**Trap culturing**

To characterize the species composition of AM fungi occurring at my sites as hyphae as well as spores, I established trap cultures using 36 of the 42 pooled soil samples (for logistic reasons, no trap cultures were established for the wet season of 1999). Trap cultures were started 1-28 d from time of soil collection from the field. All trap cultures were established at the Las Cruces Biological Station, San Vito, Costa Rica, except those for the wet season of 2000 which were established at Cornell University. Six hundred to one thousand milliliters of each pooled soil sample was mixed 2:1 (v/v) with inert material (e.g. vermiculite or perlite), sown with 25 seeds of sorghum-sudangrass (*Sorghum bicolor* (L.) Moench), and grown in 2-L pots in the greenhouse (under ambient conditions at Las Cruces; under 12-h daylength at 24° C at Cornell). The amount of soil sample, the type of inert material, and the culturing time and conditions varied from season to season, but were held constant across the six plots within a season. One control pot was established each season, using field soil that had been steam-sterilized (in a pressure cooker twice at 15 psi (~103 kPa) for 30 min at Las Cruces) or autoclaved (twice at 121° C for 1 h at Cornell).

Trap cultures were air-dried after 3-6 mo of plant growth, and the undisturbed root system and associated soil were stored in sealed plastic bags at room temperature. Roots of control plants were stained with trypan blue (Phillips and Hayman 1970, Grace and Stribley 1991) at harvest and found to be free of AM fungi. Immediately prior to spore extraction, each trap culture's root system was chopped into ~5 cm segments and mixed with associated soil.
**Spore enumeration**

To document the species composition of AM fungal communities in forest and pasture, spores were extracted by wet-sieving and sucrose-gradient centrifugation (procedure modified from Daniels 1982) from 41 of the 42 pooled field samples (the wet season 1998 Delgado forest field sample was lost) and the 36 trap cultures. Fifty to one hundred mL of each sample was weighed, and then mixed thoroughly with 0.5 g of the soil dispersant sodium hexametaphosphate (Calgon) and 500 mL of water. After soaking for 30 min, the sample was stirred again, and the water layer was poured through 850-µm and 45-µm sieves. Stirring and decanting were repeated until no fine particles could be observed in the water layer.

After a visual check for spores, the material deposited on the 850-µm sieve was discarded (only one spore ≥850 µm in dm was observed during the course of this study). Material caught on the 45-µm sieve was divided equally among four 50-mL centrifuge tubes, each containing 25 mL of water, and centrifuged at 2100 rpm for 3-5 min. The supernatant was discarded after checking that it contained no spores, and the pellets were resuspended in 60% (w/v) sucrose and centrifuged again at 2100 rpm for 3 min. The pellets were discarded after ensuring they contained no spores. The supernatant was poured through 500-µm, 250-µm, 105-µm and 45-µm sieves, and the sieves were rinsed with water to remove residual sucrose. Material on sieves was decanted into water in petri dishes and stored ≤ 72 h at 4 °C. Spores were separated from debris with fine forceps under a stereomicroscope, transferred by pasteur pipette to Whatman 50 filter paper, and mounted in polyvinyl-lactoglycerol (PVLG) and 1:1 (v/v) PVLG/Melzer's reagent on microscope slides.
Viable spores (based on surface appearance and spore contents) were identified to species using a Nikon Eclipse 600 microscope with differential interference contrast (DIC), by comparison with voucher specimens, at the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) at West Virginia University, Morgantown, West Virginia, USA. A subsample was taken from each soil sample, weighed, dried at 80°C for 48 h, and weighed again. The number of spores per species per sample was expressed as the number per 50 g of soil, calculated using the ratio of dry weight to fresh weight determined from the subsample. For *Glomus clavisporum*, which produces sporocarps (each containing >1000 spores) that typically survive the extraction process intact, I recorded the number of sporocarps rather than spores.

**Data analysis**

Effects of vegetation type, site and season on total spore abundance of AM fungi were detected by three-factor ANOVA in JMP version 3, with all independent variables treated as fixed effects (SAS Institute 1994). To satisfy model assumptions, data were log-transformed prior to analysis. Differences between plots within sites were detected by contrasts.

Because spore abundances of different AM fungal species may not be independent of one another, I first tested for effects of vegetation type, site and season on spore abundance of individual AM fungal species in field soils and trap cultures using three-factor multivariate analysis of variance (MANOVA; Bever et al. 1996). I then conducted univariate analyses of variance using reduced models that included only the factors found to be of statistical significance at $\alpha = 0.05$ in the MANOVA and their interaction terms (SAS Institute 1994). Species for which I observed ten or fewer spores, or
which occurred in fewer than five samples, were considered too rare to be included in the analysis. To satisfy model assumptions, data were ranked prior to analysis. Differences between plots within sites were detected by contrasts.

I used EstimateS version 6.0 to calculate values of the Morisita-Horn index of similarity ($C_{MH}$) for all pair-wise combinations of AM fungal spore communities from the field soils and the Jaccard index of similarity ($C_j$) for all pair-wise combinations of AM fungal spore communities from the trap cultures from the six plots (Colwell 2000).

$$C_{MH} = 2\sum (a_n \times b_n) / (\sum a_n^2 / aN^2 + \sum b_n^2 / bN^2) \times aN \times bN$$

where $aN$ is the number of individuals in plot A, $bN$ is the number of individuals in plot B, $a_n$ is the number of individuals of the $i$th species in plot A, and $b_n$ is the number of individuals of the $i$th species in plot B.

$$C_j = j / (a + b - j)$$

where $j$ is the number of shared species between plots A and B, $a$ is the number of species in plot A, and $b$ is the number of species in plot B. I used the abundance-based Morisita-Horn indices for the spore communities from field soils, but the qualitative Jaccard indices for spore communities from trap cultures, because abundance of some AM fungal species in trap cultures is likely to reflect culturing conditions rather than those species' importance in the field. Both indices assume values between zero and one, with zero indicating no similarity and one indicating 100% concordance between two plots.
RESULTS

Spore abundance of AM fungi

Spore abundance of AM fungi differed between forest and pasture (Table 1.2). Spore abundance was higher in pasture soils than forest soils at the Delgado and Ramírez sites, but did not differ between pasture and forest at the Cascante site (Figure 1.2a). AM fungal spore abundance was also higher in pasture trap cultures than forest ones for the Delgado site, but did not differ between the two vegetation types for the other two sites (Figure 1.2b). As evidenced by the size of the 95% confidence intervals, variance in spore abundance between samples was usually greater in pasture samples than in forest ones in both the field and trap cultures (Figure 1.2).

The effect of vegetation type on spore abundance of AM fungi in trap cultures differed between soils collected in the wet and dry seasons (Table 1.2). There was no difference in mean spore abundance between pasture and forest for trap cultures from soils collected during the dry season (Figure 1.3). However, spore abundance was higher in trap cultures from pasture than in those from forest for soils collected during the wet season. For trap cultures from forest soil collected during the wet season, mean spore abundance was similar to the abundance observed in both forest and pasture trap cultures for samples collected in the dry season \(t = 1.67, P = 0.1076, df = 26\). The difference in seasonality of spore abundance between vegetation types was not as evident in the field (Table 1.2).

Species composition of AM fungi

A total of 26,915 AM fungal spores in field and trap culture soil samples were identified, representing 37 species in six genera and four families (Table 1.3). Nine of these species (25%) are undescribed.