.68 $\pm$ 0.1	4.3 $\pm$ 1.3	-31.4 $\pm$ 1.4	21.0 $\pm$ 3.1
	F	15	486.8 $\pm$ 20.4	27.0 $\pm$ 9.1	0.63 $\pm$ 0.12	3.6 $\pm$ 1.4	-30.5 $\pm$ 1.2	20 $\pm$ 6.4
	G	13	484.5 $\pm$ 20.0	23.6 $\pm$ 7.2	0.70 $\pm$ 0.26	4.6 $\pm$ 1.1	-30 $\pm$ 1.2	22.0 $\pm$ 7.0
	H	9	466.7 $\pm$ 21.0	25.0 $\pm$ 6.2	0.68 $\pm$ 0.23	5.4 $\pm$ 1.3	-31.5 $\pm$ 0.7	20.0 $\pm$ 4.0
	Mean		4	471.0 $\pm$ 19.0	23.4 $\pm$ 2.0	0.65 $\pm$ 0.02	4.4 $\pm$ 0.5	-30.8 $\pm$ 0.8
III	I	8	466.3 $\pm$ 25.0	19.3 $\pm$ 3.5	0.54 $\pm$ 0.13	1.7 $\pm$ 1.0	-31.2 $\pm$ 0.8	25.0 $\pm$ 5.0
	J	10	453.2 $\pm$ 33.2	23.3 $\pm$ 7.0	0.45 $\pm$ 0.10	3.4 $\pm$ 1.0	-31.0 $\pm$ 0.8	21.3 $\pm$ 7.4
	K	7	470.5 $\pm$ 21.2	24.0 $\pm$ 7.0	0.54 $\pm$ 0.12	2.2 $\pm$ 1.0	-30.6 $\pm$ 0.8	21.1 $\pm$ 6.2
	L	7	452.5 $\pm$ 25.7	25.4 $\pm$ 12.2	0.83 $\pm$ 0.86	2.2 $\pm$ 1.4	-29.6 $\pm$ 1.4	20.4 $\pm$ 7.0
	Mean		4	467.0 $\pm$ 5.0	22.1 $\pm$ 3.0	0.50 $\pm$ 0.05	2.2 $\pm$ 0.6	-30.7 $\pm$ 0.7

Restoration								
	M	11	450.3 ± 20.0	35.4 ± 13.0	1.33 ± 0.16	2.4 ± 2.1	-29.1 ± 2.0	14.0 ± 4.0
	N	8	445.0 ± 34.5	25.1 ± 4.5	1.52 ± 0.60	3.7 ± 2.7	-27.3 ± 1.4	18.3 ± 5.0
	O	8	463.5 ± 31.3	31.6 ± 13.2	1.37 ± 0.41	2.9 ± 1.0	-29.0 ± 1.4	17.0 ± 6.1
	P	8	442.4 ± 29.0	31.5 ± 7.8	1.73 ± 0.81	3.7 ± 1.0	-29.4 ± 2.1	15.1 ± 5.2
Mean		4	453.1 ± 12.0	30.0 ± 4.0	1.31 ± 0.07	3.0 ± 0.6	-28.4 ± 0.8	14.8 ± 1.6
SG								
	P1	21	463.4 ± 25.2	19.0 ± 3.0	0.58 ± 0.22	3.0 ± 1.8	-32.4 ± 1.1	25.1 ± 4.1
	P2	14	473.5 ± 26.5	17.7 ± 4.1	0.43 ± 0.20	3.7 ± 1.7	-32.4 ± 0.6	28.5 ± 9.0
	P3	11	469 ± 17.4	18.2 ± 4.2	0.46 ± 0.11	4.5 ± 1.5	-31.5 ± 1.2	27.1 ± 6.4
	P4	12	453.0 ± 35.0	19.5 ± 6.2	0.38 ± 0.15	3.5 ± 2.0	-32.0 ± 1.0	25.4 ± 8.1
Mean		4	467.1 ± 3.7	18.6 ± 0.5	0.50 ± 0.08	4.0 ± 0.7	-32.2 ± 0.5	25.0 ± 1.2

Tree species used for the restoration of the area “restoration” in Coruripe Mill lands, Alagoas state, Brazil.

Species	Family	Given Name
<i>Himathanthus phagedaenicus</i> (Mart.) Woodson	Apocynaceae	Banana de Papagaio
<i>Samanea tubulosa</i> (Benth.) Barneby and J. W. Grimes	Fabaceae - Mimosoideae	Bordão de Velho
<i>Tabebuia serratifolia</i> (Mart.) Sandl.	Bignoniaceae	Canafístula
<i>Cassia grandis</i> (Linnaeus f.)	Fabaceae -Caesalpinoideae	Canafístula Rosa
<i>Cecropia pachystachya</i> Trec.	Cecropiaceae	Embaúba
<i>Vantanea parviflora</i> Lam.	Humiriaceae	Goiti
<i>Inga</i> sp.	Fabaceae - Mimosoideae	Ingá
<i>Tabebuia cristata</i> A.H.Gentry	Bignoniaceae	Ipê Amarelo
<i>Chamaecrista ensiformis</i> (Vell.) H.S.Irwin & Barneby	Fabaceae - Caesalpinoideae	Miolo Preto
<i>Paubrasilia echinata</i> (Lam.) E. Gagnon, H.C. Lima & G.P. Lewis	Fabaceae	Pau-Brasil
<i>Aspidosperma polyneuron</i> Müll.Arg.	Aspidosperma	Peroba Rosa
<i>Caesalpinia pluviosa</i> DC.	Fabaceae-Caesalpinoideae	Sibipiruna
<i>Bowdichia virgilioides</i> H.B.K	Fabaceae-Papilionoideae	Sucupira
<i>Vitex polygama</i> Cham.	Verbenaceae	Fruta preta