MOISTURE AND NUTRIENT CONSTRAINTS TO ECOSYSTEM PROCESSES IN A FOREST REGROWTH STAND IN EASTERN AMAZONIA, BRAZIL

By

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This work is dedicated to my wife Lívia and my daughter Cárita.

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By

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Changes in land-use and climate are likely to alter resource (e.g., moisture and nutrient) availability in tropical forest soils, but quantitative assessment of the role of resource constraints as regulators of ecosystem processes is rather limited. In this dissertation, moisture and nutrient availability were altered through dry-season irrigation and bi-weekly aboveground litter removal, respectively, to study how these resources control aboveground and belowground ecosystem processes in a forest regrowth stand in the Brazilian Amazon. Moisture availability strongly constrains soil respiration as indicated by the responses of soil carbon dioxide emissions to soil wet-up events and dry-season irrigation. Higher moisture availability in irrigated plots also increased leaf litter decomposition and slightly increased soil nitrous oxide and methane emissions, but did not alter monthly litterfall quantity and quality, and soil nitric oxide emission. Litter removal decreased carbon dioxide emissions and litterfall nitrogen concentration, but had no effects on litterfall quantity, and soil nitrogen oxides and methane emissions. Aboveground net primary productivity was constrained by moisture availability as indicated by the response of wood increment to interannual variation in dry season rainfall and to irrigation, suggesting decreased potential of carbon sequestration from forest regrowth under anticipated scenarios of reduced rainfall in Amazonia.

CHAPTER 1
INTRODUCTION

In many tropical areas, especially in the Brazilian Amazon, old-growth forests are increasingly being converted to forest regrowth—also known as secondary or successional forests—following abandonment of slash-and-burn agriculture and cattle pasture. Fearnside (1996) estimated that about 50% of the deforested Brazilian Amazon landscape was in some stage of forest regrowth in 1990. Forest regrowth provides important ecosystem services such as carbon sequestration, reestablishment of nutrient and water cycles, and maintenance of biodiversity (Brown & Lugo 1990, Markewitz *et al.* 2004, Nepstad *et al.* 2001, Sommer *et al.* 2002). In addition, these forests often represent an important source of income (woody and non-woody forest products) to local people (Brown & Lugo 1990). Adequate management of forest regrowth can represent an important alternative to reduce pressure on old-growth forest sites in the Amazon region (Brown & Lugo 1990).

Forest regrowth can play an important role in regional and global carbon (C) dynamics because of their high rates of biomass accumulation—a proxy for C sequestration (Zarin *et al.* 2001)—although the frequent clearing of regrowth results in small net C uptake compared to total emissions from deforestation in the Amazon (Steininger 2004). Efforts to determine the capacity of forest regrowth to sequester C at different spatial and temporal levels have been pursued by recent modeling efforts (Neeff 2005, Zarin *et al.* 2001). Analyses of the rates of and controls on biomass accumulation may contribute to improved modeling of the potential of forest C sequestration (Johnson *et al.* 2000). Observational studies have shown that several factors control the rate of biomass accumulation in tropical regrowth sites; these include land-use history (including disturbance type and intensity) (Gehring *et al.* 2005, Moran *et al.* 2000, Uhl *et al.* 1988, Zarin *et al.* 2005), surrounding vegetation, soil fertility (Gehring *et al.* 1999, Moran *et al.*

2000), and climate (Zarin *et al.* 2001). Such a variety of controlling factors complicates modeling efforts of forest regrowth rates, but a recent synthesis of observational studies has shown that soil texture and dry season length are strongly correlated with C accumulation by forest regrowth, possibly through their effects on the availability of soil moisture and nutrients (Zarin *et al.* 2001).

Further understanding of the processes by which moisture and nutrient availability constrain biomass accumulation rates may be critical to understand the role of forest regrowth on regional and global C dynamics under future land-use and climate change scenarios. Manipulative experiments are required to better comprehend the role of resource availability on forest ecosystem processes. Unfortunately such experiments have rarely been employed to study ecosystem processes in tropical forests in general and especially in Amazonian forest regrowth, which represents an important component of the landscape in the region (Fearnside 1996, Neeff *et al.* 2006, Zarin *et al.* 2001).

This study is part of the MANFLORA project (Manipulation of Moisture and Nutrient Availability in Young Regrowth Forests in Eastern Amazonia), which is a collaborative research program among the University of Florida, the *Universidade Federal Rural da Amazônia* (Federal Rural University of Amazonia—UFRA), and *EMBRAPA Amazônia Oriental* (EMBRAPA Eastern Amazon) initiated in 1999 at the UFRA experimental station in Castanhal, Pará, Brazil. Since 2001, moisture and nutrient availability have been altered in two separate experiments: (1) dry-season irrigation and (2) continuous litter removal. To my knowledge, the MANFLORA project represents the only long-term, large-scale (stand-level) experimental manipulation of moisture availability in tropical forest regrowth, and one of the few tropical forest regrowth nutrient manipulation studies.

In this dissertation, I examine the influence of resource availability on carbon and nutrient dynamics associated with litterfall, leaf litter decomposition, soil trace gases, and aboveground net primary productivity (ANPP). The dissertation is divided into 7 chapters, including this general introduction and literature review. The second chapter describes the study site and experimental design. The effects of moisture and nutrient availability on litterfall (Chapter 3), leaf decomposition (Chapter 4), soil trace gases (Chapter 5), and ANPP (Chapter 6) are examined separately. Conclusions are summarized in Chapter 7.

Literature Review

This literature review addresses the role of moisture and nutrient availability as constraints on ecosystem processes related to C dynamics in tropical sites, with special emphasis on forest regrowth sites in the Brazilian Amazon. The growth of adult trees is the main focus of this review since they contribute the most to stand-level C balance, although the effects of abiotic stresses on the understory [e.g., drought (Aragão *et al.* 2005, Fortini *et al.* 2003)] may be more dramatic than on overstory plants. Finally, the review is directed to moist, lowland evergreen tropical forests (Whitmore 1992), even though relevant information from dry, deciduous tropical forests is also included, since there is a great deal of literature on rainfall effects on tree growth for deciduous sites.

Moisture and Nutrient Limitations to Tropical Forests

Tropical forest formations occupy areas with limited variation in temperature but a wide range in rainfall intensity and distribution (Whitmore 1992), giving rise to differing degrees in deciduousness. Since tropical lowland evergreen forests are characterized by high annual rainfall and evergreeness, and often occur on highly weathered, dystrophic soils (Sanchez 1976), previous research has mainly overlooked the effects of moisture on forest processes and, therefore, has focused on nutrient limitations and mechanisms of nutrient conservation (Herrera *et al.* 1978, Jordan 1983). However, more recent studies have demonstrated that ecosystem processes in tropical forests, including Amazonian forests, can be substantially affected by strong seasonality in rainfall (e.g., Keller *et al.* 2004). During the dry season (monthly precipitation < 100 mm), old-growth forests in Amazonia rely on deep rooting to retain leaves (Nepstad *et al.* 1994). In extreme cases, prolonged droughts, usually associated with El Niño events, can result in higher tree mortality in tropical old-growth (Condit *et al.* 1995, Williamson *et al.* 2000) as well as regrowth forests (Chazdon *et al.* 2005), increasing forest susceptibility to fire (Nepstad *et al.* 1999).

Observational and Manipulative Experiments to Study Moisture and Nutrient Limitations in Tropical Forests

Researchers usually rely on observational studies to infer moisture and nutrient limitations to ecosystem level processes in tropical forests. Measurement of ecosystem processes during wet and dry seasons (Berish & Ewel 1988, Cornu *et al.* 1997, Dantas & Phillipson 1989, Davidson *et al.* 2000, Scott *et al.* 1992) or along rainfall gradients (Santiago 2003, Schuur & Matson 2001) has been used to study moisture constraints. An important limitation of studies based on rainfall seasonality is the lack of control over factors (e.g., light availability, vapor pressure deficit, and phenology) that covary with rainfall seasonality and may significantly affect ecosystem processes.

Nutrient constraints may be investigated by comparing ecosystem processes among different soil types varying in nutrient availability (e.g., Moran *et al.* 2000). This approach has the disadvantages of hindering the identification of the most limiting nutrient(s) at a specific site because of inherent differences in soil characteristics (e.g., soil organic matter, pH, structure, texture), as well as incomplete control over land-use history among sites. Such disadvantages may be overcome through fertilizer addition in nutrient manipulation studies. Substrate-age sequences (Vitousek & Farrington 1997) with contrasting nutrient availability have also been used to study nutrient effects on ecosystem processes, but these sequences are spatially restricted.

Although observational studies are useful for identifying general trends as well as key questions for further research, they often do not permit a process-based understanding of resource control over ecosystem dynamics. An improved understanding of moisture and nutrient limitations on ecosystem processes may be obtained through manipulative experiments. Experimental manipulations of moisture availability are usually carried through water addition or exclusion, whereas nutrient manipulation usually involves fertilizer addition or litter removal (Eviner *et al.* 2000, Hanson 2000).

Long-term, large-scale moisture manipulation studies in tropical forests include the dry-season irrigation study in the Barro Colorado Island station, Panama (Cavelier *et al.* 1999, Wieder & Wright 1995, Wright & Cornejo 1990, Yavitt & Wright 2001, Yavitt *et al.* 2004), and two throughfall exclusion studies at old-growth sites in the Brazilian Amazon (Carvalho et al. 2005, Nepstad et al. 2002). Dry-season irrigation plots are easier to establish and operate than throughfall exclusion plots, but the latter are necessary to ultimately simulate the effects of drought on ecosystem processes. To my knowledge, there are no published reports for large-scale, long-term moisture manipulative experiments in tropical forest regrowth sites, except for the studies conducted within the MANFLORA Project (Aragão *et al.* 2005, Fortini *et al.* 2003, Vasconcelos *et al.* 2004, Veluci *et al.* In preparation).

Fertilization experiments are the most common tools used to manipulate nutrient availability in forests (e.g., Davidson *et al.* 2004a, Mirmanto *et al.* 1999, Tanner *et al.* 1998) because they are relatively easy and inexpensive (Eviner *et al.* 2000). In addition, those experiments are believed to provide the most conclusive evidences of nutrient limitation in forest ecosystems (Raich *et al.* 1994). However the interpretation of fertilization experiments may be confounded by several interactions of nutrients with microorganisms and the soil (nutrient immobilization by litter microbes, adsorption, trace gas losses, volatilization, and leaching) that reduce the pool of added nutrients for the plants (Eviner *et al.* 2000) or that cause secondary effects such as nutrient imbalance or soil pH alteration (Marschner 1995).

Litter removal is another technique for manipulating nutrient availability in forests. This technique avoids the problems related to nutrient addition experiments as discussed above, but litter removal has additional and unavoidable disadvantages including indirect effects on soil moisture and temperature variation due to the lack of insulation by aboveground litter (Sayer 2005). Also, soil compaction due to trampling (in the case of litter removal by raking) and raindrop impact occurs in litter removal plots. Such effects may alter soil microorganism activity and, ultimately, affect soil nutrient availability, representing, therefore, a potential confounding factor. Microbial activity may be further influenced by reduced input of labile C (Cleveland *et al.* 2002) with litter removal.

To my knowledge there is no large-scale, long-term experimental manipulation of nutrient availability through fertilization in lowland old-growth tropical forests, but there are some reports for forest regrowth, including a short-term experiment in Costa Rica (Harcombe 1977) and three other experiments at Amazonian sites. Nutrient addition in Amazonian forest regrowth has been conducted within both short-term, small-plot (Uhl 1987) and relatively long-term, large-plot conditions (Davidson *et al.* 2004a, Gehring *et al.* 1999); however, there are no reports of litter removal studies in this region, except for the study conducted within the MANFLORA Project (Vasconcelos *et al.* 2004). Thus far, there are only two large-scale, long-term litter manipulation studies in tropical forests besides the MANFLORA Project. The first study is part of the Gigante Litter Manipulation Project (GLiMP) in Panama (Sayer 2005) and included monthly litter raking from 45 m x 45 m plots. The other study excluded litter with tents on 3 m x 3 m plots in a secondary forest in the Luquillo Experimental Forest (Puerto Rico) as part of the Soil Organic Matter Dynamics Project (Li *et al.* 2005).

Moisture Effects on Ecosystem Processes in Tropical Forests

Improved knowledge of the mechanisms by which tropical forests respond to drought stress—both at the plant and community level—is crucial to current understanding and future projections of forest dynamics, C sequestration, and fire susceptibility in the context of ongoing land-use and climate changes. Anticipated climate change for the Amazon region may include more frequent and severe dry seasons in response to global warming (IPCC 2001), deforestation (Costa & Foley 2000), and more frequent El Niño episodes (Trenberth & Hoar 1997). Large scientific research initiatives in the Amazon region since the 1980's including the ABRACOS Project (Gash *et al.* 1996) and more recently the LBA Program (Davidson & Artaxo 2004, Keller *et al.* 2004) have generated a great deal of relevant information. However, there are few observational or manipulative studies aimed at investigating moisture controls on Amazonian forest regrowth.

The understanding of how low soil moisture availability controls tropical forest ecosystem processes is not straightforward because drought has many direct and indirect effects on plant and soil organisms (Figure 1-1). Below I review some effects of low soil moisture availability on above- and belowground processes; forest floor decomposition is included in aboveground processes for the purposes of this review.

Aboveground processes

Low soil moisture availability can affect aboveground C fluxes in tropical forests in various direct and indirect interrelated ways, mainly through moisture effects on carbon dioxide (CO_2) assimilation in photosynthesis. The effects of low soil moisture availability on leaf-level photosynthesis have been the subject of many recent reviews (e.g., Chaves *et al.* 2003, Lawlor 2002).

The interacting direct effects of drought stress on C assimilation include (a) decrease in stomatal conductance in response to low soil moisture supply or high vapor pressure deficit, leading to reduced CO_2 assimilation (Flexas & Medrano 2002, Lawlor 2002, Malhi *et al.* 1998, Mulkey & Wright 1996), and (b) impairment of photosynthetic machinery (Chaves *et al.* 2003, Malhi *et al.* 1998). Drought may also affect C assimilation through indirect effects: (a) xylem cavitation during dry periods reduces hydraulic conductivity constraining stomatal conductance and CO_2 assimilation (Brodribb *et al.* 2002, Hubbard *et al.* 2001), (b) CO_2 assimilation decreases in response to phenological changes that reduce leaf area (Malhi *et al.* 1998), and (c) low soil moisture decreases nutrient availability—either directly through reducing nutrient solubility, and/or indirectly through creating less favorable conditions for the microbial activity that is responsible for the decomposition of organic matter and release of nutrients in the soil (Cornejo *et al.* 1994, Malhi *et al.* 1998)—reducing leaf nutrients and limiting CO_2 assimilation.

Some studies in the Amazon region have reported a decrease in CO_2 uptake at the leaf level during the dry season for understory forest species (Aragão *et al.* 2005, Fortini *et al.* 2003), but similar data for overstory species are scarce. Induced drought in throughfall exclusion plots reduced canopy leaf-level CO_2 assimilation for some tree species in an old-growth forest in Amazonia (Nepstad *et al.* 2002), consistent with a moisture limitation on leaf gas exchange.

Comparable studies in Amazonian forest regrowth are lacking, indicating the need for more studies of moisture limitations on canopy leaf gas exchange for these forests.

Drought effects at the leaf level may reflect processes at the individual tree and ultimately ecosystem (or stand-) levels in tropical forests (Figure 1-1). In old-growth forest sites in the Brazilian Amazon, higher stem diameter growth rates (a component of aboveground net primary productivity, ANPP) are associated with wetter periods (Higuchi *et al.* 2003, Rice *et al.* 2004, Vieira *et al.* 2004). Comparable data for regrowth sites are scarce in part because most published studies of forest regrowth in the tropics rely on one single inventory campaign in stands of different ages to represent a successional chronosequence (e.g., Saldarriaga *et al.* 1988), resulting in few available data for comparison between periods with different moisture availability for the same stand. In Costa Rican secondary rain forests, the mortality of trees (diameter at breast height ≥ 10 cm) increased significantly with lower dry-season rainfall, but not with total annual rainfall (Chazdon *et al.* 2005), suggesting that tropical forest regrowth may be extremely sensitive to rainfall seasonality.

Carbon assimilation at the stand level can also be influenced by phenological changes associated with water stress or a weakly deciduous strategy adopted by some tropical trees (Malhi *et al.* 1998). In fact, higher litterfall rates during the dry period in tropical forests have been reported in many studies (e.g., Dantas & Phillipson 1989, Scott *et al.* 1992, Wieder & Wright 1995), but irrigation during the dry season in a tropical forest in Panama did not affect the quantity or timing of litterfall (Wieder & Wright 1995). Higher litterfall rates associated with the dry season may be triggered by an increase in vapor pressure deficits (Wright & Cornejo 1990), a decrease in cloud cover and soil nutrient availability (Eamus & Prior 2001), or may reflect a genetic trait (Goulden *et al.* 2004). An important issue to consider is the production of different leaf phenotypes associated with rainfall seasonality as reported by Kitajima *et al.* (1997) in a Panamanian seasonal dry forest. Kitajima *et al.* (1997) found that leaves produced in the late wet season (measured in the dry season) had higher photosynthetic rates than those produced in the early wet and measured in the wet season.

Eddy covariance measurements of net CO_2 exchange—the balance between gross primary productivity and ecosystem respiration (Roy & Saugier 2001)—have shown different responses of net CO_2 assimilation during dry periods in the Brazilian Amazon. Malhi *et al.* (1998) reported reduced net CO_2 uptake during the dry season in central Amazonia. However, Saleska *et al.* (2003) and Goulden *et al.* (2004) found higher rates of net C sequestration during the dry season in an old-growth forest in east-central Amazonia, probably because drought reduced forest floor decomposition, but not canopy photosynthesis. Thus, litter decomposition apparently plays an important role in defining the direction of change in net ecosystem exchange due to drought stress. Litterbag and mass balance studies have shown that low moisture availability reduces litter decomposition in tropical forests (Cornejo *et al.* 1994, Cornu *et al.* 1997, Luizão & Schubart 1987, Wieder & Wright 1995), probably as a result of lower leaching and/or decomposer activity during dry periods.

Modeling studies have also predicted drought constraints on C dynamics of tropical forests. Several ecosystem modeling studies (Asner *et al.* 2000, Foley *et al.* 2002, Phillips *et al.* 1998, Potter *et al.* 2001, Potter *et al.* 2004, Prentice & Lloyd 1998, Tian *et al.* 1998) have analyzed the effects of recent El Niño-Southern Oscillation (ENSO) events on the C balance of Amazonian forests. All of these studies have indicated that the basin is a source of CO_2 (negative net ecosystem productivity, NEP) during El Niño events and a sink of CO_2 (positive NEP) during La Niña events. Changes in C balance due to ENSO events in these models are largely driven through changes in net primary productivity (NPP), and not through alterations in heterotrophic respiration (Foley *et al.* 2002, Tian *et al.* 2000). During El Niño years, lower precipitation and higher temperatures result in increased simulated annual drought stress that limits NPP.

In contrast, the dry period may represent an opportunity for C gain due to increased light availability associated with reduced cloudiness. During typical, non-ENSO rainfall years, Huete *et al.* (2006) found widespread greening in the dry season for central and eastern Amazonian old-growth forests, suggesting that sunlight may represent a stronger control over forest phenology and productivity than moisture availability. Consistent with a light limitation to forest phenology and productivity, Graham *et al.* (2003) reported increased photosynthesis, vegetative growth, and reproduction for branches of a tropical tree supplied with extra illumination during cloudy periods in Panama. Further research on the controls of water and light over tropical forest functioning is needed to better comprehend the response of these forests to climate change.

Belowground processes

Belowground processes are constrained by drought through the effects of low soil moisture availability on root and microbial dynamics. Root growth declines under low soil water potential, as shown for a temperate oak forest (Joslin *et al.* 2001), but root elongation may be stimulated by dry conditions (Akmal & Hirasawa 2004) if plants allocate a larger fraction of photosynthate to belowground biomass in response to drought. In tropical forests, fine root production decreases and mortality may increase during the dry season as shown by observational (Berish & Ewel 1988) and manipulative studies in old-growth sites (Cattânio *et al.* 2002, Cavelier *et al.* 1999, Yavitt & Wright 2001). Microbial activity is also constrained by low soil moisture availability in tropical forest soils (Cleveland *et al.* 2002, Luizao *et al.* 1992). Decreased root and/or microbial activities in the mineral soil and/or aboveground litter are likely causes of reduced soil CO_2 efflux during the dry season (Davidson *et al.* 2000, Vasconcelos *et al.* 2004).

Drought may also have an indirect effect on belowground processes if reduction in leaf C assimilation under low soil moisture conditions results in decreased export of photosynthates to roots. Such a reduced export may decrease the availability of C for root and rhizosphere microorganism activity (Högberg *et al.* 2001). However, the negative impact of low soil moisture on belowground processes can be mitigated if hydraulically lifted water makes a significant contribution to delaying soil dry-down in tropical forests. This phenomenon would allow microorganisms to remain active for longer periods (Horton & Hart 1998), therefore leading to an increase in nutrient mineralization. Da Rocha *et al.* (2004) suggested that the lack of drought stress in an eastern Amazonian old-growth forest was probably related to deep rooting and water redistribution by hydraulic lift. In the context of the Tapajós Throughfall Exclusion Experiment, Romero-Saltos *et al.* (2005) did not find evidence for hydraulically lifted water by understory/midcanopy tree species using deuterium-labeled soil water profiles, while Oliveira *et al.* (2005) showed strong evidence for the occurrence of hydraulic redistribution based on the dynamics of peaks of water recharge between shallow and deep soil layers, and sap flow data measured in tap and lateral roots.

Nutrient Effects on Ecosystem Processes in Tropical Forests

In the tropics, many soils are highly weathered and consequently dystrophic (Sanchez 1976), which has led most past research to focus on nutrient cycling and assume that nutrient availability limits tropical lowland evergreen forest productivity (Vitousek 1984). However, evidence for such a constraint is rather limited. Malhi *et al.* (2004) reported that spatial variability of coarse wood productivity of neotropical forests was apparently associated with soil fertility. Manipulative experiments involving nutrient addition are necessary to show limitation by a specific nutrient (Tanner *et al.* 1998), but such experiments are scarce in tropical lowland forests. Most studies rely on soil nutrient inventories, aboveground biomass accumulation (Zarin *et al.* 2001), leaf and litterfall nutrient concentrations (Vitousek 1984, Wood *et al.* 2005), root growth responses (Cuevas & Medina 1988), and structural properties (Herrera *et al.* 1978) to infer nutrient limitation to tropical lowland forest processes. Below I review some effects of nutrient availability on above- and belowground processes.

Aboveground processes

Many essential mineral elements are directly or indirectly involved in plant tissue growth (Marschner 1995), but a key aspect of the relation between nutrient availability and plant growth and function is the positive correlation between maximum net photosynthesis and leaf nitrogen (N) concentration (e.g., Lambers *et al.* 1998). This relationship is a consequence of the high investment of leaf nitrogen in the enzyme responsible for carboxylation (ribulose bisphosphate carboxylase, Rubisco) and in other photosynthetic enzymes (Chapin *et al.* 2002, Taiz & Zeiger 1998). However, the significance and form of the relationship between maximum net photosynthesis and leaf nitrogen concentration may depend upon the importance of other limiting nutrients including phosphorus (P) as reported for Amazonian tree species (Reich *et al.* 1994).

Phosphorus is often hypothesized to be the most limiting nutrient in old-growth and regrowth lowland tropical forests. Analyzing within-stand nutrient use efficiency and nutrient return in litterfall, Vitousek (1984) suggested that P, but not N availability, constrains fine litterfall (an important component of ANPP) in lowland tropical forests, especially at Amazonian sites. Davidson *et al.* (2004a), however, reported N co-limitation to tree growth at a forest regrowth site subjected to several cycles of slash-and-burn in the Brazilian Amazon, and associated the limitation by N with substantial losses of this element through burning. Also, in an old-growth lowland evergreen forest in Indonesia, Mirmanto *et al.* (1999) reported increased fine litterfall in plots fertilized with N, P, and N+P.

Aboveground biomass in old-growth forests in Central Amazonia has been negatively correlated with soil sand content (Laurance *et al.* 1999), probably due to the low capacity of nutrient retention by sandy soils. However, since sandy soils also have low moisture retention capacity (Brady 1989), moisture limitation may also have contributed to the results obtained by Laurance *et al.* (1999).

Belowground processes

Nutrient availability affects belowground processes by altering root and soil microorganism activities. Root responses to low nutrient availability may not be straightforward. The increased allocation of resources to belowground structures may be associated with low soil fertility (Giardina *et al.* 2004, Gower 1987). However, higher proliferation of fine roots in fertilized ingrowth cores in tropical forests suggests that root growth is limited by low soil nutrient availability (Cuevas & Medina 1988, Mcgrath *et al.* 2001, Ostertag 1998, Raich *et al.* 1994), although fine root growth did not respond to likely reduced nutrient availability in litter removal plots in an old-growth forest in Panama (Sayer *et al.* 2006). For a Panamanian lowland tropical forest, Cavelier *et al.* (1999) suggested that control of fine root production may be more complex, involving not only nutrient pulses, but also water pulses and aboveground biomass growth.

Soil microbial activity is constrained under low soil nutrient conditions. Cleveland *et al.* (2002, 2003) have shown increased microbial respiration with phosphorus addition to tropical forest soil samples in a laboratory experiment. Cleveland and Townsend (2006) reported increased *in situ* soil respiration with phosphorus and nitrogen fertilization in an old-growth forest in Costa Rica; these authors suggested that phosphorus increased microbial respiration while nitrogen probably affected soil respiration through effects on fine root dynamics.

Production of roots on top of the mineral soil has been considered as a structural characteristic of forests growing on low nutrient soils (Herrera *et al.* 1978). However a recent study has shown significant root growth in the forest floor of a relatively fertile old-growth site in Panama, suggesting that proliferation of roots on top of the mineral soil is not necessarily caused by low mineral soil nutrient levels, but may result from the availability of aboveground litter (Sayer *et al.* 2006).

Conclusions

Research on moisture and nutrient constraints to tropical forest regrowth is rather limited in quantity, and results are sometimes divergent from one study to the next. Manipulative studies to investigate soil and plant processes in tropical forest regrowth are lacking, and are an important tool for exploring the complex interactions that influence ecosystem response to resource limitations. Furthermore, these studies are needed to better understand present conditions and to project future impacts of climate and land-use changes on C dynamics in tropical forest regrowth.

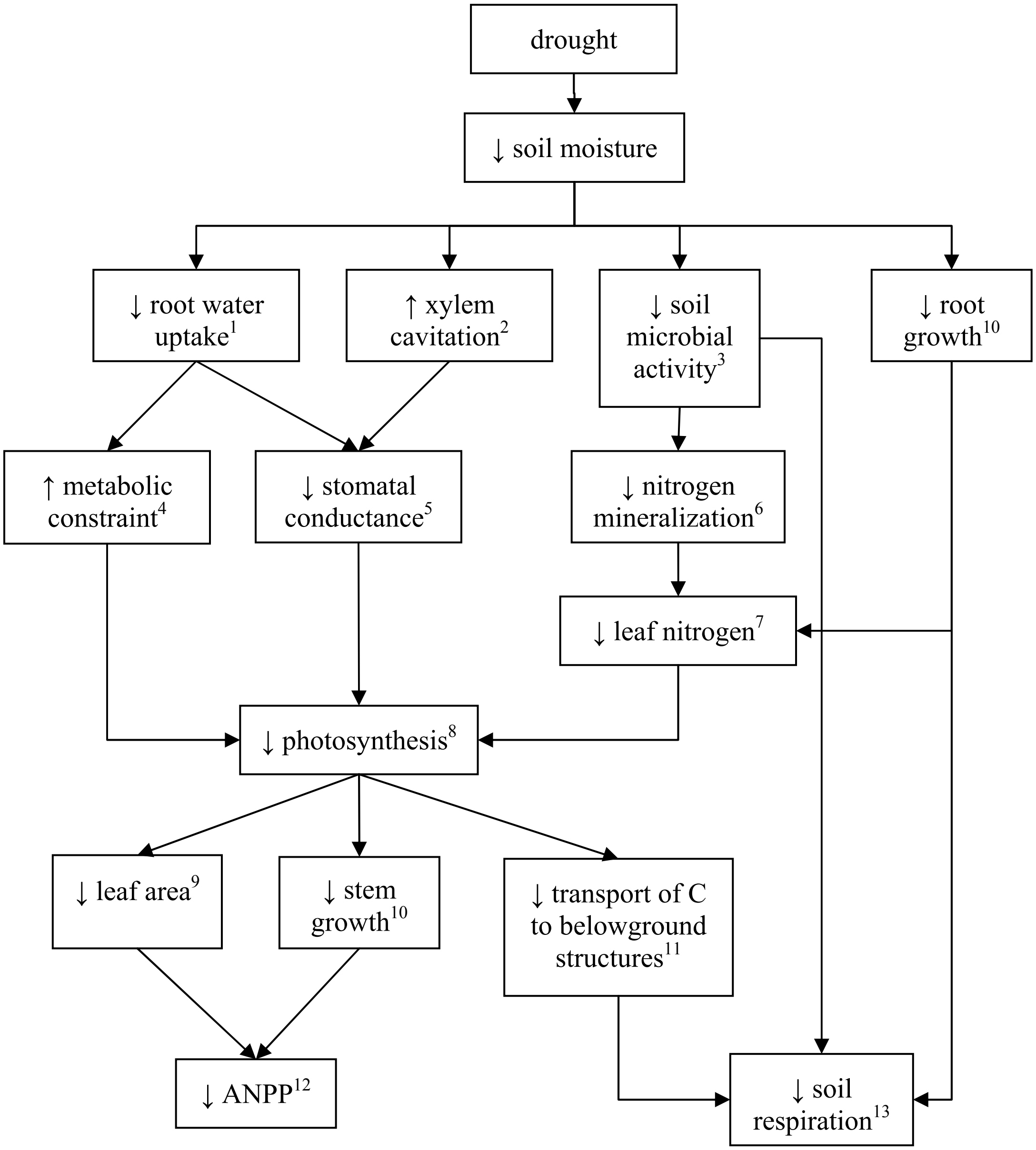

Figure 1-1. Simplified conceptual diagram of likely effects of drought on leaf- and ecosystem-level processes addressed in this dissertation. ↑ and ↓ symbols stand for increase and decrease, respectively; ANPP – aboveground net primary productivity. Numbers refer to some study evidences of drought effects on processes: [1]Eamus (2003); [2]Sperry (2000); [3]Cleveland *et al.* (2002); [4]Lawlor and Cornic (2002); [5]Brodribb *et al.* (2002), Hubbard *et al.* (2001); [6]Firestone and Davidson (1989);[7]Chapin *et al.* (2002); [8]Lawlor and Cornic (2002); [9]Rascher (2004); [10]Nepstad *et al.* (2002); [11,12]Högberg *et al.* (2001); [13]Davidson *et al.* (2000), Vasconcelos *et al.* (2004).

CHAPTER 2
STUDY SITE AND EXPERIMENTAL DESIGN

Study Site

This study was conducted at a field station belonging to the Federal Rural University of Amazonia (*Universidade Federal Rural da Amazônia*—UFRA), Brazil, near the city of Castanhal (1° 19' S, 47° 57' W) in the state of Pará. Since July 2001, daily rainfall was measured 500 m away from the experimental area using a standard rain gauge. Prior to July 2001, rainfall data reported here are from the National Agency of Electrical Energy (*Agência Nacional de Energia Elétrica*—ANEEL) network meteorological station at Castanhal (1^{o} 17' 53" S, 47^{o} 56' 56" W) located ~3 km from our site, but no longer in operation. From 70 to 90% of annual rainfall occurs between January and July, resulting in a dry period from August to December (Figure 2-1). Annual rainfall during the experimental period (Table 2-1) was consistent with the mean ± standard error value registered from 1990 to 1999 by ANEEL (2461 ± 271 mm). The number of dry months (rainfall < 100 mm $month^{-1}$) during the experimental period varied from 2 to 5; several authors (e.g., Vieira *et al.* 2004) consider dry season months as those with less than 100 mm rainfall for tropical sites.

The soils are classified as Dystrophic Yellow Latosol Stony Phase I (Tenório *et al.* 1999) in the Brazilian Classification, corresponding to Sombriustox in U.S. Soil Taxonomy. Soil granulometric composition in the first 20 cm is 20% clay, 74% sand, and 6% silt. Concretions represent 16% of the soil volume in the upper 10 cm of soil. In the surface soil (0 - 10 cm), pH is 5.0, organic C is 2.2%, organic C stock is 2.9 kg m^{-2}, total N is 0.15%, C:N is 14.4, and Mehlich-1 extractable phosphorus is 1.58 mg kg^{-1} (Rangel-Vasconcelos 2002). This level of extractable soil phosphorus suggests low availability at our study site compared to other soil types and land uses in Amazonia (Mcgrath *et al.* 2001).

Forest regrowth, annual crops, and active and degraded pastures characterize the landscape surrounding the field station. The stand under study was last abandoned in 1987 following multiple cycles of shifting cultivation, beginning in the 1940's when the old-growth forest was cleared. Each cycle of 1 to 2 years included cultivation of corn, manioc, and beans, followed by fallow. Typical shifting cultivation cycles lasted seven to ten years (G. Silva e Souza & O.L. Oliveira pers. comm.). Trees are mostly evergreen, with few species (e.g., *Annona paludosa* and *Rollinia exsucca*) showing deciduousness during the dry season. The four most abundant overstory species are *Lacistema pubescens* Mart., *Myrcia sylvatica* (G. Mey.) DC, *Vismia guianensis* (Aubl.) Choisy, and *Cupania scrobiculata* Rich., representing 71% of all stems in the stand. In November 1999, mean ± se stem density was 213 ± 19.7 individuals per 100 m^2, basal area was 13 ± 6 m^2 ha^{-1}, height was 4.9 ± 0.4 m for the stand (Coelho *et al.* 2004), and aboveground biomass was 51.1 ± 2.5 Mg ha^{-1} for trees with diameter at breast height > 1 cm.

Experimental Design

Plots were established in August 1999, when the forest regrowth was 12 years old. Each treatment plot is 20 m x 20 m with a centrally nested 10 m x 10 m measurement subplot. The area between the measurement subplot and the plot—hereafter called "outer area"—was used for some destructive samplings of soil, root, and aboveground litter. There were four replicate plots for the irrigation treatment, four plots for the litter removal treatment, and four plots left untreated as controls (Figure 2-2). Adjacent treatment plots were spaced 10 m from each other.

One tensiometer (Jet Fill Tensiometers, Soilmoisture Equipment Corp., Santa Barbara, CA, USA) was installed at a depth of 10 cm in each plot and soil water potential was recorded on a weekly basis in the morning. The number of actual replicates per treatment varied due to loss of water column tension during the dry season. Soil suction variation in response to rainfall seasonality and manipulation treatments is presented in Chapter 5.

Irrigation was applied at a rate of 5 mm day^{-1}, for about 30 minutes, during the dry seasons of 2001 to 2005 (Table 2-2) in the late afternoon. The amount of daily irrigation applied corresponds to regional estimates of daily evapotranspiration (Jipp *et al.* 1998, Lean *et al.* 1996, Shuttleworth *et al.* 1984). Irrigation water was distributed through tapes with microholes every 15 cm. In 2001, irrigation tapes were spaced 4 m from each other. In the subsequent irrigation periods we reduced the distance between tapes to 2 m to facilitate more even distribution of water.

We used rainfall and soil suction data to define approximate boundaries for the dry and wet seasons. The start of the dry season was defined by total rainfall less than 150 mm in the previous 30 days and soil suction more negative than -0.010 MPa; the end of the dry season was defined by total rainfall greater than 150 mm in the previous 30 days and soil suction less negative than -0.010 MPa. Since the soil suction data were obtained on a weekly basis, we estimate that the error in the location of seasonal boundaries is about 7 days. The lowest tension value registered was -0.092 MPa, which may reflect the limited functional range of tensiometers (Hanson 2000), although lower tensions may have occurred towards the end of dry season. The installation of tensiometers deeper in the soil and of time domain reflectrometer sensors for measurement of soil moisture content were hindered by the shallow depth to laterite in the soil profile in Apeú.

In the litter removal plots, leaf and branch fall were removed from the forest with plastic rakes every two weeks, beginning in August 2001 with the removal of the pretreatment litter layer (538 ± 35 g m^{-2}, n = 8); carbon and nitrogen stocks of the pretreatment litter layer were

222.9 ± 14.6 and 7.3 ± 0.5 g m^{-2}, respectively (n = 8). Raking maintained very low, but not entirely absent litter standing crop. Total new non-woody litterfall removed during the treatment period (from August 2001 to December 2005) was 3568 ± 136 g m^{-2} (n = 12). Carbon and nitrogen concentrations of pre-treatment litterfall were 47.9 ± 0.2 and 1.2 ± 0.02%, respectively, corresponding to a C:N ratio of 40 ± 0.7 (n = 12).

Measurements of gravimetric soil moisture content in the first 10 cm of soil for one date during the 2001 dry season indicated that irrigated plots had about twice as much moisture as control plots (22 ± 2% vs. 10 ± 2%); in the litter removal plots soil moisture was 11 ± 2%. For one date during the 2001 wet season, gravitational soil moisture content was 27 ± 2% for control and irrigated plots, and 31 ± 2% for litter removal plots (Rangel-Vasconcelos 2002). The difference in soil moisture status between control and irrigated plots was reflected in dry-season differences in pre-dawn leaf water potential for an understory species (*Miconia ciliata*); in November 2001 pre-dawn leaf water potential for control plants was about -1.2 MPa while irrigated plants were about 1 MPa less negative (Fortini *et al.* 2003).

Table 2-1. Characteristics of rainfall distribution and intensity during the experimental period in the site.

	Year						
	1999	2000	2001	2002	2003	2004	2005
Annual rainfall (mm)	2577	2399	3179	2301	2895	3038	2793
Minimum monthly rainfall (mm)	NA[b]	66	34	56	42	8	13
Maximum monthly rainfall (mm)	NA	291	489	385	499	611	476
Number of dry season months [a]	NA	3	5	4	2[c]	3	3[c]
Total dry season rainfall (mm) [d]	NA	694	304	400	647	445	615

[a] Rainfall < 100 mm month^{-1}.

[b] NA - Not available.

[c] Not consecutive months.

[d] Dry season period = August to December.

Table 2-2. Dry-season irrigation intervals and associated rainfall intensity and distribution.

Dry-season irrigation	Interval	Total rainfall (mm)	Maximum daily rainfall (mm)	Number of days without rainfall
1st	10 Aug 2001 to 16 Jan 2002	453	54	101 (63%)[a]
2nd	16 Aug 2002 to 20 Jan 2003	516	66	93 (59%)
3rd	7 Aug 2003 to 20 Dec 2003	559	74	90 (66%)
4th	23 Sep 2004 to 26 Jan 2005	547	130	81 (64%)
5th	29 Jul 2005 to 12 Dec 2005	422	66	97 (71%)

[a] Percentage of days without rainfall during dry-season irrigation period.

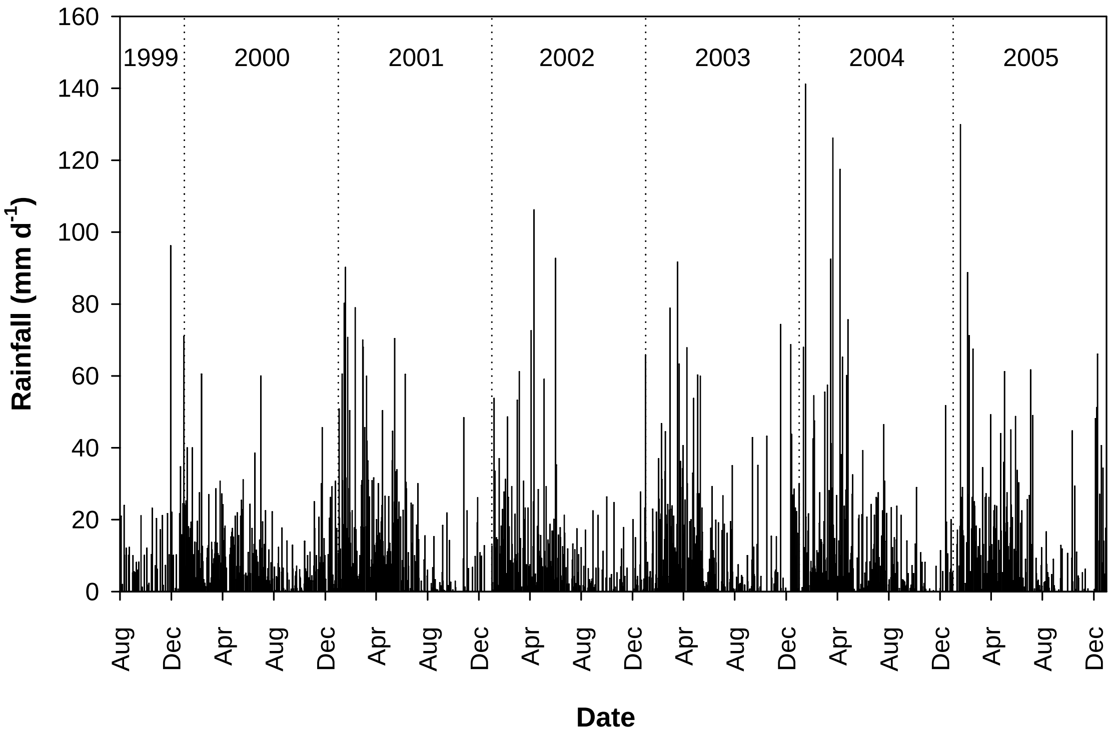

Figure 2-1. Daily rainfall during the experimental period (data prior to July 2001 are from a meteorological station about 3 km away from the study site).

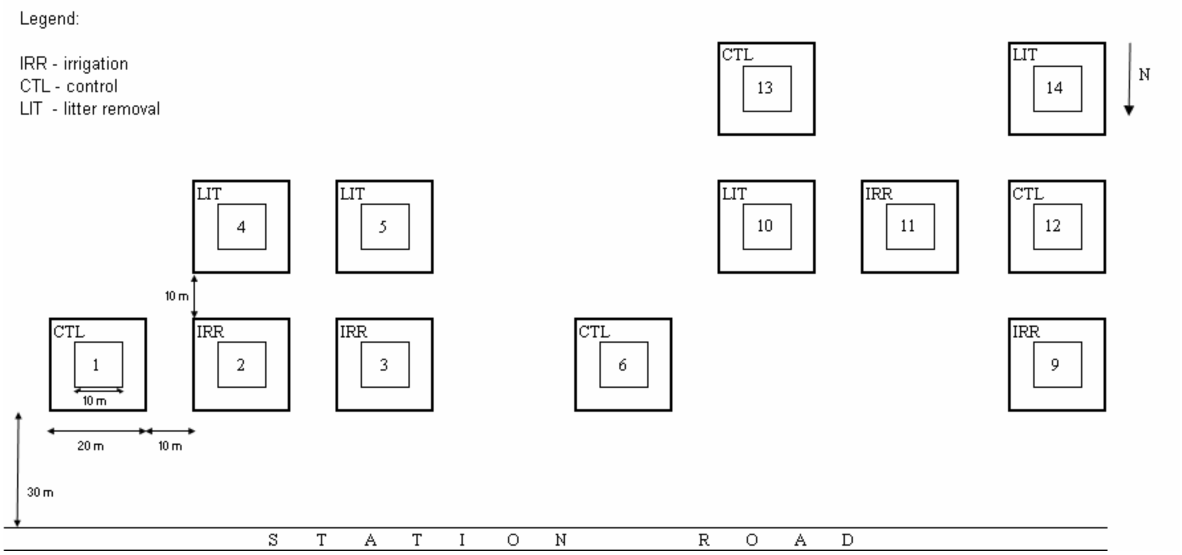

Figure 2-2. Experimental plot layout showing the arrangement of treatments.

CHAPTER 3
SEASONAL AND EXPERIMENTAL EFFECTS ON LITTERFALL QUANTITY AND QUALITY IN EASTERN AMAZONIAN FOREST REGROWTH

Introduction

Litterfall represents the major process of nutrient transfer from aboveground forest vegetation to soils (Vitousek & Sanford 1986), and fine litterfall comprises a significant fraction of aboveground net primary productivity in forests (Clark *et al.* 2001b). Litter nitrogen and phosphorus cycling are of particular importance since these nutrients usually are the most limiting for tropical forest productivity (Vitousek 1984). Low phosphorus availability is likely a common constraint for tropical forest regrowth, and nitrogen limitation appears significant for forests reestablishing after several episodes of slash-and-burn, which lead to substantial losses of nitrogen through volatilization (Davidson *et al.* 2004a, Gehring *et al.* 1999).

Litterfall quantity usually shows distinct patterns associated with rainfall seasonality, i.e., litterfall peaks during dry season (e.g., Wieder & Wright 1995). However, a direct effect of soil moisture availability on litterfall quantity and timing has not been demonstrated (Cavelier *et al.* 1999, Wieder & Wright 1995). The concentration of nutrients in leaf litterfall may also vary with rainfall seasonality in tropical forests (Wood *et al.* 2005), but a 5-year irrigation experiment in Panama did not affect litterfall nutrient concentration (Yavitt *et al.* 2004). Litterfall production has been shown to be limited by nutrient availability (Vitousek 1984), with fertilization resulting in higher litterfall rates in a dry tropical forest in Mexico (Campo & Vázquez-Yanes 2004) and a wet tropical forest in Puerto Rico (Li *et al.* 2006). Fertilization also results in increased leaf litter nutrient concentration in tropical forests (Li *et al.* 2006).

A better understanding of fluxes and pools of carbon, nitrogen, and phosphorus involved in litterfall can help to improve models of forest biogeochemistry. More appropriate quantification of the role of soil moisture and nutrients in the regulation of litterfall can facilitate predictions of carbon and nutrient dynamics under different conditions of resource availability. In this context, long-term (> 1 year) data on litterfall quantity and quality are equally important to understand interannual variability effects on carbon and nutrient dynamics, but such information is scarce for tropical forests.

The primary objective of this chapter was to investigate the effects of moisture and nutrient availability on litterfall within the context of the dry-season irrigation and litter removal experiments described in Chapter 2. We hypothesized that (a) dry-season irrigation would increase non-woody litterfall quantity and quality, and (b) litter removal would reduce non-woody litterfall quantity and quality.

Material and methods

Litterfall

From October 1999 to December 2005, litterfall was collected weekly in each of three 1 m x 1 m screen litter traps in the 10 m x 10 m measurement subplots. The weekly frequency of litterfall collection was chosen to minimize mass and nutrient losses due to leaching of trapped litter (Luizao 1989). The plant material collected in each trap was air-dried in the laboratory to remove excess moisture before storage. At 4-week intervals, material from the same collector was composited and then separated into woody and non-woody fractions. Leaves and their petioles, foliar rachises, and reproductive parts were included in non-woody litterfall. Our non-woody fraction corresponds to the “fine litter” (or “small litter”) fraction defined in several studies (e.g., Smith *et al.* 1998), except for the non-inclusion of woody material. In “fine litter”, small-diameter woody material—usually <1-2 cm diameter (Clark *et al.* 2001a, Proctor 1983)—is included assuming that this woody fraction (1) has turnover times comparable to other components of non-woody material (mostly foliar and reproductive material) and (2) may represent material produced from the current year's growth. Thus, our estimate of non-woody material may represent a slight underestimation of "fine litter".

We weighed woody and non-woody litterfall after drying at 60-70 °C until constant weight. Litterfall data for April 2003 was lost due to a malfunction of the oven that resulted in burning of litterfall samples; for this period, we used for each trap a value of litterfall estimated from the mean relative contribution of April to annual litterfall per trap as follows:

$$\mathrm{Est} = \frac{\mathrm{MC} \times \mathrm{AL}}{100 - \mathrm{MC}},$$

where:

Est = estimated litterfall for April 2003 (in g m^{-2});

MC = mean relative contribution of April to annual litterfall in 2000, 2001, 2002, and 2004 (in %), i.e.,

$$\mathrm{MC} = \frac{\sum_{i=1}^{n} (\text{April litterfall} \div \text{annual litterfall})}{n} \times 100,$$

where i = year; and

AL = total 2003 litterfall except April (in g m^{-2}).

Mean ± se MC was 6.3 ± 0.2% for all traps over four years.

Composite samples of non-woody litterfall were ground with a coffee grinder (Krups, US) and stored in 60 mL scintillation vials for subsequent analysis of carbon (C), nitrogen (N), and phosphorus (P). Carbon was determined with an elemental carbon analyzer (Carlo Erba model CNS2500) at the School of Forest Resources and Conservation (University of Florida) in samples collected from October 1999 to March 2001. We estimated that non-woody litterfall was 48% C based on the monthly non-woody litterfall C concentration (47.9 ± 0.2%, n = 18).

Nitrogen and phosphorus concentrations were determined in the Laboratory of Plant Ecophysiology and Propagation at Embrapa Amazônia Oriental (Brazil) in samples collected from January 2000 to December 2004. The Kjeldahl digestion was used to determine total nitrogen (Anderson & Ingram 1996). Phosphorus concentrations were determined colorimetrically after digestion of 0.1 g sample in sulfuric acid and peroxide (Murphy & Riley 1962). Following the criteria in Boone *et al.* (1999), all the samples were analyzed in duplicate for P, while 10% of the samples were randomly selected for duplicate analyses for N. Mean coefficient of variation in duplicate analyses was 2.1% for N (n = 542) and 4.1% for P (n = 2096). Percent error in relation to standard reference material (peach leaves, NIST SRM 1547) was -14 ± 1.6% for N (n = 22) and 2.0 ± 1.0% for P (n = 24).

To calculate N and P fluxes in non-woody litterfall (nutrient return), nutrient concentrations were multiplied by mass for each trap per month.

Litter Stock

At the end of the 2004 wet season (25-August) and dry season (29-December), we collected samples (n = 4) of forest floor litter from randomly chosen areas (25 cm x 50 cm) in each of the control and irrigated plots and processed as for litterfall. Non-woody litter stock was calculated by dividing the amount of dry material per collection area (g m^{-2}).

Statistical Analysis

We used SAS version 9.00 to run the statistical analyses. We analyzed with PROC MIXED the effects of treatment, date, and treatment-by-date interaction on the variables non-woody litterfall mass (monthly and annual), and nitrogen and phosphorus concentration and return using a repeated measures analysis with compound symmetric covariance structure. This structure assumes constant variance at all dates and equal correlations between all pairs of measures on the same experimental unit, i.e., litterfall trap for the litterfall variables and plot for litter stock. We ran separate tests to compare each of the treatments with the control. Within this analysis, significant treatment effects would have indicated temporally consistent differences between treatment and control measurements both pre- and post-treatment and across seasons, significant date effects were generally indicative of seasonal trends that affected both treatment and control measurements, and treatment-by-date effects indicated a significant difference between treatment and control measurements that occurred after the treatment was initiated. Thus, the treatment-by-date effect represents the best test of treatment effect when there were no pre-existing differences among plots prior to the treatment. We used a priori CONTRAST statements to explicitly test whether the measured variables differed between seasons and between treatments within each season (wet and dry).

When necessary, we performed log and square root transformations to meet the model assumptions of normality, based on the criteria of $P > 0.05$ in the Kolmogorov-Smirnov test, and equal variances, based on the absence of a pattern of heteroscedasticity in the plots of residual versus predicted values. Means and standard errors were calculated on the basis of untransformed data. All results are reported as significant when $P \leq 0.05$; we report marginal significance when $0.05 < P < 0.10$. Multiple comparisons of means were performed with Tukey's test ($P < 0.05$).

Results

Non-woody Litterfall

Irrigation experiment

Non-woody litterfall mass was significantly affected by date and the interaction between treatment and date (Table 3-1, Figure 3-1B). The significant effect of the interaction was not associated with consistent differences between treatments during the pre-treatment period ($P = 0.76$) or within dry-season irrigation periods ($P = 0.18$). Non-woody litterfall was significantly higher in the dry season than in the wet season (79.5 ± 1.3 and 58.5 ± 0.9 g m^{-2} $month^{-1}$, respectively; $P < 0.0001$). Annual non-woody litterfall mass was significantly affected by the interaction between treatment and date (Table 3-1); in 2003, annual non-woody litterfall mass (Figure 3-2A) in irrigated plots was significantly higher than in control plots (899.2 ± 55.3 and 742.4 ± 63.1 g m^{-2} $year^{-1}$, respectively; $P < 0.01$). Annual litterfall in the control plots was not correlated with annual rainfall ($r = 0.129$, $P = 0.808$, Pearson correlation).

Non-woody litterfall N concentration was significantly affected by date only (Table 3-1, Figure 3-3B). The effect of date was not related to a significant seasonal influence on litterfall N concentration (dry = 1.24 ± 0.01 vs. wet = 1.27 ± 0.01% N, $P = 0.86$).

The input of N in non-woody litterfall was significantly affected by date and treatment x date interaction; there was no significant effect of treatment (Table 3-1, Figure 3-4B). The significant effect of the interaction was not related to a consistent difference between treatments within dry-season irrigation periods ($P = 0.19$).

Non-woody litterfall P concentration was significantly affected by date and treatment x date interaction (Table 3-1, Figure 3-3C). Litterfall P concentration was significantly higher in control plots than in irrigated plots for some months during early- to mid-dry season (November 2001 and September 2002) and late-dry to early-wet seasons (January and February in 2002 and 2003). Litterfall P concentration was significantly lower in the dry season than in the wet season (0.38 ± <0.01 and 0.40 ± <0.01 mg P g^{-1}, respectively; $P < 0.0001$), although the difference was slight.

Phosphorus return in non-woody litterfall was significantly affected by date and treatment x date interaction (Table 3-1, Figure 3-4C). Treatment differences within dry-season irrigation periods were marginally significant ($P = 0.08$), largely due to differences during the 2003 dry-season irrigation period.

Annual return of N and P were significantly affected by date and treatment x date interaction (Table 3-1); irrigation plots showed significantly higher N and P return than control plots in 2003 (Figure 3-5).

Litter removal experiment

Non-woody litterfall mass was significantly affected only by date (Table 3-1, Figure 3-6B) and was significantly higher in the dry season than in the wet season (76.2 ± 1.2 and 58.4 ± 0.9 g m^{-2} $month^{-1}$, respectively; $P < 0.0001$). Annual non-woody litterfall mass was significantly affected by date only (Table 3-1), with the 2001 mean litterfall rates significantly higher than subsequent years, but not different from 2000 (Figure 3-2B).

Non-woody litterfall N concentration was significantly affected by treatment, date, and treatment x date interaction (Table 3-1, Figure 3-7B). During the treatment period, mean litterfall N concentration was about 12% higher for control plots than for litter removal plots (1.26 ± 0.01 and 1.13 ± 0.01% N, respectively; $P = 0.01$). This difference was not homogenous throughout the manipulation period; with the progression of litter removal, the difference between treatments in annual N concentration increased from ~ 11% in 2002 to ~ 16% in 2004, which correspond to values of ~ 5% (2002) and ~ 11% (2004) after accounting for pretreatment differences. There was also a significant effect of treatment during the pretreatment period ($P = 0.03$); however, pretreatment differences between plots did not affect the significance ($P = 0.04$) of post-treatment differences (contrast test).

The return of N in non-woody litterfall was significantly affected by date and treatment x date interaction (Table 3-1, Figure 3-8B). However, the contrast test showed that the significant effect of the interaction did not reflect consistent differences between treatments during the litter removal period ($P = 0.36$).

Non-woody litterfall P concentration was significantly affected by date only, with a marginally significant effect of treatment (Table 3-1, Figure 3-7C). Phosphorus concentration during the wet season was slightly but significantly higher than during the dry season (0.40 ± <0.01 and 0.36 ± <0.01 mg P g^{-1}, respectively; $P < 0.0001$).

The return of P in litterfall was significantly affected by date and treatment x date interaction (Table 3-1, Figure 3-8C). The significant effect of the interaction term was associated with occasionally higher values for control plots. Phosphorus return in the dry season was slightly, but significantly higher than in the wet season (0.027 ± <0.001 and 0.022 ± <0.001 g P m^{-2}, respectively; $P < 0.0001$).

Annual return of N was significantly affected by date and treatment x date interaction (Table 3-1). However, there was no detectable significant or marginally significant difference between treatment means in each year ($P > 0.10$, Tukey test), although 2001 values were generally higher than other years, and control plots tended to have higher N return than litter removal plots in 2002 (Figure 3-9A). Annual return of P was significantly affected by date only (Table 3-1), with substantially higher return rates in 2003 and 2004 than in the other years (Figure 3-9B).

Litter stock

The stock of non-woody litter (Figure 3-10) was significantly higher towards the end of the dry season (December 2004) than at the end of the wet season (August 2004) (680 ± 54 and 435 ± 36 g m^{-2}, $n = 8$, respectively; $P < 0.001$). There were no significant effects of treatment ($P = 0.203$) or treatment x date interaction ($P = 0.271$).

Discussion

Seasonal Patterns

Non-woody litterfall rates measured in this study are within the range reported for both regrowth and old-growth Amazonian and other tropical forests elsewhere (Table 3-2). The higher rates of litterfall during the dry season compared to the wet season are also consistent with other studies in tropical forests (Dantas & Phillipson 1989, Sanchez & Alvarez-Sanchez 1995, Scott *et al.* 1992, Smith *et al.* 1998, Wieder & Wright 1995). The magnitude of interannual variability over 6 years varied from 9% for irrigated plots to 16% for litter removal plots, lower than that reported for a Panamanian old-growth forest (38%) (Wieder & Wright 1995). Annual litterfall was not related to annual rainfall, suggesting that litterfall production is not controlled by rainfall intensity for this regrowth forest stand. However, Lawrence (2005) found a positive relationship between annual litterfall and annual rainfall for tropical seasonal forests at a global scale.

There were no detectable effects of rainfall seasonality on litterfall N concentration, although Yavitt *et al.* (2004) reported higher N concentration in leaf fall during the wet season for a Panamanian old-growth forest, and Wood *et al.* (2005) reported a wet season decline in leaf litterfall N concentration for a Costa Rican old-growth forest.

Non-woody litterfall P concentration was lower during the dry season than in the wet season in the present study, with some lower values of litterfall P associated with peaks in litterfall, and some higher values of P occurring during lower litterfall rates in the wet season. These results for litterfall P are consistent with data reported for a secondary dry tropical forest in Mexico (Read & Lawrence 2003) and an old-growth forest in Costa Rica (Wood *et al.* 2005).

Most annual litterfall P peaks occurred during the first 1-2 months of the wet season, when rapid decomposition of litter accumulated during the dry season could have supplied a pulse of nutrients to plants with the onset of rainfall (Lodge *et al.* 1994, Wood *et al.* 2005). Lower litterfall P concentration in irrigated plots during dry-wet season transitions (2001-02 and 2002-03), associated with the strongest dry-season irrigation periods, are consistent with the pulse hypothesis, i.e., irrigation could have prevented litter accumulation and, therefore, nutrient mineralization pulse with the onset of rainfall. However, increased soil P availability for both control and irrigation plots during wet-up events in the 2004 dry season is not consistent with irrigation effects on nutrient pulse (Veluci *et al.* In preparation). The lack of litter removal effects on litterfall P peak further suggests that the pulse hypothesis may not be applicable. Alternatively, the seasonal and treatment effects on litterfall P may be caused by differences in P resorption between treatments, and/or differences in the contribution of P-rich, reproductive litterfall (flowers and fruits) during dry-wet transitions. Reproductive litterfall has been shown to have higher P concentration than leaf litterfall for tropical forests (Scott *et al.* 1992, Zagt 1997), and to peak (number of seeds m^{-2}) during dry-wet season transitions for our experimental site, although no irrigation effects have been observed in two consecutive evaluation years (Dias 2006).

Litter stock measured in this study is within the range reported for tropical forests (Table 3-2). Increased litter stock in the dry season is consistent with higher litterfall and lower decomposition rates during this period at the study site (Chapter 4), as also reported for an old-growth forest in Panama (Wieder & Wright 1995).

Limited Impact of Dry-season Irrigation

Irrigation did not impact litterfall rates in the dry season, except for higher rates in irrigated plots for a few dates, mostly in the 2003 dry-season. These results are consistent with those found for a dry-season irrigation experiment in a semideciduous lowland forest in Panama (Cavelier *et al.* 1999, Wieder & Wright 1995), and further confirm that soil moisture availability may not trigger increased litterfall during the dry season in tropical forests. Higher dry-season litterfall rates may be linked to increased vapor pressure deficits (Wright & Cornejo 1990), decreased cloud cover, decreased soil nutrient availability (Eamus & Prior 2001), or variation in temperature (Breitsprecher & Bethel 1990). Although the exact trigger(s) of increased dry-season litterfall have not been already ascertained, it is very likely that tropical trees respond to more than one cue (Wright & Cornejo 1990).

Dry-season irrigation did not alter N and had only small effects on P concentrations in non-woody litterfall, consistent with the results from a water manipulation study in a Panamanian old-growth forest (Yavitt *et al.* 2004). The small impacts of dry-season irrigation in this study contrasts with the potential for increased N and P availability in irrigated plots due to the combination of (1) higher N and P inputs in litterfall during the dry season and (2) higher litter decomposition in irrigated plots (Chapter 4). Thus, these results suggest that low litter quality—indicated by the high C:N and lignin:N ratios of leaf litter (Chapter 4) and non-woody litterfall—may be a stronger control over N (as well as P) availability than soil water status at this site, favoring microbial immobilization of nutrients; Aerts (1997) suggest that litter chemistry (especially the lignin:N ratio) represents the most important determinant of decomposition rates in tropical regions. Furthermore, consistent with results from an irrigation study in Panama (Yavitt & Wright 1996), dry-season irrigation had no influence on soil net nitrification rates at our site (Vasconcelos *et al.* 2004).

We expected that long-term irrigation would have resulted in increased aboveground productivity and, consequently, higher non-woody litterfall rates—an index of ANPP (Clark *et al.* 2001a, Jordan 1983). However, after 5 years of dry-season irrigation, this effect has not occurred consistently. Higher litterfall rates did occur for the irrigated plots in 2003, but that was in the year with the weakest dry season over the whole experimental period, when we would have expected the least effect of dry-season irrigation on forest processes. However, increased annual litterfall in irrigated plots in 2003 may have resulted from a lag effect of the extended drought in the preceding dry season (Table 2-1), consistent with results for a temperate mixed deciduous forest (Newman *et al.* 2006). Nonetheless, the effects of the extended 2002 dry season were not sufficiently intense to affect tree mortality at the community level which actually decreased from 2002 to 2003, and remained constant in 2004 (Araújo *et al.* 2005).

Litter Removal Reduces Litterfall N Concentration

Nitrogen and phosphorus concentrations and inputs in litterfall are comparable to values reported for forests of the Brazilian Amazon and elsewhere in the tropics (Table 3-2). Increased differences in N concentrations between control and litter removal plots are consistent with the recognized role of nutrient cycling in litter as a significant source of N for tropical forest plants (Markewitz *et al.* 2004, Vitousek & Sanford 1986). Mean litterfall P concentration for the control plots in this forest regrowth stand (0.04%) coincides with the value proposed by Vitousek (1984) to distinguish between high and low P levels for tropical forests. For most months from 2000 to 2003, litterfall P concentrations were below this threshold, which may reflect the low availability of soil phosphorus, as suggested by the low soil extractable P reported for the site (Rangel-Vasconcelos 2002).

The lack of treatment effects on litterfall P concentration may be explained by sufficient supply of P from soil sources. While weathering processes are not likely a substantial source of P in highly weathered tropical soils deprived of primary P-containing minerals (Sanchez 1976), mineralization of P from soil organic matter may represent a significant source of this nutrient for plants, even after 40 months of bi-weekly litter removal. Recent studies have determined substantial amounts of labile organic-P fractions (NaOH- and $NaHCO_3$-extractable) for

Amazonian forest regrowth sites in Brazil (Frizano *et al.* 2003, Markewitz *et al.* 2004), and a simulation study concluded that N and P stored in (deeply buried) soil organic matter can sustain C accumulation rates under conditions of limited input of such nutrients in tropical forest regrowth (Herbert *et al.* 2003). In addition, some regrowth forest trees colonizing sites with low soil P availability probably present mechanisms to improve P acquisition such as mycorrhizal associations and high phosphatase exudation rates (Marschner 1995). Uhl (1987) hypothesized that high incidence of mycorrhizal infection and efficient uptake and nutrient use may be necessary for establishment of successional trees under the limiting nutrient conditions typical of abandoned lands after slash-and-burning in the tropics. Similarly, Gehring *et al.* (1999) suggested that the growth of two early successional tree species in an Amazonian forest site was not limited by soil P availability because of efficient mycorrhizal associations.

Since litter is the main source of most nutrients in tropical forests (Markewitz *et al.* 2004, Vitousek & Sanford 1986), we expected that chronic litter removal would have resulted in nutrient deficiency, and consequently reduced ANPP (Harrington *et al.* 2001). Thus, we hypothesized that non-woody litterfall rates would diminish for litter removal plots. This study thus far indicates that the quantity of non-woody litterfall was insensitive to the reduction in nutrient availability (indicated by reduced litter N concentration) imposed by the litter manipulation treatment, consistent with the results obtained by Sayer (2005) for a 2-yr litter removal study in Panama. It is possible that extending the litter removal period will further reduce nutrient concentrations in litter, leading to a critical point where productivity will be significantly constrained. Nutrient manipulation effects on ecosystem processes are usually not immediate, and litter removal studies may have slower effects on litterfall responses than fertilization studies (Campo & Vázquez-Yanes 2004, Mirmanto *et al.* 1999).

Table 3-1. F statistics and associated significance levels[a] for the effects of treatments (irrigation and litter removal), sampling date, and their interaction on non-woody litterfall mass and nutrients in a tropical regrowth forest stand in eastern Amazonia, Brazil.

	Irrigation Experiment			Litter Removal Experiment		
Non-woody litterfall	Treatment	Date	Treatment x Date	Treatment	Date	Treatment x Date
Mass	0.72^{ns}	36.76***	1.66***	0.22^{ns}	25.21***	1.34*
N concentration	0.18^{ns}	27.87***	1.00^{ns}	8.42**	29.22***	2.53***
N return	0.49^{ns}	31.94***	1.54***	0.65^{ns}	26.71***	1.40*
P concentration	0.95^{ns}	52.11***	2.56***	3.74^{ns}	53.08***	1.23^{ns}
P return	0.30^{ns}	27.37***	1.91***	0.39^{ns}	20.20***	1.36*
Annual mass	0.71^{ns}	1.78^{ns}	3.46**	0.24^{ns}	4.59***	0.74^{ns}
Annual N return	0.82^{ns}	24.47***	3.66**	0.48^{ns}	24.89***	2.67*
Annual P return	0.63^{ns}	79.92***	3.88**	0.15ns	31.99***	1.45^{ns}

[a] The level of significance is indicated (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant).

Table 3-2. Estimates of annual non-woody litterfall (mass, nitrogen, and phosphorus), non-woody litter stock, and litterfall:forest floor mass ratio (k_L) in tropical forests.

Forest description[a]	Location	Annual rainfall (mm)	Annual litterfall ($g\ m^{-2}\ yr^{-1}$)[b]	Non-woody litter ($g\ m^{-2}$)	k_L	Annual nitrogen litterfall ($g\ m^{-2}\ yr^{-1}$)	Annual phosphorus litterfall ($g\ m^{-2}\ yr^{-1}$)	Source[c]
Regrowth								
3	Brazil	2600	504					1
12	Puerto Rico	3810	820	500	1.64			2
10	Brazil	2433	690	880				3
19	Brazil	1800	890			10.4	0.28	4
3 - 18	Brazil	1940	1040 – 1300					5
30	Brazil	2830	630					6
15	Brazil	2760	783	613	1.25	9.8	0.30	7
Old-growth								
	Brazil	2100	640			8.63		8
	Brazil	2600	804					1
	Venezuela	3565	1025			12.1	0.21	9
	Brazil	2100	825			15.1	0.31	10
	Brazil	2300	928	463	2.01	11.8	0.67	11
	Panama	2600	1240					12
	Brazil	1900	970	720	1.34	11.5		13
	Indonesia	3600	710			5.84	0.16	14
	Brazil	2000		564 – 840				15
	Brazil	1800	900					16
	Brazil	2000	570 – 920					17
	Brazil	2200	890	600	1.5			18

Table 3-2. (continued)

Forest description[a]	Location	Annual rainfall (mm)	Annual litterfall (g m^{-2} yr^{-1})[b]	Non-woody litter (g m^{-2})	k_L	Annual nitrogen litterfall (g m^{-2} yr^{-1})	Annual phosphorus litterfall (g m^{-2} yr^{-1})	Source[c]
	Brazil	1800	1030			14.3	0.33	4
	Brazil	1940	1100 – 1740					5
	Brazil	2000	1380 – 1460					19

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[a] For the forest regrowth sites, age (years after abandonment) is presented. Regrowth includes sites classified as secondary forests, while old-growth refers to primary and mature forest sites.

[b] Litterfall dry mass estimated as two times litterfall carbon for the studies without direct report of mass.

[c] For each source number, details of coordinates, soil type, and authors of each study are presented below:

1 - 1° 44' S, 47° 9' W, Capitão Poço, Brazil, unspecified soil, Dantas and Phillipson (1989)
2 - 18° 19' N, 65° 49' W, Puerto Rico, Ultisol, Cuevas *et al.* (1991)
3 - 2° 25' S, 59° 50' W, Manaus, Brazil, Oxisol, Mesquita *et al.* (1998)
4 - 2° 59’ S, 47° 31’ W, Paragominas, Brazil, Haplustox, Markewitz *et al.* (2004)
5 - Southwestern Amazonia, Brazil, Ultisols with patches of Oxisols, Salimon *et al.* (2004)
6 - 1° 18' 6" S, 48° 26' 35" W, Belém, Brazil, Yellow Latosol, Oliveira (2005)
7 - 1° 19’ S, 47° 57’ W, Apeú, Brazil, Distrophic Yellow Latosol, this study
8 - 2° 34’ S, 60° 7’ W, Manaus, Brazil, Yellow Latosol, Klinge and Rodrigues (1968)
9 - 1° 54’ N, 67° 3’ W, San Carlos de Rio Negro, Oxisol, Cuevas and Medina (1986)
10 - 2° 34’ S, 60° 7’ W, Manaus, Brazil, Yellow Latosol, Luizao (1989)
11 - Maracá Island, Brazil, Scott *et al.* (1992)
12 - 9° 09’ N, 79° 51’ W, Barro Colorado Island, Panama, Alfisol, Wieder and Wright(1995)
13 - Curuá-Una Forest Reserve, Brazil, Oxisol, Smith *et al.* (1998)
14 - 0° 6’ S, 113° 56’ E, Kalimantan, Indonesia, “Yellow sandy soil”, Mirmanto *et al.* (1999)
15 - Tapajós National Forest, Brazil, Ultisols and oxisols, Silver *et al.* (2000)
16 - 2° 59’ S, 47° 31’ W, Paragominas, Brazil, Haplustox, Davidson *et al.* (2000)
17 - 2.897° S, 54.952° W, Tapajós National Forest, Haplustox, Nepstad *et al.* (2002)
18 - 2° 35’21.08’’ S, 60° 06’ 53.63’’ W, Manaus, Brazil, Oxisol, Luizao *et al.* (2004)
19 - 2° 64’S, 54° 59’W, Tapajós National Forest, Brazil, Oxisols and Ultisols, Silver *et al.* (2005)

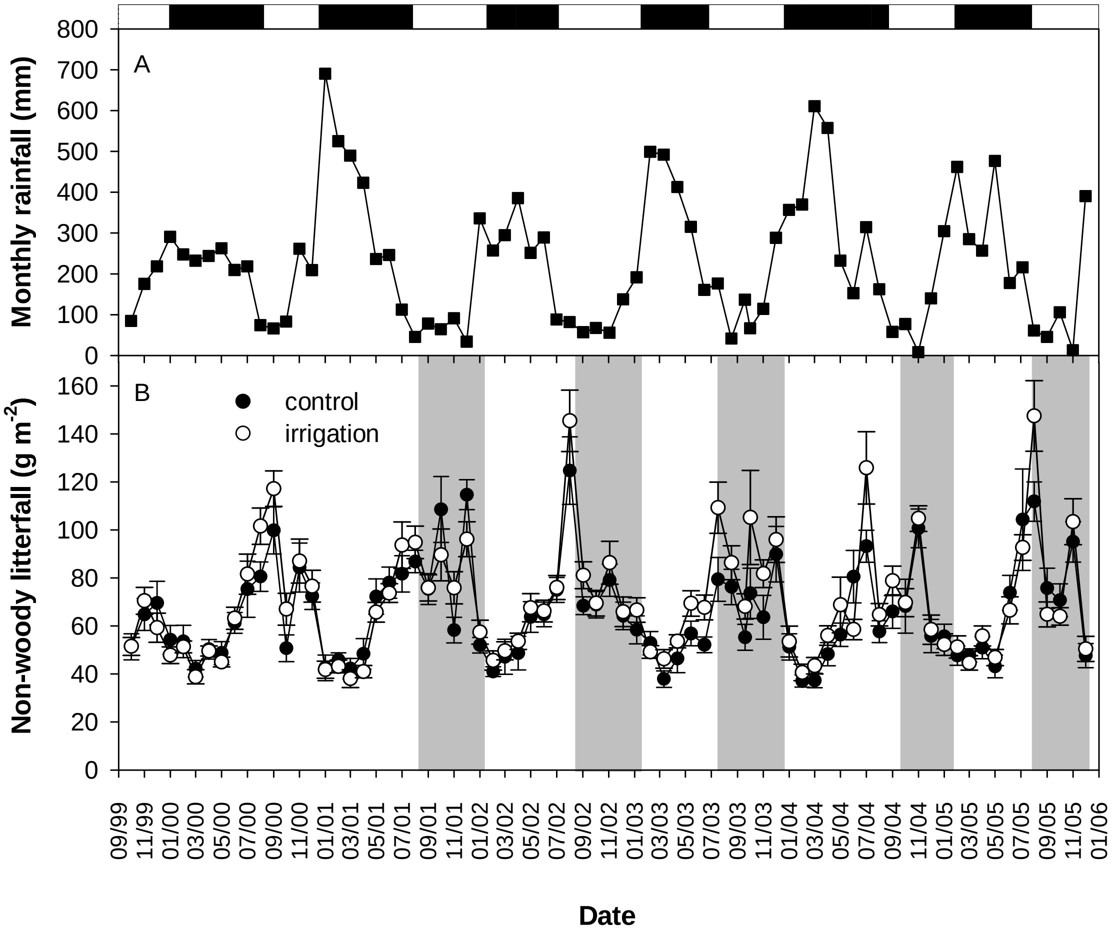

Figure 3-1. Effects of rainfall patterns and dry-season irrigation on non-woody litterfall in an Amazonian forest regrowth stand, Brazil. A) Monthly rainfall. B) Monthly non-woody litterfall for control and irrigation plots. In Figure 3-1B, each symbol represents the mean ± standard error, n = 12. Vertical gray bars indicate the irrigation periods. White and black horizontal bars mark the dry and wet seasons, respectively.

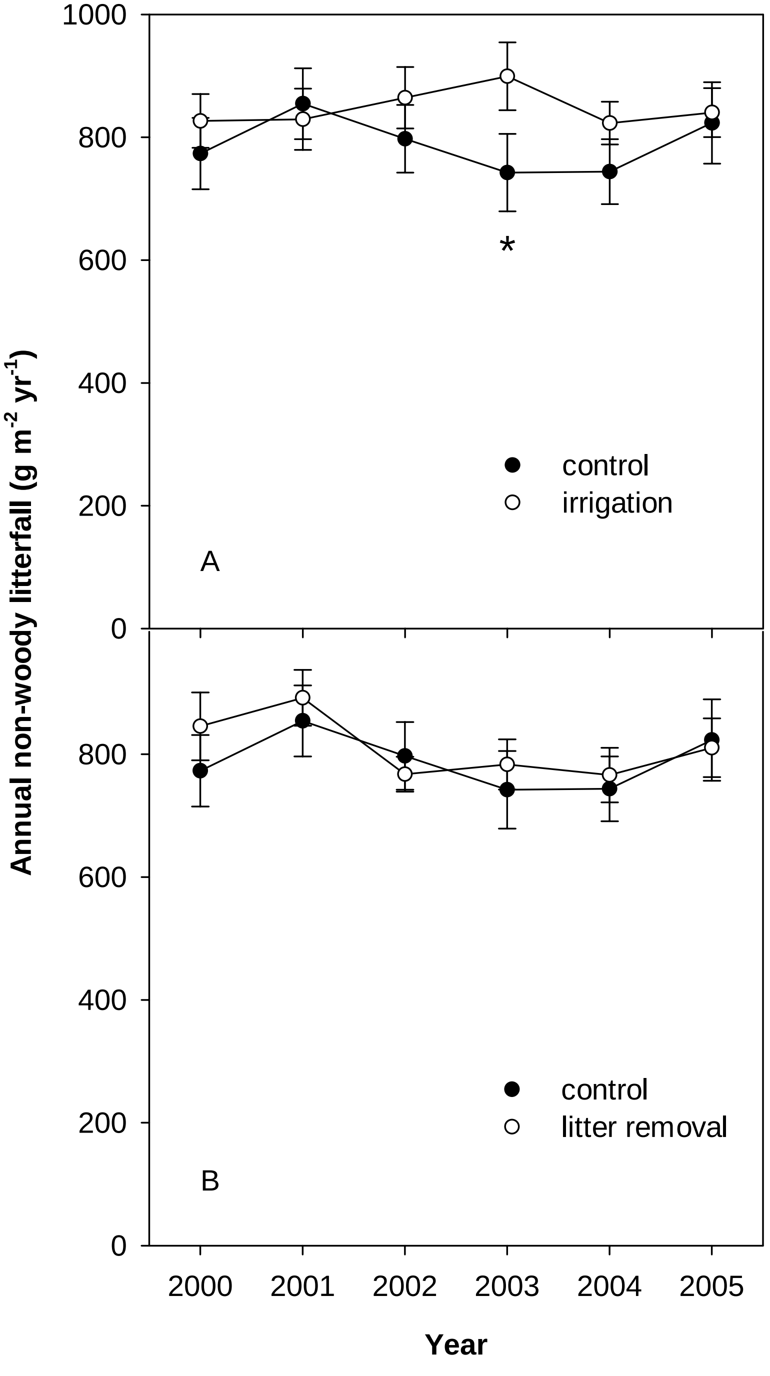

Figure 3-2. Effects of dry-season irrigation and litter removal on annual non-woody litterfall in an Amazonian forest regrowth stand, Brazil. A) Non-woody litterfall for control and irrigation plots. B) Non-woody litterfall for control and litter removal plots. Each symbol represents the mean ± standard error, n = 12. Treatments began in August 2001. Asterisk indicates significant treatment difference ($P < 0.05$).

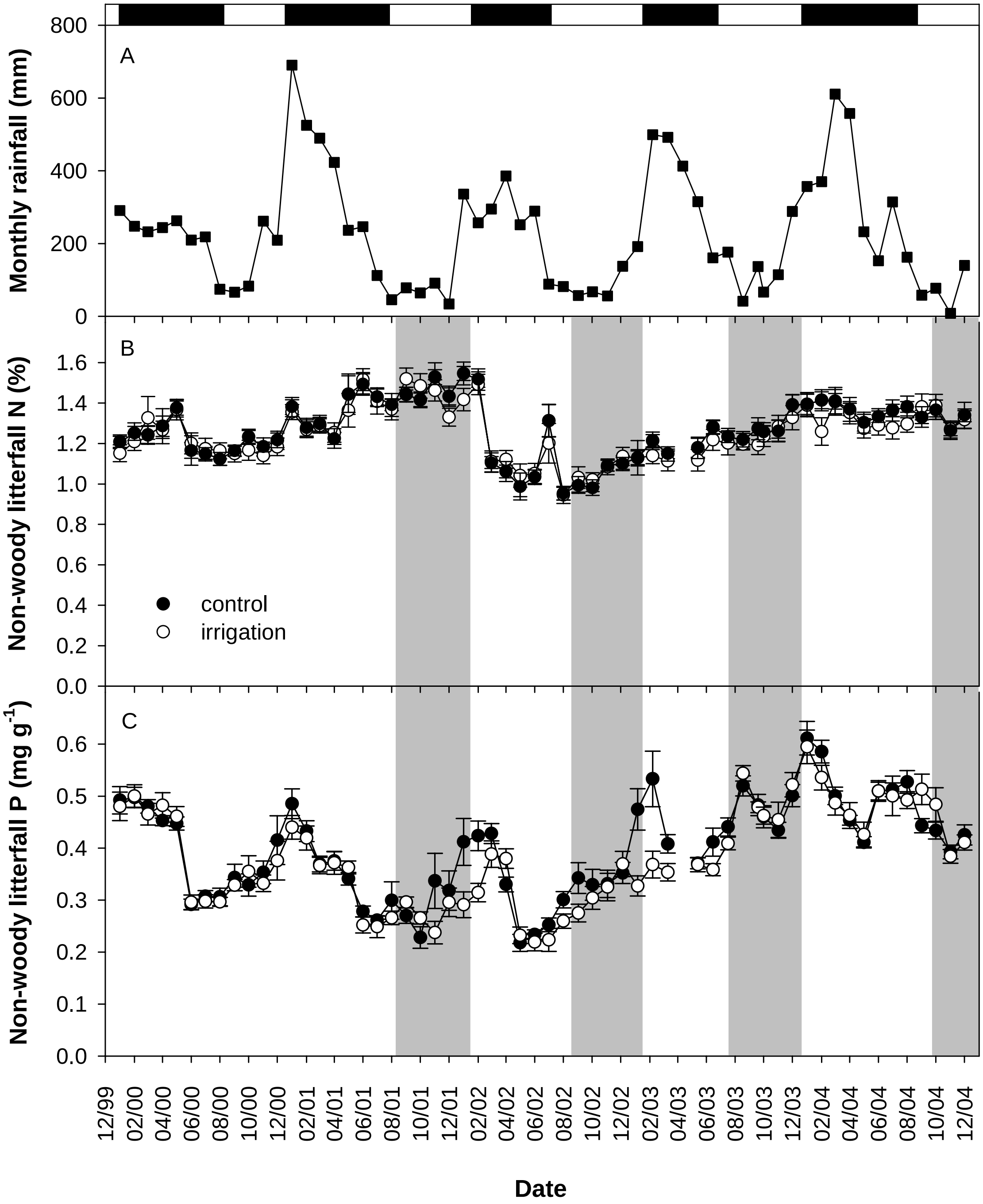

Figure 3-3. Effects of rainfall patterns and dry-season irrigation on non-woody litterfall nutrient concentrations in an Amazonian forest regrowth stand, Brazil. A) Monthly rainfall. B) Nitrogen (N) concentration for control and irrigation plots. C) Phosphorus (P) concentration for control and irrigation plots. In Figures 3-3B-C, each symbol represents the mean ± standard error, n = 12. Vertical gray bars indicate the irrigation periods. White and black horizontal bars mark the dry and wet seasons, respectively.

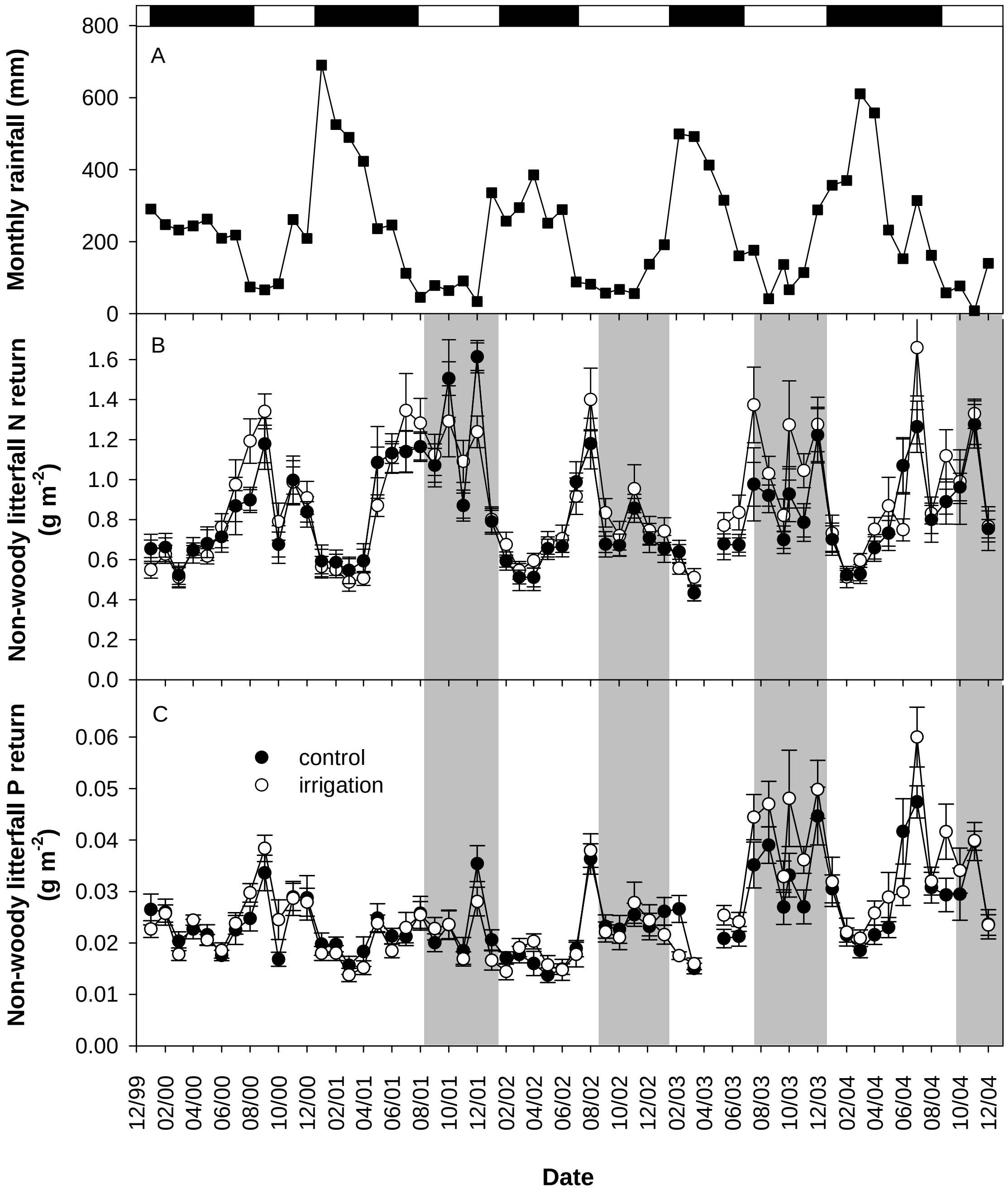

Figure 3-4. Effects of rainfall patterns and dry-season irrigation on non-woody litterfall nutrient return in an Amazonian forest regrowth stand, Brazil. A) Monthly rainfall. B) Nitrogen (N) return for control and irrigation plots. C) Phosphorus (P) return for control and irrigation plots. In Figures 3-4B-C, each symbol represents the mean ± standard error, n = 12. Vertical gray bars indicate the irrigation periods. White and black horizontal bars mark the dry and wet seasons, respectively.

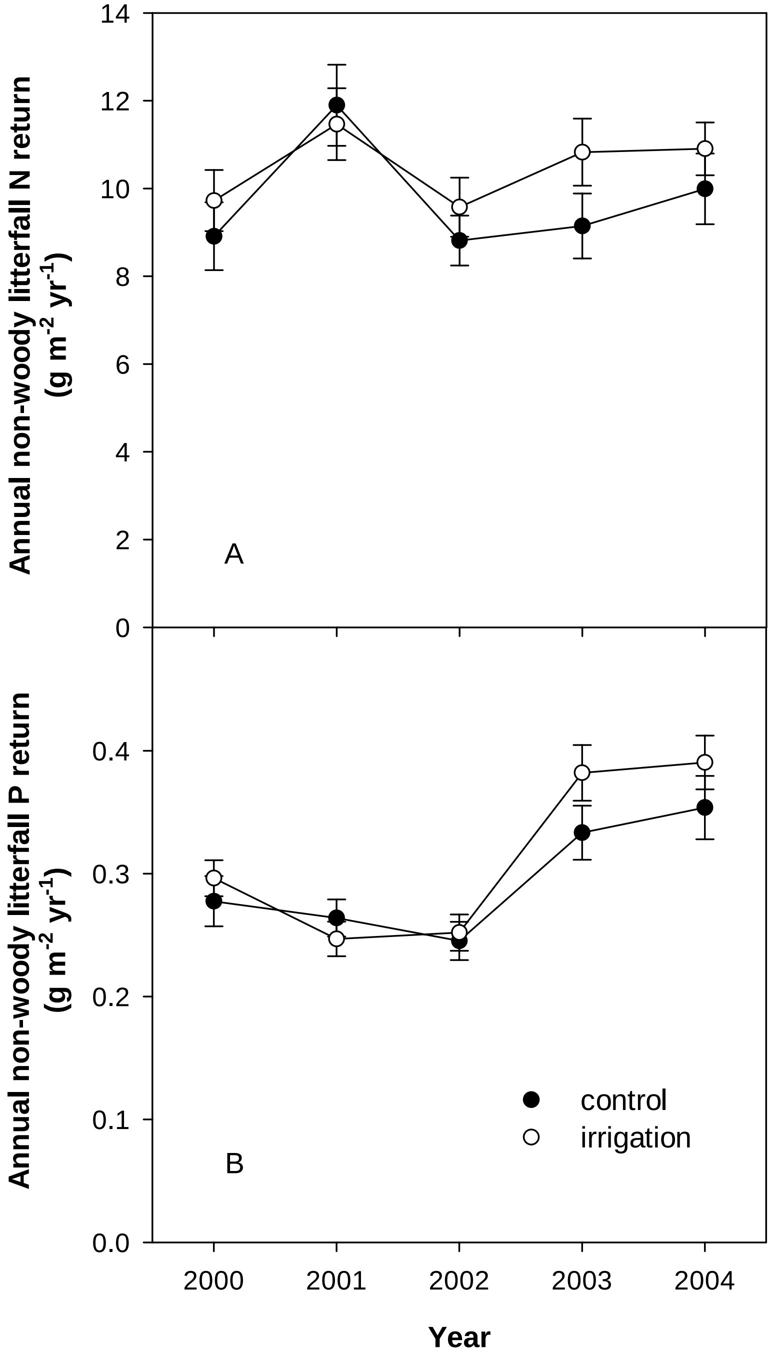

Figure 3-5. Effects of dry-season irrigation on annual non-woody litterfall nutrient return in an Amazonian forest regrowth stand, Brazil. A) Nitrogen (N) return for control and irrigation plots. B) Phosphorus (P) return for control and irrigation plots. Each symbol represents the mean ± standard error, n = 12. Treatments began in August 2001.

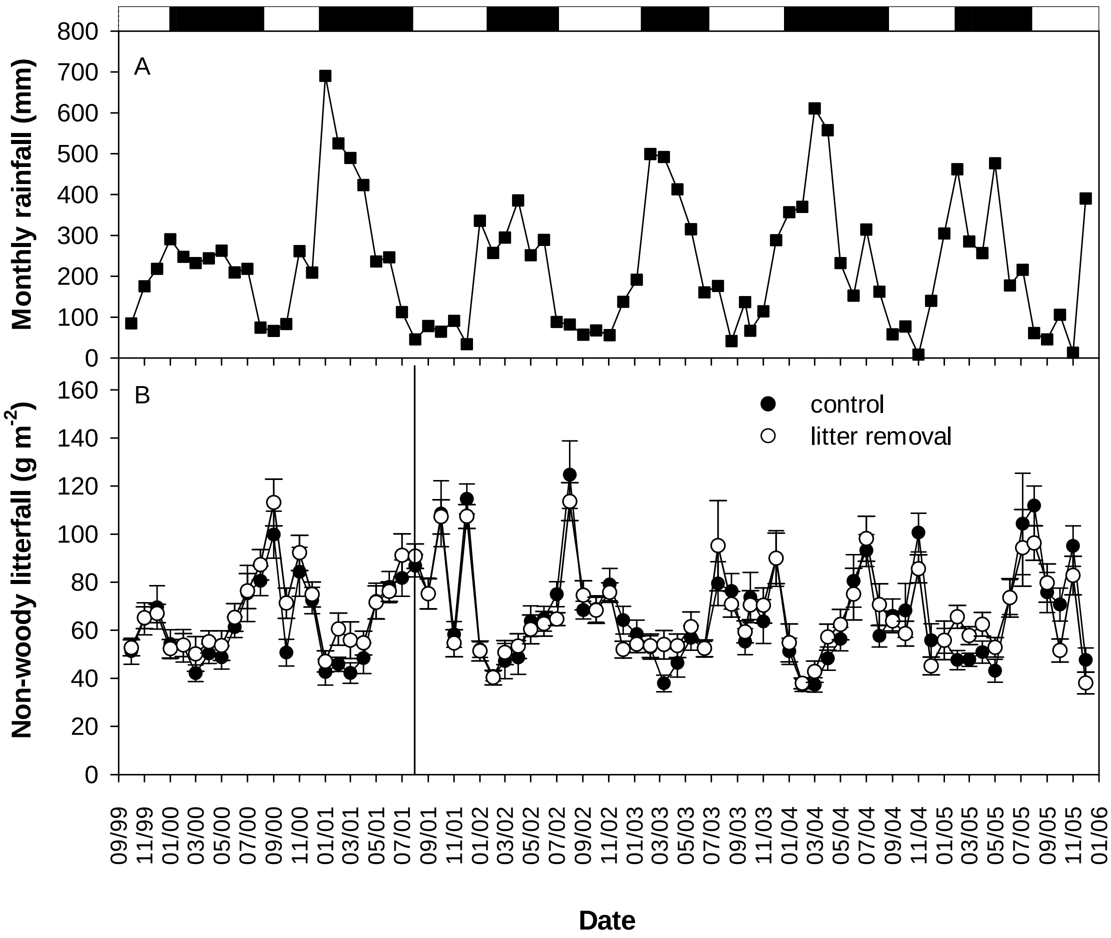

Figure 3-6. Effects of rainfall patterns and litter removal on non-woody litterfall in an Amazonian forest regrowth stand, Brazil. A) Monthly rainfall. B) Monthly non-woody litterfall for control and litter removal plots. In Figure 3-6B, each symbol represents the mean ± standard error, n = 12. The vertical line indicates the beginning of the litter removal treatment. White and black horizontal bars mark the dry and wet seasons, respectively.

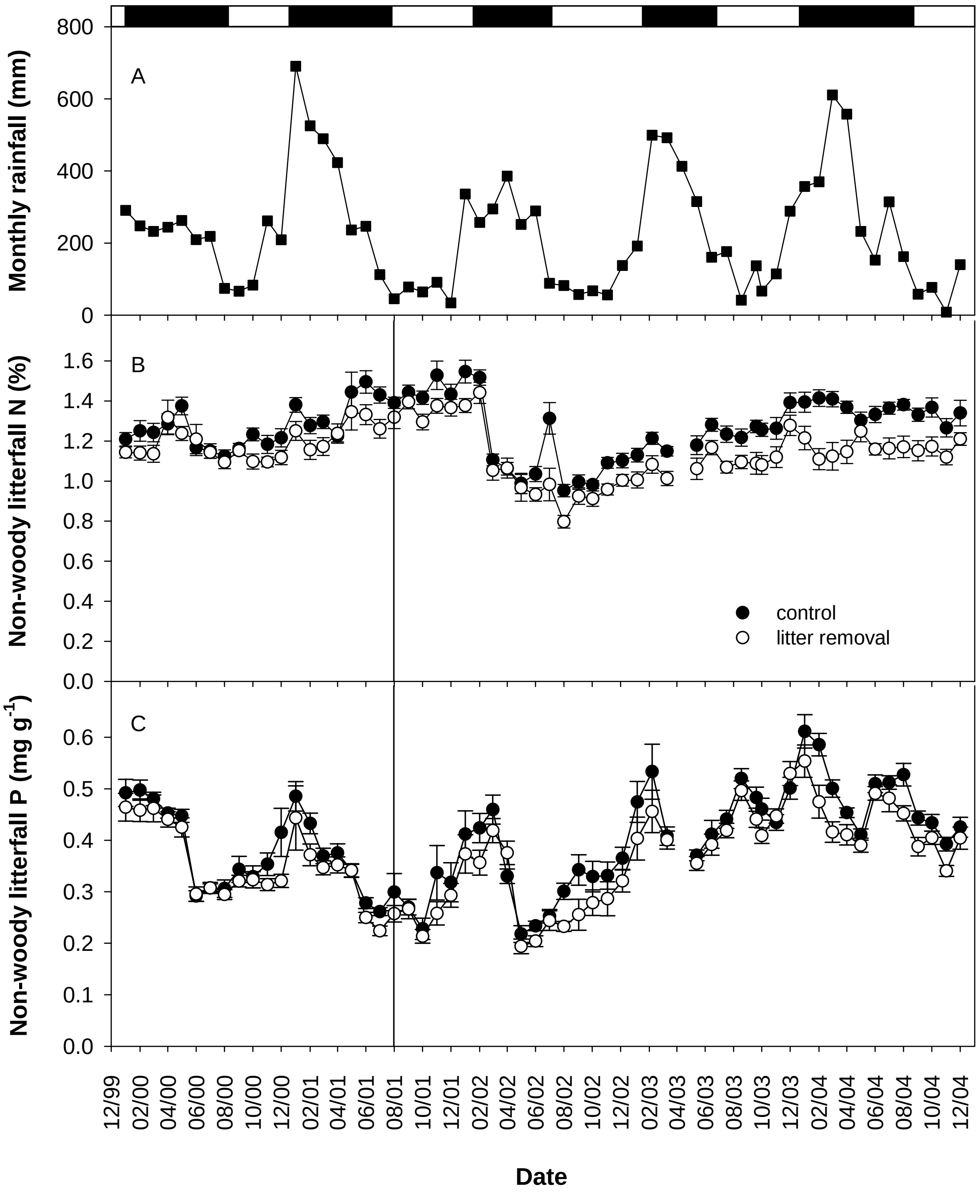

Figure 3-7. Effects of rainfall patterns and litter removal on non-woody litterfall nutrient concentrations in an Amazonian forest regrowth stand, Brazil. A) Monthly rainfall. B) Nitrogen (N) concentration for control and litter removal plots. C) Phosphorus (P) concentration for control and litter removal plots. In Figures 3-7B-C, each symbol represents the mean ± standard error, n = 12. The vertical line indicates the beginning of the litter removal treatment. White and black horizontal bars mark the dry and wet seasons, respectively.

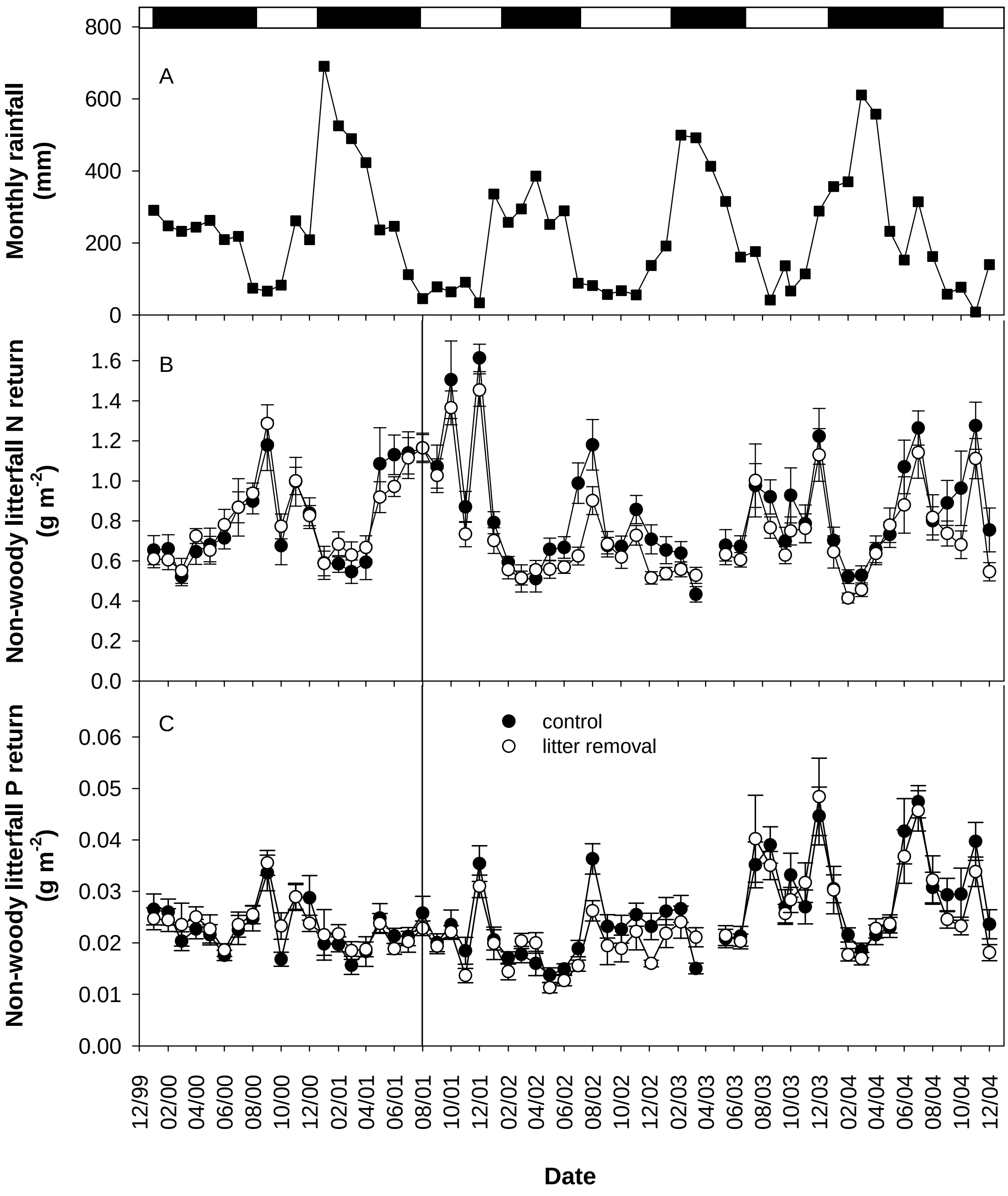

Figure 3-8. Effects of rainfall patterns and litter removal on non-woody litterfall nutrient return in an Amazonian forest regrowth stand, Brazil. A) Monthly rainfall. B) Nitrogen (N) return for control and litter removal plots. C) Phosphorus (P) return for control and litter removal plots. In Figures 3-8B-C, each symbol represents the mean ± standard error, n = 12. The vertical line indicates the beginning of the litter removal treatment. White and black horizontal bars mark the dry and wet seasons, respectively.

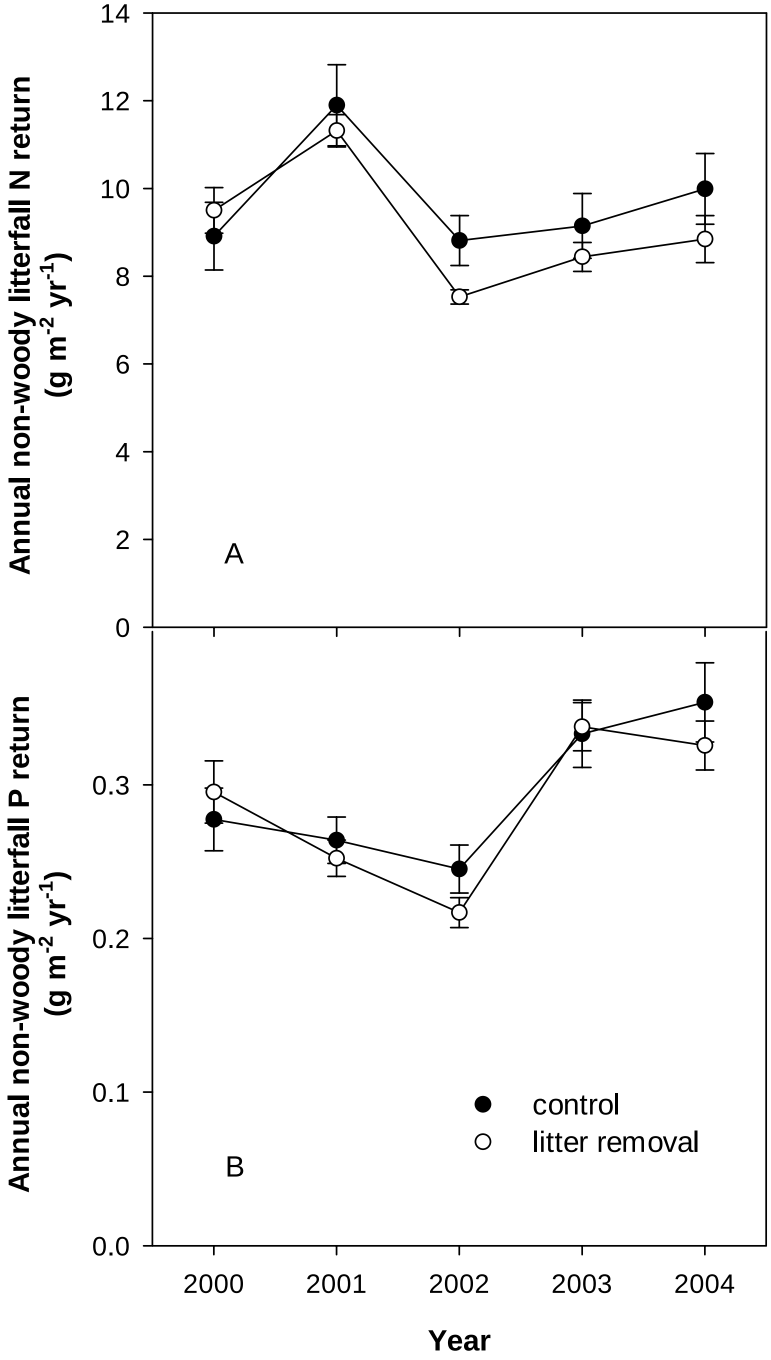

Figure 3-9. Effects of litter removal on annual non-woody litterfall nutrient return in an Amazonian forest regrowth stand, Brazil. A) Nitrogen (N) return for control and litter removal plots. (B) Phosphorus (P) return for control and litter removal plots. Each symbol represents the mean ± standard error, n = 12. Treatments began in August 2001.

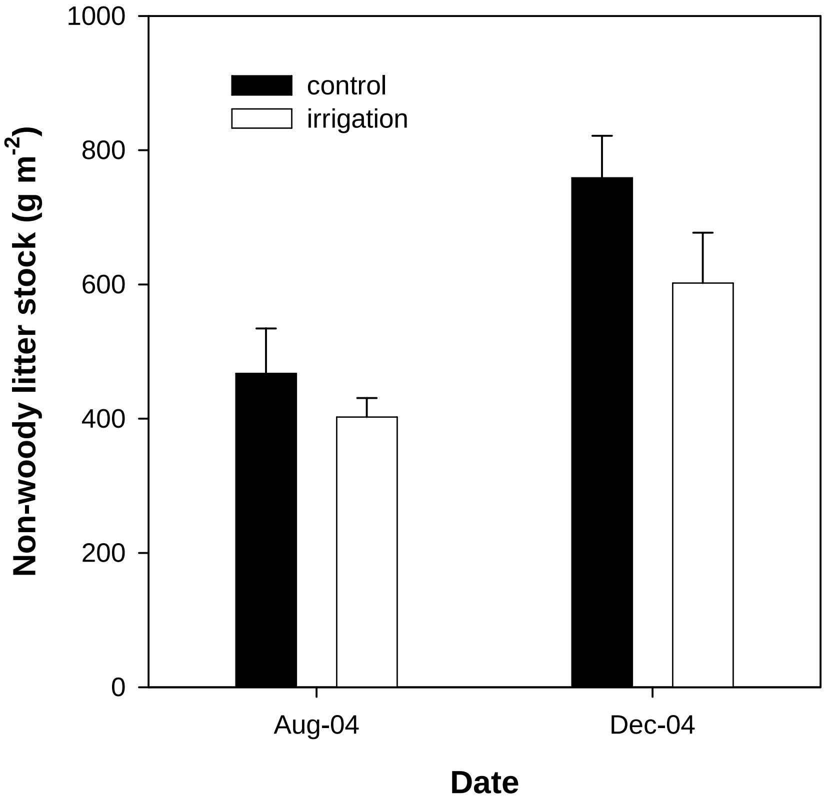

Figure 3-10. Non-woody litter stock for control and irrigation plots in an Amazonian forest regrowth stand, Brazil. Each bar represents the mean ± standard error, n = 4.

CHAPTER 4
LEAF DECOMPOSITION IN A DRY SEASON IRRIGATION EXPERIMENT IN EASTERN AMAZONIAN FOREST REGROWTH

Introduction

Litter decomposition is a major nutrient cycling process in terrestrial ecosystems and is particularly important for forest ecosystems on low fertility soils, including many tropical forests (Golley 1983, Swift *et al.* 1979). The rate of litter decomposition is controlled by interactions of litter quality, environmental conditions, and soil organisms (Swift *et al.* 1979). Litter quality is defined as the amount and types of organic carbon compounds, nutrient concentrations, and ratios between carbon compounds and nutrients in litter; low-quality litter (e.g., low nutrient, high carbon compounds:nutrient ratio) usually shows lower decomposition rates than high-quality litter (e.g., high nutrient, low carbon compounds:nutrient ratio) (Loranger *et al.* 2002, Mesquita *et al.* 1998, Songwe *et al.* 1995). For moist tropical forests moisture and temperature are assumed to be non-limiting, and litter quality is thought to be the dominant control on decomposition rates (Aerts 1997), although seasonal drought (Cornejo *et al.* 1994) and excessive moisture (Schuur 2001) may retard decomposition at some tropical forest sites. Water manipulation studies may help to clarify seasonal drought effects on litter decomposition.

A considerable number of studies have investigated decomposition responses of leaf litter from several plant species to rainfall seasonality in tropical forests (e.g., Cornu *et al.* 1997, Cuevas & Medina 1988, Luizão & Schubart 1987), but there are few such data for tropical regrowth sites (Mesquita *et al.* 1998). The effects of water manipulation on decomposition rates have been examined for old-growth tropical forests in Brazil (Nepstad *et al.* 2002) and Panama (Cornejo *et al.* 1994, Wieder & Wright 1995), but related studies are lacking for regrowth stands. Studies to quantify decomposition rates and their controls can help to improve understanding of carbon and nutrient cycling in tropical forest regrowth sites.

The primary objective of this chapter was to investigate the effects of moisture availability on litter decomposition within the context of the dry-season irrigation experiment described in Chapter 2. We hypothesized that (a) decomposition rates would be faster under higher moisture availability in the wet season and during dry-season irrigation periods in the treatment plots, and (2) decomposition rates would be faster for species with higher quality leaves.

Study Site and Experimental Design

Study site and experimental design are described in Chapter 2.

Material and Methods

Leaf Litter Decomposition

The litterbag method (Harmon *et al.* 1999) was used to study leaf litter decomposition. This method is the most frequently employed for examining litter decomposition in terrestrial ecosystems (Wieder & Lang 1982), although it has several limitations that can significantly influence decomposition rates including alteration of litter microclimate and exclusion of certain decomposer organisms (but see Prescott 2005). However, the litterbag method is adequate for studies comparing species, sites, and the effects of experimental manipulations on decomposition (Heal *et al.* 1997, Wieder & Lang 1982).

Traps were put outside the treatment plots to collect leaves for the decomposition study. Fresh fallen leaves of *Lacistema pubescens* Mart., *Ocotea guianensis* Aubl., *Stryphnodendron pulcherrimum* (Willd) Hochr., and *Annona paludosa* Aubl. were collected every week for 3-4 months prior to installing each of 3 separate decomposition experiments (described below). Collected leaves were dried under ambient conditions and stored. *L. pubescens* was chosen because it is the most common species in the study area (Araújo *et al.* 2005) and *A. paludosa*, *O. guianensis*, and *S. pulcherrimum* were selected because they represent a wide range in leaf texture; *O. guianensis* leaves are thick and appears to be recalcitrant, while *A. paludosa* and *S.*

pulcherrimum possess leaves that appear to decompose more rapidly. After the collection period, 6-8 subsamples of 10 g each were oven dried at 65-70 ^{o}C until constant weight and the dry weight conversion factor (air-dry mass:oven-dry mass) was calculated. These subsamples were processed following procedures described for litterfall (Chapter 3) to determine the initial leaf litter chemical composition (described below). For the second and third decomposition experiments, *S. pulcherrimum* was replaced by *Vismia guianensis* (Aubl.) Choisy because we noticed that some leaflets of the former species were smaller than the openings in the litterbag screen, which could overestimate decomposition rates. *V. guianensis* is another common pioneer species in the study area (Araújo *et al.* 2005).

Bags of polypropylene with openings of 1 mm x 0.8 mm and measuring 20 cm x 20 cm received about 10 g of air-dried material of only one species. In each of the control and irrigation plots, 18 litterbags of each species were randomly placed in the surface of the litter layer. After 30, 60, 120, 180, 240, and 360 days (Experiments 1 and 2) and 13, 31, 45, 61, and 90 days (Experiment 3), three bags of each species were retrieved in each plot. After retrieval, litterbags were air dried to facilitate the removal of adhering soil particles and roots gently using forceps and small, soft brushes (Tigre, medium size, Brazil). Then, the material was oven dried and weighed to calculate remaining leaf mass. Samples for the last collection in Experiment 2 were discarded because it was not possible to separate out soil particles from leaf material.

To investigate the effects of dry-season irrigation on the remaining mass of leaf litter under different stages of decomposition, the experiments had different installation and duration periods. Experiments 1 and 2 lasted 12 months and were installed in the beginning of the 2002 wet (February 7) and 2003 dry (July 27) seasons, respectively, in order to determine the effect of seasonality on initial and later stages of decomposition. For an improved temporal resolution of dry-season irrigation effects on decomposition, Experiment 3 was carried out exclusively during three months in the 2004 dry season (September 24 to December 23), with more frequent sampling during that period than for Experiments 1 and 2.

To assess seasonal effects on leaf litter decomposition irrespective of treatment, we compared the remaining leaf mass of control plots at 60 days in Experiment 1 and at 61 days in Experiment 2, which corresponded to wet and dry seasons, respectively. Total rainfall in these wet and dry seasons was 1311 and 359 mm, respectively. For this analysis we used data for *A. paludosa*, *L. pubescens*, and *O. guianensis*.

Remaining leaf mass (percent) was calculated as

$$\frac{X_t}{X_0} \times 100,$$

where X_t is the dry litter mass at the time *t* and X_0 is the initial dry litter mass.

Initial Leaf Litter Chemistry

Phosphorus concentrations were determined colorimetrically after digestion of 0.1 g sample in sulfuric acid and peroxide (Murphy & Riley 1962). Carbon and nitrogen were determined with an automated dry combustion instrument (LECO Model CNS-2000). Lignin and cellulose were determined by a sequential digestion of fibres (Anderson & Ingram 1996).

Specific Leaf Area

The specific leaf area (SLA) was measured in individuals of *Annona paludosa* (n = 3), *Lacistema pubescences* (n = 4), *Ocotea guianensis* (n = 4), and *Vismia guianensis* (n = 4) located in the control plots. In each tree, three young, fully expanded leaves were chosen from different branches, and three discs (1.11 cm^2) were collected from each leaf. The discs were dried at 65 ^{o}C for 48 h and individually weighed to the nearest 0.0001 g.

Statistical Analysis

We used SAS System version 9.00 to run the statistical analyses. Decomposition rates (*k*) were calculated by fitting the observed data (i.e., remaining leaf mass) to the single exponential model proposed by Olson (1963) using the PROC NLIN procedure. In the single exponential model, $X_t = X_0 e^{-kt}$; where X_t and X_0 are the litter mass at the times *t* and 0 (initial), respectively, and *k* is the decomposition rate (yr^{-1}). Although this model makes unrealistic assumptions (e.g., treats litter as a uniform, homogeneous substance) regarding the decomposition of litter, *k* values calculated with this model are useful for interpreting short-term (first year decomposition), comparative experiments (Paustian *et al.* 1997) such those in this study. For these analyses, data ($X_t \div X_0$) from three litterbags were averaged per plot for each sampling date because we considered individual plots as the experimental units, resulting in n = 4 for each combination of species, treatment, and sampling date. The effects of species, treatments, and the species x treatment interaction on *k* values were analyzed with a two-way ANOVA using PROC ANOVA. PROC CORR was used to analyze the correlation between *k* and initial litter quality parameters.

The effects of species on initial litter chemistry were analyzed with one-way ANOVA using PROC ANOVA. The TTEST procedure was used to compare seasonal effects on remaining leaf mass for control plots. The statistical analyses were carried out using the mean SLA calculated for each leaf per species. Means and standard errors were calculated on the basis of untransformed data. All results are reported as significant when $P \leq 0.05$. Multiple comparisons of means were performed with Tukey's test.

Results

Dry-season irrigation had no significant effects (Table 4-1) on leaf litter decomposition rates (*k*) obtained by fitting curves to all of the collection data in the twelve-month experiments (Table 4-2, Figure 4-1). However, in Experiment 2, *k* values obtained from curve fitting to the dry season data only were significantly higher in irrigated than in control plots (1.04 ± 0.06 and 0.86 ± 0.06 yr^{-1}, respectively; $P < 0.01$) (Table 4-1). In the three-month dry-season experiment, *A. paludosa* showed significantly higher decomposition rates than the other species under irrigation, which did not differ significantly among them (Table 4-3), and within species, decomposition rates were significantly higher in irrigated plots than in control plots (Table 4-3).

All of the experiments showed significant effects of species on *k* (Table 4-1). Overall, *A. paludosa* showed the highest decomposition rates (Table 4-2, 4-3). There were significant differences ($P < 0.0001$) in specific leaf area, carbon, nitrogen, phosphorus, lignin, and cellulose concentrations, lignin:N ratio, and C:N ratio among species (Table 4-4), but there were no significant correlations between *k* and leaf quality parameters (Table 4-5, Figure 4-2).

The analysis of seasonal effects on decomposition showed that remaining leaf mass was significantly ($P < 0.001$) higher in the dry season than in the wet season for *L. pubescens* (87.6 ± 0.9 and 76.0 ± 0.9%, respectively) and *O. guianensis* (88.5 ± 1.1 and 77.5 ± 0.9%, respectively); there was a marginally significant effect ($P < 0.052$) of season on *A. paludosa* remaining leaf mass (dry = 80.4 ± 3.1% vs. wet = and 71.8 ± 1.7%).

Discussion

Decomposition rates measured in this study are within the range reported for tropical forests (Table 4-6). Decomposition was faster during the wet season than the dry season, as observed in other studies in tropical forests in Amazonia (Cornu *et al.* 1997, Luizão & Schubart 1987) and elsewhere (Cornejo *et al.* 1994, Wieder & Wright 1995). Moisture constraints on decomposition were further confirmed by higher mass loss rates in dry-season irrigated plots, except when irrigation was applied to litter previously exposed to field conditions for 180 days; in this case, the greater proportion of recalcitrant compounds in advanced stages of litter decay

(Swift *et al.* 1979, Wieder & Lang 1982) probably conferred less susceptibility to decomposition in response to increased moisture availability.

Although leaf decomposition is significantly constrained during the dry season, the greatest difference between mass loss in control and irrigated plots was 10 to 13% only, and between dry and wet seasons was 7 to 12% only. Such small differences could be due to exclusion of macrofauna activity in leaf decomposition in the 1 mm x 0.8 mm opening bags. Using 1-mm mesh litterbags with additional openings of about 10 mm, Luizão and Schubart (1987) suggested that surface fine root penetration and macroarthropod activity determined the great difference in leaf mass loss between the dry and wet seasons for 3-yr-old forest regrowth in central Amazonia. It is not likely that fine root colonization has been constrained in our litterbags as we did observe fine root adhered to leaves. However, the 1 mm x 0.8 mm opening bags likely restricted macroarthropods to access leaf material and this could have contributed to the small differences between dry and wet as well as control and irrigation percent leaf mass losses in this study.

Leaf chemical and structural traits in this study are also consistent with other studies in tropical forests (Table 4-7). The range of lignin concentration (42.9 to 51.7%) found for the species investigated in this regrowth forest is high in comparison to reported values for old-growth forest tree species in Panama (Table 4-6), but similar to results of Mesquita *et al.* (1998) and Vasconcelos and Laurance (2005), who found lignin concentrations of about 53% for regrowth forest species in two central Amazonian sites.

The lack of correlation between decomposition rates and leaf quality parameters may result from the reduced number of leaf litter species tested in this study, as also observed by Fonte and Schowalter (2004) who investigated 8 different litter species for a Puerto Rican forest. For tropical forests, the lignin:N ratio was found to be the strongest predictor of decomposition in a Panamanian old-growth forest (Santiago 2003), while decomposition rates decreased with higher tannin concentration for a regrowth forest site in Amazonia (Mesquita *et al.* 1998). However, the strongest leaf quality predictor may change according to the stage of the decomposition process (Loranger *et al.* 2002).

Despite the lack of correlation between decomposition rates and litter quality parameters, the highest decomposition rates observed in *Annona paludosa* are probably explained by their higher leaf quality, i.e., high concentrations of nitrogen and phosphorus, the lowest concentration of lignin, and thin leaves (high specific leaf area). The low decomposition rates of *Ocotea guianensis* and *Vismia guianensis* leaves are associated with low N and P concentrations, high lignin concentration, the highest C:N and lignin:N ratios (> 50), and thicker leaves (low specific leaf area). Interestingly, *Lacistema pubescens* was often the "outlier" interfering with a strong linear relationship between *k* and litter quality; decomposition rates of *Lacistema* are lower than would be predicted by regressing the data from the other species, suggesting that decomposition of *Lacistema* leaf litter may be strongly controlled by some litter quality parameter not determined in this study. One potential explanation is the pubescent habit of its leaves.

Overall, moisture effects on *k* were comparatively higher than those related to litter quality; while *k* was on average 2.4 times higher in irrigated than in control plots during the three-month dry-season experiment, the greatest difference between species maximum/minimum *k* was 1.5 considering all of the experiments.

Table 4-1. F statistics and associated significance levels (in parentheses) for the effects of treatments (control and irrigation), species, and their interactions on leaf litter decomposition rates in a tropical regrowth forest in eastern Amazonia, Brazil.

Experiment	Treatment	Species	Treatment x Species
1 (started in wet season)	3.66^{ns}	15.96***	0.64^{ns}
2 (started in dry season; full period included in analysis)	0.91^{ns}	10.26***	1.70^{ns}
2 (started in dry season; dry season only included in analysis)	10.47**	11.56***	1.64^{ns}
3 (started in dry season; frequent sampling)	194.92***	10.15***	3.20*

Table 4-2. Decomposition rates (mean ± standard error) for overstory species in a tropical regrowth forest stand in eastern Amazonia, Brazil (n = 8).

	Experiment 1 (started in wet season)	Experiment 2 (started in dry season)	
		full period included	dry season only
Species		k (yr^{-1})	
A. paludosa	0.97 ± 0.05[1] a	1.26 ± 0.09 a	1.21 ± 0.09 a
L. pubescens	0.91 ± 0.03 ab	1.02 ± 0.03 bc	0.93 ± 0.04 b
O. guianensis	0.78 ± 0.03 bc	0.85 ± 0.04 c	0.73 ± 0.07 b
S. pulcherrimum	0.65 ± 0.04 c	-	-
V. guianensis	-	1.08 ± 0.04 ab	0.91 ± 0.07 b

[1] Within a column, different letters indicate that means differ at P < 0.05 (Tukey's test).

Table 4-3. Decomposition rates (mean ± standard error) for overstory species under control and irrigated plots (Experiment 3) in a tropical regrowth forest stand in eastern Amazonia, Brazil ($n = 4$). This experiment started in the dry season and encompasses frequent sampling during this period.

	Control	Irrigation
Species	k (yr^{-1})	
A. paludosa	0.59 ± 0.04[1] Aa	1.52 ± 0.08 Ba
L. pubescens	0.49 ± 0.06 Aa	1.14 ± 0.05 Bb
O. guianensis	0.48 ± 0.03 Aa	1.00 ± 0.06 Bb
V. guianensis	0.39 ± 0.03 Aa	1.02 ± 0.14 Bb

[1] Within columns and rows, different lower- and upper-case letters, respectively, indicate that means differ at $P < 0.05$ (Tukey's test).

Table 4-4. Initial quality parameters (mean ± standard error) of leaves incubated in litterbags for decomposition studies in a tropical regrowth forest in Eastern Amazonia, Brazil ($n = 6$-8).

	Annona paludosa	*Lacistema pubescens*	*Ocotea guianensis*	*Vismia guianensis*
Carbon (%)	50.77 ± 0.12[1] a	53.32 ± 0.17 b	52.55 ± 0.08 c	52.65 ± 0.07 c
Nitrogen (%)	1.05 ± 0.02 a	1.66 ± 0.01 b	0.90 ± 0.02 c	1.02 ± 0.01 a
Phosphorus (mg g^{-1})	0.45 ± 0.01 a	0.50 ± 0.01 b	0.32 ± 0.01 c	0.4 ± 0.01 d
Lignin (%)	42.9 ± 0.7 a	46.3 ± 0.6 b	47.4 ± 0.3 b	51.7 ± 1.1 c
Cellulose (%)	37.9 ± 0.3 a	42.0 ± 0.4 b	29.4 ± 1.1 c	41.8 ± 0.8 d
Carbon : nitrogen	48.51 ± 0.89 a	32.16 ± 0.21 b	58.57 ± 1.18 c	52.65 ± 0.27 d
Lignin : nitrogen	40.98 ± 1.03 a	28.05 ± 0.33 b	52.82 ± 1.15 c	50.24 ± 0.90 c
Specific leaf area (cm^2 g^{-1})	165 ± 13 a	191± 15 a	66 ± 2 b	122 ± 6 c

[1] Within a row, different letters indicate that means differ at $P < 0.05$ (Tukey's test).

Table 4-5. Pearson correlation coefficients between decomposition rate (*k*) and initial quality parameters of leaves of overstory tree species incubated in litterbags (Experiment 2) in a tropical regrowth forest in Eastern Amazonia, Brazil (n = 4).

Leaf litter quality parameter	Pearson coefficient
Nitrogen (%)	0.06206 (0.9379)[1]
Phosphorus (mg g^{-1})	0.56678 (0.4332)
Lignin (%)	-0.44339 (0.5566)
Cellulose (%)	0.55625 (0.4437)
Carbon : nitrogen	-0.27987 (0.7201)
Lignin : nitrogen	-0.32252 (0.6775)
Specific leaf area (cm^2 g^{-1})	0.62462 (0.3754)

[1] Numbers in parentheses are significance values

Table 4-6. Decomposition rates estimated from litterbag studies for some tropical forest sites.

Site	Decomposition rate (yr^{-1})	Source
Regrowth		
Manaus, Brazil	0.47 – 0.61	Mesquita *et al.* (1998)
Guadeloupe, French West Indies	0.41 – 2.39	Loranger *et al.* (2002)
Apeú, Brazil	0.39 – 1.52	This study
Old-growth		
San Carlos de Rio Negro, Venezuela	0.58 – 5.00	Cuevas and Medina (1988)
Maracá Island, Brazil	2.01	Scott *et al.* (1992)
Southern Bakundu Reserve Forest, Cameroon	1.55 – 4.6	Songwe *et al.* (1995)
Barro Colorado Island, Panama	3.2	Wieder and Wright (1995)
Maracá Island, Brazil	0.61 – 2.58	Luizão *et al.* (1998)

Table 4-7. Chemical composition of leaf litter for some tropical forest sites.

Site	Carbon (%)	Nitrogen (%)	Lignin (%)	Celllulose (%)	C:N	Lignin:N	SLA (cm^2 g^{-1})	Source
Venezuela[a]	49 – 57	1.12 – 1.71	14.2 – 26.3	17.3 – 39.4			62 – 77	Cuevas and Medina (1988)
Panama[a]							69.2 – 122.3	Cornejo *et al.* (1994)
Venezuela[a]							78 – 114[c]	Reich *et al.* (1995)
Brazil[b]	47.4 – 48.0	1.2 – 1.3	53 – 54					Mesquita *et al.* (1998)
Guadeloupe[b]		1.1 – 2.5	22.8 – 29.5	19.2 – 20.9		11.7 – 20.7		Loranger *et al.* (2002)
Panama[a]	47.3 – 43.2	0.90 – 1.22	16.0 – 13.7	18.4 – 18.0	58.4 – 39.2	11.8 – 19.9		Santiago *et al.* (2003)
Brazil[d]	48.4	0.94	53.5		51.7	44.0		Vasconcelos and Laurance (2005)

[a] old-growth forest

[b] regrowth forest

[c] mid successional species

[d] mixed leaf litter of successional species

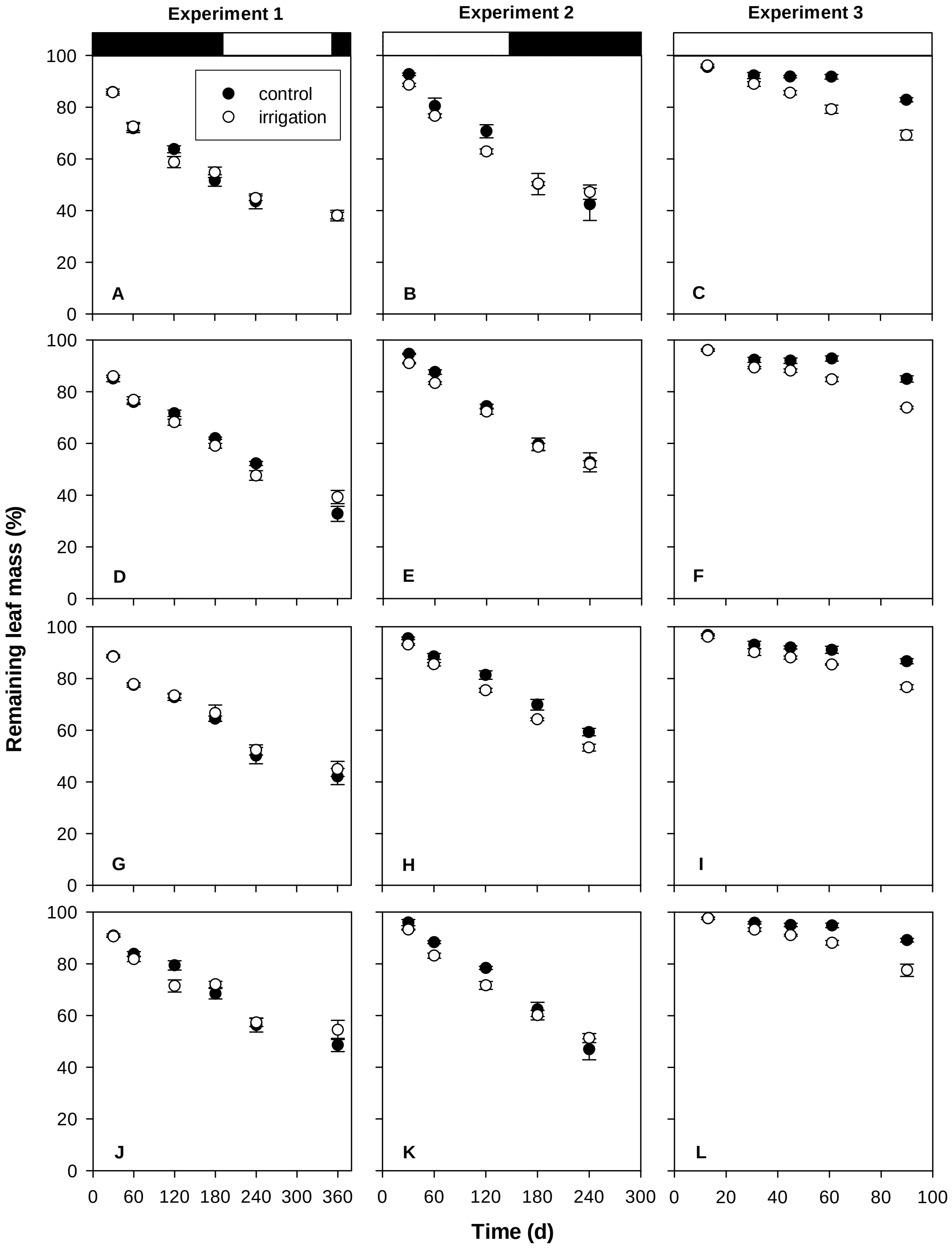

Figure 4-1. Effects of dry-season irrigation on leaf litter decomposition in a forest regrowth stand in eastern Amazonia, Brazil. Remaining leaf mass of (A, B, C) *Annona paludosa*, (D, E, F) *Lacistema pubescens*, (G, H, I) *Ocotea guianensis*, (J) *Stryphnodendron pulcherrimum*, and (K, L) *Vismia guianensis*. Each symbol represents the mean ± standard error (n = 4). White and black horizontal bars mark dry and wet seasons, respectively. Note different scales on the x-axes. Experiment 1 started in the wet season, while Experiments 2 and 3 started in the dry season; Experiment 3 had more frequent sampling than the other experiments.

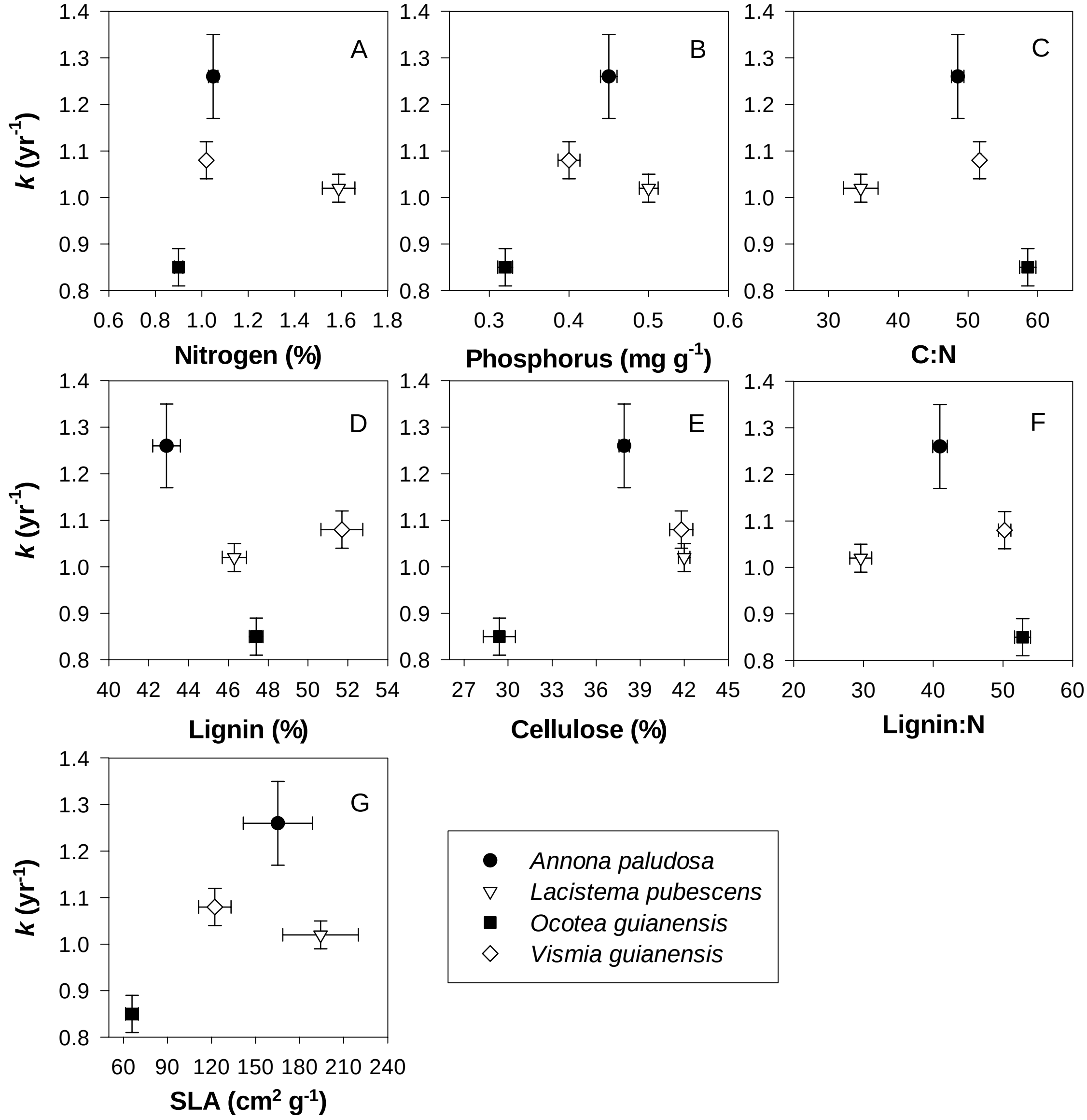

Figure 4-2. Relation between decomposition rate (k) and initial leaf litter characteristics for tree species in a forest regrowth stand in eastern Amazonia, Experiment 2. A) Nitrogen concentration. B) Phosphorus concentration. C) Carbon:nitrogen ratio. D) Lignin concentration. E) Cellulose concentration. F) Lignin:nitrogen ratio. G) Specific leaf area. Each symbol represents the mean ± standard error for the y-axis (vertical error bar) and the x-axis (horizontal error bar); n = 8 for k and n = 6-8 for litter quality.

CHAPTER 5
MOISTURE AND SUBSTRATE AVAILABILITY CONSTRAIN SOIL TRACE GAS FLUXES IN AN EASTERN AMAZONIAN REGROWTH FOREST

Introduction

Tropical forests represent an important source of atmospheric greenhouse gases including carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), along with nitric oxide (NO), a precursor to the photochemical production of tropospheric ozone (Vitousek & Matson 1992). The production and consumption of these gases are strongly linked to the availability of both soil moisture and decomposable substrate. However, seasonal cycles of precipitation, litterfall, and decomposition are often confounded in ways that limit our ability to quantify the relative importance of these interacting factors from seasonal observations of gaseous fluxes.

Observational studies in tropical forests have shown that higher soil moisture availability during the wet season usually increases soil CO_2 and N_2O effluxes, decreases NO efflux, and decreases CH_4 consumption rates (Davidson *et al.* 2000, Fernandes *et al.* 2002, Garcia-Montiel *et al.* 2001, Kiese & Butterbach-Bahl 2002, Kiese *et al.* 2003, Verchot *et al.* 2000, Verchot *et al.* 1999). Fewer studies have evaluated the response of soil trace gas fluxes to experimental manipulation of soil moisture availability in tropical forests. In a throughfall exclusion experiment in the Tapajós National Forest, Brazil, emissions of N_2O and CH_4 were reduced by the exclusion of about 50% of annual throughfall, but no treatment effect was observed for NO or CO_2 emissions (Davidson *et al.* 2004b). Addition of water to dry soil in short-term, small-scale field studies has resulted in increased emissions of CO_2, NO, and N_2O in wet (Garcia-Montiel *et al.* 2003b, Nobre *et al.* 2001) and seasonally dry (Davidson *et al.* 1993) tropical forest soils.

Regarding manipulative experiments of substrate availability (i.e., addition or removal of aboveground litter), there are only two reports of long-term, large-scale field studies (Li *et al.*

2004, Sayer 2005) that have assessed emissions of soil CO_2 in tropical forests in addition to this study. In both studies, reduction of substrate availability through litter removal decreased soil CO_2 efflux, which is consistent with several related studies in temperate forests (Bowden *et al.* 1993, Jandl & Sollins 1997, Rey *et al.* 2002, Sulzman *et al.* 2005), but we encountered no published reports of litter removal effects on NO, N_2O, and CH_4.

Measurements of soil CO_2 efflux and non-woody litterfall can be used to estimate total belowground carbon allocation (TBCA) in forests (Raich & Nadelhoffer 1989). For mature forests, TBCA is about two times aboveground litterfall, while for regrowth forests, TBCA is about three times aboveground litterfall (Davidson *et al.* 2002, Raich & Nadelhoffer 1989), indicating that regrowth forests allocate a relatively larger proportion of C to belowground structures than mature forests (Davidson *et al.* 2002). Although TBCA represents the single largest flux of C in forest ecosystems aside from canopy C assimilation (Davidson *et al.* 2002), little is known about this flux of C in tropical forests.

A better understanding of how trace gas emissions from tropical forest soils are affected by moisture and substrate availability can help to improve current biogeochemical models that predict impacts of changes in climate and land-use practices on the atmospheric concentrations of these gases (Potter & Klooster 1998). Such data, together with more estimates of total belowground C allocation in tropical forests are also needed to better understand carbon dynamics in regrowth forests (Johnson *et al.* 2000). Few such data are available for Amazonian regrowth forests, a significant and dynamic component of forest landscapes in this region (Fearnside 1996, Zarin *et al.* 2001).

Our primary objective in this study was to quantify the effects of moisture and substrate availability on soil trace gas emissions in an Amazonian regrowth forest stand. In one experiment, dry-season moisture limitation was reduced by irrigation. In the other experiment, substrate limitation was provoked by litter removal. The dry-season irrigation and litter removal experiments are described in Chapter 2.

Material and Methods

Field Measurements

Since July 2001, daily rainfall has been measured 500 m away from the experimental area using a standard rain gauge. Prior to July 2001, rainfall data reported here are from the National Agency of Electrical Energy (ANEEL) network meteorological station at Castanhal (01^{o} 17' 53" S, 47^{o} 56' 56" W) which is no longer in operation and that was about 3 km away from our site.

One tensiometer (Jet Fill Tensiometers, Soilmoisture Equipment Corp., Santa Barbara, CA, USA) was installed at a depth of 10 cm in each plot and soil water potential was recorded on a weekly basis in the morning. The number of actual replicates per treatment varied due to loss of water column tension during the dry season.

Soil CO_2 efflux was generally measured bi-weekly, beginning in March 2000, with an LI-6400 portable photosynthesis system fitted with an LI-6400-09 soil CO_2 flux chamber (LI-COR Inc., Lincoln, NE, USA). The chamber was fit into circular polyvinyl chloride (PVC) collars (11.5 cm internal diameter x 5.5 cm deep), which were installed approximately 2 cm into the soil. Each plot contained three soil collars, spaced at least 1 m apart, totaling 12 collars per treatment and sampling date. No live vegetation was contained within the collars. Measurements were taken between 0630 and 1100 hours.

To better understand the results of CO_2 flux analyses within the context of stand-level C dynamics, we also collected data on litterfall (Chapter 3). We estimated that non-woody litterfall was 48% C based on the monthly non-woody litterfall C concentration (47.9 ± 0.2%)

during the period of October 1999 to March 2001. Non-woody litterfall was 80 to 90% of total litterfall. Woody litterfall data are not reported here because of its much smaller impact on short-term trace gas emissions due to its slow turnover rate.

Two additional PVC collars with 20 cm diameter and 10 cm height were installed within each plot (total of 8 collars per treatment and sampling date) and inserted approximately 2-3 cm into the soil for measurement of soil NO, N_2O, and CH_4 gas fluxes. During the measurements, a vented PVC cover made from the end cap of a 20-cm diameter PVC pipe was fit into the collars. On average, NO, N_2O, and CH_4 flux measurements were made every two months, beginning in August 1999. The flux measurement technique for NO used a chemiluminescence detector (Scintrex LMA-3, Scintrex Limited, Concord, ON, Canada) as described by Verchot *et al.* (1999). N_2O and CH_4 fluxes were measured by gas chromatography analyses of four syringe samples extracted from the same chambers at 10-minute intervals (Verchot *et al.* 2000, Verchot *et al.* 1999). The PVC collars used for soil trace gas measurements were left in place throughout the course of the experiments.

To augment our understanding of the N gas fluxes (NO and N_2O), we also include here results of potential soil nitrification determined with a variation of the aerobic incubation method (Hart *et al.* 1994). Nitrification is the precursor to the denitrification process, and both processes produce NO and N_2O (Firestone & Davidson 1989). For each plot, we analyzed one composite sample made of four samples collected at a depth of 10 cm in October 2001. We estimated net N nitrification from changes in nitrate concentrations during 7-day incubation of soil. We corrected soil gravimetric moisture to 75% field capacity before sample incubation at about 28 ^{o}C in an incubator (Isuku FR24BS, Isuku Seisakusho Ltd., Tokyo, Japan). We did extractions of samples in 2 M potassium chloride (KCl) three days after collection in the field and in incubated samples. We kept samples under refrigeration (4 ^{o}C) prior to the initial extraction. We filtered extracts through Whatman No. 42 filter paper before analysis of nitrite/nitrate using a flow-injection system on a Lachat QuikChem AE autoanalyzer (Lachat Instruments, Milwaukee, WI, USA). Prior to the extractions, we dried subsamples of soil for 24 hours at 105 ^{o}C to determine actual moisture content.

Statistical Analyses

We used the SAS System version 9.00 to run the statistical analyses. We analyzed with PROC MIXED the effects of treatment, date, and treatment-by-date interaction on the variables trace gas flux, soil water potential, and non-woody litterfall using a repeated measures analysis with compound symmetric covariance structure. This structure assumes constant variance at all dates and equal correlations between all pairs of measures on the same experimental unit, i.e., collar, tensiometer, or trap for the soil trace gases, soil water potential, and litterfall variables, respectively. We ran separate tests to compare each of the treatments with the control. Within this analysis, significant treatment effects would have indicated temporally consistent differences between treatment and control measurements both pre- and post-treatment and across seasons (none were observed), significant date effects were generally indicative of seasonal trends that affected both treatment and control measurements, and treatment-by-date effects indicated a significant difference between treatment and control measurements that occurred after the treatment was initiated. We used CONTRAST statements to explicitly test whether the measured variables differed between seasons and between treatments within each season (wet and dry). We used the TTEST procedure to compare treatments and control means for soil nitrification.

When necessary, we performed log and square root transformations to meet the model assumptions of normality, based on the criteria of $P > 0.05$ in the Kolmogorov-Smirnov test, and equal variances, based on the absence of a pattern of heteroscedasticity in the plots of residual versus predicted values. Means and standard errors were calculated on the basis of untransformed data. All results are reported as significant when $P \leq 0.05$; we report marginal significance when $0.05 < P < 0.10$.

We estimated annual soil C efflux by linear interpolation between sampling dates using the EXPAND procedure. To estimate annual soil C efflux, we assumed that the variation in soil CO_2 efflux with time of day was minimal as previously reported by Davidson *et al.* (2000) for an eastern Amazonian primary forest. We tested for interannual and between treatment differences in annual soil C efflux and annual litterfall C values for control and irrigated plots in 2001 and 2002 using the PROC MIXED procedure. For the litter removal vs. control plot comparison of annual soil C efflux and annual litterfall C we used the TTEST procedure for 2002 data only; we did not include the 2001 data in the litter removal vs. control comparison because the treatment regime was not initiated until August 2001. We estimated the relative contribution of aboveground litter to soil respiration by subtracting litter removal soil CO_2 efflux from control soil CO_2 efflux.

Results

Irrigation Experiment

Rainfall declined from mid-July to early-January (dry season) during each year of the study (Figure 5-1A), resulting in lower soil water potential during this period (Figure 5-1B). The dry-season irrigation resulted in significantly ($P < 0.0001$) less negative soil water potential in control plots for most of the dates in 2001 and 2002 (Figure 5-1B). During the 2001 dry season, soil water potential was -0.052 ± 0.003 and -0.024 ± 0.002 MPa in control and irrigated plots, respectively; corresponding values for the 2002 dry season were -0.046 ± 0.003 and -0.013 ± 0.002 MPa.

There was a significant effect of date and the interaction between treatment and date on soil CO_2 efflux (Table 5-1). Soil CO_2 efflux for irrigated plots was significantly higher than for control plots during the dry-season irrigation ($P < 0.0001$, Figure 5-1C). There was also a significant effect of date and the interaction between treatment and date on soil CO_2 efflux for the pretreatment period ($P < 0.0001$); however, pretreatment differences between plots did not affect the significance of the dry-season irrigation effect. In the 2001 dry season irrigation period, soil CO_2 efflux values were 3.91 ± 0.13 and 5.54 ± 0.19 μmol CO_2 m^{-2} s^{-1} for control and irrigated plots, respectively; corresponding values for the 2002 dry season were 4.76 ± 0.19 and 6.21 ± 0.25 μmol CO_2 m^{-2} s^{-1}.

The lowest mean soil CO_2 efflux rate (2.33 ± 0.19 μmol CO_2 m^{-2} s^{-1}), which occurred in the control treatment on 24 October 2001 (Figure 5-1C), coincided with a successive decrease in soil water status (to -0.084 MPa) caused by a long dry spell of 24 days without rain out of a total of 31 days, with total precipitation of only 9 mm during the 31-day period. A 93% increase in the control plot soil CO_2 efflux in the subsequent measurement coincided with an increase in soil water status (to -0.008 MPa) following two consecutive rainy days (19 and 26 mm) after the long dry spell, and immediately prior to the soil respiration measurement; no increased soil CO_2 efflux was observed for irrigated plots. The pulse in soil CO_2 efflux was then followed by a decrease in CO_2 emissions associated with another dry period.

Annual soil C efflux was significantly higher in 2002 than in 2001 ($P < 0.0001$) (Table 5-2). The effects of treatment and the interaction between treatment and date were marginally significant ($P < 0.07$ and $P < 0.10$, respectively). Annual litterfall C was not affected by treatment or year (Table 5-2); although the interaction between treatment and year was marginally significant ($P < 0.053$) it is not readily attributable to a treatment effect.

The significant effect of date on NO efflux (Table 5-1, Figure 5-2B) was largely due to a single value measured in the end of July 2002; wet vs. dry season contrasts indicated non-significant seasonal differences in NO efflux. For N_2O (Figure 5-2C), the wet season efflux was significantly higher than the dry season efflux (5.62 ± 0.50 and 2.41 ± 0.47 μg N m^{-2} h^{-1}, respectively; $P< 0.0001$). During dry-season irrigation, treatment vs. control contrasts indicated that N_2O efflux in irrigated plots was significantly higher than in control plots (4.18 ± 0.87 and 2.34 ± 0.75 μg N m^{-2} h^{-1}, respectively; $P < 0.05$).

Date was again the only factor to have a significant effect on CH_4 efflux (Table 5-1, Figure 5-2D). Methane efflux in the dry season was significantly lower than in the wet season (-0.348 ± 0.118 and 0.128 ± 0.118 mg CH_4 m^{-2} d^{-1}, respectively; $P < 0.0001$). During dry-season irrigation, treatment vs. control contrasts indicated that CH_4 efflux in irrigated plots was also significantly higher than in control plots (0.226 ± 0.361 and -0.526 ± 0.185 mg CH_4 m^{-2} d^{-1}, respectively; $P < 0.01$). The net CH_4 emissions were generally close to zero, with most chambers generally showing net uptake of CH_4 (77% in control plots and 80% in irrigated plots). The range of CH_4 efflux for the whole experimental period was -5.00 to 22.03 mg CH_4 m^{-2} d^{-1}. Two chambers with very high effluxes (5.93 and 9.97 mg CH_4 m^{-2} d^{-1}) drove the large variability in the mean efflux for the control plot in March 2001, while the high variability for the irrigation means in September and October 2001 was driven by one chamber (9.08 and 10.30 mg CH_4 m^{-2} d^{-1}). The apparent high mean net production of CH_4 for irrigated plots in September (0.884 ± 1.353 mg CH_4 m^{-2} d^{-1}) and October (0.879 ± 1.187 mg CH_4 m^{-2} d^{-1}) 2001 becomes net consumption (-0.461 ± 0.172 and -0.292 ± 0.223 mg CH_4 m^{-2} d^{-1}) if we exclude the high efflux chambers from the calculation of means.

There was no significant effect of irrigation on net nitrification rates for control and irrigated plots (0.11 ± 0.02 and 0.11 ± 0.03 μg N g^{-1} soil d^{-1}, respectively).

Litter Removal Experiment

Soil water potential (Figure 5-3B) was significantly less negative in the wet season than in the dry season ($P < 0.0001$). Soil CO_2 efflux during the pretreatment period (Figure 5-3C) for litter removal and control plots did not differ significantly (4.18 ± 0.12 and 4.24 ± 0.08 μmol CO_2 m^{-2} s^{-1}, respectively; $P = 0.87$). During the litter manipulation period, soil CO_2 efflux in litter removal plots was significantly lower than in control plots (3.54 ± 0.17 and 4.90 ± 0.18 μmol CO_2 m^{-2} s^{-1}, respectively; $P < 0.001$). This difference was not homogeneous throughout the experimental period and followed a trajectory that can be divided in three phases. In the first phase, corresponding with the dry season and the early rainy season, the difference between treatment and control measurements was apparent for nearly all of the measurements made during the first six months of litter removal. The second phase, from 6-10 months after the beginning of litter removal, corresponded with the mid to late rainy season. During this phase, there were fewer measurements in which the difference between treatment means was significant. In the third phase, corresponding with the following dry season, the difference in soil CO_2 efflux between treatments was uniformly significant, and persisted through the end of the measurement period.

Aboveground litter respiration represented 22 ± 2% of total soil respiration for the whole litter removal period and was 22 ± 2, 16 ± 4, and 28 ± 2% of total soil respiration during the first, second and third phases, respectively. Annual soil C efflux was significantly lower (P <

0.05) in litter removal than in control plots in 2002 (Table 5-2). There was no significant difference in annual litterfall C between control and litter removal treatments in 2002 (Table 5-2).

The significant interaction effect on N oxide emissions (Table 5-1) was not related to a consistent effect of litter removal on either NO or N_2O effluxes (Figures 5-4B-C, respectively); the difference between treatments for both gases during the litter removal period was non-significant. Emissions of CH_4 (Figure 5-4D) in the dry season were significantly lower than in the wet season (-0.420 ± 0.164 and 0.287 ± 0.113 mg CH_4 m^{-2} d^{-1}, respectively; $P < 0.01$).

Mean net nitrification rates in control plots were marginally higher ($P = 0.06$) than in litter removal plots (0.11 ± 0.02 and 0.07 ± 0.01 μg N g^{-1} soil d^{-1}, respectively).

Discussion

Soil CO_2 Efflux and Belowground C Allocation

The soil CO_2 efflux rates measured in our study are within the range of data reported for tropical forests and are consistent with several other studies in Amazonian forests (Cattânio *et al.* 2002, Davidson *et al.* 2004b, Davidson *et al.* 2000, Fernandes *et al.* 2002, Nepstad *et al.* 2002, Salimon *et al.* 2004, Verchot *et al.* 2000) and in tropical forests elsewhere (Ishizuka *et al.* 2002, Kiese & Butterbach-Bahl 2002) that reported higher emissions of CO_2 during the wet season than in the dry season. We have also shown strong pulses of CO_2 efflux in response to rain events during dry periods (soil wet-up events), as observed in old-growth forests in the Brazilian Amazon (Davidson *et al.* 2000, Sotta *et al.* 2004) and in Costa Rica (Schwendenmann *et al.* 2003). Our dry-season irrigation experiment further demonstrates the constraint that moisture availability exerts on soil CO_2 efflux.

Soil CO_2 efflux as measured in the field mainly integrates root and microbial respiration, and we have not determined if the reduction in soil respiration in the dry season was caused by decreased activity of microbes, roots or both. However, a laboratory study with soil from the same site showed a significant increase in microbial basal respiration during the 2001 wet season compared to the previous dry season (Rangel-Vasconcelos 2002), as observed in other tropical forests (Cleveland *et al.* 2003, Luizao *et al.* 1992). Although microbial respiration rates determined under laboratory conditions cannot be compared to rates obtained in the field with chamber techniques, those results suggest that reduction in soil microbial activity during the dry season likely contributed to the observed lower rates of soil respiration during this period at our site. Likewise, reduced activity of microbes in decomposing aboveground litter during the dry season could have contributed to lower soil CO_2 efflux in non-irrigated plots. Borken *et al.* (2003) have recently shown that microbial respiration of the O horizon can contribute significantly to CO_2 pulses after soil wet-up events in a temperate forest and Goulden *et al.* (2004) reported that increased soil respiration after a rainfall during the dry season was associated with surface litter rehydration in an Amazonian old-growth forest. Wieder and Wright (1995) have also observed higher litter mass loss under irrigation compared with no irrigation in a tropical forest in Panama. Finally, lower soil CO_2 efflux during the dry season could also have resulted from constrained root respiration due to decreased root growth (Cattânio *et al.* 2002) or decreased flux of photosynthates to roots, which limits root respiration itself (Högberg *et al.* 2001) and/or rhizospheric microbial respiration (Kuzyakov & Cheng 2001). Further research on differentiating root from microbial respiration and aboveground litter from soil respiration are needed to better understand how moisture constrains CO_2 efflux from tropical forest soils, especially because likely concomitant and opposite variations in root and microbial dynamics under dry conditions (Davidson *et al.* 2004b) make it difficult to understand the mechanisms by which moisture controls total soil respiration.

The variation in the size of the difference of soil CO_2 efflux between control and litter removal throughout the manipulation period followed a trajectory that can be linked to altered substrate availability and variation in soil water status due to the seasonality of rainfall. In the first phase of this trajectory, the early impact of litter removal on soil respiration suggests that CO_2 efflux associated with microbial decomposition of aboveground litter and superficial root respiration represents a substantial proportion (about 22 % in the present study) of total soil respiration (Raich & Schlesinger 1992). During the second phase, an interaction between substrate availability and rainfall seasonality appears to influence the variation in soil CO_2 efflux. The difference between control and litter removal plots decreased during some dates in the second phase, suggesting that the contribution of belowground respiration was relatively higher during the wet season. The third phase may be characterized by the depletion of labile soil carbon and, therefore, an increase in the difference in soil CO_2 efflux between treatments. Although this phase is also coincident with the 2002 dry season, its length and consistency (i.e., lack of responsiveness to dry-season wet-up events) lead us to suspect that, due to the removal of the litter layer, substrate availability has become a larger constraint on soil respiration than reduced moisture availability. In 2002, litter removal resulted in a 28% reduction in soil CO_2 efflux, which is very similar to the 27% found in Costa Rica after 2 years of litter removal (Sayer 2005), but lower than the 54% reduction after 7 years of litter removal in Puerto Rico (Li *et al.* 2004).

The estimated annual soil C efflux measured in our control plots is comparable to another estimate for eastern Amazonian forests in Brazil (Davidson *et al.* 2000) and is higher than estimates for tropical old-growth forests elsewhere (Ishizuka *et al.* 2002, Schwendenmann *et al.* 2003); annual fluxes measured in other tropical forest sites are given in Table 5-3. We observed lower total rainfall and higher annual soil C efflux in 2002 than in 2001, suggesting that the interannual variability in soil C efflux was not caused by differences in annual rainfall. Pulses of CO_2 associated with rainfall events observed in this study are consistent with the hypothesis that rainfall distribution, rather than total rainfall, may better explain annual variability in soil C efflux. Differences in annual soil C efflux between irrigation and control plots are also consistent with a substantial moisture constraint on soil respiration.

Based on our annual soil C efflux and non-woody litterfall C estimates (Chapter 3), we can calculate a C efflux : litterfall C ratio of 4.0 – 5.2 for our control plots, consistent with the mean value of 4.16 reported by Davidson *et al.* (2002) for young forests. Total belowground carbon allocation (TBCA) estimated by the difference between annual basis C fluxes in soil respiration and litterfall (Raich & Nadelhoffer 1989) is underestimated for regrowth forests if C storage in roots and soil is not accounted for (Davidson *et al.* 2002). However, simple calculation of TBCA based only on soil respiration and litterfall can provide a lower limit of TBCA for regrowth forests. For our site, the ratio between annual soil C efflux and annual litterfall C indicates that TBCA relative to litterfall is similar to values for other regrowth forest site in the eastern Amazon (Davidson *et al.* 2002) and higher than those of mature forests (Davidson *et al.* 2002, Raich & Nadelhoffer 1989), consistent with increased allocation of C to belowground structures as a mechanism by which regrowth forests cope with the demands for water and nutrients (Davidson *et al.* 2002).

Differences in annual soil C efflux between litter removal and control plots are consistent with a substantial substrate constraint on soil respiration. In 2002, the amount of carbon in litterfall (368 ± 14 g C m^{-2} yr^{-1}) was well within one standard error of the mean difference in soil C efflux between control and litter removal (559 ± 291 g C m^{-2} yr^{-1}). This substantial difference in soil C efflux also suggests that ~20% of total soil C efflux is due to litter respiration, with the remaining ~80% due to belowground respiration; this is consistent with results obtained in litter removal studies in forest ecosystems in the tropics (Li *et al.* 2004, Sayer 2005) and other climatic regions (Bowden *et al.* 1993, Jandl & Sollins 1997, Rey *et al.* 2002).

Nitrogen Oxide Emissions

Nitric and nitrous oxide effluxes measured in this study both in wet or dry seasons are among the lowest reported for either regrowth or old-growth tropical forests in the Brazilian Amazon (Cattânio *et al.* 2002, Davidson *et al.* 2004b, Garcia-Montiel *et al.* 2001, Nepstad *et al.* 2002, Verchot *et al.* 1999) and tropical forests elsewhere (Erickson *et al.* 2001, Ishizuka *et al.* 2002, Palm *et al.* 2002). These low N oxide effluxes may result from low rates of N cycling, as indicated by the very low net nitrification rates we found in both seasons compared to other studies for Amazonian forests (Garcia-Montiel *et al.* 2003a, Neill *et al.* 1997, Palm *et al.* 2002). The thin concretionary soils of this site, along with the recent history of repeated slash-and-burn cycles and the high litterfall C:N ratios, are consistent with a very conservative nitrogen cycle and low rates of both nitrification and denitrification. Although fluxes were consistently low, slightly higher efflux of N_2O in the wet season compared to the dry season reported here has also been observed in other tropical forests (Cattânio *et al.* 2002, Erickson *et al.* 2002, Garcia-Montiel *et al.* 2001, Kiese & Butterbach-Bahl 2002, Nepstad *et al.* 2002, Verchot *et al.* 1999). Consistent with the results obtained by Nobre *et al.* (2001), we also found a significant effect of irrigation on N_2O efflux. Higher N_2O efflux associated with wetter soil conditions during both the wet season and dry-season irrigation periods likely resulted from increased denitrification (Davidson 1991).

The effects of litter removal on N oxide fluxes are not clear and difficult to interpret since the fluxes are inherently very low at our site. If N trace gas emissions were already limited by N availability in this infertile soil, the removal of litter might be expected to decrease emissions further. However, that decrease would be difficult to detect relative to the naturally low emissions that were already frequently near detection limits prior to litter removal.

Methane Emissions

Methane fluxes measured at our site are in the lower range of both net consumption and net production fluxes found for tropical forests (Kiese *et al.* 2003, Palm *et al.* 2002, Verchot *et al.* 2000). Higher net consumption of CH_4 in the dry season and lower net consumption (or even small net production) in the wet season observed in our study is consistent with the pattern of CH_4 emissions measured in other Brazilian Amazonian forests (Cattânio *et al.* 2002, Nepstad *et al.* 2002, Verchot *et al.* 2000) and tropical forests elsewhere (Kiese *et al.* 2003). Increased net CH_4 production during the wet season as well as during the irrigation period in our study suggests that higher soil water status decreased soil aeration leading to an increase in methanogenesis (Davidson & Schimel 1995). Although decreased aeration during the wet season could have resulted in higher efflux of CH_4 and N_2O, higher soil CO_2 efflux associated with wetter soil conditions could also have contributed to the increased efflux of CH_4 and N_2O because of the consumption of O_2 in the respiration process (Palm *et al.* 2002, Verchot *et al.* 2000).

Conclusions

We conclude that soil CO_2 efflux is strongly linked to soil moisture and substrate availability as indicated from the responses of CO_2 emissions to soil wet-up events, dry-season irrigation, and litter removal for this tropical regrowth stand. On an annual basis, this regrowth stand allocates a large amount of C to belowground structures relative to litterfall C. Relieving dry season water limitation increased soil respiration by about 40 and 30% in the two dry seasons studied, corresponding to annual increases of 27 and 13% in 2001 and 2002, respectively. Removing aboveground litter reduced annual soil respiration by 28% in 2002.

In general, N oxide emissions were very low, probably due to the inherently low rates of nitrogen cycling at this site. Emissions of N_2O and CH_4 were constrained by low moisture availability, while emissions of NO were not affected by irrigation. We were unable to detect more severe substrate limitation induced by the litter removal treatment on N oxide and CH_4 emissions.

The substantial impacts of soil moisture and aboveground litter on soil CO_2 efflux shown in this study suggest that alterations in the availability of these resources that may result from climate and land-use changes in tropical regions could have significant effects on regional CO_2 fluxes.

Table 5-1. F statistics and associated significance levels for the effect of treatments (irrigation and litter removal), sampling date, and their interaction on soil trace gas fluxes and non-woody litterfall in a tropical regrowth forest stand in eastern Amazonia[a] (PROC MIXED, SAS System version 9.0). Significant "treatment" effects (not observed) would indicate temporally consistent differences between treatment and control both pre- and post-treatment and across seasons, significant "date" effects are generally indicative of seasonal trends that affect both treatment and control measurements, and "treatment x date" effects indicate a significant difference between the treament and control measurements that occurs after the treatment was initiated.

	Irrigation experiment			Litter removal experiment		
Variable	Treatment	Date	Treatment x Date	Treatment	Date	Treatment x Date
CO$_2$ efflux	2.55ns	9.48***	5.02***	3.10ns	9.63***	3.24***
NO efflux	0.04ns	5.46***	1.50ns	3.29ns	7.65***	2.21*
N$_2$O efflux	0.93ns	4.20***	1.00ns	0.32ns	6.42***	1.68*
CH$_4$ efflux	0.91ns	2.14**	1.22ns	< 0.01^{ns}	2.21**	0.77^{ns}
Litterfall	0.24ns	45.27***	1.62**	0.27^{ns}	32.91***	1.18^{ns}

[a] The level of significance is indicated (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant).

Table 5-2. Annual soil carbon efflux and non-woody litterfall carbon for control, irrigated and litter removal plots in a tropical regrowth forest stand in eastern Amazonia (mean ± se, n = 12 per treatment).

Treament	Soil C efflux (g m^{-2} yr^{-1})		Non-woody litterfall C (g m^{-2} yr^{-1})	
	2001	2002	2001	2002
Control	1593 ± 74	1988 ± 126	410 ± 28	383 ± 27
Irrigation	2021 ± 154	2237 ± 158	398 ± 24	415 ± 24
Litter removal	-	1429 ± 165	-	368 ± 14

Table 5-3. Estimates of annual soil carbon (C) efflux in old-growth and regrowth tropical forests.

Forest Location	Rainfall (mm yr^{-1})	Soil type	Soil C efflux (g C m^{-2} yr^{-1})	Reference
Old-growth[1]				
Pará, Brazil (2^o 59' S, 47^o 31' W)	1800	Haplustox	2000	Davidson *et al.* (2000)
Sumatra, Indonesia (1^o 05.164' S, 102^o 05.702' E)	2060	Ultisol	560-820	Ishizuka *et al.* (2002)
La Selva, Costa Rica (10^o 20' N, 83^o 50' W)	4200	Typic Haploperox "old alluvium	1060	Schwendenmann *et al.* (2003)
Pará, Brazil (2.8968 oS, 54.9519 oW)	2000	Haplustox	1000	Davidson *et al.* (2004b)
Acre, Brazil	1940	dystrophic and eutrophic Ultisols with patches of Oxisols	1700	Salimon *et al.* (2004)
Barro Colorado, Panama	2600	Oxisol	1740	Sayer (2005)
Pará, Brazil (2^o 64' S, 54^o 59' W)	2000	clayey (Ultisols and Oxisols)	1084	Silver *et al.* (2005)
		sandy (Ultisols)	1363	Silver *et al.* (2005)
Regrowth[2]				
Pará, Brazil (2^o 59' S, 47^o 31' W), 20-year-old	1800	Oxisol and alfisol	1800	Davidson *et al.* (2000)
Gran Sabana, Venezuela (5^o 0' S, 61^o 0' W)	2200	Acrohumox	896 (tall) 1241 (medium) 1024 (low)	Priess and Fölster (2001)
Acre, Brazil, 3-18-yr-old	1940	dystrophic and eutrophic Ultisols	1600	Salimon *et al.* (2004)
Pará, Brazil		Oxisol	1790	This study

[1] Includes sites classified as mature and primary forests.
[2] Includes sites classified as secondary forests.

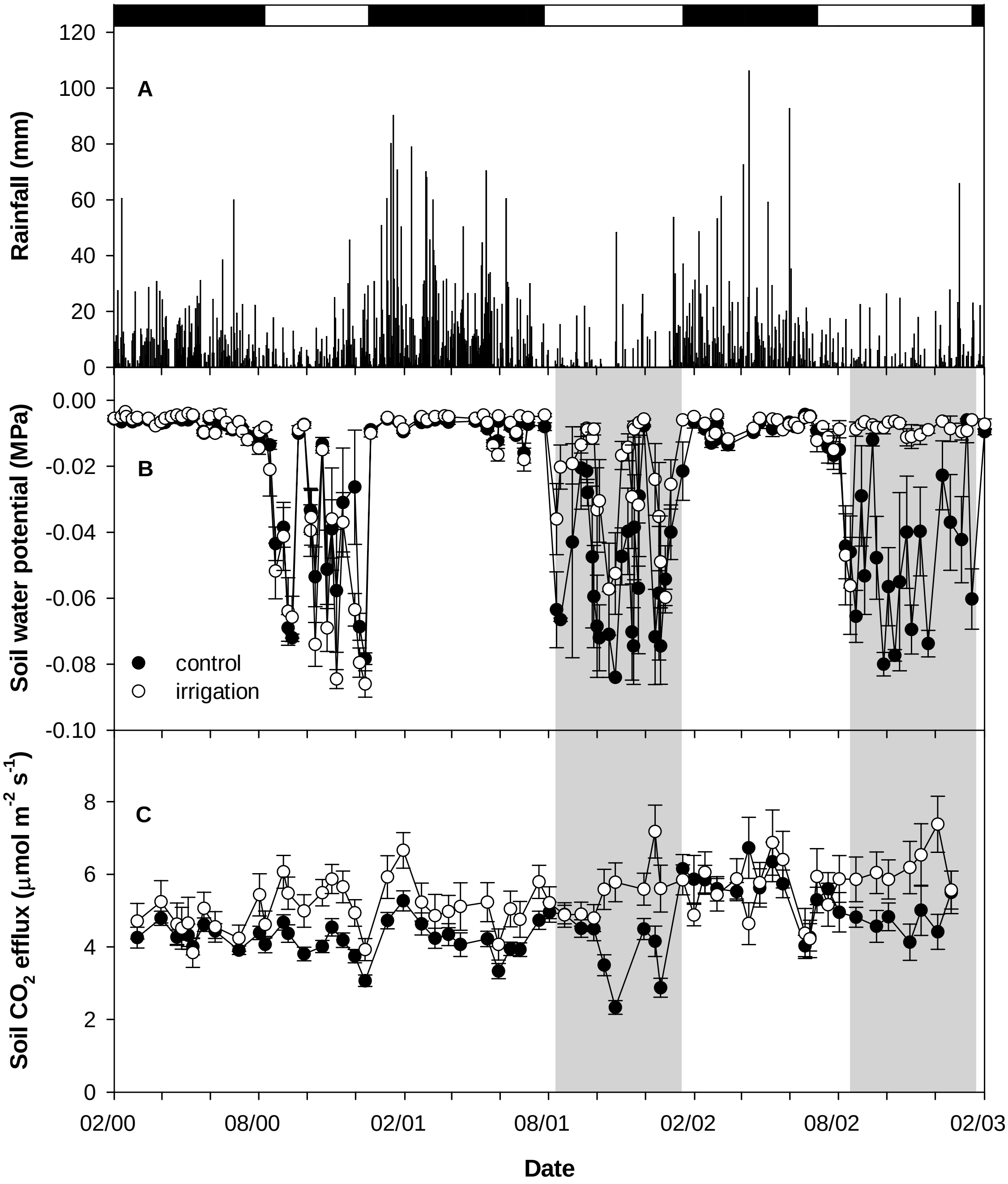

Figure 5-1. Effects of rainfall patterns and dry-season irrigation on soil moisture status and soil respiration in an Amazonian regrowth forest stand, Brazil. A) Daily rainfall at the study site. B) Soil water potential. C) Soil carbon dioxide (CO_2) efflux. In Figures B-C, circles represent means (± se); n = 4 for soil water potential and n = 12 for soil CO_2 efflux per sampling date. Gray-shaded areas indicate the dry season irrigation periods. White and black horizontal bars mark dry and wet seasons, respectively.

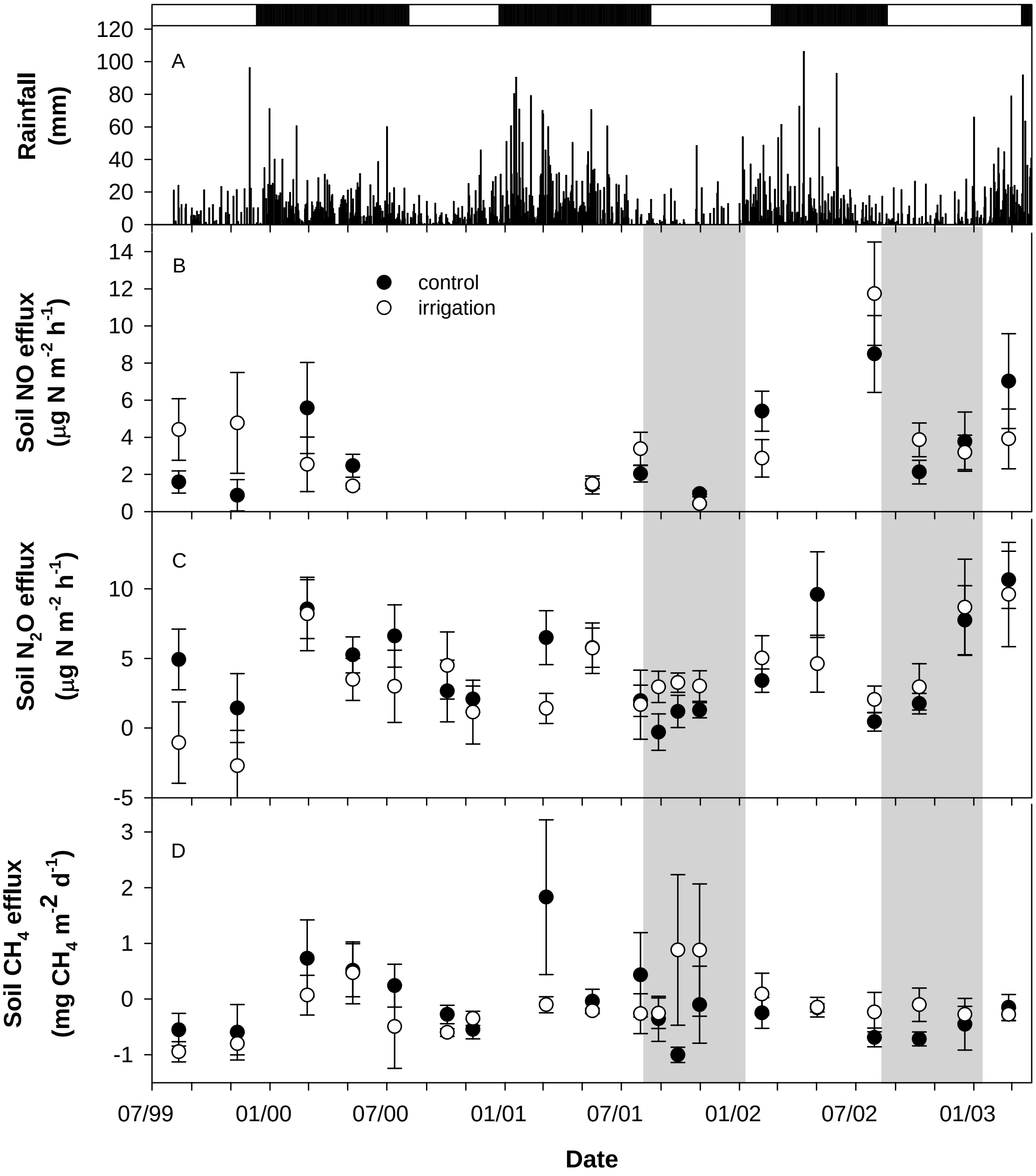

Figure 5-2. Effects of rainfall patterns and dry-season irrigation on soil nitrogen oxide and methane effluxes in an Amazonian regrowth forest stand, Brazil. A) Daily rainfall at the study site. B) Soil nitric oxide (NO) efflux. C) Soil nitrous oxide (N_2O) efflux. D) Soil methane (CH_4) efflux. In Figures B-D, closed and open circles represent means (± se) for control and irrigation treatments, respectively (n = 8 per sampling date). Gray-shaded areas indicate the dry season irrigation periods. White and black horizontal bars mark dry and wet seasons, respectively.

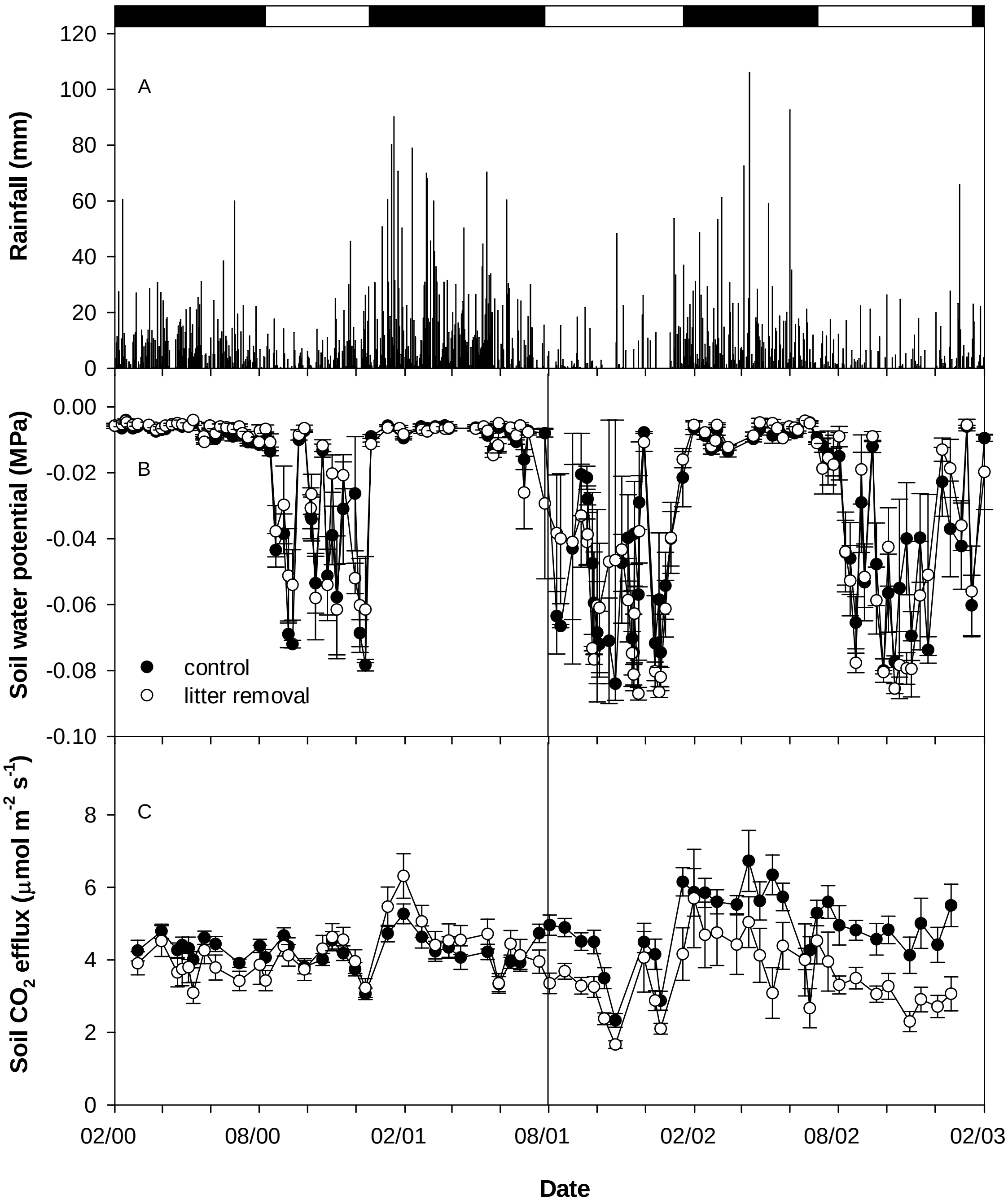

Figure 5-3. Effects of rainfall patterns and litter removal on soil moisture status and soil respiration in an Amazonian regrowth forest stand, Brazil. A) Daily rainfall at the study site. B) Soil water potential. C) Soil carbon dioxide (CO_2) efflux. In Figures B-C, circles represent means (± se); n = 4 for soil water potential and n = 12 for soil CO_2 efflux per sampling date. The vertical line indicates the beginning of the litter removal treatment. White and black horizontal bars mark dry and wet seasons, respectively.

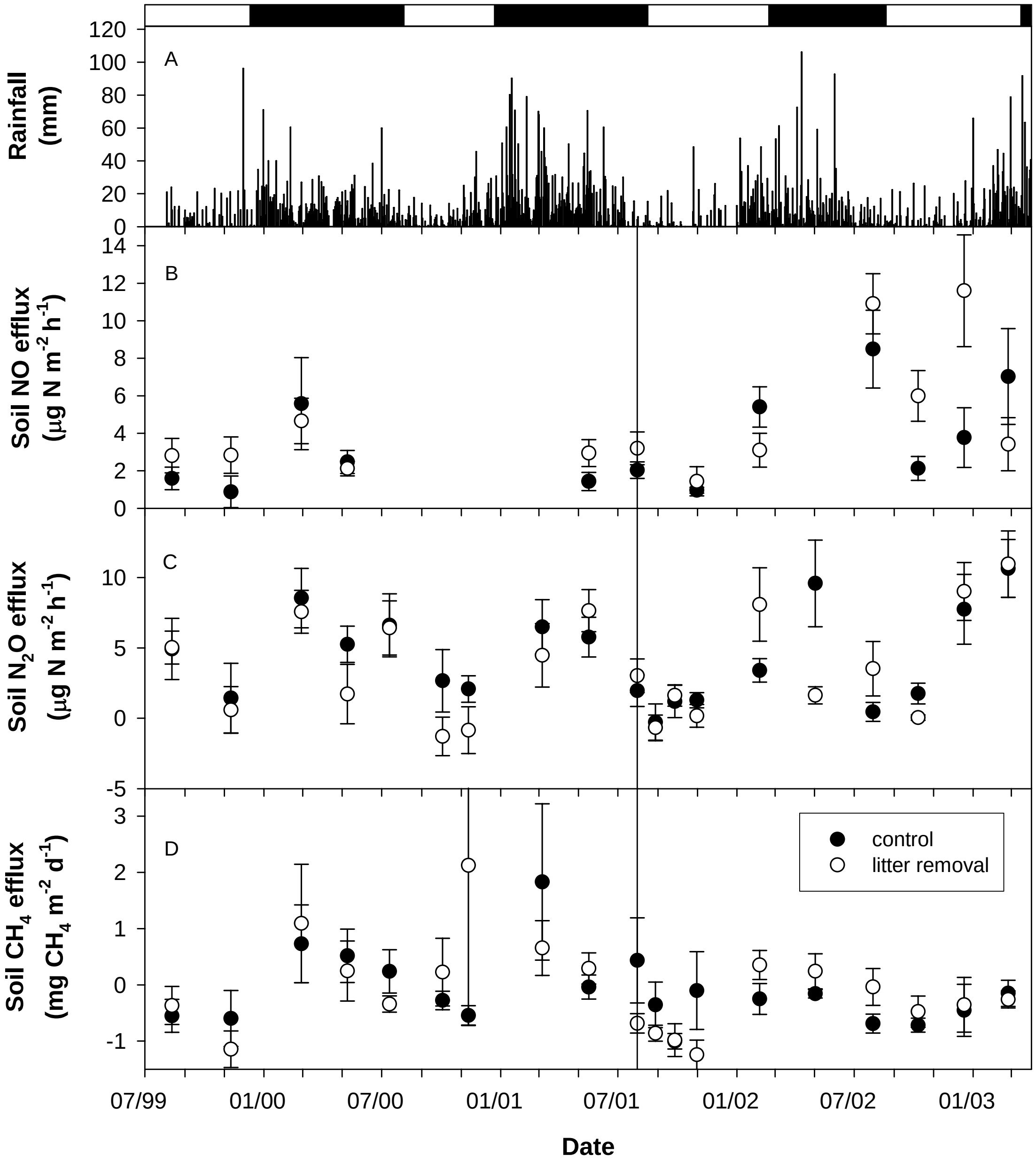

Figure 5-4. Effects of rainfall patterns and litter removal on soil nitrogen oxide and methane effluxes in an Amazonian regrowth forest stand, Brazil. A) Daily rainfall at the study site. B) Soil nitric oxide (NO) efflux. C) Soil nitrous oxide (N_2O) efflux. D) Soil methane (CH_4) efflux. In Figures B-D, closed and open circles represent means (± se) for control and litter removal treatments, respectively (n = 8 per sampling date). The vertical line indicates the beginning of the litter removal treatment. White and black horizontal bars mark dry and wet seasons, respectively.

CHAPTER 6
MOISTURE CONSTRAINTS TO ABOVEGROUND NET PRIMARY PRODUCTIVITY IN EASTERN AMAZONIAN FOREST REGROWTH

Introduction

Net primary productivity (NPP) is considered to be the best integrator measure of resource effects on ecosystem processes (Chapin & Eviner 2005). Improved understanding of temporal shifts in NPP may aid predictions of ecosystem response to ongoing climate and land-use changes (Tian *et al.* 1998). In tropical forests, reliable estimates of NPP mostly involve measurements of aboveground net primary productivity (ANPP) components; due to methodological difficulties belowground NPP is rarely measured (Clark *et al.* 2001b). For tropical forest regrowth (e.g. following agricultural conversion and abandonment), there is a paucity of data even on ANPP, in part because these sites are very rarely measured over multiple years.

Aboveground biomass increment in live trees (i.e., wood increment) and non-woody litterfall (a proxy for leaf production) are commonly used to estimate ANPP; both aboveground biomass increment and non-woody litterfall can be relatively easily measured and represent two significant components of total ANPP (Clark *et al.* 2001a). Stem diameter and height measures are usually used to estimate aboveground biomass (AGB) through allometric equations (e.g., Ducey *et al.* Submitted). Despite several reports on AGB for tropical forest regrowth (Gehring *et al.* 2005, Saldarriaga *et al.* 1988, Zarin *et al.* 2001), repeated measures of AGB and litterfall are rare and calculations of ANPP for these forests are therefore lacking.

Observational and manipulative experiments suggest that moisture availability may be an important control over ANPP in tropical forests. At old-growth forest sites in the Brazilian Amazon, higher diameter growth rates are associated with wetter periods (Higuchi *et al.* 2003, Rice *et al.* 2004, Vieira *et al.* 2004). Nepstad *et al.* (2002) have previously shown that soil moisture depletion during a partial throughfall exclusion experiment reduced ANPP in an old-growth Amazonian forest. Conversely, excessive soil moisture may also decrease ANPP (Schuur & Matson 2001). Analogous data from both observational and manipulative studies are lacking for tropical forest regrowth, even though recent estimates indicate that there are ~38 million ha of regrowth in Latin America alone, and the area is growing as unproductive deforested land is abandoned (ITTO 2002).

The primary objective of this chapter was to investigate the response of ANPP to experimentally increased dry-season moisture availability and inter-annual variability in dry-season precipitation during a four-year irrigation experiment described in Chapter 2. We hypothesized that dry-season irrigation would increase ANPP, and that ANPP would also be positively correlated with dry-season precipitation.

Study Site and Experimental Design

Study site and experimental design are described in Chapter 2.

Material and Methods

Aboveground Net Primary Productivity

Aboveground net primary productivity (ANPP) was estimated as the sum of annual increases in aboveground biomass (AGB) of trees (diameter at breast height ≥ 1 cm) and non-woody litterfall (Clark *et al.* 2001a, Grace *et al.* 2001) between July 2001 and July 2005. To estimate AGB, we used site-specific mixed-species and species-specific allometric equations based on diameter measurements (Table 6-1; Ducey *et al.* In preparation). Diameter increments have previously been published, in part, by Araújo *et al.* (2005). Non-woody litterfall data are reported on Chapter 3.

Aboveground biomass increment (AGBI, in Mg ha^{-1} yr^{-1}) was calculated for each plot as follows (Clark *et al.* 2001a):

ABGI = (Σ increments of surviving trees) + (Σ increment(s) of ingrowth),

where the increment of surviving trees was calculated as the AGB in year_{x+1} minus the AGB in the previous year (i.e., year_x), and the increment of ingrowth was calculated as the AGB in the ingrowth year minus AGB relative to the minimum diameter (1 cm).

This method of calculating AGBI may underestimate its actual value if trees exhibit significant growth between their last measurement and their death. In a separate study, the increment in diameter at breast height (DBH) of trees with DBH ≥ 5 cm was measured every 1-2 months from November 2003 to December 2005 using dendrometer bands fabricated with aluminium tapes (data not presented). We observed that several months prior to tree death, stem increment was consistently equal to zero, suggesting that unaccounted diameter increment prior to tree death (Clark *et al.* 2001a) may have little impact on biomass increment estimates.

Statistical Analysis

We used the SAS System version 9.00 to run the statistical analyses. We analyzed with PROC MIXED the effects of treatment, date, and treatment-by-date interaction on ANPP using a repeated measures analysis with compound symmetric covariance structure. This structure assumes constant variance at all dates and equal correlations between all pairs of measures on the same experimental unit, i.e., plot. We used PROC NLIN for linear regression analysis between ANPP and rainfall (current- and previous-year annual rainfall and dry-season rainfall); annual rainfall corresponds to total rainfall in the interval between yearly diameter measurements. All results are reported as significant when $P \leq 0.05$; we report marginal significance when $0.05 < P < 0.10$. Multiple comparisons of means were performed with Tukey's test.

Results

ANPP was significantly affected by date ($P = 0.034$) and treatment ($P = 0.026$), with marginally significant effects of treatment x date interaction ($P = 0.059$). In the annual periods from July 2002 to July 2003, and July 2003 to July 2004, ANPP was significantly higher in irrigated plots than in control plots (Figure 6-1). ANPP was also positively correlated with previous-year dry-season rainfall (Figure 6-2; $R^2 = 0.45$; $P < 0.01$).

Discussion

Aboveground net primary productivity range calculated for this site (12.3 ± 0.5 to 16.6 ± 2.1 Mg ha^{-1} yr^{-1}, n = 4, control plots) is equivalent to the highest values reported by Clark *et al.* (2001b) for old-growth tropical forests. Our estimate of ANPP for this site represents a lower bound, because it only includes wood increment and non-woody litterfall.

Although our calculated ANPP values are relatively high, the aboveground biomass accumulated about 12 years after land abandonment (51.5 ± 2.6 Mg ha^{-1}) is 13% lower than the value obtained (59.2 Mg ha^{-1}) with a model developed to predict aboveground biomass accumulation by Amazonian regrowth forests (Zarin *et al.* 2001), and substantially lower (> 70%) than the value predicted by the model developed for regrowth forests recovering from first-cycle slash-and-burn in central Amazonian regrowth forests (Gehring *et al.* 2005). Lower biomass compared to model predictions may result from (a) the history of repeated burning events (Zarin *et al.* 2005), (b) the inherent low fertility of the concretionary soil, and (c) relatively distinct dry season periods at the study site (Apeú). While the difference from Zarin *et al.*'s (2001) is within the model error, the great discrepancy in relation to Gehring *et al.*'s (2005) work may be due to less severe dry seasons and more fertile soils for central Amazonian regrowth forests (Gehring *et al.* 2005).

While the results of our dry-season irrigation experiment demonstrate the constraint of moisture availability on ANPP at this site, reduced ANPP associated with lower previous-year dry-season rainfall indicates a lag effect of the influence of drought on ANPP. A recent dendrochronological study also found a lag effect of rainfall on stem growth for a tropical tree species in Bolivia (Brienen & Zuidema 2005). Possible explanations for lag effects on tree growth include rainfall controls on bud preformation (Critchfield 1960), storage of reserves under favorable conditions (Dünisch *et al.* 2003), and long-term water table storage (Borchert 1994). Decreased moisture availability can reduce productivity through effects on leaf hydration, stomatal conductance, and, ultimately, leaf photosynthesis (Chaves *et al.* 2003, Malhi *et al.* 1998, Mulkey & Wright 1996). In our study, irrigation may have stimulated plant productivity directly through decreasing drought limitations on photosynthesis and/or indirectly through enhancing nutrient availability due to moisture effects on litter decomposition (Cornejo *et al.* 1994, Wieder & Wright 1995); irrigation may have also allowed plants to better utilize higher light availability during less cloudy days typical of the dry season. Leaf water potential and gas exchange for *Vismia guianensis*—a common species at the study area (Araújo *et al.* 2005)—showed less negative water potential (higher leaf hydration) and sustained higher photosynthetic capacity for some dates under irrigation (Vasconcelos *et al.* 2002). Photosynthetic capacity in the understory at the site has also been shown to respond positively to both irrigation and dry-season rainfall events (Fortini *et al.* 2003) and to be sensitive to interannual differences in dry-season rainfall (Aragão *et al.* 2005).

Wood increment was more sensitive than litterfall to altered moisture availability, consistent with greater reductions in stemwood growth than fine litterfall in throughfall exclusion plots previously reported for an old-growth Amazonian forest (Nepstad *et al.* 2002). Greater sensitivity of stemwood increment to altered moisture availability may have important implications for the carbon balance of tropical forest regrowth, since stemwood represents a large pool of carbon with a low turnover rate.

Anticipated climate change for the Amazon region may include more frequent and severe dry seasons in response to global warming (IPCC 2001), deforestation (Costa & Foley 2000) and more frequent El Niño episodes (Trenberth & Hoar 1997). Our results indicate that the potential of forest regrowth to sequester carbon will decrease under that projected scenario.

Table 6-1. Allometric equations used to estimate tree biomass in a tropical regrowth forest stand in eastern Amazonia, Brazil.

Species	Equation[1]
Abarema jupunba	Biomass = $0.014978 \times DBH^{3.5763}$
Casearia javitensis	Biomass = $0.32982 \times DBH^{1.7336}$
Lacistema pubescens	Biomass = $0.044856 \times DBH^{3.1285}$
Myrcia sylvatica	Biomass = $0.14988 \times DBH^{3.093}$
Ocotea guianensis	Biomass = $0.098412 \times DBH^{2.6117}$
Poecilanthe effusa	Biomass = $0.28772 \times DBH^{2.2747}$
Vismia guianensis	Biomass = $0.2897 \times DBH^{2.0468}$
All other taxa	Biomass = $0.18598 \times DBH^{2.3155}$

[1] Biomass = dry weight in kg; DBH = diameter at breast height in cm

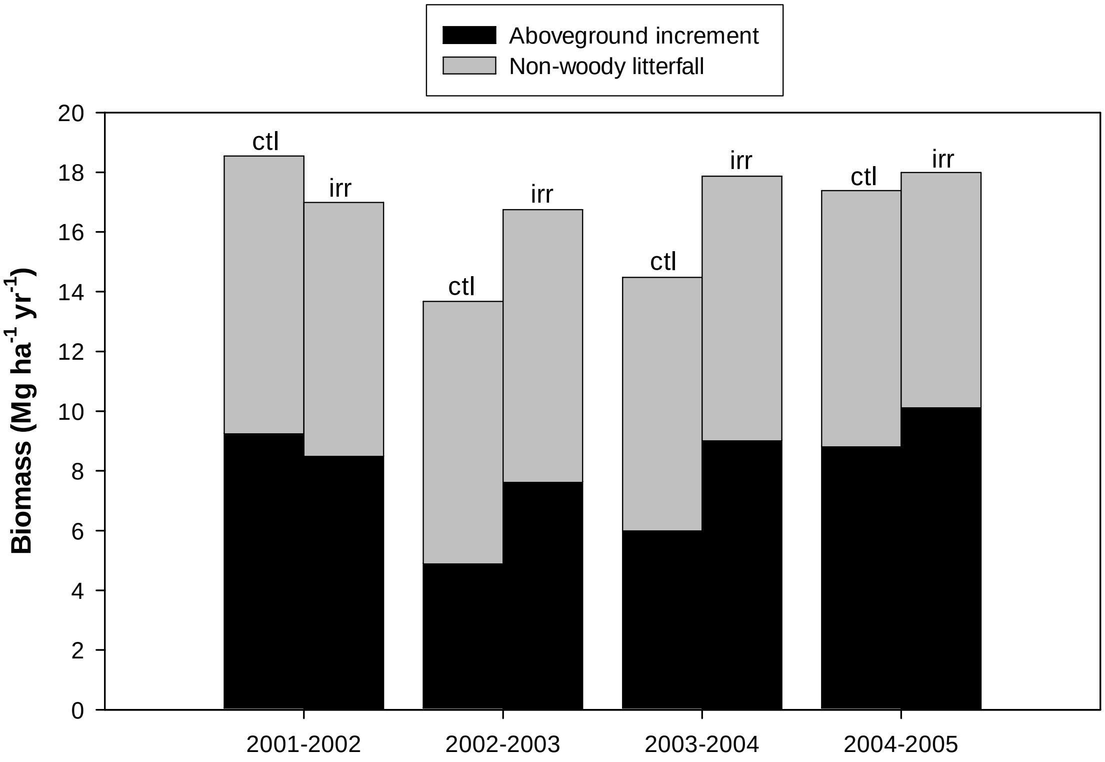

Figure 6-1. Effects of dry-season irrigation on aboveground increment and non-woody litterfall for a tropical forest regrowth stand in eastern Amazonia, Brazil. Each stacked bar represent means (n = 4). Ctl and irr refers to control and irrigation plots, respectively.

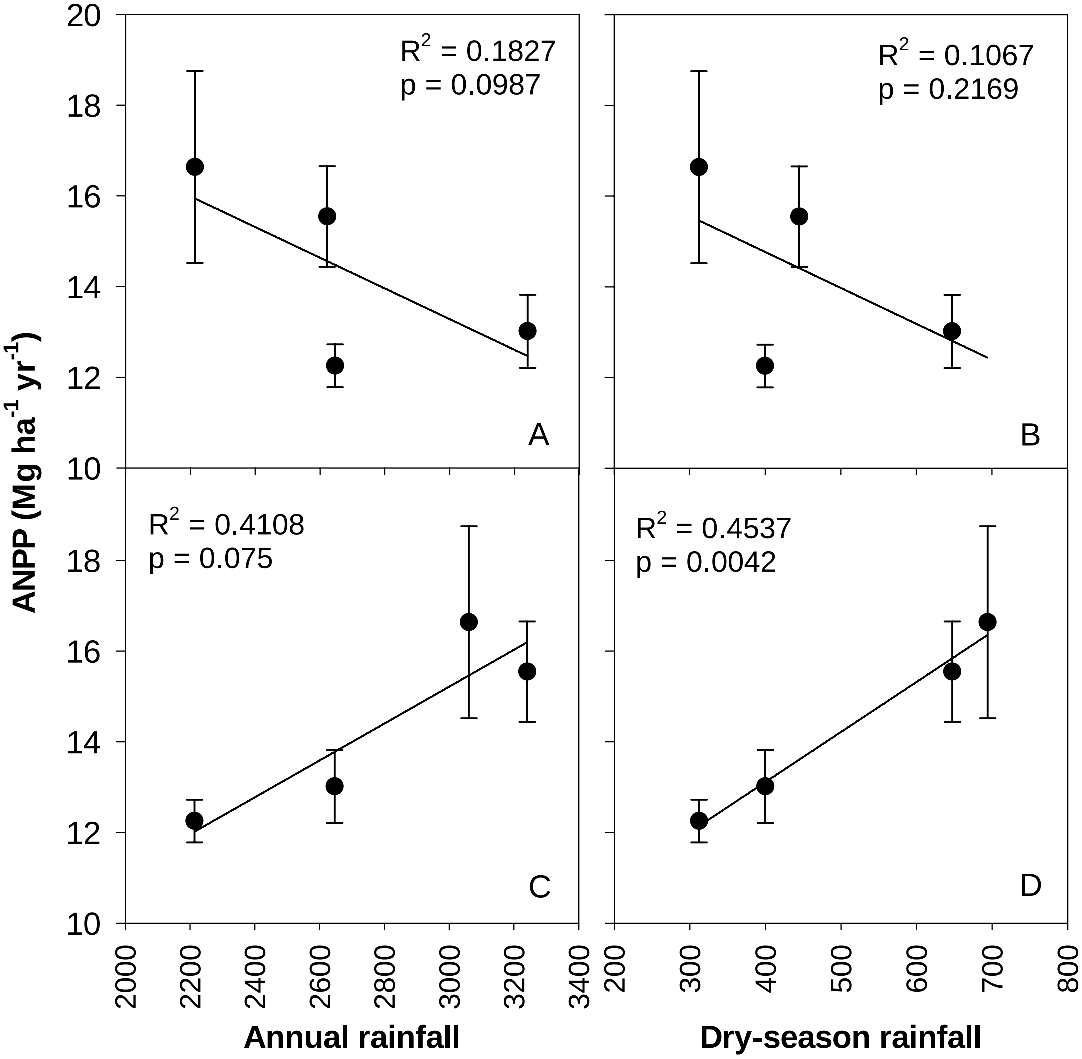

Figure 6-2. Relationship between aboveground net primary productivity (ANPP) and rainfall in an Amazonian forest regrowth stand, Brazil. A) Current-year annual rainfall. B) Current year dry-season rainfall. C) Previous year annual rainfall. D) Previous year dry-season rainfall. Symbols are means ± se for control plots (n = 4).

CHAPTER 7
CONCLUSIONS

This long-term (five continuous years), stand-level resource manipulative experiment—consisting of daily dry-season irrigation and bi-weekly removal of aboveground litter—demonstrated moisture and nutrient limitations to carbon and nutrient dynamics associated with above and belowground ecosystem processes for a forest regrowth site in eastern Amazonia, Brazil. Aboveground (litterfall quantity and quality, and litter decomposition) and belowground (soil trace gas fluxes) processes showed marked intrannual variation associated with rainfall seasonality (Table 7-1). Soil carbon dioxide (CO_2) efflux and litter decomposition rates were strongly linked to moisture availability as indicated from their responses to rainfall seasonality and dry-season irrigation; differential decomposition rates among tree species were linked to leaf chemical and physical properties. Soil emissions of nitrous oxide (N_2O) and methane (CH_4) were slightly increased by dry-season irrigation, but soil nitric oxide (NO) emissions were not sensitive to changes in soil moisture availability in irrigated plots. Aboveground net primary productivity – an index that integrates resource effects on ecosystem processes – was constrained by moisture availability as indicated by the response of wood increment to interannual variation in dry season rainfall and to irrigation.

The early impacts of aboveground litter removal on soil CO_2 efflux are consistent with a substantial contribution of microbial decomposition of aboveground litter (especially non-woody material) and superficial root respiration to soil CO_2 efflux. Altered nutrient availability due to litter removal was detected as increasingly reduction of nitrogen concentration in non-woody litterfall over time, consistent with the importance of litter cycling as source of nitrogen in forest ecosystems. However, net primary productivity (non-woody litterfall quantity) has not been constrained by reduced nitrogen availability so far, suggesting some capacity of trees to sustain the same levels of biomass production with reduced leaf nitrogen. Non-woody litterfall phosphorus concentration was less sensitive to chronic litter removal probably because plants compensated for removed phosphorus by accessing soil organic sources. Nitrogen oxide emissions and methane emissions were not affected during the initial period (first 18 months) of litter removal.

In general, this forest regrowth stand showed high resistance to altered nutrient availability, which may be linked to mechanisms that allow trees to mobilize nutrients (e.g., phosphorus) from soil organic sources, and to maintain productivity even under reduced litterfall nitrogen in litter removal plots. On the other hand, reduced ANPP associated with moisture availability suggests decreased potential of carbon sequestration from forest regrowth under anticipated scenarios of reduced rainfall in Amazonia. Our results may help to improve predictions of forest regrowth in the Brazilian Amazonia derived from process-based models such as CARLUC (Hirsch *et al.* 2004).

Table 7-1. Summary of ecosystem process responses to intrannual and interannual variability effects (for control plots) and resource manipulation (dry-season irrigation and litter removal) effects. Intrannual variability refers to variations associated with rainfall seasonality. The degree of resource manipulation effects is relative to control.

Process/Variable	Intrannual variability	Interannual variability	Dry-Season Irrigation	Litter Removal
Soil water availability	Yes	NA	++	o
Litterfall quantity	Yes	Yes	+	o
Litterfall nitrogen concentration	No	NA	o	--
Litterfall phosphorus concentration	Yes	NA	o	o
Leaf litter decomposition	Yes	NA	++	NA
Soil CO_2 efflux	Yes	Yes	++	--
Soil NO efflux	Yes	NA	o	o
Soil N_2O efflux	Yes	NA	+	o
Soil CH_4 efflux	Yes	NA	+	o
ANPP	NA	Yes	+	NA

Yes: presence of variability
No: absence of variability
+: slight, but significant increase
++: significant increase
--: significant decrease
o: no significant variation
NA: not available

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BIOGRAPHICAL SKETCH

Steel Silva Vasconcelos was born on August 4, 1972, in Rio de Janeiro, RJ, Brazil. He attended the University Federal Rural do Rio de Janeiro (UFRRJ) where he graduated in agronomic engineering in 1995. Continuing at the same university, Steel received a master's degree in soil science in 1997 studying the tolerance of rice plants to aluminium toxicity. After graduation, he moved with his wife Lívia and daughter Cárita to Pará, north of Brazil, where he worked for about two years on the selection of maize genotypes suitable for slash-and-mulch agriculture. In June 1999, Steel started to work as a research assistant for a forest ecology project coordinated by Dr. Daniel Zarin—the MANFLORA project based out of Castanhal, Pará. Then, in 2002, he moved to Gainesville, FL, with his family to start his Ph.D. program in the School of Forest Resources and Conservation at the University of Florida (UF) under the supervision of Dr. Zarin. His doctoral research was developed at the MANFLORA experimental site. During his Ph.D. program, Steel was hired by the Brazilian Agricultural Research Center (EMBRAPA) as a researcher to study soil-plant-water relationships at the Eastern Amazon Research Center in Belém, Pará. Upon completing his Ph.D. program, he intends to continue working with tropical forest ecology as part of his research duties at EMBRAPA.