EFFECTS OF FERTILISATION ON SOIL NUTRIENT CHARACTERISTICS AND THE GROWTH OF TREE STAND IN SECONDARY SEASONALLY DRY TROPICAL FORESTS IN MEXICO

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Received September 2011

CAMPO J, SOLÍS E & GALLARDO JF. 2012. Effects of fertilisation on soil nutrient characteristics and the growth of tree stand in secondary seasonally dry tropical forests in Mexico. Soil properties and stem growth were studied in dominant tree species in secondary tropical dry forests growing on limestone in Yucatán Peninsula, México. A young forest (10 years old, site A) with phosphorus (P)-poor soil and an older forest (60 years old, site B) with soil having higher available P contents were selected. Tree growth of representative species of each site was studied in relation to soil nitrogen (N) and P concentrations. Four independent plots (12 m × 12 m) in each forest were either left intact (controls) or fertilised with N, P or with N + P for three years. In the young forest soil concentrations of NO_3^- were higher than those of NH_4^+, indicating that nitrification proceeded fast. Application of N and N + P increased the concentration of soil NO_3^- at site A; fertilisation treatments at this site increased trunk growth only in *Lysiloma latisiliquum*. In contrast, N or P fertilisation at site B did not significantly affect soil extractable N concentrations. At this site, fertilisation increased N transformations and P addition promoted growth in *Acacia gaumeri* and *Bursera simaruba*. These findings indicated that nutrient limitation occurred in the secondary forest.

Keywords: Calcareous soils, nutrient limitation, forest succession

CAMPO J, SOLÍS E & GALLARDO JF. 2012. Kesan pembajaan terhadap ciri-ciri nutrien tanah dan pertumbuhan dirian pokok di hutan tropika kering sekunder bermusim di Mexico. Ciri-ciri tanah dan pertumbuhan batang dikaji pada spesies pokok dominan di hutan tropika kering sekunder yang tumbuh atas batu kapur di Semenanjung Yucatán, México. Hutan muda (10 tahun, tapak A) yang mempunyai sangat kurang kandungan fosforusnya (P) serta hutan yang lebih tua (60 tahun, tapak B) yang lebih kaya kandungan P dipilih untuk kajian. Pertumbuhan pokok yang mewakili setiap tapak dikaji kandungan nitrogen (N) dan P tanahnya. Empat plot (12 m × 12 m) di setiap hutan dibiar tanpa sebarang rawatan hutan (kawalan) atau diberi baja N, P atau N + P selama tiga tahun. Di tapak A, kandugan NO_3^- tanah lebih tinggi daripada NH_4^+. Ini menunjukkan bahawa proses nitrifikasi berlaku dengan pantas. Aplikasi N dan N + P meningkatkan kandungan NO_3^- di tapak A. Rawatan pembajaan di tapak ini menambahkan pertumbuhan batang hanya dalam *Lysiloma latisiliquum*. Sebaliknya pembajaan N atau P di tapak B tidak memberi kesan kepada kandungan N boleh ekstrak dalam tanah. Di tapak ini pembajaan meningkatkan transformasi N dan penambahan P menggalakkan pertumbuhan *Acacia gaumeri* dan *Bursera simaruba*. Keputusan ini menunjukkan pengehadan nutrien berlaku di hutan sekunder.

INTRODUCTION

Nitrogen (N) and phosphorus (P) are essential to a variety of plant functions and play important roles in photosynthesis (Lambers et al. 2008). Their restricted availability often limits plant carbon acquisition and growth in tropical forests (Elser et al. 2007, Reich et al. 2009). Research has shown that during primary succession, nutrient limitation of plant growth changes from limited N availability in the early stages to limited P availability in later stages (Vitousek & Farrington 1997). Much less attention has been paid to the implications of nutrient limitation for plants and ecosystems in disturbed tropical dry areas. The accumulation of P in biomass is one of the most important effects of fallow vegetation in the Yucatán Peninsula in south-eastern Mexico (Campo & Dirzo 2003). However, although soil nutrient availability varies with forest successional

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stage and spatial variation in species composition, the overall situation of regenerating secondary seasonally dry tropical forests (SDTFs) of the Yucatán Peninsula is that plants are nutrient-limited (Campo & Dirzo 2003). The most common tree species in a young secondary forest responded to the addition of nutrients by increasing their foliar P concentration, whereas trees in older stands increased only their foliar N concentrations (Campo & Dirzo 2003).

The main objective of the present study was to assess how variation in nutrient availability affected the growth of dominant tree species in these secondary SDTFs of Yucatán. Fertilisation experiments were conducted with N and P to test whether tree growth and soil N transformation rates during forest regeneration were directly related to the supply of either of these mineral nutrients. Nutrient limitation of these forests was explored by combining fertilisation manipulations with measurements of the growth (increment in diameter at breast height, dbh) of dominant tree species and with measures of potential soil N mineralisation and nitrification in control and fertilised plots in a young (10 years) and an older (60 years) secondary SDTFs. Our hypothesis was that if forest production was limited by natural supplies of N and/or P, then fertilising the soil would result in an increased amount of uptake, enabling a greater total accumulation of carbon, as reflected by an increase in the trunk growth of representative species of these secondary forests. On the other hand, if microbial activity was constrained by nutrient availability, changes in supplies of N and/or P would result in greater N transformation rates in the forest soils.

MATERIALS AND METHODS

Study sites

The experiment was carried out in two areas of a secondary SDTF in the Dzibichaltún National Park region in the Yucatán Peninsula (21° 06' N, 89° 17' W). The whole area was previously used for the cultivation of henequén (*Agave fourcroydes*) and for slash-and-burn agriculture. One sector of this area was abandoned approximately 10 years ago (young secondary forest area—site A) while the other, 60 years ago (older secondary forest area—site B). A detailed description of the study sites, forest ecosystems and fertilisation experiments was reported in Gamboa et al. (2010). Only the procedures unique to this study are given here.

The mean annual temperature is 25.8 °C, with a difference of less than 5.0 °C between the coolest and warmest months. The average annual precipitation during the study period (June 1997–May 2001) was 946 ± 111 mm (mean ± SE), most of which fell between June and October, contributing to 75% of the total precipitation.

The landscape consists of large flat areas (less than 10 m above sea level). The predominant lithology includes late Pliocene material, with numerous areas of exposed limestone. Soils comprising Lithic Haprendolls are mainly shallow and organic-rich and directly overlie weathered calcium carbonate. The depth of the Ah horizon varies between -50 and -100 mm and its bulk density ranges from 0.4 to 0.7 Mg m^{-3} (Table 1). Although the soil of both areas can be regarded as P-poor at the beginning of rainy season (site A = 8 ± 1.0 mg P kg^{-1}, site B = 9 ± 1.0 mg P kg^{-1}), the older forest area has soils with higher availability of P than younger forest soils in the dry season (Table 1). However, absolute contents of available P in site B at the onset of the experiment (dry season) duplicated those in site A.

The average soil organic C concentration and soil pH at the study sites were similar to those described for tropical seasonal forest soils derived from limestone in Jamaica (Kelly et al. 1988) and Puerto Rico (Murphy & Lugo 1986).

Experimental design

In each forest site, we established sixteen 12 m × 12 m plots with 8-m spatial buffers between them in March 1997. Site B had fewer trees (dbh > 2.5 cm, 4015 ± 321 trees ha^{-1}) than site A (4328 ± 282 trees ha^{-1}). Basal area was greater in the former compared with the latter, i.e. 15.0 ± 1.3 m^2 ha^{-1} and 9.4 ± 0.8 m^2 ha^{-1} respectively. Floristically, Leguminosae were the most important family in both forest sites (Ceccon et al. 2002).

The plots were randomly assigned to one of four treatments, namely, control, added N, added P and added N + P. Each treatment was replicated four times. Fertilisers ((urea $(NH_2)_2CO$) or triple superphosphate ($Ca(H_2PO_4)_2.H_2O$) or both) were added in two pulses for three consecutive years (1998–2000): at the end of the dry season (May, 60% of the total annual amount of added

Table 1 Organic C, total and inorganic N and total and extractable P contents in soils (0–5 cm soil depth) of 10- (site A) and 60-year-old (site B) secondary forests of Yucatán, Mexico

Parameter	Site A	Site B	p
Soil bulk density (Mg m^{-3})	0.5 ± 0.1	0.6 ± 0.1	ns
pH (H_2O)	7.3 ± 0.1	7.4 ± 0.1	ns
Soil organic C (mg C g^{-1})	229 ± 12	118 ± 16	***
Total N (mg N g^{-1})	21.7 ± 0.6	17.4 ± 0.8	*
C:N ratio	10.5 ± 0.3	6.7 ± 0.5	***
NH_4^+ (mg N kg^{-1})	50 ± 3.6	34 ± 1.5	**
NO_3^- (mg N kg^{-1})	73 ± 6.3	100 ± 15.4	ns
Total inorganic N (mg N kg^{-1})	123 ± 21	134 ± 18	ns
Total P (mg P kg^{-1})	1704 ± 608	2934 ± 133	***
P–$NaHCO_3$ (mg P kg^{-1})	11 ± 1.2	19 ± 3.1	***
Organic C (Mg C ha^{-1})	57 ± 2.7	35 ± 1.9	***
Total N (Mg N ha^{-1})	5.4 ± 0.2	5.2 ± 0.2	ns
NH_4^+ (kg N ha^{-1})	14 ± 1.8	10 ± 0.4	**
NO_3^- (kg N ha^{-1})	20 ± 5.2	30 ± 2.4	ns
Total inorganic N (kg N ha^{-1})	34 ± 1.7	40 ± 2.5	**
Total P (kg P ha^{-1})	425 ± 61	870 ± 101	**
P-NaHCO3 (kg P ha^{-1})	2.8 ± 0.3	5.7 ± 0.7	**

Values for each forest are mean ± SE; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant $p > 0.05$

fertiliser) and in the middle of the rainy season (September, 40%). The annual concentrations of fertilisers used were 220 kg N ha^{-1} and 75 kg P ha^{-1}. No significant differences in soil N and P concentrations were found between treatment plots before the experiment (Ceccon et al. 2002).

Soil sampling and analysis

We collected eight samples of surface soils (depth 0–5 cm) at random from each of the plots in May 2001 (at the end of the experiment). Soils were collected after the first rainfall event in the region (in the first week after rainy season started) as representative of conditions at the onset of the rainy season. In SDTFs, concentrations of nutrients were lowest in soil during the rainy period (Saynes et al. 2005).

Soil samples were promptly transported to the laboratory, stored at 4 °C and processed within two days of collection. In the laboratory, soil samples were sieved (to pass a 2-mm mesh) and a subsample was dried until constant weight for moisture determination. The remaining soil was used in potential soil N transformation (i.e. potential soil N mineralisation and potential soil nitrification) assays and measurements of available P and inorganic N concentrations (NH_4^+ and NO_3^-). Inorganic N concentrations were measured by extracting 20 g subsample in 100 mL 2 M KCl. The soil KCl solution was shaken for 1 hour, allowed to settle overnight and subsequently filtered (Whatman No. 42 filter paper). Nitrogen mineralisation and nitrification potentials were measured during one week of aerobic incubation (Hart et al. 1994). A second 20-g subsample was saturated to field water holding capacity with distilled water, maintained at field capacity moisture (gravimetric method) and incubated at 27 °C for 1 week before extracting with KCl (final inorganic N concentration). Potential soil mineralisation was estimated from the difference between inorganic N at the beginning and end of the incubation and results were expressed based on weekly inorganic N production. Likewise, potential soil nitrification was determined from the difference in NO_3^- concentration between the beginning and the end of incubation and results were expressed in similar units.

Available P was extracted from a 0.5-g subsample of fresh soil (i.e. not dried soil)

with bicarbonate solution ($NaHCO_3$, 0.5 N) according to Watanabe and Olsen (1965). This extractable P can be considered as short-term available P to plants (Cross & Schlesinger 1995). Subsequently, we also performed digestion with sulphuric acid and ammonium persulphate in order to determine the total soil P in the extract (APHA 1992). All extracts and standards were analysed colorimetrically using an NP analyser. We calculated mean nutrient concentrations and potential soil N transformation (i.e. potential soil mineralisation and potential soil nitrification) for each treatment of four plots (Tables 2 and 3).

Tree growth measurements

In all plots, species identity, density, frequency and basal area of individuals based on the work by Ceccon et al. (2002) were used to select the three dominant species with dbh ≥ 5 cm at each forest area. The species selected were *Acacia gaumeri* (basal area compared with total basal area in the forest = 11.7%), *Leucaena leucocephala* (10.8%) and *Lysiloma latisiliquum* (15.4 %) at site A and *A. gaumeri* (8.3%), *Pithecellobium dulce* (12.8%) and *Bursera simaruba* (12.3%) at site B. With the exception of *B. simaruba* (Burseraceae), all species were members of the Leguminosae. We measured dbh of all selected species in all plots within the 10 m × 10 m central area at one-year intervals during the four years of the study. For each forest site, the three selected species were represented in all nutrient treatments, except for *A. gaumeri* that was not present at site B and was therefore absent from the N-fertilised plots of that forest. At site A, there were between 4 and 11 trees per plot of each selected species while at site B, there were between 3 to 8 trees per plot of the corresponding selected species. Mean growth increments for each species per plot were calculated and these values were used for statistical analysis with n = 4 plots per treatment. For both sites, the increment in tree diameter between 1998 and 2001 were reported, thereby minimising any lags or transient effects that could occur immediately after the initial fertiliser application.

Statistical analysis

Statistical analyses were carried out on effects of fertilisation treatments on soil nutrient concentrations and potential N transformations and on growth rates for each dominant tree species. All statistical tests involved analysis of variance (ANOVA) by site. The honestly significant difference (HSD) test was used when statistical differences ($p < 0.05$) were observed.

Table 2 Soil N and P parameters and trunk growth of dominant tree species under four nutrient-addition treatments in a 10-year-old secondary forest in Yucatán, Mexico

Parameter	Control	N	P	N + P	Significance
Soil (depth 0–5 cm) characteristics in 2001					
NO_3^- (mg N kg^{-1})	98 ± 10.3	162 ± 13.8	71 ± 14.6	143 ± 10.3	N, N + P
NH_4^+ (mg N kg^{-1})	69 ± 10.1	54 ± 16.7	62 ± 14.5	84 ± 14.6	ns
Total inorganic N (mg N kg^{-1})	157 ± 10.8	216 ± 13.6	133 ± 14.6	227 ± 12.6	N, N + P
Potential soil N mineralisation (mg N kg^{-1} $week^{-1}$)	4.1 ± 0.5	1.6 ± 0.7	2.3 ± 0.9	1.7 ± 0.8	N, N + P
Potential soil nitrification (mg N kg^{-1} $week^{-1}$)	8.0 ± 1.9	6.3 ± 0.9	2.8 ± 1.6	6.1 ± 1.2	P
P–$NaHCO_3$ (mg P kg^{-1})	9.7 ± 0.8	4.3 ± 0.2	14.3 ± 2.9	10.6 ± 1.0	N
Diameter growth in 1998–2001 (mm $year^{-1}$)					
Acacia gaumeri	1.6 ± 0.4	1.8 ± 0.4	2.2 ± 0.3	2.2 ± 0.2	ns
Leucaena leucocephala	1.7 ± 0.7	1.7 ± 0.6	2.3 ± 0.3	2.0 ± 0.1	ns
Lysiloma latisiliquum	1.8 ± 0.6	4.2 ± 1.1	3.9 ± 0.5	4.0 ± 0.7	N, P, N + P

Values for each treatment are means ± SE of four plots; ns = not significant $p > 0.05$; N = nitrogen addition, P = phosphorus addition, N + P = addition of both N and P

Table 3 Soil N and P parameters and trunk growth of dominant tree species under four nutrient-addition treatments in a 60-year-old secondary forest in Yucatán, Mexico

Parameter	Control	N	P	N + P	Significance
Soil (depth 0–5 cm, characteristics in 2001)					
NO_3^- (mg N kg^{-1})	45 ± 10.8	60 ± 5.9	67 ± 7.3	68 ± 11.7	ns
NH_4^+ (mg N kg^{-1})	47 ± 12.7	29 ± 5.4	31 ± 3.2	45 ± 8.7	ns
Total inorganic N (mg N kg^{-1})	92 ± 11.9	89 ± 5.4	98 ± 3.4	113 ± 9.8	ns
Potential soil N mineralisation (mg N kg^{-1} $week^{-1}$)	2.1 ± 0.4	1.8 ± 0.3	3.7 ± 0.5	3.3 ± 0.7	P
Potential soil nitrification (mg N kg^{-1} $week^{-1}$)	3.2 ± 0.6	3.0 ± 0.7	4.8 ± 0.6	4.7 ± 0.9	P
P–$NaHCO_3$ (mg P kg^{-1})	9.2 ± 1.4	7.6 ± 0.8	17.5 ± 2.7	16.9 ± 2.1	P, N + P
Diameter growth in 1998–2001 (mm $year^{-1}$)					
Acacia gaumeri	0.5 ± 0.4	na	1.6 ± 0.6	1.8 ± 0.5	P, N + P
Pithecellobium dulce	1.1 ± 0.3	1.0 ± 0.3	0.9 ± 0.2	1.6 ± 0.4	ns
Bursera simaruba	4.3 ± 0.8	4.3 ± 1.1	8.1 ± 1.3	6.6 ± 0.8	P, N + P

Values for each treatment are means ± SE of four plots; na = not available, ns = not significant $p > 0.05$; P = phosphorus addition, N + P = addition of both N and P

RESULTS

Site A

Concentration of soil NO_3^- was 42% higher than NH_4^+ in control plots of site A (Table 2). The addition of N and N + P consistently increased soil NO_3^- and total inorganic N concentration relative to the control ($F = 18.7$, $p < 0.001$). However, the application of fertilisers did not have significant effects on soil NH_4^+ concentration ($F = 1.06$, $p = 0.403$). In contrast, N addition reduced the extractable P concentration by 56% compared with control plots ($F = 9.61$, $p = 0.002$); no other nutrient addition had significant effect. N enrichment (alone or combined with P) significantly reduced potential soil mineralisation ($F = 5.14$, $p = 0.016$). Notably, the application of P significantly decreased the potential soil nitrification ($F = 16.8$, $p < 0.001$).

The increase in dbh in the young forest was very consistent across species in the unfertilised plots (Table 2). The dbh variation across species was about 9%. No significant differences ($F = 0.43$–0.71, $p > 0.05$) in trunk growth of each species were found between the plots the year before fertilisers were added.

In general, the additions of P or N + P did not affect the growth rate of the leguminous trees relative to the control ($F = 0.559$–1.24, $p > 0.05$). Nevertheless, the trunk growth of *L. latisiliquum* approximately doubled in all three fertilisation treatments ($F = 4.43$, $p = 0.026$).

Site B

The difference between the NH_4^+ and NO_3^- concentrations detected in the soils of the young forest was not observed in the older forest (Table 3). Fertilisation had no significant effect on NH_4^+ ($F = 1.87$, $p = 0.188$) and NO_3^- ($F = 2.28$, $p = 0.132$) concentrations in this forest. However, P and N + P additions increased the extractable P concentrations compared with the control ($F = 9.26$, $p = 0.002$). In addition, P fertilisation increased soil N transformation ($F = 5.32$, $p = 0.015$ for potential soil mineralisation, and $F = 3.73$, $p = 0.042$ for potential soil nitrification). The combined nutrient addition (N + P) did not have significant effect on either soil N transformation, i.e. potential soil mineralisation and potential soil nitrification.

The trunk growth in unfertilised plots differed considerably between species in the older site (Table 3). During the year before fertilisers were added, the differences between the set of plots for each species were not significant ($F = 0.59$–0.80, $p > 0.05$). The addition of P or N + P increased

trunk growth in *A. gaumeri* (leguminous) ($F = 5.07$, $p = 0.033$) and *B. simaruba* (non-leguminous) ($F = 6.91$, $p = 0.006$). In contrast, the application of fertilisers did not have significant effect on trunk growth in *P. dulce* (leguminous tree with lower leaf-N concentration than *Acacia*) ($F = 1.18$, $p = 0.357$).

DISCUSSION

Forest soils from Yucatán have high organic C and total N concentrations because of the high carbonate content in the area (Gamboa et al. 2010). Although total N concentration was significantly lower in older forest soils in comparison with the younger stand, the absolute N contents in both forest soils were similar between sites (Table 1). This result was consistent with the low variation in foliar N concentration in *A. gaumeri* at both sites (leaf N concentration = 26.4 mg N g^{-1} and 23.4 mg N g^{-1} in sites A and B respectively, Campo & Dirzo 2003).

The concentration of extractable-NH_4^+ at site A was higher than that at site B or those reported for other SDTF soils (Erickson et al. 2002, Tripathi & Singh 2008). Soil NH_4^+ contents at sites A and B were the same at the onset of the experiment. In our study, young forests have shallow soils that were frequently waterlogged (J Campo, personal observation) and exhibited increases in NH_4^+, whereas in older soils evapotranspiration may have helped avoided this effect. The decrease in NH_4^+ concentration observed at site B may be due to the absence of water logging (aerobic conditions) but further investigation is needed to ascertain this. This decrease could reflect the proposed high nitrification as it could be a consequence of higher N immobilisation by soil microbes as a consequence of the decreased abundance of N-fixing trees in older secondary forest (Ceccon et al. 2002), or it could result from the effects of both factors.

The available P concentrations in both forest soils studied here (i. e. the values in the control plots), especially in the younger forest, were the lowest reported for SDTF soils (Murphy & Lugo 1986). These low extractable P concentrations could result from the basic soil pH (precipitation as Ca phosphates) and these P concentrations were in the lower range of data reported for different soil units (Cross & Schlesinger 1995). The absolute available P contents in the older forest soils were similar to younger forest during the dry season. This finding suggested that the accumulation of P during the period without rain was an important source of nutrients for plants and microbes when the rainy season started, much more in the older than in younger forest soils. The available P value at site B could be sufficient for tree nutrition in this type of tropical forest, where mycorrhizal symbiosis occurred (Huante et al. 1995). However, it is not enough for site B where available P can be too low to satisfy the demands of the growing trees. These findings were consistent with the low leaf P concentration and the high leaf N:P ratio value reported for *A. gaumeri* in a young forest (Campo & Dirzo 2003).

Our findings for the extractable NO_3^- and NH_4^+ concentrations in both forest soils provided evidence of high nitrification at site A (indicated by the high values of potential soil nitrification). At site B, nitrification is activated only upon the addition of P. This observation suggested a new determination of the soil N subcycle by P availability in the older forest. The suggestion that microbial N transformation in a young forest could be limited by soil P availability corroborated the hypothesis of Cole and Heil (1981), which indicated that there was limitation of soil N transformation by the availability of soil P. On the other hand, our data were consistent with the evidence suggesting that P exerted a broader level of control over soil N dynamics in tropical forests (Cleveland et al. 2011).

Potential soil mineralisation values are usually higher than those of potential soil nitrification. However, in this case, the nitrification *in vitro* was so intense and fast that soil ammonia was exhausted because of rapid transformation to nitrate. As a result, values of potential soil nitrification higher than those of potential soil mineralisation were found in the young forest. Furthermore, the potential soil nitrification was higher than the potential soil mineralisation in control soil and in soils fertilised with N (alone or combined with P).

In contrast to the findings reported above, the addition of P to soil had no significant effect on N transformation in tropical rainforests in Colombia (Cavelier et al. 2000) or in Brazil (Davidson et al. 2007), probably because these forest ecosystems had acid soils and the added P was quickly adsorbed by soil sesquioxides (Fuentes et al. 2008). The patterns found in Brazil and Colombia were similar to responses observed in the more organic soils of site A in this study. Although the effects seemed comparable,

the causes were probably different because soil sesquioxides were not expected to play a major role in 'organic' soils. Conversely, these soils were generally rich in carbonates and Ca (Gamboa et al. 2010). Under these conditions, P could also be fixed at high pH values (Lugo & Murphy 1986).

At site A, the younger forest stand, an increase in soil NO_3^- occurred as a result of N fertilisation. In this stand, the trunk growth response only increased in *L. latisiliquum*; the leguminous species had the lowest nutrient demand. However, *L. latisiliquum* also responded to P addition. This finding suggested that N and P were limiting at site A. In contrast, the soil inorganic N concentrations did not change significantly at site B. This explained the unresponsiveness of the trunk growth rate to N fertilisation. However, two of the most common tree species in this forest increased their trunk growth in response to P and N + P additions. This finding was consistent with the microbial responses indicated by the stimulation of N transformation in the soil and suggested that P was limiting in this older forest. P availability probably limited soil microorganisms in this forest as well.

Acacia gaumeri was the only species that occurred in both forest stands. The effects of adding P and N + P in the younger forest (an ecosystem with low soil P availability) and in the older forest (where higher P was available) exhibited a striking contrast. Surprisingly, however, fertilisation of the stand in the young forest did not affect the growth of *Acacia*. However, this species responded to P fertilisation in the older forest where the soil P content was higher and *Acacia* trees tripled their trunk growth in response to the P and N + P additions. This observation proved that the young forest stand was N- and P-limited. Moreover, the intense microbial activity in the soil immobilised all added N and P, as expected in this situation of nutrient scarcity. The addition of P could produce indirect positive effects on trunk growth in the older forest, even for the non-leguminous trees, through positive activation of N dynamics. An increase in *Acacia* leaf P concentration has also been reported after addition of N + P in the younger but not in the older forest (Campo & Dirzo 2003) of the present study.

Results of the current study suggested that in an ecosystem with low P supply and trees with high leaf N:P concentrations (*Acacia* leaf N:P ratio = 33 at site A), increase in foliar nutrient concentration was a more evident response to nutrient additions than the increase in tree growth. However, in an ecosystem with higher soil P availability and where higher leaf P concentration had been reached (*Acacia* leaf N:P ratio = 14 at site B), the leaf P concentration did not change. Rather, tree growth increased in response to the indirect effect of P addition.

Further research on this and other SDTF ecosystems is needed. The microbiological and enzymatic activities of the forest soil should be studied carefully to corroborate the hypothesis postulated here regarding the activation of microorganisms by P additions to the soil and the positive influence of the resulting microbial activity on N nutrition of trees.

The evidence of nutrient limitation in these Yucatán secondary forests is only partly consistent with previous observations that have suggested P limitation of forest growth in tropical regions (Vitousek 1984). However, our results also agree, in part, with the findings of Ceccon et al. (2002) and Campo and Dirzo (2003), who have presented indirect evidence based on soil and plant analysis respectively. This evidence suggested that P limitation occurred during recovery of the SDTFs in Yucatán. However, the limitation applies essentially to soil microbiology, not to all tree species.

CONCLUSIONS

Despite high natural variability, this study provided evidence of nutrient limitation in secondary forest stands for both tree growth and soil microbial function. The significant responses to fertilisation in tree growth, plant uptake and microbial retention of soil N at site A suggested that N and P limitations were important in the relatively nutrient-poor younger forest site. However, the initial N and P limitation indicated by the growth responses of *L. latisiliquum* (in addition to the increase of foliar P in all dominant leguminous trees at site A) indicated shifts over time to a microbial P limitation, especially for nutrient-demanding trees as found in the older site B.

ACKNOWLEDGEMENTS

The first author was partially supported by sabbatical fellowships from the Consejo Nacional de Ciencia y Tecnología (138403), the Dirección

General de Apoyo al Personal Académico, Universidad Nacional Autónoma de México (Mexico) and the Spanish Ministry of Science and Innovation. This work was supported by the Consejo Nacional de Ciencia y Tecnología grant CB 60429 and by one grant offered by the Universidad Nacional Autónoma de México, PAPIIT 220610 to the first author.

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