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Nutrient status and plant growth effects of forest soils in the Basin of Mexico

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Plant response to N deposition may be limited by P limitation in forests growing on Andisol soils in the Basin of Mexico.

Abstract

The nutrient status of forest soils in the Mexico City Air Basin was evaluated by observing plant growth responses to fertilization with N, P or both nutrients combined. P deficiency was the most frequent condition for soil from two high pollution sites and N deficiency was greatest at a low N deposition site. Concentrations of Pb and Ni, and to a lesser extent Zn and Co, were higher at the high pollution sites. However, positive plant growth responses to P and sometimes to N, and results of wheat root elongation bioassays, suggest that heavy metal concentrations were not directly phytotoxic. Further studies are needed to determine if heavy metal toxicity to mycorrhizal symbionts of eucalyptus (*Eucalyptus camaldulensis* Dehnh.) from high pollution sites may explain the P deficiency and stunted growth. P deficiency is expected to limit the capacity for biotic N retention in N saturated forested watersheds in the Basin of Mexico dominated by Andisols.
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Keywords: Andisols; Nitrogen deposition; Phosphorus deficiency; Nitrate leaching; Mexico City Air Basin

1. Introduction

Forests to the south and southwest of Mexico City are exposed to elevated levels of ozone, nitrogen and sulfur pollutants (Fenn et al., 2002c). Ozone causes typical injury symptoms of *Pinus hartwegii* Lindl. in these forests (Miller et al., 2002) and a severe decline of *Abies religiosa* Schl. stands was first reported in the 1980s in the Desierto de los Leones National Park in the southwestern end of the Basin (Alvarado and Hernández, 2002). Air pollution is believed to play a role in the decline. Forests to the east of Mexico City are exposed to comparatively low levels of air pollution (Fenn et al., 2002c). Earlier studies suggested that the relatively N rich volcanic soils of forests in the Basin of Mexico may not respond to N deposition with a positive growth response (Fenn et al., 2002b). Because Andisols have a characteristically high phosphorus (P) fixation capacity, P is commonly a limiting nutrient in both natural ecosystems and crop systems supported by Andisols (Shoji et al., 1993). Forests in the Basin of Mexico reportedly respond variably to either N or P fertilization (Marín et al., 2002) and low P levels in soils of the Basin have

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been reported (Cruz-Cisneros and Valdés, 1990; Manske et al., 2000; Saldivar-Castillo, 1982).

Evaluating the nutrient status of forest soils in the Basin of Mexico is of practical importance in understanding the effects of air pollution on nutrient cycling and forest decline, and to better understand the conditions under which these watersheds are most susceptible to elevated nitrate export and associated water quality concerns. Nitrate concentrations in streams and springs in forested watersheds in the Basin correspond with N deposition levels (Fenn et al., 1999, 2002b). Nitrogen deposition could more readily lead to high levels of nitrate export if vegetation in these watersheds has a limited capacity to biologically assimilate N from atmospheric deposition because of naturally high soil N availability and because of inherent P limitation. Thus, the primary objective of this study was to evaluate the nutrient status of forest soils in the Basin of Mexico at sites with varying levels of atmospheric deposition. To this end, short-term fertilization studies of three indicator species cultivated in native forest soils were carried out and are herein reported. Because of unusually poor growth of *Eucalyptus camaldulensis* Dehnh. seedlings planted in soil from Ajusco (a highly polluted forest site), another objective became to determine the soil factors causing this stunted growth.

2. Materials and methods

2.1. Study locations and soil sampling

Soil sampling was initially carried out in May 2001 at three study sites in the Basin of Mexico with differing levels of N deposition (Fig. 1). The Desierto de los Leones National Park site (DL) is a highly polluted site located in the direction of the prevailing winds from Mexico City. Nitrogen deposition in throughfall in 1996 was reported to be 18.5 kg ha^{-1} yr^{-1} at DL in a relatively open stand of *P. hartwegii* (Fenn et al., 1999). Nitrogen deposition in the Ajusco (AJ) area has not been reported but based on previous studies, this site is known to also experience high air pollution exposure (Fenn et al., 2002c). Nitrogen deposition in throughfall at the low deposition site used in this study (Zoquiapan, ZOQ) was 5.5 kg ha^{-1} yr^{-1} in 1996 (Fenn et al., 1999). Streamwater nitrate concentrations correspond with the levels of N deposition, with high nitrate concentrations from springs and streams at DL and AJ and low concentrations in streams at ZOQ and surrounding areas (Fenn et al., 2002b). Initially, a second low pollution site, located in the Izta-Popo National Park (Paso de Cortes) was included in this study, but permission

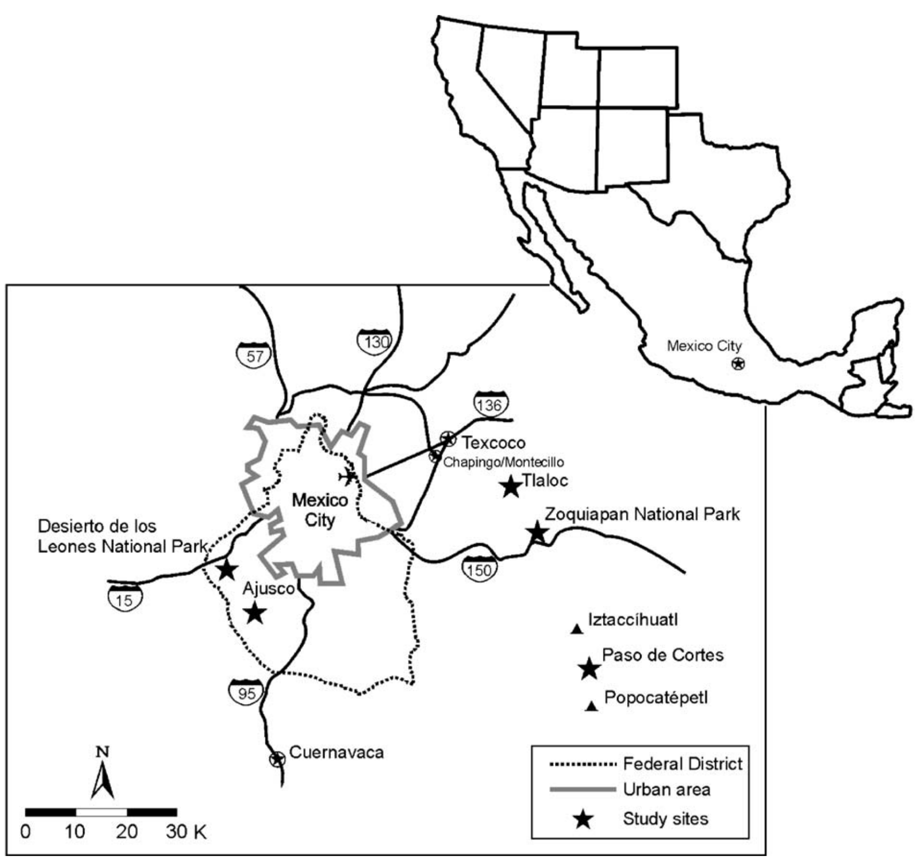

Fig. 1. Map showing the locations of the forest sites (indicated by star symbols) within the Basin of Mexico from which soils were collected for the experiments in this study.

to continue research in the park was later denied because of possible danger from volcanic activity in the area. Studies of plant growth responses to fertilization were done using soils from ZOQ, AJ and DL. In October 2003 soils were collected from these three sites again and from Tlaloc (TL) for nutrient and heavy metal analyses and phytotoxicity bioassays. Tlaloc is a low N deposition site located approximately 70 km upwind (east) of Mexico City (Fig. 1).

At each of the three plant-response study sites, ten soil samples were taken at a 0–30 cm depth to obtain a composite sample. The composite soil samples were collected from *P. hartwegii* stands over an area of approximately 250 m^2. These soils were used for the plant growth response studies described below. These soils do not have a significant organic layer and so only the mineral soil was sampled. Total C, N and S were determined by combustion analysis (Carlo Erba Instruments, Milan, Italy; Model NA 1500, Series 2 (Table 1).

2.2. Greenhouse bioindicator study of soil growth effects: eucalyptus seedlings

The nutritional status of the forest soils was evaluated based on the fertilization responses of fast-growing *Eucalyptus camaldulensis* seedlings planted in the composite soil samples collected from the three pine forest sites as described above. Eucalyptus seeds were first planted into a steam-sterilized potting soil mixture composed of 50% sand, 25% perlite and 25% vermiculite in May 2001. Four weeks after germinating, the seedlings were transplanted into 3.9 L pots containing native forest soil that was previously passed through a 3 mm sieve.

Fertilization treatments were applied 15 weeks after transplanting into the native soils when the plants were on average 3.5, 4.9 and 13.8 cm in height at AJ, DL and ZOQ, respectively. The seedlings were fertilized by spreading the previously weighed fertilizer evenly over the soil surface. Fertilization treatments consisted of added N, P, NP or NPK. Nitrogen was applied as ammonium sulfate, P as calcium phosphate, and K as potassium sulfate. Nitrogen, P and K were added at a rate equivalent to 200, 120 or 160 kg ha^{-1}. Six replicate plants were included within each fertilization treatment for each site. The plants were maintained in a greenhouse at the Postgraduate Agricultural College in Montecillo, State of Mexico, Mexico. Height growth of the plants ($n = 6$) was evaluated every 4–6 weeks, and at the end of the experiment the dry weight of foliage, stems and roots ($n = 3$) was determined. Foliar N and P concentrations were determined by Kjeldahl digestion and subsequent colorimetric analysis. Replication for dry weight and foliar nutrient determinations was less than that for height growth because half of the plants were sacrificed for evaluation of symbiotic root fungi associations (Perea-Estrada et al., 2005).

Table 1
Sulfur, nitrogen and carbon concentrations (g kg^{-1}) and C:N ratio of pine forest soils

Site	S	N	C	C:N
0–5 cm soil depth				
Zoquiapan	0.41	3.73	69.0	18.5
Tlaloc	0.62	6.57	94.9	14.4
Ajusco	0.70	5.71	90.2	15.8
Desierto de los Leones	0.75	7.00	103.2	14.7
5–15 cm soil depth				
Zoquiapan	0.25	2.00	34.7	17.3
Tlaloc	0.39	4.60	59.3	12.9
Ajusco	0.53	4.47	71.0	15.9
Desierto de los Leones	0.55	5.09	80.2	15.8

2.3. Greenhouse bioindicator study of soil growth effects: lettuce and radish

Romaine lettuce (*Lactuca sativa* L. var. *longifolia* Lam.; Parris Island Cos variety) and radish (*Raphanus sativus* L.; Champion variety) seedlings were grown in soils collected in July 2003 from under pine canopies at a depth of 0–30 cm at DL, AJ and ZOQ. Soils were sieved to 3 mm. Two to three seeds were planted per container in 0.35 L styrofoam cups with drain holes in the bottom on November 7, 2003, and maintained in an air conditioned greenhouse in Riverside, California, USA. On November 17 (5 days after germination) the seedlings were fertilized with N, P or a NP combination at the same rates used in the eucalyptus experiments except that K was not included in the radish and lettuce experiments. Nitrogen was applied as ammonium nitrate and P was applied in the form of calcium phosphate. All fertilizers were in granular form. Six replicate pots were utilized per fertilizer treatment/site/plant species combination. However, due to mortality from damping off disease, the average number of surviving replicates was 4.4 for lettuce and 5.2 for radish. No supplemental lighting was used in the radish and lettuce bioassays. All the plants were harvested on December 15, 2003, and dried to obtain dry weight data. Lettuce plants were divided into foliage and root fractions for determining dry weights. Radish seedlings were separated into foliage, tuber and root fractions for dry weight determination.

2.4. Root elongation bioassay and heavy metal concentrations in soil

Soils from two high-pollution sites (DL and AJ) and two low pollution sites (ZOQ and Tlaloc) were composited and analyzed for Pb, Ni, Co, Cr, Cu, Zn, Mn and Al, and a root elongation bioassay was performed to evaluate the potential for heavy metal toxicity of the soils (ASTM, 2003; Green, 2001). Soils were collected in October 2003 at a depth of 0–5 cm and 5–15 cm from under pine canopies. All soils were sieved to 2 mm. Heavy metal concentrations in the soils were determined by the microwave digestion technique described by Fenn et al. (2002a) as modified from Millward and Kluckner (1989). A single composite soil sample from the 0–5 cm depth and the 5–15 cm depth from each site was microwave digested using concentrated HNO_3 and HF according to US EPA method 3052 (US EPA, 1995). Total metal concentrations in the digests were determined by atomic absorption spectrophotometry (Fenn et al., 2002a).

The root elongation bioassays were done by pregerminating wheat (*Triticum aestivum* L. cv. Yecora Rojo) seeds in Petri dishes containing three paper filter disks and 15 ml of 10 mM $CaCl_2$ (Green, 2001). Two days later viable seeds were planted in the native pine soils (80 g soil per container) in cone-shaped planting containers. Two seeds were planted in each of 12 containers for each site and soil depth combination. Four days after planting, the seedlings were harvested, soil was washed from the roots with water, and the length of all the roots was measured. The length of the longest root was used as the test variable. The experiment was repeated two additional times.

2.5. Statistical analyses

Statistical analyses were performed using SigmaStat statistical software, version 2.03 for Windows 95 (Jandel Scientific Software, San Rafael, California). The data in Figs. 2, 5 and 6 were analyzed with one-way ANOVA followed by Tukey's all pairwise multiple comparisons procedures ($p < 0.05$). The plant height data in Fig. 4 was analyzed with one-way Friedman repeated measures ANOVA on ranks followed by Tukey's all pairwise multiple comparisons procedures. For the eucalyptus height growth and biomass data for plants grown in soil from Zoquiapan, the NP treatment was omitted due to an experimental error in transplanting this set of seedlings. For plants growing in soil from Ajusco, two of the six plants in the N fertilization treatment died a few weeks after fertilization; therefore all height growth data for these two plants was deleted. Plant biomass data for these two plants were also deleted from Fig. 2.

3. Results

3.1. Greenhouse bioindicator study of soil growth effects

Total N and S concentrations in soil were higher at DL and AJ than at ZOQ (Table 1), yet the total dry weight of eucalyptus seedlings grown in unfertilized soil from ZOQ was 2.6 and 7.5 times greater than seedlings grown in soil from DL and AJ (Fig. 2). The height growth of eucalyptus seedlings was 1.5 and 3.0 times greater after 38 weeks of growth in unfertilized native forest soil from ZOQ compared to seedlings grown in soil from DL and AJ (Figs. 3 and 4). Height growth of seedlings in soil from ZOQ did not respond to added N or P when these were added singly, but when N and P were added simultaneously height growth tended to increase in the final weeks of the experiment, although the increase was not statistically significant overall ($p = 0.12$; Fig. 4). The total dry weight of eucalyptus plants in soil from ZOQ responded to fertilization with N and NPK, although the response was not as dramatic as for seedlings planted in soil from DL and AJ (Fig. 2).

Height growth of eucalyptus seedlings in soil from DL decreased with N treatment alone, although the difference was not statistically significant, increased with P treatment, and nearly doubled in the NP and NPK treatments (Fig. 4). Total dry weight of seedlings in soil from DL was not affected by N, but the P or N plus P treatments increased growth by 2–3-fold compared to the unfertilized control or the N treatment (Fig. 2). Growth responses to fertilization for seedlings from AJ followed a similar pattern as those from DL, although absolute values for height were lower for eucalyptus seedlings growing in soil from AJ (Fig. 4). Nitrogen fertilization tended to depress biomass production at AJ compared to the control. In fact, two of the six seedlings planted in soil from AJ died seven weeks after the N fertilizer was added. Fertilization with P increased biomass production at AJ five-fold, but total seedling dry weight in the P treatment was still 1.5 and 2.0 times higher at DL and ZOQ compared to AJ (Fig. 2). The dry weight of eucalyptus seedlings in the NPK treatment was significantly greater at ZOQ than at AJ or DL.

Foliar N concentrations increased in the eucalyptus seedlings growing in soil from all three sites in all the fertilization treatments containing N. At DL and AJ but not at ZOQ, foliar N concentrations decreased significantly in the P treatment (Fig. 5). Foliar P decreased in the N treatment for seedlings growing in soil from ZOQ and adding P alone did not increase the P concentration in foliage. Furthermore, P levels in foliage of plants treated with NPK at ZOQ were lower than seedlings treated with P alone (Fig. 5). For seedlings grown in soil from AJ and DL foliar P increased in all treatments that included P, although the increased foliar P at DL in the P only treatment was not significantly greater than the unfertilized control.

Lettuce seedlings from AJ responded with greater biomass production when fertilized with P (2.8 times greater than the control; Fig. 6). In contrast, the N and P treatments did not affect lettuce seedling growth at DL or ZOQ. Because of damping off disease, an insufficient number of replicate lettuce plants survived in the NP treatments at AJ and ZOQ; thus no data are

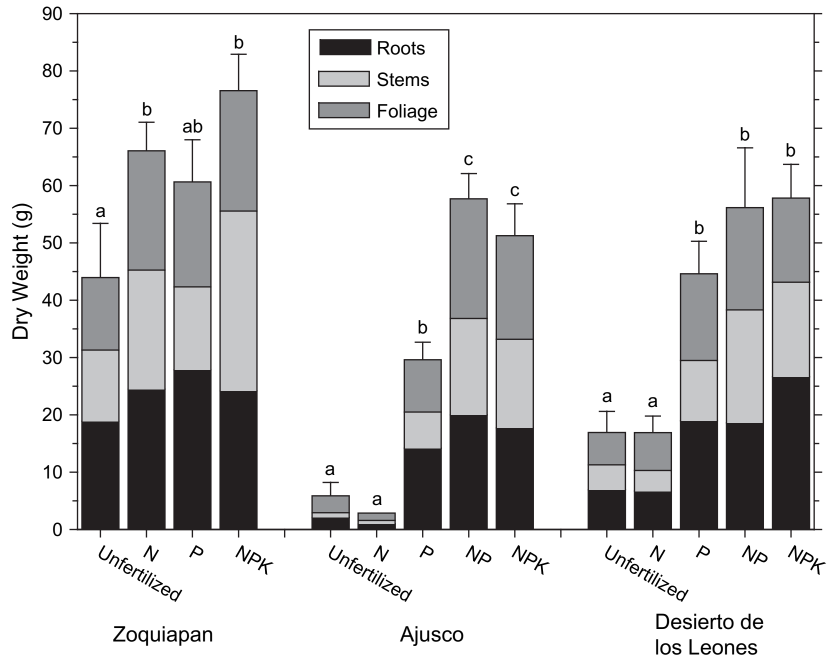

Fig. 2. Eucalyptus foliage, stem and root biomass and total biomass ($n = 3$) after 38 weeks of growth in pine forest soil. Error bars represent the standard error of the mean of total seedling biomass (roots, stems and foliage combined). Letters above the bars indicate significant differences between fertilization treatments within a site based on one-way ANOVA followed by Tukey's all pairwise multiple comparisons procedures ($p < 0.05$).

presented for the NP treatment at these sites. Lettuce seedling survival rates suggest that N fertilization increased damping off disease occurrence. Out of a total number of 12 seedlings planted per site/fertilization treatment combination, 1–4 seedlings survived in the N and NP treatments and 8–10 survived in the P and control treatments (all sites included). Radish growth at AJ responded positively to either P or NP treatments, while at DL and ZOQ radish growth was greater in the NP treatment compared to the other treatments (Fig. 6).

3.2. Heavy metal concentrations in soil and root elongation bioassay

Concentrations of Pb were 2.2–9.1 times higher in surface soil (0–5 cm) at AJ and DL compared to TL and ZOQ and 1.6–7.7 times higher in the 5–15 cm depth (Table 2). The highest Pb concentrations were measured at DL in forest clearings or under fir canopies. Concentrations of Ni were 1.0–3.9 times higher at AJ and DL compared to TL and ZOQ. The highest Ni concentrations were found in soil from AJ, while those from DL were only slightly higher than at ZOQ or TL (Table 2). Concentrations of Zn in soil were 3–4 times higher at DL than ZOQ or TL in several instances and Zn concentrations at AJ were generally 2–3 times higher than at ZOQ or TL. In soil from TL, concentrations of Mn and Cu were generally several fold greater than the other three sites (Table 2). Chromium concentrations were highly similar among the sites except for low concentrations in soil from Tlaloc. Cobalt concentrations were generally 1–2 times higher at AJ and DL than ZOQ or TL, while Al concentrations were highly similar among the four sites. Although statistical analyses of differences in pH were not done because replicate composite soil samples were not analyzed, there appears to be a clear trend of lower soil pH values at AJ and DL. In every site/canopy cover/soil depth combination listed in Table 2 the lowest absolute pH values were in soil from either DL, AJ or both sites.

Among the three wheat seedling root elongation bioassays, no consistent evidence of greater phytotoxicity of the soils from any given site was found. In the first experiment root length was decreased at AJ and DL, but only in the 5–15 cm depth and only in soil from forest clearings (Table 3). In the second bioassay (only soil from the 0–5 cm depth was used), the only difference between sites was the finding of lower root elongation at ZOQ and TL in soil from under fir canopies. In the third experiment, only the pine soil a the 0–5 cm depth was tested and root length was slightly lower in soil from DL compared to ZOQ and TL (Table 3).

4. Discussion

4.1. Nutrient status of forest soils in the Valley of Mexico

With eucalyptus seedlings at AJ and radish at ZOQ and DL, the NP treatment resulted in the greatest

Fig. 3. Photograph showing the difference in growth rates of eucalyptus seedlings after growing for 38 weeks in unfertilized soil from Ajusco, Desierto de los Leones and Zoquiapan (from left to right, respectively).

growth response (Figs. 2 and 6). Trees grown in some forest soils have been shown to grow best when N and P are applied simultaneously (Fisher and Binkley, 2000). At AJ the growth response to added P of eucalyptus, lettuce, and to a lesser degree radish, was striking; demonstrating the severe P limitation at AJ. In eucalyptus growing in soil from AJ and DL and radish at ZOQ and DL, seedlings fertilized with P responded to a further biomass increase when N was also added to the soil (although not statistically significant at DL, the biomass increase in eucalyptus in the NP and NPK treatments at DL support the larger trend across species and sites). This suggests that with an adequate supply of P, N then becomes the growth limiting nutrient in these soils. It should also be noted that the P treatments were applied as calcium phosphate. Thus calcium (Ca) was also added in the P treatments and could cause a positive growth response. However, we consider that to be unlikely, because Ca levels in forest soils in the Basin of Mexico appear to be adequate (Marín et al., 2002). López-López et al. (1998) concluded that at DL Ca was not a limiting nutrient for growth of *A. religiosa*, and that although levels of Ca in soil at DL were lower than those found over the geographic range of this species, Ca concentrations in soil were still at sufficient levels.

Foliar nutrient responses to fertilization confirm the foliar biomass response findings. Added N caused foliar P concentrations to decrease by approximately 50% at ZOQ, presumably as a result of N-induced growth increase and dilution of foliar P concentrations. At both AJ and DL, N concentrations increased significantly in the N fertilization treatment, but biomass did not increase, again demonstrating that N is not growth-limiting at these high pollution sites. When P was added, foliar N decreased by more than half compared to the control, presumably as a result of growth dilution. At AJ and DL, both growth and foliar P concentrations increased in all the treatments that included P, demonstrating P limitation at AJ and DL. The results from this study support the hypothesis that chronic N deposition may more fully alleviate N limitation and increase P limitation of plant growth, although data from additional sites and from long term field fertilization studies are needed to determine possible relationships between N deposition and plant nutrient status in the Mexico City Air Basin.

The above findings concur with previous reports that forest production in the Basin of Mexico can be either P- or N-limited (Marín et al., 2002), although P limitation appears to be more common, as would be expected because of the high P-fixation capacity of Andisols (Brady and Weil, 2002; Shoji et al., 1993). These findings also suggest that notwithstanding the inherently N-rich status of these soils, plus the chronic N deposition and the excess nitrate exported from watersheds at DL (Fenn et al., 2002b), N can become a limiting nutrient for plant growth when these forest soils are supplemented with plant-available P. However, since P fertilization is not the norm, and because of the inherently high N fertility of these soils even with low levels of N deposition (Fenn et al., 1999, 2002b; Table 1; A. Gomez-Guerrero, personal communication, and M.E. Fenn, unpublished data), N deposition probably does not have a consistent or major growth-promoting effect on plants at forest sites across the Basin of Mexico (Fenn et al., 1999, 2002b). To some extent, the response of plant growth to chronic N deposition in the Basin of Mexico may be a species-specific response, based on the ability of a given species and its mycorrhizal symbionts to acquire P from a limited pool of available P. The infrequent or weak plant growth response to added N (Table 4) is in contrast with most N fertilization studies in temperate forests of the northern hemisphere where N limitation is the norm (Vitousek and Field, 2001).

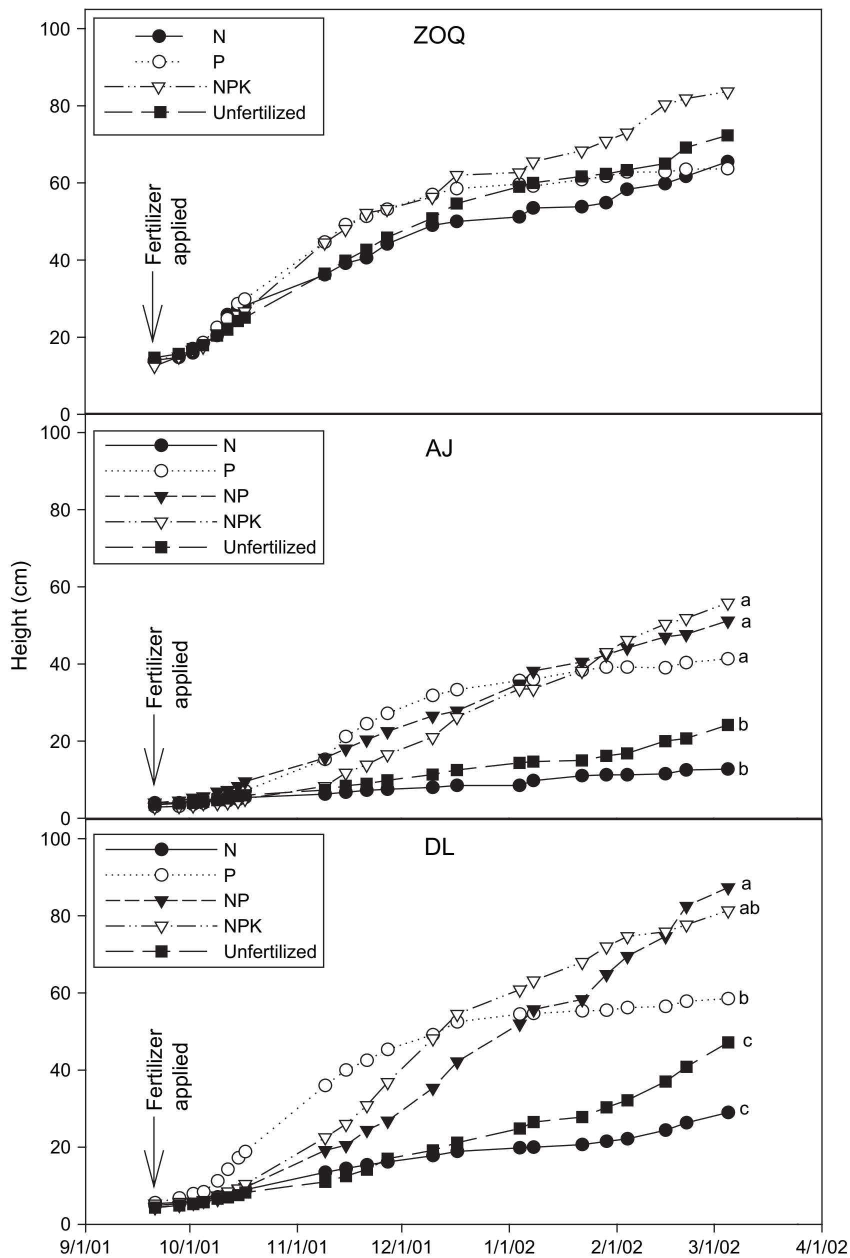

Fig. 4. Eucalyptus height growth rates of seedlings ($n = 6$) growing in soil from three pine forest sites within the Basin of Mexico. Fertilization treatments were added 15 weeks after transplanting the seedlings into the pine soils. Significant differences between the fertilization treatments within a given site were determined using one-way Friedman repeated measures ANOVA on ranks followed by Tukey's all pairwise multiple comparisons procedures. Letters at the end of the lines indicate significant differences between fertilization treatments.

4.2. Cause of differential growth response of eucalyptus to forest soils across the Basin

Differences in growth response was much greater than expected for eucalyptus seedlings grown in native pine forest soils from the three study sites. Particularly striking was the stunted growth of eucalyptus seedlings grown in unfertilized soil from AJ compared to that from ZOQ. We used eucalyptus as a fast-growing bioindicator plant, useful for evaluating soil nutrient supply characteristics in short-term experiments. Three factors were evaluated for their possible role in contributing to the stunted eucalyptus growth in the soil from AJ: (1) nutrient deficiencies; (2) toxicity due to heavy metal accumulation; or (3) inadequate symbiotic fungal populations. Studies on the latter of the three factors has been reported elsewhere (Perea-Estrada, 2003; Perea-Estrada et al., 2005). The percent eucalyptus roots that were mycorrhizal was low in this study, particularly at AJ and DL. The percent ectomycorrhizal was 5–10% at AJ and DL compared to 20% at ZOQ (Perea-Estrada et al., 2005). The percent endomycorrhizal was 10–20%

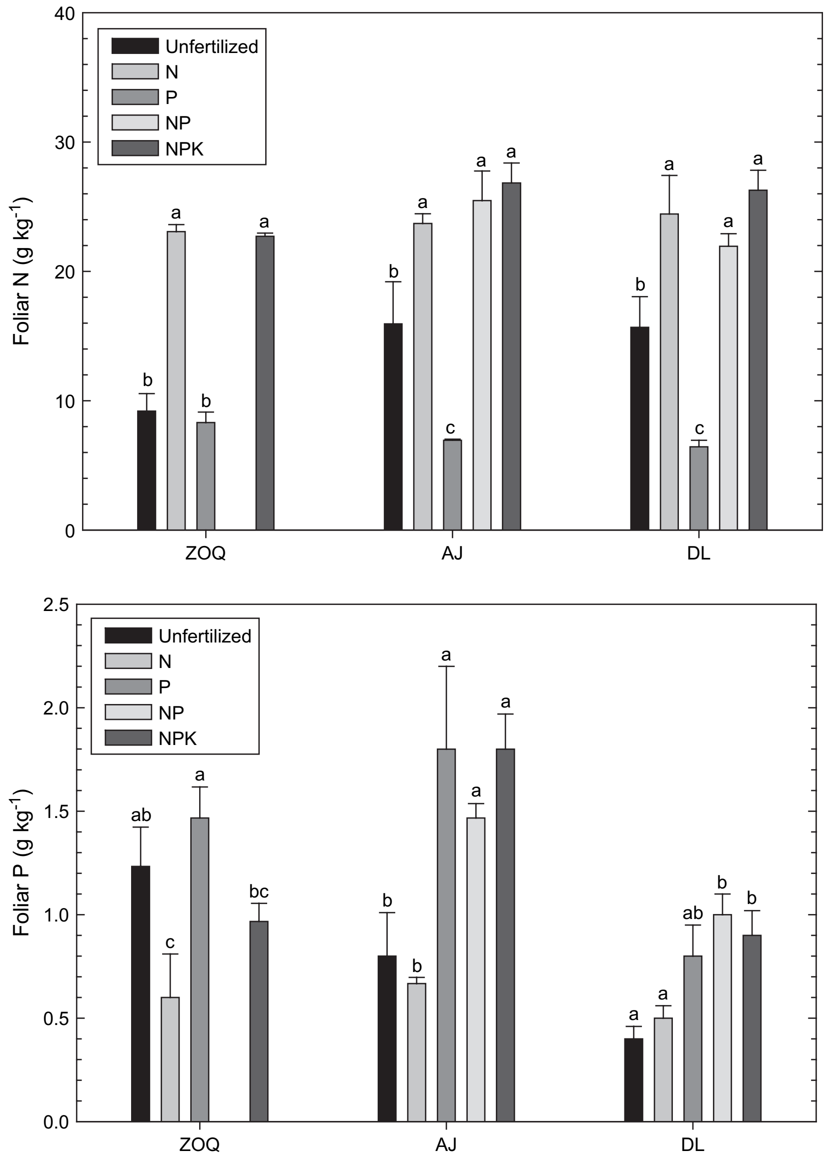

Fig. 5. Concentrations of N and P in eucalyptus foliage from seedlings growing in soil from three pine forest sites within the Basin of Mexico. Letters above the bars indicate significant differences between fertilization treatments within a site based on one-way ANOVA followed by Tukey's all pairwise multiple comparisons procedures ($p < 0.05$).

at AJ and DL compared to 25% at ZOQ. It was also found that many of the short roots were colonized by endophytic fungi often associated with abundant external mycelium. These non-mycorrhizal root fungi are also believed to be symbiotic, but have been little studied to date (Addy et al., 2000; Girlanda et al., 2002; Grünig et al., 2001). Again, the number of roots colonized by these putative symbiotic fungi in association with external mycelium was significantly greater for seedlings grown in soil from ZOQ (as high as 45%) than from AJ or DL. Only after fertilization with P or N + P did these root-associated fungi increase to an equivalent amount at all three sites (Perea-Estrada et al., 2005).

The fertilization studies suggest that a primary P deficiency and a secondary N deficiency were the causes of low eucalyptus biomass production at AJ. However, the elevated heavy metal concentrations in soil at AJ and low levels of symbiotic root fungal associations in seedlings grown in soil from AJ, suggests a possible connection that warrants further study. This hypothesis purports that the nutrient deficiency in eucalyptus at AJ was particularly severe because metal toxicity was causing deleterious effects on symbiotic fungi (Kim et al., 2004), thus inhibiting fungal acquisition and transfer of N and P to the host plant. Lower soil pH at AJ and DL may also increase the bioavailability of trace metals at these sites compared to ZOQ. That heavy metals were not directly toxic to the plant species tested in this study is evidenced by the strong positive growth responses to P, N or N + P, and the lack of consistent

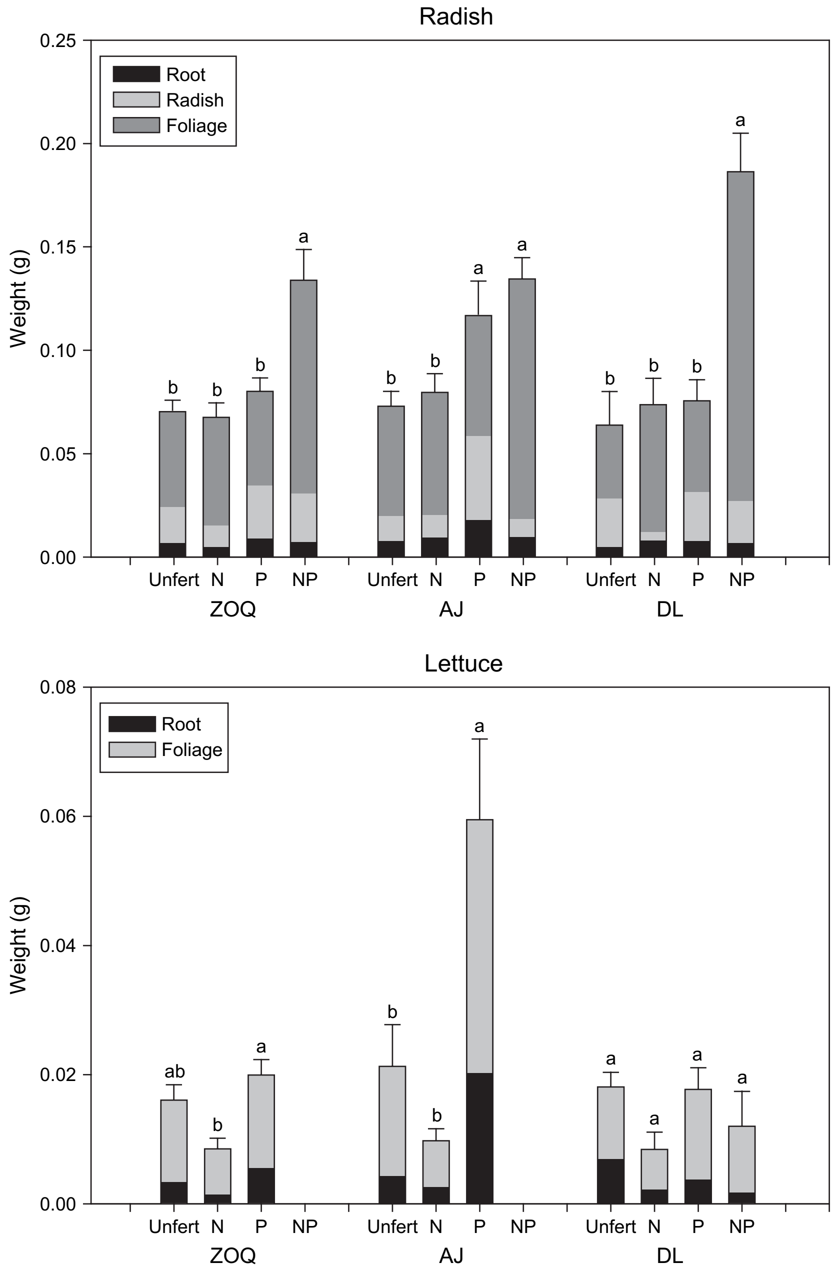

Fig. 6. Growth responses to fertilization of lettuce and radish seedlings grown in pine forest soil from three sites in the Basin of Mexico. Letters above the bars indicate significant differences between fertilization treatments within a site based on one-way ANOVA followed by Tukey's all pairwise multiple comparisons procedures ($p < 0.05$).

evidence of phytotoxicity in the wheat root elongation bioassays. These assays have been shown to detect toxic levels of metals in soil (Ahlrichs, 1990; ASTM, 2003; Green, 2001), although elongation of lettuce (*Lactuca sativa* L.) roots (ASTM, 2003) or some microbial assays (Green, 2001) are in some cases more sensitive to metal toxicity than elongation of wheat roots. Although less likely, it is also possible that the fertilization treatment reduced the bioavailability of the heavy metals, thus increasing growth at AJ. Heavy metal concentrations in this study were similar to those reported for high-pollution sites in previous studies in the Basin of Mexico (Quiroz and Ilangovan, 1999) and in a review of this subject it was concluded that metal concentrations in soil and plant foliage were not likely to be a phytotoxic levels, based on literature studies of heavy metal toxicity tests (Fenn et al., 2002a). However, lead and cadmium concentrations in soil and foliage of overstory and understory species were higher at DL and AJ than at ZOQ (Fenn et al., 2002a).

Table 2
Soil pH and heavy metal concentrations in soil (mg kg^{-1})[a] under *Pinus hartwegii* and *Abies religiosa* canopies and in open areas at four sites in the Valley of Mexico

Site	Canopy cover	Pb	Ni	Co	Cr	Cu	Zn	Mn	Al	pH
0–5 cm depth (mg kg^{-1}, except Al is %)										
ZOQ	Clearing	8.0	15.2	6.5	18.1	10.9	22.2	198.9	2.3	6.08
TL	Clearing	16.5	9.6	5.1	12.5	48.4	37.6	466.4	2.7	5.61
AJ	Clearing	56.0	29.9	7.9	18.4	27.0	68.0	196.1	2.1	5.67
DL	Clearing	73.0	21.4	8.4	21.3	20.9	89.9	327.2	2.5	5.26
ZOQ	*P. hartwegii*	13.3	16.1	6.9	21.0	15.2	32.8	207.2	2.4	5.84
TL	*P. hartwegii*	16.7	10.8	5.6	13.1	32.0	54.5	667.4	2.7	6.46
AJ	*P. hartwegii*	48.1	41.6	9.9	21.3	31.9	74.0	265.4	1.9	5.34
DL	*P. hartwegii*	37.6	21.1	8.6	20.7	15.9	64.8	277.3	2.7	5.73
ZOQ	*A. religiosa*	12.5	16.9	6.9	20.4	15.1	33.1	335.2	2.4	6.21
TL	*A. religiosa*	11.5	10.0	5.1	13.5	69.5	47.7	740.1	2.6	6.53
AJ	*A. religiosa*	45.9	16.9	5.7	16.3	30.1	65.1	201.9	2.2	5.99
DL	*A. religiosa*	86.0	22.8	8.1	21.6	27.9	136.2	402.4	2.5	5.74
5–15 cm depth										
ZOQ	Clearing	10.7	15.2	6.7	19.1	12.3	27.5	220.2	2.3	5.80
TL	Clearing	11.9	13.4	5.1	12.4	40.5	32.9	443.6	2.9	5.78
AJ	Clearing	13.7	25.2	7.3	16.9	15.1	29.5	159.7	1.9	5.48
DL	Clearing	83.0	22.8	8.8	22.0	22.6	91.3	330.9	2.8	5.53
ZOQ	*P. hartwegii*	7.3	15.4	6.7	19.7	13.1	24.3	212.5	2.3	5.93
TL	*P. hartwegii*	10.1	9.6	4.5	11.0	28.2	35.5	534.7	2.5	6.54
AJ	*P. hartwegii*	32.1	36.8	9.4	19.6	21.2	49.1	242.4	1.9	5.36
DL	*P. hartwegii*	15.6	21.3	8.6	20.5	12.6	35.1	262.6	2.7	5.74
ZOQ	*A. religiosa*	8.8	14.5	6.9	19.6	13.1	30.5	289.6	2.7	5.80
TL	*A. religiosa*	8.6	9.5	4.2	10.6	33.5	38.7	607.3	2.2	6.52
AJ	*A. religiosa*	29.7	16.1	5.9	15.7	19.3	47.0	189.5	2.4	5.75
DL	*A. religiosa*	25.7	20.1	7.8	18.5	14.1	49.7	184.0	2.5	5.58

Tlaloc and Zoquiapan are low pollution sites; Desierto de los Leones and Ajusco are highly polluted sites.
[a] Data are single chemical analyses of composite soil samples made up from seven soil samples collected under pine or fir trees and in open areas.

4.3. Implications of these nutrient status studies

A major implication of P limitation in forested watersheds in the Basin of Mexico is the expectation that with continuing or increasing chronic N deposition from the greater Mexico City metropolitan area, N saturation symptoms (e.g., watershed nitrate export) will likely increase in severity due to the limited capacity of vegetation to assimilate the added N. Fenn et al. (2002b) reported that foliar N concentrations in *P. hartwegii* trees did not increase after fertilization with high levels of N at four study sites across the Basin. Foliar biomass growth responses to N fertilization in this same study were not statistically significant except at DL where the response was negative (Fenn et al., 2002b). These results support the contention that vegetative demand for added N in forests within the Basin of Mexico is limited. Thus, it would not be surprising if increasing N deposition results in increased nitrate concentrations in runoff from these watersheds.

The results of the present study suggest that fertilization with P may result in greater tree growth and storage of N and C within woody biomass and litter, and possibly lower nitrate export in streams and to groundwater. This conclusion is supported by the finding of much greater total foliar N content in eucalyptus biomass in the NP treatment compared to the N treatment at AJ and DL (58 and 2.4 times greater, respectively; Perea-Estrada, 2003). Foliar N content was not different among these treatments at ZOQ, where N was limiting but P was not. However, longer-term fertilization studies in the Basin are needed to determine how broadly this conclusion may hold true across sites and plant species. If this hypothesis of limited biotic N retention because of P deficiency is correct, fertilization with P could provide a potential management option for reducing nitrate losses to groundwater and streamwater. However, it is not expected that forest managers in the Basin of Mexico would choose to apply P fertilization at the catchment scale to improve water quality, especially in parks, reserves and recreational areas. Even if P fertilization was demonstrated to increase vegetative N uptake in N saturated areas and reduce N losses, this would probably be of limited practical use considering

Table 3
Length of wheat roots after 5 days of growth in root elongation bioassays

Site	Canopy cover	Root length by soil depth and experiment number[a]			
		0–5 cm, Exp. 1	5–15 cm, Exp. 1	0–5 cm, Exp. 2	0–5 cm, Exp. 3
ZOQ	Clearing	10.8 (0.86)	13.33 (0.88)a	12.10 (1.14)	
TL	Clearing	12.0 (0.82)	12.75 (0.73)a	10.88 (1.85)	
AJ	Clearing	10.0 (0.91)	9.60 (1.18)b	12.48 (2.19)	
DL	Clearing	9.75 (1.18)	8.33 (1.67)b	13.50 (1.24)	
ZOQ	*P. hartwegii*	11.39 (0.87)	10.00 (1.34)	11.99 (1.14)	14.38 (0.91)a
TL	*P. hartwegii*	12.50 (0.75)	10.67 (0.67)	10.37 (1.34)	14.72 (0.88)a
AJ	*P. hartwegii*	10.13 (0.55)	12.83 (0.61)	9.38 (1.72)	13.20 (0.90)ab
DL	*P. hartwegii*	11.44 (0.68)	9.75 (1.36)	10.51 (0.91)	11.43 (1.03)b
ZOQ	*A. religiosa*	10.78 (0.92)	11.50 (0.76)	7.90 (1.77)ab	
TL	*A. religiosa*	11.00 (0.54)	9.80 (0.98)	6.71 (1.39)a	
AJ	*A. religiosa*	11.89 (0.66)	10.83 (0.28)	13.05 (1.12)bc	
DL	*A. religiosa*	11.94 (0.73)	11.50 (0.78)	14.78 (1.01)c	

[a] Values in parentheses are standard errors of the mean. Lowercase letters following the standard error data indicate significant differences between sites within a given canopy cover/soil depth combination.

that forest fertilization is not generally practiced in the Basin of Mexico.

The development of N saturated forest conditions as a result of elevated N deposition has been reported to lead to P limitation of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the Netherlands (Houdijk and Roelofs, 1993; Mohren et al., 1986). Although in contrast to these studies in the Netherlands, P limitation occurs in the Basin of Mexico without elevated N deposition or symptoms of N saturation, more detailed studies at a greater number of sites in the Basin would be required to determine if in some areas N deposition might alleviate N deficiency and result in more acute P deficiency. In this study there were no instances of increased growth with N fertilization accompanied by even greater growth in the NP treatment. Thus, we found no clear evidence of N fertilization-induced P limitation. However, the response to P fertilization of plants growing in soils from DL and AJ, where elevated N deposition occurs, are consistent with a pattern of increased P limitation in response to alleviated N deficiency because of chronic atmospheric N deposition. This pattern merits further investigation, and soil type distribution and the amount of forested lands in the Basin that are predominantly P or N limited warrants further study. Marín et al. (2002) reported that forest soils in the Basin of Mexico are mainly Andisols and Inceptisols and that pines respond mainly to N but also to P. Radish plants growing in soil from DL and ZOQ did not respond to either N or P alone, but growth increased significantly in the NP treatment. However, the role of each of these two nutrients in causing growth increases with the combined treatment cannot be discerned from these results alone.

Table 4
Summary of fertilization growth responses of eucalyptus, radish and lettuce seedlings grown in forest soil from *P. hartwegii* stands[a]

Site	Species	Growth response to fertilization			
		N	P	NP	NPK
Zoquiapan	Eucalyptus	+	0	0	+
	Radish	0	0	+	
	Lettuce	−	0		
Ajusco	Eucalyptus	−	+	+	+
	Radish	0	+	+	
	Lettuce	0	+		
Desierto de los Leones	Eucalyptus	0	+	+	+
	Radish	0	0	+	
	Lettuce	0	0	0	

[a] No growth response is indicated by 0, positive growth response by +, and a negative growth response by −. Absence of any symbol indicates that data is not available for that site/treatment combination, either because the NPK treatment was not applied in the lettuce and radish experiments or because of missing data.

5. Conclusions

Phosphorus was the most common nutrient deficiency for vegetation growing in native soil from highly-polluted *P. hartwegii* stands (AJ and DL) in the Basin of Mexico, although N alone stimulated growth of eucalyptus at ZOQ (a low pollution site). More commonly, however, N appeared to limit growth once P deficiency was alleviated. Eucalyptus seedlings growing in unfertilized soil from the high-pollution Ajusco site were particularly stunted compared to Zoquiapan, a low pollution site.

Fertilization of soils from Ajusco with P, stimulated eucalyptus growth, but adding N did not, unless N and P were both applied to soil. Concentrations of Pb, Ni and Zn were highly elevated in soils from DL and AJ, but do not appear to be directly phytotoxic. However, based on previously published results of this study (Perea-Estrada et al., 2005) further research is needed to see if metal toxicity could be affecting the function of symbiotic root fungi at AJ and thus impeding the capacity for N and P uptake from soil. If P deficiency is common in watersheds in the Basin of Mexico this may facilitate nitrate leachate loss and export from the watershed under conditions of chronic atmospheric N deposition because of limited biotic capacity for N retention.

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