Ecological Applications, 14(4) Supplement, 2004, pp. S150–S163

NITROGEN AND PHOSPHORUS LIMITATION OF BIOMASS GROWTH IN A TROPICAL SECONDARY FOREST

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Abstract. Understanding secondary successional processes in Amazonian terrestrial ecosystems is becoming increasingly important as continued deforestation expands the area that has become secondary forest, or at least has been through a recent phase of secondary forest growth. Most Amazonian soils are highly weathered and relatively nutrient poor, but the role of nutrients as a factor determining successional processes is unclear. Soils testing and chronosequence studies have yielded equivocal results regarding the possible role of nutrient limitation. The objective of this paper is to report the first two years' results of a nitrogen (N) and phosphorus (P) fertilization experiment in a 6-yr-old secondary forest growing on an abandoned cattle pasture on a clayey Oxisol. Growth of remnant grasses responded significantly to the N + P treatment, whereas tree biomass increased significantly following N-only and N + P treatments. The plants took up about 10% of the 50 kg P/ha of the first year's application, and recovery in soil fractions could account for the rest. The trees took up about 20% of the 100 kg N/ha of the first year's application. No changes in soil inorganic N, soil microbial biomass N, or litter decomposition rates have been observed so far, but soil faunal abundances increased in fertilized plots relative to the control in the second year of the study. A pulse of nitric oxide and nitrous oxide emissions was measured in the N-treated plots only shortly after the second year's application. Net N mineralization and net nitrification assays demonstrated strong immobilization potential, indicating that much of the N was probably retained in the large soil organic-N pool. Although P availability is low in these soils and may partially limit biomass growth, the most striking result of this study so far is the significant response of tree growth to N fertilization. Repeated fire and other losses of N from degraded pastures may render tree growth N limited in some young Amazonian forests. Changes in species composition and monitoring of long-term effects on biomass accumulation will be addressed as this experiment is continued.

Key words: Amazon Basin; arthropods; Brazil; deforestation; nitric oxide; nitrogen cycle; nitrous oxide; nutrient cycling; phosphorus cycle; soil; soil fauna; succession.

INTRODUCTION

Most of the >500 000 km² of deforested land in the Brazilian Amazon Basin has been converted to agricultural use, at least temporarily, and much is then abandoned to secondary succession. In shifting agricultural practices, a fallow phase between crop cycles is important for accumulation of nutrient stocks in regrowing forest vegetation. These nutrients accumulated by the fallow vegetation are then released to the next crop by clearing and burning. In some cases, this system of crop rotation has gone on for decades, while, in other cases, the farmers abandon the land. Cattle pastures are also often abandoned once grass productivity declines and woody invaders cannot be easily controlled, leaving these ''degraded lands'' to secondary succession. Hence, the area that has become secondary forest or at least has been through a recent phase of secondary forest growth is large and is likely to expand as deforestation continues.

A rationale based on the changes in soil chemical and physical properties during soil genesis has been used to explain N limitation of net primary productivity (NPP) on young soils, P limitation on highly weathered old soils, and colimitation of N and P on intermediate aged soils in mature tropical forests (Vitousek and Farrington 1997, Harrington et al. 2001). Weathering of rocks under young soils releases P, but the soils and ecosystems have not yet accumulated N that is derived mostly from the atmosphere. In contrast, sources of P from weathering of primary minerals are often mostly spent in old soils and much of the soil P is occluded with secondary minerals, while the soils and ecosystems have accumulated significant stocks of N. Most

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Manuscript received 1 October 2001; revised 3 July 2002; accepted 9 July 2002; final version received 2 August 2002. Corresponding Editor: A. R. Townsend. For reprints of this Special Issue, see footnote 1, p. S1.

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PLATE 1. Photographs of (left) one of the control plots and (right) one of the plots fertilized with N and P in the secondary forest in May 2002, two years after the first fertilization treatment. Note the proliferation of remnant pasture grasses in the fertilized plot. The study plots are located at Fazenda Vitoria, near Paragominas, Pará, Brazil. Photo credit: Ricardo Figueiredo.

Amazonian soils are old, highly weathered, and relatively nutrient poor, suggesting a likelihood of P limitation. Indeed, mature Amazonian terra firme forests cycle P very efficiently (Herrera et al. 1978, Vitousek 1984). The P cycle is conservative due to a combination of low P inputs from precipitation, low soil P stocks in sandy Spodosols and high capacities for P fixation in the clays of Oxisols and Ultisols. Nitrogen is abundant relative to phosphorus in most of these mature tropical forests (Vitousek 1984, Martinelli et al. 1999).

The applicability of these generalizations about nutrient limitation to NPP is not clear in secondary forests. The strong capacity of most Amazonian soils to fix P in forms that are mostly unavailable to plants suggests that P should also be limiting in cleared lands. Indeed, fertilization with P is often necessary for good pasture productivity in the eastern Amazon (Dias-Filho et al. 2001). Extractable-P indices of plant-available P are low for soils of all land uses in Amazonia, including secondary forests (McGrath et al. 2001). In addition to concerns about P, however, a large fraction of biomass N is volatilized during clearing and burning of mature forests, secondary forests, and cattle pastures (Kauffman et al. 1995, 1998, Hölscher et al. 1997), and N–P–K fertilization is common in some types of row crop agriculture. Therefore, N could also become limiting to NPP in secondary lands in Amazonia because of management practices that reduce stocks of plant-available N (McGrath et al. 2001). We hypothesized that N and/or P limitation could be important factors determining the rate of biomass accumulation, species composition, and other biogeochemical processes in Amazonian secondary forests.

The evidence for this hypothesized role of nutrients in tropical secondary succession is equivocal. Legumes are present in most Amazonian secondary forests, but they tend not to be the fastest growing early successional species, leaving open the question of the importance of biological N fixation and the possibility of N limitation to plant growth. Buschbacher et al. (1988) found no statistically significant correlations between chemical soil analyses and rates of forest regrowth after pasture abandonment on clayey Oxisols in eastern Amazonia. Instead, they attributed variability in forest regrowth rates to the intensity of previous land use that may have affected seed sources and soil physical properties. A forest chronosequence study on land abandoned from shifting agriculture in eastern Amazonia revealed no evidence of lower soil or foliar nutrient concentrations in secondary forests compared to a primary forest, except for labile P in the top 5 cm of soil, which was higher in the primary forest (Johnson et al. 2001). In contrast, Gehring et al. (1999) found species-specific responses to fertilizer treatments and an overall increase in biomass in response to N and P fertilization in young forest regrowth following slash-and-burn agriculture on sandy Ultisols of eastern Amazonia. In another secondary forest chronosequence study at five locations across the Amazon Basin, Moran et al. (2000) showed faster height growth on relatively Ca + Mg-rich Alfisols, and no significant variation among the remaining sites on Spodosols, Ultisols, and Oxisols. It is possible that other confounding factors among these regions, such as the type and intensity of previous land use, could have accounted for the taller forests observed on the Alfisol sites and/or the absence of differences among the other soil types.

Here we report on the first two years' results of a nutrient addition experiment that tests the effects of N and P additions on biomass accumulation after pasture abandonment on clayey Oxisols. The purpose is not to demonstrate an approach for remediation of degraded lands, but rather to experimentally test the role of nutrient limitation on biomass accumulation. We stratify biomass measurements by life-form and trees species because nutrient availability can also affect competi-

TABLE 1. Means (± 1 SE) of selected soil chemical and physical properties of the study area in Pará, Brazil.

Depth (cm)	pH (in H_2O)	K (mol_c/m^3)	Ca (mol_c/m^3)	Ca + Mg (mol_c/m^3)	Al (mol_c/m^3)	Bulk density (g/cm^3)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)
2.5	5.7 (± 0.06)	773 (± 60)	57 (± 5)	72 (± 5)	1 (± 0.3)	0.60 (± 0.01)	4.3	4.3	30.0	61.3
5.0	5.4 (± 0.09)	476 (± 61)	25 (± 2)	33 (± 3)	3 (± 0.5)	0.96 (± 0.06)	2.0	3.7	18.3	76.0
10.0	5.2 (± 0.10)	355 (± 43)	19 (± 2)	26 (± 2)	3 (± 0.7)	1.05 (± 0.02)	2.0	4.0	14.7	79.3
15.0	5.3 (± 0.09)	318 (± 41)	17 (± 2)	22 (± 2)	3 (± 0.8)	1.03 (± 0.04)	2.0	3.0	15.0	80.0
20.0	5.3 (± 0.10)	284 (± 38)	17 (± 2)	22 (± 2)	2 (± 0.6)	1.05 (± 0.09)	1.0	3.0	15.3	80.7

tion. Similarly, we are measuring several of the possible competing fates of applied nutrients, such as foliar uptake, microbial immobilization, soil retention, litter decomposition, and loss via trace gas emissions, to help explain the magnitude of biomass growth responses and possible cascading effects of nutrient addition in ecosystem processes. The first year's results of chemical analyses are presented. The study will be continued to determine if longer-term results will differ from the initial responses that are documented here.

MATERIALS AND METHODS

Site description

The study is being conducted at Fazenda Vitória, located 6.5 km northwest of the town of Paragominas, Pará State, Brazil, in eastern Amazonia (2°59′ S, 47°31′ W; see Plate 1), an area of extensive land-use change since the 1960s (Nepstad et al. 1991). Average annual rainfall is 1800 mm and is highly seasonal, with <250 mm falling from July to November (Jipp et al. 1998). Both mature and secondary forests remain evergreen throughout the dry season by extracting water from deep in the soil profile, where roots have been observed at 18 m depth (Nepstad et al. 1994, Jipp et al. 1998). Some early successional species are also evergreen while others shed a substantial portion of their leaves during the dry season.

Soils in the region developed on Pleistocene terraces cut into the Belterra clay and Tertiary Barreiras formations (Sombroek 1966, Clapperton 1993). These sediments consist primarily of kaolinite, quartz, and hematite and are widespread at elevations below 200 m in the Amazon basin (Clapperton 1993). The soils were classified by Sombroek (1966) as Kaolinitic Yellow Latosols (Haplustox, according to USDA Soil Taxonomy), and they contain 60–80% clay content. Pasture soil pH in water was 5.2 to 5.7 (Table 1).

The study was undertaken in a 7.25-ha area of abandoned pasture that was originally planted in 1971 with the grass *Panicum maximum* and abandoned in 1984. The pasture was grazed by cattle in a rotation regime, with 1–4 animals/ha, and was periodically burned and herbicided. Accidental fires have burned the area at least three times since abandonment, the last time being 1993. There was still some remaining grass cover at the beginning of this experiment, but herbs, shrub, and trees dominated the vegetation. Of the trees >2 cm in diameter at breast height (dbh), maximum and median heights were 7.5 m and 3.1 m, respectively; maximum and median diameters were 11.4 cm and 3.5 cm, respectively.

Fertilization experimental design

Four plots, 20 × 20 m, were established in each of three replicate blocks in November 1999, which is the end of the dry season. The vegetation was inventoried at that time. The four plots within each block received the following treatments at the beginning of the rainy season in January 2000 and again in February 2001: (1) control without fertilization, (2) 100 kg N/ha as urea, (3) 50 kg P/ha as simple superphosphate, and (4) N + P at these same rates.

Species identification and biomass measurements

All individual trees in each plot with dbh ≥ 2cm were identified taxonomically and were measured for height and dbh. Foliar and woody biomass were estimated from the allometric equations developed by Uhl et al. (1988) at this same ranch:

$$\ln(\text{woody biomass}) = -2.17 + 1.02\ln(\text{diameter}^2) - 0.39\ln(\text{height})$$

$$\ln(\text{foliar biomass}) = -0.66 + 1.43\ln(\text{diameter}^2) - 2.10\ln(\text{height})$$

where diameter is measured in centimeters and height in meters.

The biomass of plants <2 cm dbh was measured destructively in two miniplots (2 × 1 m) in each plot. All aboveground plant parts were harvested and were separated by life-form (grass, herb, shrub, tree, and dead material). Samples were dried at 60°C before weighing.

Soil physical and chemical properties

Selected physical and chemical properties of the soil were determined prior to the experiment to help characterize the site (Table 1). In each block, four sets of soil samples were removed from areas outside the plots for initial characterization of soil bulk density and texture. A bulk-density corer, with 5 cm diameter stainless steel rings as an inner sleeve, was manually inserted into the soil to collect samples from the following depth intervals (cm): 0–2.5, 2.5–5, 5–10, 10–15, and 15–20. After a core from a depth interval was removed, the surrounding soil was excavated to the same depth to prevent contamination of the sample to be taken from the next depth interval. Four samples from each block were then composited (keeping the depth intervals separate) and air dried. Soil texture was determined by the pipette method (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA] 1997). For soil chemical properties, three samples were collected from within each plot before fertilization for the same depth increments listed above. A composite sample was made for each depth and each plot and then air dried. Soil pH was measured in a 1:2.5 water slurry. Total N concentrations of 0.2-g subsamples were determined by micro-Kjeldahl digestions (Bremner and Mulvaney 1982). An index of plant-available P was measured using the Mehlich-I dilute double acid extraction procedure (Amacher 1996), using 2 g dry soil in 20 mL of acid (EMBRAPA 1997). Total P concentrations were determined by digestion of 0.2 g subsamples in sulfuric acid and peroxide, and concentrations of both available P and total P extracts were determined colorimetrically (Murphy and Riley 1962). Exchangeable K^+ was extracted in 0.05 N HCl and quantified by flame photometry (EMBRAPA 1997). Exchangeable Al and Ca^{++} were determined by extraction in 1 mol/L KCl and analyzed by atomic absorption spectrophotometry (EMBRAPA 1997). Exchangeable Ca + Mg was measured in these extracts by titration with 0.0125 mol/L EDTA in the presence of Eriochrom Black (EMBRAPA 1997).

Three samples of the 0–20 cm depth increment were also collected from each plot in January, February, and May 2000 for analysis of extractable NH_4^+ and NO_3^- and for assays of net N mineralization and net nitrification (May samples only). These samples were transported to the laboratory on ice and were stored at 4°C until analyzed. Subsamples were passed through a 2-mm sieve, extracted in 2 mol/L KCl, and then filtered through Whatman No. 42 filter paper. Net N mineralization and net nitrification were estimated from changes in NH_4^+ and NO_3^- concentrations during 7-d aerobic incubations at 27°C of 20-g subsamples (Hart et al. 1994). All extracts were stored frozen until analyzed. Ammonium concentrations were determined colorimetrically by the salicylate/nitroprusside method (Mulvaney 1996). Nitrate was reduced to nitrite using a nitrate reductase kit (Nitrate Elimination Company, Lake Linden, Michigan, USA), and nitrite was analyzed colorimetrically by the Griess-Illosvay procedure, using sulfanilamide and N-(1-napthyl)ethylenediamine (Snell and Snell 1949).

Soil microbial biomass and activity

Three additional soil samples were collected from each plot and from each of the five depth increments for determination of microbial biomass C and N. Samples were transported on ice and refrigerated for at most one week before analysis. Samples were passed through a 2-mm sieve and allowed to stand at ambient temperature for 24 h prior to analysis. Chloroform fumigation (72 h) and direct extraction (agitation at 180 rpm on a benchtop shaker in 0.5 mol/L K_2SO_4, followed by filtration through Whatman No. 42 paper) was performed on 20-g subsamples (Vance et al. 1987). Unfumigated subsamples were similarly extracted. Gravimetric water content was determined by drying other subsamples at 105°C for 24 h so that results could be expressed on a dry mass basis. The C present in fumigated and unfumigated extracts was determined colorimetrically by adaptation of a method described by Islam and Weil (1998). Aliquots of 2 mL were mixed with 0.75 mL of 0.17 mol/L $K_2Cr_2O_7$ and 2 mL H_2SO_4 in a 75 mL Pyrex digestion tube. The covered tubes were manually agitated and then heated to 150°C for exactly 10 min with glass beads. The samples were allowed to cool, 10 mL distilled water was added, and then the samples were analyzed spectrophotometrically at 590 nm, comparing concentrations to a standard curve with samples containing 0–2 mg C as sucrose dissolved in 0.5 mol/L K_2SO_4. Microbial biomass C was estimated from the chloroform-labile C, using a K_{ec} factor of 0.35 (Voroney et al. 1991). Organic N in the extract was determined by micro-Kjeldahl procedure, followed by titration with 0.025 mol/L H_2SO_4. Microbial biomass N was estimated from the chloroform-labile N, using a k_N factor of 0.68 (Brookes et al. 1985).

Foliar nutrient concentrations

Samples of foliar tissues were collected in May 2000, at the end of the first post-fertilization growing season, from the following species: *Panicum pilosum* (grass), *Borreria verticilata* (herb), *Desmodium canum* (herb), *Rolandra argentea* (herb), *Cordia multispicata* (shrub), *Rollinia exsucca* (tree), and *Zanthoxyllum rhoifolium* (tree). Two or three individuals (depending on their presence) of each species were sampled within each of the 12 plots. Fully expanded leaves were collected from the middle part of the plant in the case of herbs and from the middle part of the canopy in the case of bushes and trees. Because the canopy is not yet closed in this young forest, most of the leaves, including those that we sampled, are frequently exposed to full sunlight. Tissues were dried at 70°C for 48 h and ground in a Wiley mill (40 mesh). Nitrogen concentrations of 0.1-g subsamples were determined by micro-Kjeldahl digestions (Bremner and Mulvaney 1982). Phosphorus concentrations of 0.2-g subsamples were determined by digestion in sulfuric acid and peroxide (Murphy and Riley 1962). Reference samples (from the National Institute of Standards and Technology, Standard Reference Materials, Gaithersburg, Maryland, USA) of peach leaves (NIST 1547) and pine needles (NIST 1575) were analyzed at the same time by the same methods.

Leaf area index

Leaf area index (LAI) was measured at 12 points arranged in a grid within each plot using a Li-Cor LI-2000 instrument (Li-Cor, Lincoln, Nebraska, USA). Measurements were made at a height of 60 cm above the soil surface either between 0600 and 0900 hours or between 1600 and 1800 hours. Measurements were repeated in May and December 2000 to obtain estimates at the end of the wet and dry seasons, respectively.

Soil trace gas emissions

Chamber measurements have been explained in detail for N_2O and NO (Verchot et al. 1999), CH_4 (Verchot et al. 2000), and CO_2 (Davidson et al. 2000). Briefly, N_2O and CH_4 fluxes were measured in vented static chambers placed over the soil, with syringe samples of headspace gas collected at 0, 10, 20, and 30 min after placement of the chamber over the soil. The syringe samples were analyzed by gas chromatography within 48 h of sampling. Fluxes of NO and CO_2 were measured in the field with battery-operated Scintrex LMA-3 (Scintrex Limited, Concord, Ontario, Canada) and Li-Cor 6252 analyzers, respectively. Air was circulated between the vented chamber headspace and the gas analyzers at 0.5 L/min for about 5 min. For all gases, fluxes were calculated from linear regressions of the concentration increase, chamber height, and air temperature and pressure.

Three chamber bases made of PVC pipe were placed in each of the 12 plots in November 1999. Fluxes were measured in November 1999 and twice in January 2000, about one week before and one week after the fertilization application. Measurements were repeated in April, September, and December 2000, and a similar schedule was followed in 2001.

Litter decomposition and soil faunal activity

Decomposition of litter was measured in large mesh (1 cm) and small mesh (1 mm) nylon mesh bags (20 × 20 cm). The small mesh excludes macrofauna (body size > 2 mm). Each nylon bag was filled with 10 g of a mixture of air-dried leaves of seven plant species (*R. exsucca*, *Z. rhoifolium*, *Vismia guianensis*, *Banara guianensis*, *Metrodorea flavida*, *Cordia multispicata*, and *Rolandra argentea* sampled from the area outside our study plots prior to fertilization. A total of 12 litterbags (six for each mesh size) were distributed in each of the 12 plots in April 2000. After 60, 180, 380, and 440 d, one litterbag of each mesh size was removed from each plot and brought to the laboratory. The litter was removed from bags and dried at 60°C for 72 h and weighed.

Soil fauna were collected in nine pitfall traps (Delabie et al. 2000) in each plot, placed at 0.5-m intervals along a transect. Each trap consisted of a glass receptacle (3.5 cm diameter) with liquid preservative (70% alcohol) placed with its top at the soil level. The pitfall traps were left in the field for 7 d. Ant specimens were initially identified by genus and later by morphospecies. Other arthropods were identified by order using taxonomic keys.

Statistical analyses

The assumption of normality was tested for all datasets using the one-sample Kolmogorov-Smirnov test offered in Systat statistical software (Systat, Richmond, California, USA). Logarithmic transformations were necessary for data on grass biomass, soil P concentrations, and NO emissions. Changes in biomass were analyzed both by repeated measures design, using data from all three sampling dates (1999, 2000, 2001), and by one-way ANOVA of the difference in biomass between the 1999 and 2001 dates. The one-way ANOVA permitted testing of block effects, which were not significant, and pairwise comparisons of fertilizer treatments. Because the trends for growth observed between 1999 and 2000 were very similar to those observed between 2000 and 2001, these two approaches yielded nearly identical statistical results. Results from only the one-way ANOVA are presented here. In the case of trees with diameter >2 cm, the initial November 1999 biomass of each plot was found to be highly significant as a covariate in the ANOVA. In other words, plots that started with higher tree biomass accumulated additional biomass faster than those that started with less tree biomass, and this source of variation had to be partitioned in the ANOVA in order to reveal fertilizer treatment effects. Foliar nutrient concentrations were tested with a two-way ANOVA, using fertilizer treatment and species as main effects.

RESULTS

Biomass accumulation

The average total aboveground biomass for all plots in November 1999, prior to fertilization, was 13.4 Mg/ha, with 5% as grass, 5% vines, 15% herbs, 7% woody plants <2 cm diameter, 7% dead material, 36% as wood in trees >2 cm diameter, and 25% as foliage of trees >2 cm diameter. The fertilization treatments were then applied early in the rainy season. Total biomass increased in all plots by the end of the rainy season in June 2000 and continued to grow in 2001 (Fig. 1). Although there was a trend of enhanced grass growth in the P-only plot, the difference was not statistically significant. Grass biomass increased significantly only in the N + P treatment. Proliferation of remnant grasses in this treatment may have shaded herbs, causing a significant decrease in herb biomass in that treatment (Table 2; also see Plate 1). Few dif-

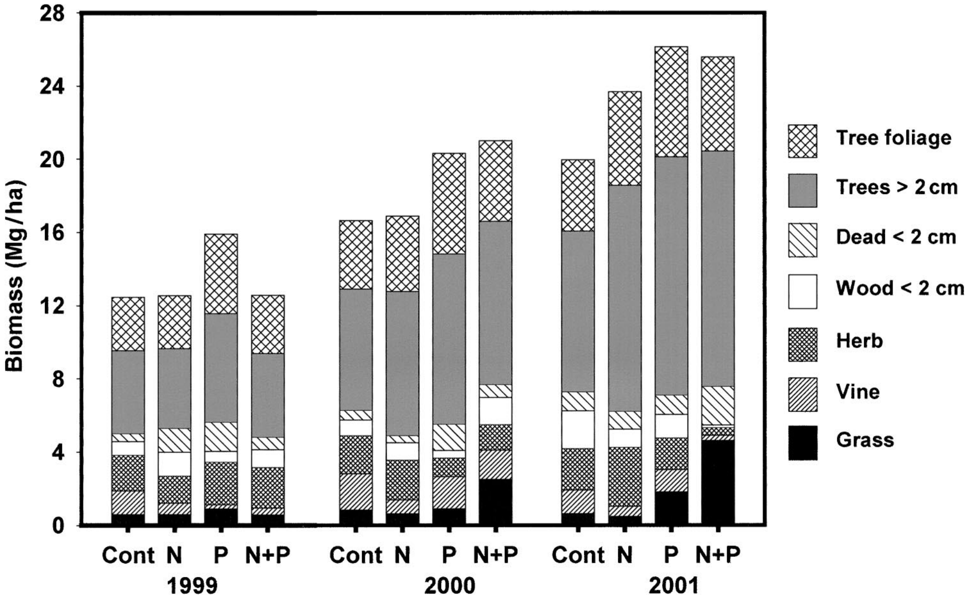

FIG. 1. Mean biomass by life-form for each fertilizer treatment measured in three successive years. Standard errors of the means of growth rates and statistical tests are given in Tables 2 and 3.

ferences among treatments were found for the other non-tree life-forms.

In contrast to the grasses, woody tree biomass increased in all plots, but grew faster in the N-only and N + P treatments (Table 2). The arithmetic means by treatment are shown in Table 2, but the least-square means shown in Table 3, which are adjusted for the highly significant covariance of pretreatment 1999 biomass with biomass growth ($P < 0.01$), show that the responses of tree biomass accumulation to N-only and N + P were similar and significantly larger than the response to P only. The adjusted least-square means of tree biomass accumulation were 4.6, 8.6, 5.7, and 8.6 Mg/ha for control, N, P, and N + P treatments, respectively, with a mean-square error of 1.8.

The number of new individuals recruited into the >2 cm diameter class during the two-year study period was 54, 66, 51, and 75 for the control, N, P, and N + P treatments, respectively. Hence, the growth of small trees appears to follow the same pattern of a positive response to N addition as was measured for total woody biomass. Mortality during the study period was 22, 6, 12, and 8 individuals for the control, N, P, and N + P treatments, respectively. This result suggests that nutrient addition may have reduced mortality, but this sample size is probably too small to make strong inferences in this regard.

A total of 588 individual trees (>2 cm diameter) from 39 species were identified and measured in the 12 plots in November 1999. *R. exsucca* made up 63% of the tree biomass, *Z. rhoifolium* made up 14%, and the other 24% was made up of the remaining 37 tree species. These three groups accounted for 60%, 17%, and 22% of the tree woody biomass accumulation, respectively. The least-square means of the ANOVAs for these two most abundant species indicate that *R. exsuc-*

TABLE 2. Change in biomass (Mg/ha) during the two growing seasons between November 1999 (prefertilization) and June 2001, Fazenda Vitória, Paragominas, Pará.

Treatment	Grasses	Vines	Herbs	Bushes	Dead	Trees > 2 cm		All biomass
						Wood	Foliage	
Control	$0.0^{a} \pm 0.4$	0.0 ± 1.0	$0.3^{ab} \pm 0.0$	1.3 ± 0.6	$0.6^{ab} \pm 0.3$	$4.2^{a} \pm 2.5$	1.0 ± 0.5	7.5 ± 3.0
N	$-0.1^{a} \pm 0.4$	0.0 ± 0.4	$1.7^{a} \pm 0.4$	-0.3 ± 1.2	$-0.3^{a} \pm 0.3$	$8.0^{b} \pm 1.9$	2.2 ± 0.2	11.1 ± 1.2
P	$0.9^{a} \pm 0.6$	1.0 ± 0.7	$-0.6^{bc} \pm 0.5$	0.7 ± 0.9	$-0.5^{a} \pm 0.4$	$7.1^{a} \pm 2.2$	1.7 ± 0.6	10.2 ± 2.7
N + P	$4.1^{b} \pm 0.7$	-0.1 ± 0.0	$-1.8^{c} \pm 1.1$	-0.9 ± 0.0	$1.4^{b} \pm 0.6$	$8.3^{b} \pm 2.9$	2.0 ± 0.2	13.0 ± 3.2

Notes: Means (± 1 SE) are given for the three plots per fertilizer treatment. For those life-forms where the fertilizer treatment effect was significant in a one-way ANOVA ($P < 0.05$), lowercase letters indicate pairwise comparisons by Fisher's protected least significant difference ($P < 0.05$). For life-forms where no letters are shown, the fertilizer treatment effect was not significant. In the case of woody biomass of trees > 2 cm dbh, the initial biomass measured in 1999 was a significant covariate in the ANOVA (see *Discussion*), so the adjusted least-square means (see Table 3) are used for the pairwise comparisons of fertilizer treatments.

TABLE 3. Least-square means and mean-square errors from one-way ANOVAs of changes in tree woody biomass (Mg/ha) between November 1999 (pre-fertilization) and June 2001 for each of the two most abundant tree species and for all tree species combined.

Fertilizer treatment	*Rollinia exsucca*	*Zanthoxyllum rhoifolium*	All species
Control	2.3^{a}	1.7	4.6^{a}
N	5.2^{b}	1.0	8.6^{b}
P	4.1^{ab}	1.1	5.7^{a}
N + P	4.9^{b}	1.6	8.6^{b}
MSE	1.1	0.4	1.8

Notes: Initial biomass was a significant covariate in these ANOVAs. The fertilizer treatment effect was significant ($P < 0.05$) for *Rollinia exsucca* and for all species combined. Means followed by the same letter are not significantly different ($P < 0.05$) in a post hoc comparison using Fisher's protected least significant difference.

ca showed a strong response to N-only and N + P; but that fertilizer treatments did not affect growth of the less abundant *Z. rhoifolium* (Table 3).

Foliar N and P concentrations

Phosphorus fertilization (P and N + P treatments) increased the foliar P concentrations of the grass species, two herb species, and the shrub species selected for foliar analyses (Table 4). Foliar P of the grass species doubled or tripled in response to P fertilization, and grass was also the only non-woody life-form that showed greater biomass growth with N + P fertilization (Table 2). The foliar P concentration of one tree species, *R. exsucca*, did not appear to respond to P fertilization, whereas the P concentration of another tree species, *Z. rhoifolium*, did increase with P and N + P fertilization (Table 4). Although growth of these two tree species responded differently to N-fertilizer treatment (Table 3), they both showed clear increases in foliar N concentration in response to the N and N + P treatments (Table 5). One herb species (*Rolandra argentea*) showed a similar foliar N response (Table 5). The shrub species, the other two herb species, and the grass species did not show clear differences in foliar N concentration among treatments, which is consistent with the lack of response of biomass accumulation to N fertilization for these life-forms (Table 2).

Leaf area index

Leaf area index (LAI) was significantly higher in the N + P treatment than in the other treatments at both the May 2000 (end of rainy season) and December 2000 (end of dry season) measurement dates (Fig. 2). Higher LAI in the N + P treatment is consistent with a large increase in grass growth in the N + P plots (Table 2). Grass comprised about 20% of the total biomass in the N + P plots despite its lack of woody tissue, and it probably contributes a larger fraction of the LAI. The LAI decreased by 25–50% during the dry season of 2000 (Fig. 1), indicating leaf shedding and senescence. Nevertheless, the higher LAI in the N + P plots compared to other treatments persisted through the dry season of 2000.

Soil physical and chemical properties

Most of the fertilizer P was recovered in either Mehlich-I extractions or total-P analyses of soil samples collected in May 2000 (Table 6). The P-only and N + P plots had significantly elevated P concentrations relative to the soils of the control and N-only fertilized plots. One of the N + P plots had particularly high concentrations, causing large standard errors about that treatment's means. The mean P concentrations in Table 6 were converted to P stocks using measured bulk density values (Table 1). Stocks of P were then summed for the top 20 cm of soil (rightmost column, Table 6). Subtracting the average soil P stocks for the control and N-only plots from the average P stocks for the P and N + P treatments (Table 6), we estimate that 18 and 62 kg P/ha of the first year's P application was recovered in the Mehlich-I and total-P pools, respectively, in the plots that received P fertilizer. This is obviously an overestimate of recovery in the total P pool, because the target application rate was 50 kg $\mathrm{P \cdot ha^{-1} \cdot yr^{-1}}$, but this difference is within the expected

TABLE 4. Foliar phosphorus concentrations (g/kg) in May 2000 (4 mo post-fertilization), Fazenda Vitória, Paragominas, Pará.

Species	Control	N	P	N + P	Species means
Panicum pilosum (grass)	0.79	0.74	1.88	1.67	1.27^{a}
Borreria verticilata (herb)	2.18	1.80	3.10	2.00	2.27^{b}
Desmodium canum (herb)	1.23	1.18	1.40	1.57	1.35^{a}
Rolandra argentea (herb)	1.60	1.51	2.29	2.40	1.95^{b}
Cordia multispicata (shrub)	1.82	1.41	3.16	2.47	2.21^{b}
Rollinia exsucca (tree)	1.03	1.12	1.14	1.02	1.08^{a}
Zanthoxyllum rhoifolium (tree)	1.15	1.21	1.52	1.51	1.35^{a}
Treatment means	1.40^{A}	1.28^{A}	2.07^{B}	1.81^{B}	

Notes: Means of three samples (one per replicate plot) are presented for each fertilizer treatment. Pairwise statistical comparisons (Fisher's least significant difference; $P < 0.05$) are given for means for treatment (uppercase letters) and species (lowercase letters) main effects. The R^2 for the model was 0.65.

TABLE 5. Foliar nitrogen concentrations (g/kg) in May 2000 (4 mo post-fertilization), Fazenda Vitória, Paragominas, Pará.

Species	Control	N	P	N + P	Species means
Panicum pilosum (grass)	8.5	8.0	9.0	9.7	8.8^{a}
Borreria verticilata (herb)	19.6	17.5	17.3	16.7	17.8^{d}
Desmodium canum (herb)	15.1	16.9	17.1	16.9	16.5^{cd}
Rolandra argentea (herb)	14.3	18.0	13.1	17.6	15.8^{c}
Cordia multispicata (shrub)	17.7	17.2	12.7	18.3	16.5^{cd}
Rollinia exsucca (tree)	12.9	14.9	12.4	13.2	13.3^{b}
Zanthoxyllum rhoifolium (tree)	15.0	20.3	13.5	16.8	16.4^{cd}
Treatment means	14.7^{AB}	16.1^{C}	13.6^{A}	15.6^{BC}	

Notes: Means of three samples (one per replicate plot) are presented for each treatment. Pairwise statistical comparisons (Fisher's least significant difference; $P < 0.05$) are given for means for treatment (uppercase letters) and species (lowercase letters) main effects. The R^2 for the model was 0.76.

margin of error caused by spatial heterogeneity. Most importantly, these data demonstrate that these soils have strong P retention capacity.

Soil N stocks in the top 20 cm of soil are on the order of 4000 kg N/ha, and no effect of application of 100 kg N/ha on soil N stocks was expected or observed. Inorganic-N concentrations are about 100 times lower than total N concentrations, however, and so effects of fertilizer-N on soil ammonium and nitrate might have been observed. Although the dynamic inorganic-N concentrations changed rapidly from one sampling date to the next, there was surprisingly no observable fertilizer treatment effect (Table 7).

Soil microbial biomass and activity

Microbial biomass C and N measured at the end of the wet season in May 2000 showed no statistically significant differences among treatments for any soil depth increment. Mean microbial biomass across treatments ranged from 700 to 1500 mg C/kg dry soil and 70 to 80 mg N/kg dry soil in the top 2.5 cm soil, and from 280 to 460 mg C/kg dry soil and 50 to 60 mg N/kg dry soil in the 15–20 cm depth interval.

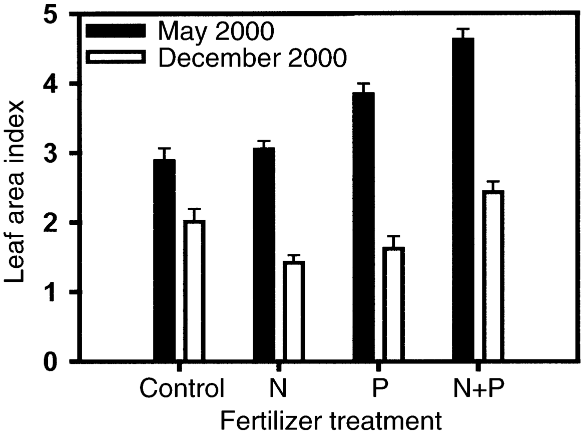

FIG. 2. Effects of fertilizer treatment on leaf area index (LAI) measured in May and December 2000, which is the end of the rainy and dry seasons, respectively. For each date, pairwise comparisons using Fisher's least significant difference shows that the LAI value of the N + P treatment is significantly different ($P < 0.01$) from the LAI of the other treatments.

Soils from all treatments in May 2000 showed negative values for mean net N mineralization and net nitrification (Table 8), indicating net immobilization of both ammonium and nitrate. Although there is a trend for higher immobilization rates in the N and N + P plots, the treatment effect was not statistically significant in a one-way ANOVA.

Soil trace gas emissions

The only significant effect of fertilizer treatment on emissions of soil gases was observed on 23 February 2001, two days after the second fertilization application, when NO and N_2O emissions increased in the N-only and N + P plots (Fig. 3). We do not know why a clear response to N fertilization did not occur in January 2000, although the measurements were made 6 d after fertilization in January 2000 instead of 2 d post-fertilization in 2001. The pulse of NO and N_2O emissions may be very short-lived if the microbial and plant sinks for immobilization of added N are strong, as appears to be the case. Consistent with previous studies of pastures and forests on this ranch, emissions of CO_2 and N_2O were highest during the wet season (January through May) and lowest during the dry season (July through December). Negative CH_4 fluxes indicate net consumption of atmospheric CH_4 by methanotrophic soil bacteria, mostly during the dry season, whereas net production of CH_4 (positive fluxes in Fig. 3) by methanogenic bacteria in wet soil sometimes occurs during the rainy season (Verchot et al. 2000). Fluxes of CH_4 were spatially highly variable, and their apparent increase in the P-amended plot in 2001 is not statistically significant.

Litter decomposition and soil faunal activity

No significant differences in arthropod abundances were observed among treatments in 2000 ($P = 0.05$),

TABLE 6. Soil Mehlich-I-extractable phosphorus and total phosphorus measured in May 2000 (post-fertilization) by soil depth increment.

	Mehlich-I phosphorus (mg P/kg dry soil)				Total phosphorus (mg P/kg dry soil)			
Soil depth (cm)	Control	N	P	N + P	Control	N	P	N + P
0–2.5	3.5 ± 0.5	3.2 ± 0.2	28.2 ± 7.9	26.3 ± 18.6	257 ± 21	250 ± 9	396 ± 28	349 ± 72
2.5–5	2.9 ± 0.4	3.1 ± 0.4	8.5 ± 1.6	17.2 ± 12.6	213 ± 16	222 ± 30	239 ± 14	272 ± 47
5–10	2.8 ± 0.4	3.0 ± 0.2	6.5 ± 1.0	22.5 ± 18.1	206 ± 15	224 ± 27	231 ± 22	259 ± 53
10–15	2.8 ± 0.4	2.5 ± 0.2	4.4 ± 0.6	13.7 ± 10.3	178 ± 7	217 ± 37	209 ± 6	219 ± 33
15–20	2.1 ± 0.3	2.4 ± 0.3	4.0 ± 0.7	10.1 ± 7.2	144 ± 17	198 ± 17	197 ± 17	188 ± 26
P stocks (0–20 cm, kg P/ha)	5.2 ± 0.6	5.4 ± 0.4	14.1 ± 2.0	32.4 ± 20.0	367 ± 19	426 ± 34	451 ± 13	467 ± 64

Notes: Means ± 1 SE of three plots per fertilizer treatment are presented. Fertilizer treatment and depth were significant ($P < 0.05$) in analyses of covariance of log-transformed data. The R^2 values for Mehlich-I and total-P models were both 0.54. P stocks were calculated using bulk density measurements.

although there was a nonsignificant trend of lower abundances for several taxa and for the total number of arthropods (excluding ants) in the control plots (Table 9). This trend became statistically significant in 2001, with more than twice as many arthropod individuals encountered in P and N + P plots than in control plots. Counts were generally lower in 2001 than 2000, indicating some interannual variation, but more data will be required to detect any sort of a trend with succession. Because ants are social insects, numbers of individuals found in traps is less meaningful than the number of species. The mean number of ant species encountered ranged from eight to 14, and there were no significant differences among treatments.

The litterbag studies conducted during the first two years of this experiment utilized foliage collected prior to fertilization. Hence, the results test whether the fertilizer treatments affected decomposition of existing litter, which they apparently did not. No significant treatment effects on litter decomposition rates were observed (Fig. 4). Nor was there a significant effect of mesh size on decomposition. About 40–65% of the litter mass was lost during the first 60 d. In most cases, 70–85% of the mass had been lost after 440 d, although variability among individual bags was large, probably due to the large variation in soil surface microclimate in this young secondary forest.

New foliage formed after fertilization has since been collected from each plot for new litterbags for additional decomposition studies, which will address the question of whether changes in foliar tissues caused by the fertilization treatments affect decomposition rates. Likewise, the changes in faunal abundances observed in pitfall traps in the second year may affect decomposition in large-meshed litterbags in subsequent years.

DISCUSSION

Plant responses

This experiment is being conducted on an abandoned cattle pasture that received moderate to heavy use. This same site was the subject of a study of initial secondary succession following the last fire in 1993 (Vieira et al. 1994). At that time, growth of tree species was associated with presence of the shrub, *Cordia multispicata* (Boraginaceae), which apparently provides a favorable microclimate for germination. Many of the species now found on the site, such as *R. exsucca*, *Banara guianensis*, *Casearia grandiflora*, *Solanum crinitum*, *Vismia guianensis*, *Cecropia palmata*, and *Z. rhoifolium*, first appeared under canopies of *C. multispicata*, which has since decreased in importance. *R. exsucca* and *Z. rhoifolium*, which are now the most dominant species, have small seeds that are often carried into abandoned pastures by small (<50 g) fruit-eating birds (Silva et al. 1996). This type of seed dispersion is common and important in many degraded lands, where seed banks and live root stocks are depleted.

Total aboveground biomass in 1999 was 13.4 Mg/ha, yielding an average annual accumulation rate of ~2.2 Mg/ha since the last fire in 1993. This rate of biomass accumulation is intermediate between the 0.6

TABLE 7. Extractable inorganic N (2 mol/L KCl; mean ± 1 SE) in the top 20 cm of soil pre- and post-fertilization in 2000, in a pasture at Fazenda Vitória, Paragominas, Pará, Brazil.

	Ammonium (mg N/kg)			Nitrate (mg N/kg)		
Treatment	Jan (pre-fertilization)	Feb (post-fertilization)	May (post-fertilization)	Jan (pre-fertilization)	Feb (post-fertilization)	May (post-fertilization)
Control	18.4 ± 1.4	7.3 ± 0.8	15.7 ± 1.7	0.1 ± 0.0	0.6 ± 0.1	3.8 ± 0.7
N	20.1 ± 2.5	7.1 ± 0.7	13.9 ± 1.8	0.1 ± 0.0	0.5 ± 0.0	4.0 ± 2.3
P	20.6 ± 2.7	6.7 ± 1.3	11.0 ± 1.9	0.1 ± 0.0	0.7 ± 0.1	2.5 ± 0.5
N + P	15.7 ± 2.1	6.1 ± 1.5	13.5 ± 6.1	0.1 ± 0.0	0.7 ± 0.1	5.5 ± 1.7

TABLE 8. Net N mineralization and net nitrification (mg N·kg^{-1} dry soil·7 d^{-1}) measured in aerobic laboratory incubations of soils sampled in May 2000.

Treatment	Net mineralization	Net nitrification
Control	-4.4 ± 0.6	-1.5 ± 0.9
N	-6.2 ± 2.7	-2.1 ± 2.6
P	-2.3 ± 1.3	-0.4 ± 0.7
N + P	-8.7 ± 2.4	-2.7 ± 1.5

Notes: Means ± 1 SE are reported for six incubation samples per treatment. Negative values indicate net N immobilization and net nitrate consumption.

and 5.0 Mg·ha^{-1}·yr^{-1} rates reported by Uhl et al. (1988) for secondary forests in the Paragominas area, including this same ranch, that had received heavy and moderate prior land use, respectively. During the growing seasons of 2000 and 2001, we measured increases of about 2.8 Mg·ha^{-1}·yr^{-1} tree biomass (>2 cm diameter, foliage + wood) and ~3.8 Mg·ha^{-1}·yr^{-1} of total biomass in the control treatment. The rates of tree biomass accumulation nearly doubled in the N-only and N + P treatments. Surprisingly, P fertilization caused only a small and not statistically significant increase in tree growth compared to the control treatment (Table 3).

In a study of secondary vegetation regrowing after slash and burn agriculture on sandy soils, Gehring et al. (1999) found no increased biomass accumulation one year after a single fertilizer application (60 kg N and 26 kg P/ha plus other nutrients), but found a significant increase after the dose was doubled in the second year. Our study used larger doses and exhibited increased growth rates by the end of the first rainy season.

The grasses and herbs and some shrubs had higher foliar P concentrations after P and N + P treatments, and the grasses grew better in the N + P treatment. The rapidly growing grasses may shade the herbs and other small statured vegetation, precluding the latter's effective use of added P nutrition. Gehring et al. (1999) and Uhl (1987) also observed a significant grass growth response by fallow vegetation to fertilizers. The observed response of grasses to P fertilization is consistent with ranching practices in the area, where only P fertilization (usually 50 kg P/ha) is commonly applied once every 5–10 yr to active pastures. However, significant grass growth only in the N + P treatment of this experiment suggests that N may also be colimiting for grasses in the soils of this degraded abandoned pasture. Oliveira et al. (2001) also reported growth responses of grasses (*Brachiaria* spp.) to N + P fertilization in degraded cattle pastures of the Cerrado region of Brazil, with N being the most important limiting nutrient.

The two most common tree species had elevated foliar N concentrations in response to the N and N + P treatments. However *R. exsucca* grew better in response to these N additions, whereas *Z. rhoifolium* exhibited the greatest increase in foliar N concentrations but no growth response. This result contrasts somewhat from those of Gehring et al. (1999), who found that *R. exsucca* responded to a full complement of fertilizer nutrients (N, P, K, Ca, Mg, S, and micronutrients) and responded almost as well when N or P was left out of the complement. Gehring et al. (1999) also found increased foliar concentrations a more consistent response to fertilization than increased growth.

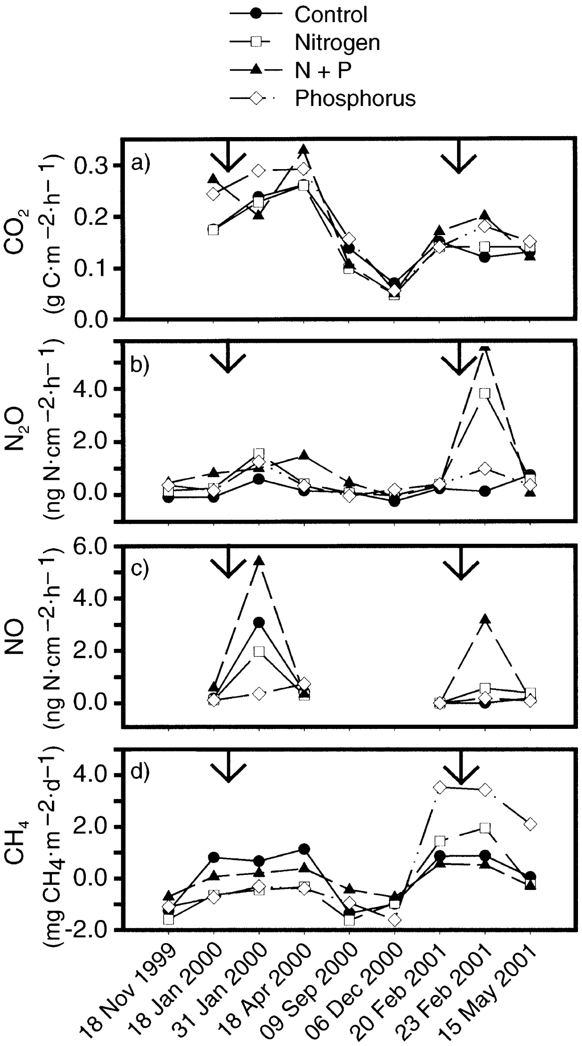

FIG. 3. Soil emissions of CO_2, N_2O, NO, and CH_4 for each fertilizer treatment. Arrows indicate dates of fertilization. Each point is a mean of nine chamber flux measurements per treatment and date. The large standard errors of the means are not included as error bars in these plots for the sake of visual clarity. The only date when fluxes were statistically significantly different among treatments ($P < 0.05$) was 23 February 2001, when NO and N_2O emissions were greater in the plots that received N and N + P compared to the control. Equipment failure caused missing data for NO measurements on three dates.

The trees were an important fate of ~20% or more of the fertilizer N. Foliar N stocks were calculated by

TABLE 9. Mean (± 1 SE) number of arthropods and ants collected in pitfall traps distributed in three plots ($n = 3$) per each fertilization treatment in April 2000 and April 2001.

Arthropods	April 2000 N	April 2000 P	April 2000 N + P	April 2000 Control	April 2001 N	April 2001 P	April 2001 N + P	April 2001 Control
Order								
Aranae	63 ± 22	54 ± 14	79 ± 20	30 ± 5	25 ± 8	21 ± 5	23 ± 5	21 ± 1
Blattodea	36 ± 12	25 ± 9	16 ± 9	13 ± 9	0	1 ± 1	2 ± 0	0
Colembola	185 ± 74	205 ± 70	498 ± 49	188 ± 93	42 ± 24	94 ± 19	90 ± 13	28 ± 9
Coleoptera	191 ± 22	209 ± 64	203 ± 49	135 ± 59	94 ± 12	123 ± 13	120 ± 25	40 ± 12
Dermaptera	3.7 ± 0.9	2.7 ± 1.2	5.7 ± 0.7	1.3 ± 0.3	0	0	0	0.3 ± 0.3
Diptera	25 ± 3	35 ± 8	44 ± 1	28 ± 11	14 ± 5	23 ± 6	23 ± 2	9 ± 3
Hemiptera	1.7 ± 0.9	0	0.3 ± 0.3	0.7 ± 0.7	0	0	0	0
Hymenoptera (except ants)	0.3 ± 0.3	0	0.3 ± 0.3	0.3 ± 0.2	0	0	0	0.3 ± 0.3
Homoptera	1.7 ± 0.9	3.0 ± 1.5	1.7 ± 1.7	0.3 ± 0.3	0.7 ± 0.3	1.6 ± 0.9	1 ± 0	0
Lepidoptera	0	0	0	0	0.7 ± 0.3	0	0	0
Orthoptera	12 ± 4	12 ± 4	12 ± 5	9 ± 2	7 ± 1	10 ± 2	9 ± 1	5 ± 2
Protura	0	0	0	0	0	0.7 ± 0.7	0.7 ± 0.3	0
Thysanura	0	0.7 ± 0.3	1.0 ± 0.6	0	0	0	0	0
Not identified	6.3 ± 3.3	8.0 ± 1.5	8.0 ± 2.6	5.3 ± 2.8	8.0 ± 3.5	5.0 ± 1.5	7.3 ± 0.9	1.0 ± 0.6
All arthropods	542 ± 137	562 ± 269	878 ± 132	418 ± 240	195 ± 22	284 ± 38	284 ± 27	107 ± 22
Ants								
No. species/plot	13 ± 2	13 ± 2	10 ± 2	14 ± 1	9 ± 1	8 ± 2	11 ± 1	9 ± 2
No. individuals/plot	37 ± 2	32 ± 5	22 ± 4	30 ± 3	21 ± 4	17 ± 2	22 ± 2	22 ± 4
Total no. ant species collected	24	21	19	25	16	17	21	18

multiplying measured foliar N concentrations by estimated foliar biomass (from allometry). Foliar concentrations were measured for the two most important species, *R. exsucca* and *Z. rhoifolium*, and the mean of these two species' foliar N concentrations was used for the ''other'' category. Due to tree growth in the control plot during the 2000 rainy season, we calculate that tree foliar N increased from 39 to 50 kg N/ha. A similar increase of about 11 kg tree foliar N/ha was observed in the P treatment, due primarily to growth and not to changes in N concentration. In the N and N + P treatment, however, tree foliar N increased by 28 and 21 kg N/ha, respectively. Therefore, 10–17 kg N/ha of the first year's fertilizer N was recovered in foliar biomass. Nearly half of that sink was attributable to the relatively large increase in foliar concentrations of *Z. rhoifolium*, while the other half was mostly attributed to a more modest increase in foliar concentration and considerable enhanced growth by *R. exsucca* in the N-fertilized plots. Although we did not measure N concentrations in wood, assuming a typical value of 0.2 mg N/g dry wood and no change in the concentration due to fertilization, enhanced growth of the woody biomass alone would account for a sink of ~3–5 kg N/ha in the N-fertilized plots. Roots were not measured, so these estimates of 13–22 kg N/ha of the 100 kg fertilizer-N/ha recovered in tree wood and foliage represent a lower limit of plant uptake of fertilizer N.

Similar calculations for P indicate that the foliage of the trees in the P and N + P treatments may have sequestered about 1 kg P/ha more than the control and N-only plots. Because P concentrations are generally <0.1 g P/kg dry wood, the difference in woody biomass P among treatments is trivial. The increased foliar P and stimulated grass growth in the N + P treatment resulted in an increased sink of 4 kg P/ha in the grass biomass of that treatment.

Soil responses

While the plants appear to have responded to fertilization treatments, the soil microorganisms did not shown signs of changed activity according to the indices that we measured. No statistically significant fertilizer treatment effects have yet been observed for litter decomposition, soil microbial biomass C and N, net N mineralization, net nitrification, and emissions of CO_2 and CH_4 from the soil surface. A pulse of N_2O and NO emissions was measured only once, 2 d after the second year application of N. The lack of at least a week-long increase in N_2O and NO after the first year N fertilization is somewhat surprising, but is consistent with the observed absence of changes in inorganic-N concentrations. There was no persistent long-term effect of N addition on N gas emissions in either year. Hence, the post-fertilization increases in nitrification and/or denitrification that would be responsible for pulses of NO and N_2O emissions must be brief.

The dominance of ammonium over nitrate as the most common form of inorganic N, along with the strong net immobilization capacity of the soil observed in this study and previous studies (Verchot et al. 1999), indicate a strongly conservative N cycle in this de-

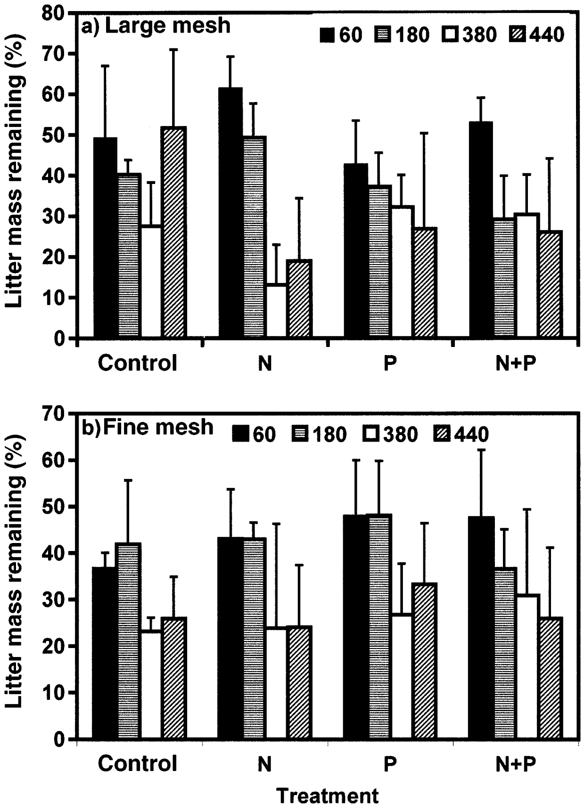

FIG. 4. Decomposition of leaf litter using (a) large mesh (1-cm) nylon bags and (b) fine mesh (1-mm) nylon bags. Means + 1 SE are shown for three bags per treatment and sampling date (60, 180, 380, and 440 d after placement in the field). No significant difference in mass remaining ($P = 0.05$) was found for either fertilizer treatment or mesh size.

graded abandoned pasture. No increase in microbial biomass N was measured in the N-fertilized plots in May 2000, but the microbial biomass N may have been transformed into soil organic matter by then. Because the total soil N pool is large and spatially variable relative to the 100 kg N/ha fertilizer application, we cannot measure a detectable change in the total N pool to infer the fate of the fertilizer N.

Unlike N, P fertilizer application rates were similar in magnitude to the total soil P stocks of the top 20 cm soil. Over 35% of the added P was recovered in the Mehlich-I fraction and the recovery in the total-P fraction could account for all of the fertilizer P. Therefore, recovery in grass and tree foliage accounts for about 10% of the added P, and the rest appears to have been retained in the soil.

Implications for N and P limitation of secondary forest growth

Previous work has shown that impoverishment of the seed bank in the pasture soil affects species composition of the regenerating forest (Vieira et al. 1994), and now this work demonstrates that impoverishment of plant-available forms of nutrients may limit the rate of growth of these trees. The first two years' results from this nutrient manipulation experiment indicate that the remnant grasses responded to N + P fertilization, while the trees responded mostly to N fertilization. Based on the P-fixing capacity of these highly weathered, kaolinitic soils, we hypothesized that availability of phosphorus would affect rates of biomass accumulation and other biogeochemical processes in this young secondary forest growing on an abandoned pasture. This hypothesis was partially supported by observed increases in grass growth in the N + P treatment. However, an important role of N limitation in addition to P limitation of grass growth and a response to N alone for trees was somewhat surprising. Mineralization of large soil organic N stocks and possible inputs from biological N fixation might have met plant demands for N. However, the dominant species in this young successional forest are not N fixers, and low availability of soil P may prevent N fixers from having a significant advantage. Gehring et al. (1999) found increased growth of the nodulated legume, *Inga macrophylla*, only when P was included in the fertilizer complement. Moreover, the frequent entry of fire into this abandoned cattle pasture may have volatilized the most available forms of N (and perhaps P) that were actively cycling in this ecosystem. The relatively large total N stocks in soils may belie a limitation of the much smaller actively cycling, plant-available N pool in the ecosystem.

These results differ from a fertilization experiment by Uhl (1987) at San Carlos, Venezuela, where N + P + K application shortly after tilling an old field favored grasses and forbs but did not enhance tree establishment and growth during the following six months. Uhl (1987) concluded that nutrient availability is not a critical factor for tree establishment early in old field succession. In contrast, our results show that, once the trees are established a few years later in the successional process, their subsequent growth may be nutrient limited. Harcombe (1977) also did not find increased biomass growth after fertilizing a recently cleared forest in Costa Rica. A critical difference, besides the more fertile volcanic soils of Costa Rica, is that an agricultural phase with repeated fire that volatilizes substantial N stocks did not occur in the Costa Rican study. In contrast, nutrient impoverishment is a common reason for agricultural abandonment in Amazonian landscapes.

Studies that correlate results of soil testing with plant growth (e.g., Bushbacher et al. 1988, Johnson et al. 2001) assume that the soil tests, which were derived primarily for agricultural plants, are also good indices of nutrient availability for native forest species. Mycorrhizal associations and root exudates of native species may render forms of soil nutrients available to these plants that are not readily available to agricultural species. We used soil and foliar analyses to track the fate of fertilizers, but not as correlative factors to infer causes of variation in plant growth. The experimental design of fertilizer manipulations provides direct evidence for the effect of nutrient availability on species composition and growth. Although there are differences in details, our results generally agree with those of Gehring et al. (1999), where nutrient amendment experiments conducted after slash-and-burn agriculture on sandy soil also showed growth responses of a four-year-old secondary forest to fertilizer applications. Taken together, it appears that nutrient limitation may be a common feature in Amazonian secondary forests where previous land use practices have depleted stocks of readily available plant nutrients.

Many questions remain that will require further monitoring of this experiment to address. For example, how long will added P remain in the plant available Mehlich-I fraction of the soil, and will the additional P in the total soil P fraction eventually become available to plants? When grasses decrease in dominance as the tree canopy closes, will the P that the grass sequestered become available for other plant uptake, or will it remain bound in organic form so that the biomass gain in the grass component will not be transferred to other life-forms? Will the increase in grasses owing to fertilization change the trajectory of species composition during succession? Although we have not yet observed significant changes in decomposition and soil microbial processes, and we found treatment effects on soil fauna abundances only in the second year of fertilization, will the effects of changes in foliar nutrient concentrations on rates of decomposition and related ecosystem processes eventually become clearer? Will the rate of biomass accumulation in the control plots ‘‘catch up’’ to the fertilized plots once this relatively modest input of nutrient addition is diluted by usual successional growth, or will the observed boost in growth persist?

For the moment, we can conclude that the vegetation, six years after the last fire and 15 years since pasture abandonment, responded to nutrient amendments by either increasing foliar concentrations or increasing growth or both. Although P availability is low in these soils and may partially limit biomass growth, the most striking result of this study so far is the significant response of tree growth to N fertilization. Repeated fire and other losses of N from degraded pastures may render tree growth N limited in these young Amazonian forests. If sustained, these early results indicate that nutrient limitation is an important factor determining rates of biomass accumulation in secondary forests growing on clayey Oxisols of abandoned cattle pastures that had moderate to heavy prior land use.

ACKNOWLEDGMENTS

This research was supported by Grant No. NCC5–332 of NASA’s Terrestrial Ecology Program and is part of the Large-scale Biosphere–Atmosphere (LBA) project. We thank Dr. Moacyr Dias-Filho for assisting with the initial fertilizer application design, Dra. Regina Möller for assistance with soil bulk density measurements, and Elizabeth Belk for assistance with trace gas measurements. We also thank Carlos Alberto Silva, Karina de Fátima Rodrigues Pantoja, and Michele Maria Correa for their assistance in the laboratory and in the field. We thank CNPq’s Programa de Bolsa for the LBA project for supporting bolsistas Pantoja, Correa, and Sabá. We also thank two anonymous reviewers for their helpful comments.

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