Figure 3.2  a) Mean percent root colonization by AM fungi, b) mean shoot phosphorus concentration, c) mean shoot nitrogen concentration and d) mean total shoot nitrogen after six months of growth for *Terminalia amazonia* seedlings inoculated with forest AM fungi ($n_{\%RC} = 16$), with pasture AM fungi ($n_{\%RC} = 15$), or no AM fungi ($n_{\%RC} = 13$). For all leaf nutrient measurements, $n = 8$. Means labeled with the same letter do not differ at $\alpha = 0.05$. Error bars indicate 95% confidence intervals.
Root colonization by AM fungi differed among the three types of inocula ($F_{2,32} = 143.56, P < 0.0001$). The pasture AM fungal community was able to colonize *T. amazonia* seedlings, but did so to a lesser extent than did the forest AM fungal community (Figure 3.2a). Seedlings inoculated with forest AM fungi had an average of 66% of the root system colonized by AM fungi at harvest, while, on average, only 19% of the root system was colonized by AM fungi in seedlings inoculated with pasture AM fungi ($F_{1,32} = 99.71; P < 0.0001$). Root colonization in uninoculated seedlings was negligible.

Shoot phosphorus concentration differed among the treatments ($F_{2,12} = 30.25, P < 0.0001$), in a pattern similar to that observed for root colonization by AM fungi. At harvest (ie. six months after inoculation), mean phosphorus concentration of seedlings inoculated with the forest AM fungal community was almost twice that of seedlings that were inoculated with the pasture AM fungal community ($F_{1,12} = 23.42, P = 0.0004$; Figure 3.2b). Mean phosphorus concentration of seedlings inoculated with pasture AM fungi was slightly higher than the mean phosphorus concentration of uninoculated seedlings ($F_{1,12} = 8.146, P = 0.0145$).

Both nitrogen concentration and total nitrogen content of shoots differed among the three inocula ($F_{2,12} = 14.25, P = 0.0007, F_{2,12} = 7.35, P = 0.0083$, respectively). Mean shoot nitrogen concentration was lowest for seedlings inoculated with forest AM fungi ($t_{23} = 5.01, P = 0.0003$) and differed little between seedlings inoculated with pasture AM fungi and uninoculated seedlings ($t_{23} = 1.86, P = 0.0882$; Figure 3.2c). In contrast, mean shoot nitrogen content was highest for seedlings inoculated with forest AM fungi and lowest for uninoculated seedlings (Figure 3.2d).
Concentrations of other nutrients measured (K, Ca, Mg, Cu, Zn) did not differ among seedlings from different inoculum types (data not shown). However, boron concentration did differ among seedlings from the three inoculum types ($F_{2,12} = 9.23, P = 0.0037$), in a manner similar to nitrogen. There was no difference in boron concentration between uninoculated seedlings and seedlings inoculated with pasture AM fungi ($t_{23} = 1.52, P = 0.1540$), but boron concentration was lower in seedlings inoculated with forest AM fungi than in the other two treatments ($t_{23} = 4.01, P = 0.0017$).

Of the three potentially phytotoxic elements (Al, Fe, Mn) that frequently occur at high levels in soils at the field sites (Table 2.2), manganese did not show a difference in concentration in shoots of seedlings from the three inoculum types ($F_{2,12} = 0.96, P = 0.4087$; data not shown). However, concentrations of aluminum and iron did differ among seedlings from the three inoculum types ($F_{2,12} = 6.43, P = 0.0127$, and $F_{2,12} = 6.36, P = 0.0131$, respectively). Mean shoot aluminum and iron concentrations were lower for seedlings inoculated with forest AM fungi than for seedlings in the other two inoculum types ($t_{23} = 3.59, P = 0.0037$ and $t_{23} = 3.55, P = 0.0040$; Figure 3.3). Mean shoot concentrations of aluminum and iron did not differ between seedlings inoculated with pasture AM fungi and uninoculated seedlings ($t_{23} = 0.02, P = 0.9869$ and $t_{23} = 0.39, P = 0.7004$, respectively).

**DISCUSSION**

This study found that AM fungal spore inocula from forest and pasture trap cultures differed in their ability to benefit seedlings of a tropical forest tree species. Seedlings inoculated with forest AM fungi exhibited higher levels of root colonization by AM fungi and phosphorus uptake, greater leaf area and
Figure 3.3 Mean shoot a) aluminum concentration and b) iron concentration after six months of growth for *Terminalia amazonia* seedlings inoculated with forest AM fungi, pasture AM fungi, and no AM fungi (*n* = 8). Means labeled with the same letter do not differ at $\alpha = 0.05$. Error bars indicate 95% confidence intervals.
biomass after six months of growth, and lower aluminum and iron uptake than either uninoculated seedlings or seedlings inoculated with pasture AM fungi. Although the simplified pasture AM fungal community used in this study was able to colonize seedlings, seedlings inoculated with these fungi differed only slightly from uninoculated seedlings in phosphorus uptake and not at all in biomass.

The primary benefit of mycorrhizas to seedlings inoculated with forest AM fungi in this experiment appears to have been improved phosphorus uptake. Both the concentration and total amount of phosphorus in shoot tissue tended to be much higher in seedlings inoculated with forest AM fungi. Phosphorus uptake has been shown to be greatly improved for mycorrhizal seedlings relative to nonmycorrhizal seedlings in previous studies of tropical forest tree species (e.g., Siqueira and Saggini-Júnior 2001, de Grandcourt et al. 2004).

There was some evidence of either nitrogen limitation in seedlings inoculated with forest AM fungi or luxury consumption by seedlings in the other two treatments. Both boron and nitrogen concentrations were higher in seedlings inoculated with pasture AM fungi and uninoculated seedlings, while total amounts of these nutrients in the shoots did not differ, suggesting that all seedlings may have exhausted the available supplies of these nutrients.

The seedlings inoculated with pasture AM fungi were intermediate between the controls and the seedlings inoculated with forest AM fungi with respect to root colonization and phosphorus supply. Yet, they did not differ from uninoculated seedlings in growth. This result suggests that pasture AM fungi may be more expensive (in terms of carbon cost per unit gain of phosphorus) for these seedlings to maintain than forest AM fungi. Although AM fungi are generally regarded as mutualists, they have been shown to differ
in both cost and benefit to their hosts (van der Heijden et al. 1998a) (Johnson et al. 1997).

While the biology of the other AM fungal species used in this study has not been explored, pot studies with *S. calospora* (the most abundant species in pasture inoculum) have found that this species takes up phosphorus only at short distances from the root and may be represent a larger carbon cost to plant hosts than other AM fungi (Jakobsen et al. 1992, Smith et al. 2000). At least some *Acaulospora* species (*Acaulospora* was present in both forest and pasture inocula) may be capable of transporting phosphorus over longer distances than *Scutellospora* (Jakobsen et al. 1992). In a chronosequence study of temperate old field succession, Johnson et al. (1991) found that spore abundance of *S. calospora* decreased with increasing time since field abandonment. They regarded *S. calospora* as an early successional species, which may make it relatively ineffective as a mutualist of tree seedlings.

Alternatively, inoculation with forest AM fungi may have provided some additional benefit to the seedlings beyond phosphorus uptake. Levels of aluminum and iron are high in soils at the sites of origin (Table 2.2) and were high in the mix derived from these soils and used in this experiment (described in methods). Forest AM fungi may play a role in protecting seedlings from accumulating excessive levels of phytotoxic elements during phosphorus uptake. In addition to boosting phosphorus uptake, inoculation of seedlings with forest AM fungi appeared to lower concentrations of nutrients found at excessive levels in autoclaved field soil. The protection of plants from metal toxicity by AM fungi is well-documented, but the mechanisms are not fully understood (Clark and Zeto 2000, Entry et al. 2002). Differences between AM
fungal isolates in plant benefit under acidic soil conditions have been observed previously (Clark et al. 1999).

Differences in plant benefit between the two AM fungal inocula used in this study may be related to differences in efficacy of AM fungi for different plant hosts. Both type of AM fungi and plant host species appear to have strong effects on plant response to mycorrhiza formation (van der Heijden et al. 1998b, Hart and Reader 2002a, Klironomos 2003). A comparison of forest tree seedlings and pasture plants with AM fungal inocula of forest and pasture may find that pasture AM fungi provide greater benefit to pasture plants than forest AM fungi.

Although the spore communities used in this study were derived from AM fungal communities observed in the field, they did not include all of the species observed in the field. Trap cultures are not equally effective for all members of an AM fungal community (Brundrett et al. 1999). A future study would need to use multiple years of trap cultures, capturing different AM fungal species as they became active, in order to create inocula more representative of the total AM fungal community in each habitat type.

These results suggest that the shift in the AM fungal community as a result of conversion of forest to pasture may have a strong effect on the ability of _T. amazonia_ seedlings to recolonize pasture sites. The extent to which changes in the mycorrhizal fungal community can limit colonization of pastures by forest plant species urgently needs study. In conserving biodiversity in tropical regions, the importance of conserving diversity of belowground organisms, such as mycorrhizal fungi, should not be overlooked.