Links between tree species, symbiotic fungal diversity and ecosystem functioning in simplified tropical ecosystems

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Summary

• We studied the relationships among plant and arbuscular mycorrhizal (AM) fungal diversity, and their effects on ecosystem function, in a series of replicate tropical forestry plots in the La Selva Biological Station, Costa Rica.

• Forestry plots were 12 yr old and were either monocultures of three tree species, or polycultures of the tree species with two additional understory species. Relationships among the AM fungal spore community, host species, plant community diversity and ecosystem phosphorus-use efficiency (PUE) and net primary productivity (NPP) were assessed.

• Analysis of the relative abundance of AM fungal spores found that host tree species had a significant effect on the AM fungal community, as did host plant community diversity (monocultures vs polycultures). The Shannon diversity index of the AM fungal spore community differed significantly among the three host tree species, but was not significantly different between monoculture and polyculture plots. Over all the plots, significant positive relationships were found between AM fungal diversity and ecosystem NPP, and between AM fungal community evenness and PUE. Relative abundance of two of the dominant AM fungal species also showed significant correlations with NPP and PUE.

• We conclude that the AM fungal community composition in tropical forests is sensitive to host species, and provide evidence supporting the hypothesis that the diversity of AM fungi in tropical forests and ecosystem NPP covaries.

Key words: *Acaulospora morrowiae*, *Acaulospora scrobiculata*, *Acaulospora spinosa*, arbuscular mycorrihzas, *Cedrela odorata*, *Cordia alliodora*, *Hyeronima alchorneoides*, tropical trees.

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Introduction

Arbuscular mycorrhizal (AM) fungi are an important component of tropical soil microflora, enhancing mineral nutrient uptake of their hosts, particularly phosphorus, and host plant growth (Janos, 1980a; Lovelock *et al.*, 1996; Gehring, 2003). Experiments in mesocosms and pots indicate that AM fungi are likely to have a large influence on the maintenance of diversity and productivity of plant communities (Grime *et al.*, 1987; van der Heijden *et al.*, 1998a, 1998b; Kiers *et al.*, 2000; Klironomos *et al.*, 2000; Pringle & Bever, 2002; Bever, 2003), and that high levels of plant diversity will, in turn, enhance fungal diversity (Bever *et al.*, 1996; Bever, 2003). Although AM fungi are not host-specific, different fungal isolates have been observed to be more beneficial to some hosts than others (Gange *et al.*, 1993; Kiers *et al.*, 2000), and AM fungal communities have been observed to vary significantly depending on host species (Sieverding, 1989; Bever *et al.*, 1996; Eom *et al.*, 2000; Husband *et al.*, 2002a; Lovelock *et al.*, 2003).

In tropical forests, an improved understanding is developing of the diversity of AM fungi (Picone, 2000; Husband *et al.*, 2002a; Lovelock *et al.*, 2003); and of how AM fungal communities are distributed temporally (Husband *et al.*, 2002b) and with host species over the landscape. However, our understanding of how different fungal communities might affect ecosystem function in tropical forests is still not well developed, and is based largely on studies with seedlings (Janos, 1980a; Lovelock *et al.*, 1996; Kiers *et al.*, 2000; Gehring, 2003). From the negative-feedback model (Bever, 2003) we would expect that enhancing host species diversity would lead to enhanced AM fungal diversity. This was observed recently in temperate forests (Landis *et al.*, 2004) and grassland plant communities (Burrows & Pfleger, 2002). Additionally, based on the theories of Tilman *et al.* (1997), we expect that enhanced diversity of AM fungi should lead to increased nutrient capture by the AM fungal community (particularly of limiting P, which should be manifest as an increase in the efficiency of P use) and ultimately to enhanced ecosystem productivity.

Sited within La Selva Biological Reserve in north-east Costa Rica, our project is aimed at investigating the relationship between plant diversity and sustainable productivity in tropical forests. On the edge of an undisturbed tropical forest we have replicate monocultures of each of three commercially important tree species: *Cedrela odorata* L. (Meliaceae); *Cordia alliodora* (R. & P) Cham. (Boraginaceae); *Hyeronima alchorneoides* Allemão (Euphorbiaceae). The same three tree species are also grown in combination with two species with life forms: a palm, *Euterpe oleracea* Mart (Arecaceae); and a giant perennial herb, *Heliconia imbricate* (Kuntze) Baker (Heliconiaceae). We used these closed-canopy experimental plots, which are all on the same soil type and have been established for 12 yr before sampling, to investigate whether AM fungal communities are sensitive to host tree species and to plant diversity within the plots. Additionally we performed some exploratory regression analyses to investigate how the characteristics of the AM fungal community are related to the ecosystem phosphorus-use efficiency (PUE) and net primary productivity (NPP) of the plots. These analyses are preliminary because causality cannot be inferred from statistical significance of regressions among variables. However, they may provide insights from which we can generate hypotheses for future work.

We characterized the AM fungal community in the forestry plots with a one-time sampling of AM fungal spores, with identification of spores based on morphological characteristics. Using the relative abundance of AM fungal spores to characterize the AM fungal community is controversial (Sanders, 2004) because not all fungi present may be sporulating at the time of sampling (Bever *et al.*, 1996), and the relative abundance of spores may not reflect the true abundance of the fungi hyphae (Clapp *et al.*, 1995; Merryweather & Fitter, 1998a, 1998b). Additionally, the morphology of spores may underestimate the diversity of the AM fungal community because spores have relatively few distinguishing morphological characters, despite large genetic variation (Rodriguez *et al.*, 2005). An alternative method of enumerating AM fungal communities is by using molecular tools. However, these methods are also controversial because of high levels of polymorphism in AM fungal rDNA (Sanders, 2002), and because results are strongly influenced by the choice of methods used (discussed by Landis *et al.*, 2004). We justify our use of spores to identify the AM fungal community in this study because spores are an important feature of the life history of AM fungi. Moreover, we have a species list based on morphological characteristics for this site (Lovelock *et al.*, 2003), and many of the species are within the genus *Acaulospora*, members of which have spore-wall ornamentation that facilitates identification. Characterizing the AM fungal community by its spores is similar to characterizing a forest plant community from collections of seeds or other plant parts. Although this is a common approach in paleontological research (Burnham *et al.*, 1992), we have no evidence that the relative abundance of spores correlates with the relative abundance of the AM species within the hyphal network in soils and roots. Because of this limitation, we anticipate that the species diversity reported here is an underestimate of the true diversity. However, enumerating spore communities has been used successfully to distinguish differences in AM fungal communities across broad gradients in hosts (Bever *et al.*, 1996; Eom *et al.*, 2000; Lovelock *et al.*, 2003) and environmental conditions (Egerton-Warburton & Allen, 2000). Therefore the use of spores as a surrogate for the AM fungal community should be sufficiently sensitive to test whether host species, and the diversity of host species, affect the AM fungal community, and whether differences in the AM fungal community can be linked to resource use and productivity of forest communities.

Materials and Methods

Arbuscular mycorrhizal fungal spores were collected in September 2000 from soil within experimental plots at La Selva Biological Station (10°26′ N, 83°59′ W) of the Organization for Tropical Studies in north-east Costa Rica. The experimental plots were established in 1991 using a randomized block design on an abandoned cacao plantation (Haggar & Ewel, 1994). The soils of the experimental plots are recent alluvium (Haggar & Ewel, 1994, 1997; Sollins *et al.*, 1994; Hiremath & Ewel, 2001) and are thus relatively fertile compared with other tropical forests (13.7 g g^{-1} potassium chloride-extractable N; and 14.4 g g^{-1} acid ammonium fluoride-extractable P, soil depth 0–10 cm). Annual rainfall at the site is ≈4 m distributed fairly evenly throughout the year, with the exception of a short dry season in February and March (McDade *et al.*, 1994). Three replicate plots (40 × 60 m) of *C. odorata*, *C. alliodora* and *H. alchorneoides* were established. Trees were planted so that each individual was 2 m from its six nearest neighbors, resulting in a density of 2887 trees ha^{-1}. Each of the nine plots was divided in half. One half was left as a monoculture of trees, while the other was underplanted with the palm *E. oleracea*

and the giant herb *H. imbricata* in an additive design to create polycultures. In the polycultures, palms were planted in alternate rows, in alternate spaces between trees (i.e. one-fourth the tree density); heliconias were planted in rows that were not planted with palms, in every available space between trees (i.e. half the tree density). By mid-1993 canopy closure had occurred. After mid-1993 NPP (above- and below-ground biomass increments plus litterfall), nutrient uptake, and soil nutrient supply were monitored until 2000. From these data the ecosystem efficiency of nutrient use was calculated (Hiremath & Ewel, 2001, after Bridgham *et al.*, 1995):

Ecosystem PUE = NPP/rate soil P supply

Soil P supply was measured as the concentration of extractable P (Hiremath & Ewel, 2001).

Arbuscular mycorrhizal communities were characterized in May 2001 from samples from seven 10 cm deep soil cores (2.5 cm diameter) taken randomly from within each of the three replicate 40 × 30 m plots, for each of three tree species growing alone, or with the two other species (polycultures including the palm and herb). Leaf litter was not included in the cores.

Isolation of spores, identification and enumeration of AM fungal morphospecies

Spores were isolated from 50 ml of the collected soils. Extraction of spores was by wet sieving to 45 μm followed by centrifugation on a 20 : 60% sucrose gradient (Daniels & Skipper, 1982). The supernatant was washed in water and placed in Petri dishes from which spores were extracted under a dissecting microscope. Spores are often degraded in field material, making identification difficult. For this reason live spores were collected; parasitized spores were collected only when there appeared to be sufficient characters remaining to allow identification. Preliminary groupings and identifications were made on fresh material, but all spores were mounted in Melzer's reagent (which differentially stains wall structures and is diagnostic for AM species; Morton, 1998) and examined under a compound light microscope at ×100 to ×1000 magnification to confirm identifications, and as a further confirmation of whether spores were alive or dead.

Identification was made following the species descriptions provided by the International Culture Collection of Arbuscular- and Vesicular-Arbuscular Fungi (http://invam.caf.wvu.edu). Because of the generally poor state of field material (spores differ in age and state of degradation), and the low relative abundance of some morphospecies, we chose to use a conservative approach to identifying species, naming a morphospecies only if there were sufficient spores in good enough condition to be certain of their identity. Consequently the number of species reported here is likely to be lower than that actually present (Bever *et al.*, 2001). For the genera *Paraglomus* and *Glomus*, spores of only one species were conclusively identified (*Glomus tortuosum*); the rest were placed in one of four working groups: (1) *Paraglomus* '*occultum*-like', likely to be *Paraglomus occultum*, but could be an amalgam of similar morphospecies (*Archeospora trappii*, *Entrophospora schenckii* or *Glomus spurcum*); (2) *Glomus* '*clarum*/*intradicies*-like'; (3) *Glomus* '*etunicatum*/*geosporum*-like'; and (4) *Glomus* 'other' for all other unidentified *Glomus* spores. Additionally, three undescribed morphospecies of *Acaulospora* were found. *Acaulospora* sp. 1 is similar to *Acaulospora morrowiae* (deep purple stain in Melzer's reagent) but is larger, pale yellow when live, with a distinct rough ornamentation pattern that is similar to, but not the same as, that of *Acaulospora rehmii*. *Acaulospora* sp. 2 is similar to *Acaulospora* sp. 1 but with more pronounced, deeper ornamentation. *Acaulospora* sp. 3 is similar to *Acaulospora denticulata*.

Data analysis

Comparison of taxonomic composition of the arbuscular mycorrhizal community among all possible pairs of soil samples was calculated using the Bray–Curtis dissimilarity coefficient (Bray & Curtis, 1957), which has been shown to be one of the most robust coefficients for the analysis of taxonomic composition data (Faith *et al.*, 1987). Spore abundance data, using both numbers of spores of each species, were transformed to their square roots before the analysis to reduce the influence of occasional large abundance values for some taxa (Field *et al.*, 1982). The transformed abundance values for each taxon were standardized by the maximum attained by that taxon. This standardization equalizes the potential contributions of taxa to the overall dissimilarity in composition. Without standardization by taxon, the Bray-Curtis values are dominated by those taxa that attain high abundance (Faith *et al.*, 1987).

To test the significance of taxonomic differences due to host tree species and the diversity of the plant community (monocultures or polycultures of trees and the palm and herb), the Bray–Curtis matrix was subjected to the analysis of similarities procedure (ANOSIM) devised by Clarke (1993) using the computer program PRIMER 5 (Plymouth Marine Laboratory, UK; Clarke & Warwick, 1994). We used the two-way crossed analysis of similarity (ANOSIM), where host tree and plant diversity of plots were the two factors. The advantage of the ANOSIM test is that it does not assume any underlying distribution to the data, and it avoids using the Bray–Curtis index directly to compare sets of assemblages. Instead it is a nonparametric test, based only on the rank order of the matrix values. We performed this test on both relative abundance and presence–absence data.

Global nonmetric multidimensional scaling (GNMDS; Kruskal, 1964), which has been shown to be one of the most effective methods available for the ordination of taxonomic composition data (Minchin, 1987), was used to provide a visual summary of the pattern of Bray–Curtis values for the comparison of spore composition of soil samples. This method was chosen over other ordination techniques because it makes

no assumptions about the underlying distribution of the data. Each GNMDS was run with 10 random starting configurations, and proceeded through 400 iterations for each of three dimensions. Stress values for the two-dimensional MDS were <0.15. Sample points closest together on the resulting scatter plot represent host trees with the most similar AM fungal spore abundances. In the approach taken, each ordination has an associated ANOSIM test statistic, making interpretation of the plots unambiguous. Patterns of arbuscular mycorrhizal diversity among host tree species and plots with differing diversity were compared using diversity metrics calculated using the PRIMER 5 program (Clarke & Warwick, 1994): number of species (S); the Shannon diversity index ($H' = -\Sigma_i p_i \log(p_i)$, where p_i is the proportion of the total count arising from the ith species); and Pielou's Evenness Index ($J' = H' \log^{-1} S$). These values were subjected to ANOVA using the statistical computing package DATA DESK 6.1 (Data Descriptions, NY, USA). In the analysis of the effect of host tree and plant species diversity, host species was considered a random effect and diversity was considered fixed. Inspecting residual plots assessed the suitability of ANOVA models.

Exploratory analysis of the relationship among the Shannon diversity index, Pielou's evenness of the AM fungal community and relative abundance of the dominant AM fungal species, and ecosystem NPP and PUE, was done by assessing the correlation over all plots; for analysis of the influence of host tree species and the mono- and polyculture treatments, by using mixed models in the generalized linear model module of DATA DESK 6.1. We chose to designate AM fungal community parameters as the independent variables, but this is not intended to imply a causal relationship. Adequacy of the models was assessed by inspecting residual plots. Full details of the data collection, and calculations of plot-level NPP and PUE parameters, are provided by Hiremath & Ewel (2001).

Results

A total of 3065 live spores were examined, and 16 morphospecies of fungi were identified (Table 1). The major components of the community were *Acaulaspora morrowiae*, *A. scrobiculata*, *A. spinosa*, *A. mellea*, *P.* '*occultum*-like', and *G.* '*etunicatum/geosporum*-like'. Together these six species accounted for >90% of the spores. Similar numbers of spores were recovered from all mono- and polyculture plots (25.3 spores per 50 ml soil ± SE 1.7, $N = 141$).

Higher mean numbers of fungal species were found under *H. alchorneoides* (5.25 ± SE 0.25) than under *C. odorata* or *C. alliodora* (3.76 ± SE 0.24 and 3.82 ± SE 0.23, respectively; main effect of host tree species, $F_{2,12} = 11.47$, $P = 0.0016$). Using relative abundance of AM fungal spores, the composition of AM fungal communities also differed significantly depending on the tree species (Fig. 1a, global R statistic for the factor host in the ANOSIM, 0.235, $P = 0.063$; Table 2). Arbuscular mycorrhizal fungal communities associated with host species *H. alchorneoides* and *C. alliodora* were significantly different (global R statistic, 0.574, $P = 0.01$). Analysis of the data using only presence or absence of AM fungal species found no significant differences in the fungal community over host species.

Both monoculture and polyculture plots had similar numbers of AM fungal species (monocultures, 4.17 ± SE 0.22; polycultures, 4.00 ± SE 0.21 per 50 ml soil) despite vastly different densities of fine roots (monocultures 0.07–0.27 cm cm^{-3}, polycultures 0.62–0.94 cm cm^{-3}, J.J.E., unpublished data).

Table 1 Relative abundance of arbuscular mycorrhizal fungal spores from plots at La Selva Biological Station, Costa Rica, planted with three tree species, either alone or in combination with a perennial herb and a palm

AM fungal species	Spores	
	Number	Percentage of total
Acaulospora morrowiae Spain & Schenck	723	23.6
Acaulospora scrobiculata Trappe	718	23.4
Acaulospora spinosa Walker & Trappe	532	17.4
Acaulospora mellea Spain & Schenck	392	12.8
Acaulospora foveata Trappe & Janos	62	2.0
Acaulospora sp. 1	24	0.8
Acaulospora sp. 2	26	0.8
Acaulospora sp. 3	4	0.1
Scutellaspora pellucida (Nicol. & Schenck) Walker & Sanders	51	1.7
Scutellaspora castanea Walker	2	0.1
Scutellaspora scutata Walker & Diederichs	2	0.1
Paraglomus 'occultum-like'	232	7.6
Glomus 'etunicatum/geosporum-like'	167	5.4
Glomus 'clarum/interadicies-like'	98	3.2
Glomus tortuosum Schenck & Smith	12	0.4
Glomus 'other'	20	0.7
Total	3065	

Spores are from seven 50 ml soil samples from each of 18 plots.

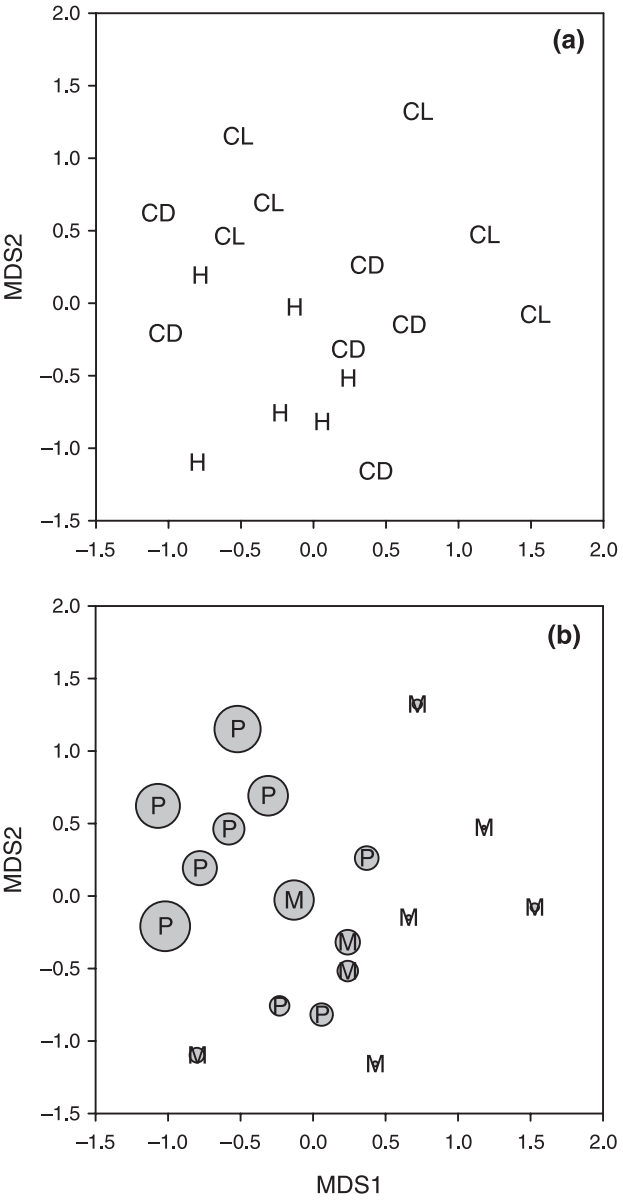

Fig. 1 (a) Ordination plot of multidimensional scaling of arbuscular mycorrhizal communities recovered from tropical soils associated with three different host tree species: *Cedrela odorata*, CL; *Cordia alliodora*, CD; *Hyeronima alchorneoides*, H. (b) Host trees species were grown either in monoculture (M) or with two other species (P): the palm *Euterpe oleracea* and herb *Heliconia imbricata*. The relative abundance of the arbuscular mycorrhizal fungal species *Acaulospora spinosa* is represented by the size of circles overlain on the symbols. Global *R* statistic for the factor host was 0.235, $P = 0.063$. When comparing host species H and CL, *R* statistic was 0.574, $P = 0.01$. Global *R* statistic for the factor diversity was 0.321, $P = 0.046$. The two-dimensional multidimensional scaling plot has a stress value of 0.15.

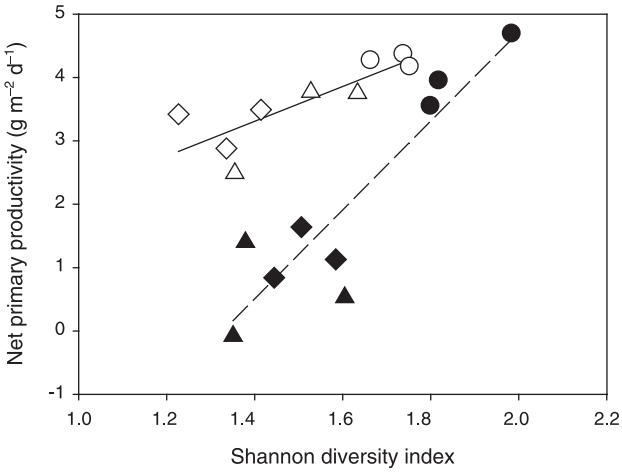

Fig. 2 Relationship between net primary production and the Shannon diversity index of arbuscular mycorrhizal fungal spore communities ($r^2 = 0.24$) under plots planted with tropical tree species *Cedrela odorata* (triangles), *Cordia alliodora* (diamonds) and *Hyeronima alchorneoides* (circles). Trees were planted either as monocultures (open symbols) or as polycultures coplanted with a palm and giant herb (closed symbols). Lines of best fit for the monoculture (solid line) and polyculture (dashed line) plots.

Polyculture plots also had Shannon diversity index values similar to plots where trees were growing alone (trees only, $0.96 \pm$ SE 0.05; multispecies, $1.10 \pm$ SE 0.05, $P = 0.089$). The composition of AM fungal communities differed significantly between monoculture and polyculture plots (Fig. 1b, ANOSIM global *R* statistic, 0.321, $P = 0.046$). Plots with only one tree species had abundant *A. morrowiae* (57.3%) and low levels of *A. spinosa* (12.4%), whereas in polyculture plots *A. spinosa* was abundant (46.7%) and *A. morrowiae* was less abundant (23%). This effect was stronger for *Cedrela* and *Cordia* than for *Hyeronima* (compare Fig. 1a,b). In contrast, analysis of presence or absence of AM fungal species indicated no significant differences in AM fungal communities betweeen mono- and polyculture plots.

To explore the strength and direction of the relationships between the AM fungal community and the PUE and NPP of the plots, we assessed the correlation among variables and used mixed linear models. There was no significant relationship between AM fungal diversity and ecosystem PUE of the plots. The regression of NPP and AM fungal diversity was significant (Fig. 2a, $r^2 = 0.236$, $P = 0.026$). This relationship was influenced by the relatively higher productivity and AM fungal diversity associated with *H. alchorneoides*. Additionally, the slope of the diversity–NPP relationship was significantly greater for polycultures compared with monocultures (Shannon diversity index × mono–polyculture interaction in a mixed model, $F_{1,14} = 8.175$, $P = 0.0126$).

Over all the experimental plots, the relationship between community evenness of the AM fungi and ecosystem PUE was significant (Fig. 3, $r^2 = 0.203$, $P = 0.032$). However, host tree species did not conform to the same relationship (Pielou's evenness–tree species interaction in the mixed model, $F_{2,12} = 5.597$, $P = 0.0192$), with experimental plots planted with *Cordia* and *Hyeronima* exhibiting a strongly positive relationship between AM fungal community evenness and PUE,

Table 2 Relative abundance of arbuscular mycorrhizal fungal spores for each species from plots at La Selva Biological Station, Costa Rica, planted with three tree species, either alone (single) or in combination with a herb and a palm (multispecies)

	Cedrela odorata		*Cordia alliodora*		*Hyeronima alchorneoides*	
AM fungal species	Single	Multi	Single	Multi	Single	Multi
Acaulospora morrowiae Spain & Schenck	0.384 ± 0.053	0.175 ± 0.136	0.360 ± 0.163	0.038 ± 0.017	0.262 ± 0.127	0.176 ± 0.074
Acaulospora scrobiculata Trappe	0.222 ± 0.022	0.253 ± 0.059	0.218 ± 0.056	0.363 ± 0.051	0.163 ± 0.058	0.202 ± 0.043
Acaulospora spinosa Walker & Trappe	0.040 ± 0.005	0.348 ± 0.119	0.005 ± 0.003	0.331 ± 0.071	0.153 ± 0.087	0.145 ± 0.050
Acaulospora mellea Spain & Schenck	0.216 ± 0.095	0.071 ± 0.068	0.001 ± 0.001	0.079 ± 0.057	0.214 ± 0.088	0.135 ± 0.050
Acaulospora foveata Trappe & Janos	0.008 ± 0.008	0.041 ± 0.033	0.003 ± 0.003	0.005 ± 0.002	0.031 ± 0.007	0.034 ± 0.025
Acaulospora sp. 1	0.010 ± 0.007	0	0.022 ± 0.014	0.030 ± 0.027	0.004 ± 0.002	0.002 ± 0.002
Acaulospora sp. 2	0	0.008 ± 0.005	0.008 ± 0.008	0.012 ± 0.009	0.014 ± 0.014	0.004 ± 0.002
Acaulospora sp. 3	0	0.004 ± 0.004	0	0	0	0.004 ± 0.004
Scutellaspora pellucida (Nicol. & Schenck) Walker & Sanders	0.002 ± 0.002	0.004 ± 0.002	0.020 ± 0.003	0.003 ± 0.003	0.016 ± 0.004	0.048 ± 0.024
Scutellaspora castanea Walker	0.003 ± 0.003	0	0	0	0.002 ± 0.002	0
Scutellaspora scutata Walker & Diederichs	0	0	0	0.003 ± 0.003	0	0.002 ± 0.002
Paraglomus '*occultum*-like'	0.054 ± 0.013	0.070 ± 0.026	0.053 ± 0.021	0.041 ± 0.017	0.102 ± 0.034	0.137 ± 0.046
Glomus '*etunicatum/geosporum*-like'	0.040 ± 0.033	0.020 ± 0.012	0.274 ± 0.161	0.085 ± 0.044	0.010 ± 0.007	0.020 ± 0.010
Glomus '*clarum/interadicies*-like'	0.011 ± 0.004	0	0	0	0.023 ± 0.017	0.083 ± 0.083
Glomus tortuosum Schenck & Smith	0.004 ± 0.004	0.002 ± 0.002	0.011 ± 0.011	0	0.002 ± 0.002	0.007 ± 0.005
Glomus 'other'	0	0.004 ± 0.004	0.017 ± 0.009	0.009 ± 0.009	0.006 ± 0.003	0.001 ± 0.001

Spores are from seven 50 ml soil samples from each of three plots per tree species.

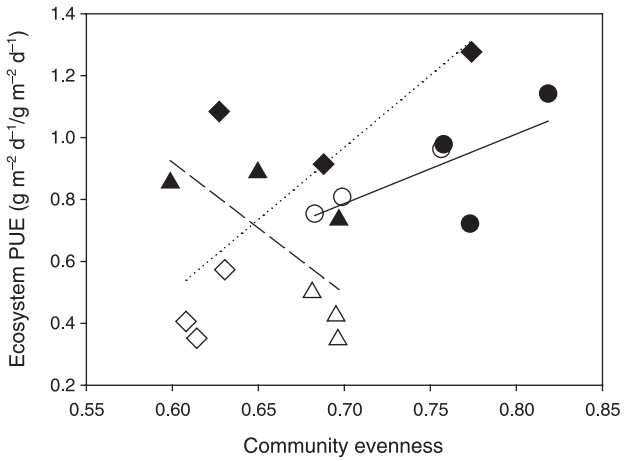

Fig. 3 Relationship between ecosystem phosphorus-use efficiency (PUE) and community evenness of arbuscular mycorrhizal fungal spore communities (r^2 = 0.20) under plots planted with tropical tree species *Cedrela odorata* (triangles), *Cordia alliodora* (diamonds) and *Hyeronima alchorneoides* (circles). Trees were planted either as monocultures (open symbols) or as polycultures coplanted with a palm and giant herb (closed symbols). Lines of best fit for each tree species: *Hyeronima* (solid line), *Cedrela* (dashed line) and *Cordia* (dotted line).

while the opposite was observed for *Cedrela*. Over all the experimental plots, there was no significant relationship between community evenness and NPP.

The relative abundance of dominant fungal species also showed significant relationships with NPP and PUE over the plots. As the relative abundance of *A. spinosa* increased, NPP declined (Fig. 4a, r^2 = 0.425, P = 0.002). This effect was dominated by the polyculture plots (% *A. spinosa* × mono–polyculture interaction in the mixed model, $F_{1,14}$ = 10.245, P = 0.0064). Additionally, increasing relative abundance of *A. spinosa* was also associated with increasing PUE (Fig. 4b, r^2 = 0.311, P = 0.009). This trend was strongest for the monoculture plots (% *A. spinosa* × mono–, polyculture interaction in the mixed model, $F_{1,14}$ = 5.578, P = 0.033), but one monoculture plot of *Hyeronima* was largely responsible for the significance of the interaction.

There was no significant relationship between the relative abundance of *A. morrowiae* and NPP, however, ecosystem PUE declined with increasing relative abundance of *A. morrowiae* (Fig. 4c, r^2 = 0.276, P = 0.015). Neither host tree species nor the inclusion of the palm and herb significantly affected this relationship. In addition to *A. spinosa* and *A. morrowiae*, spores of *A. scrobiculata* were also highly abundant over the plots (Table 1). No significant correlations were found between the relative abundance of *A. scrobiculata* and ecosystem PUE or NPP.

Discussion

The AM fungal community described here, from recent alluvial soils, is dominated by fungal species of the genus *Acaulospora*, as has been found in a study of the older, less fertile Oxisols at La Selva (Lovelock *et al.*, 2003). The community differs, however, in that *A. spinosa* and *A. scrobiculata* were rare on the Oxisols but are common in the more fertile plots studied here. AM fungal communities dominated by *Acaulospora* have been reported in other forested ecosystems (Helgason *et al.*, 1998; Merryweather & Fitter, 1998a, 1998b).

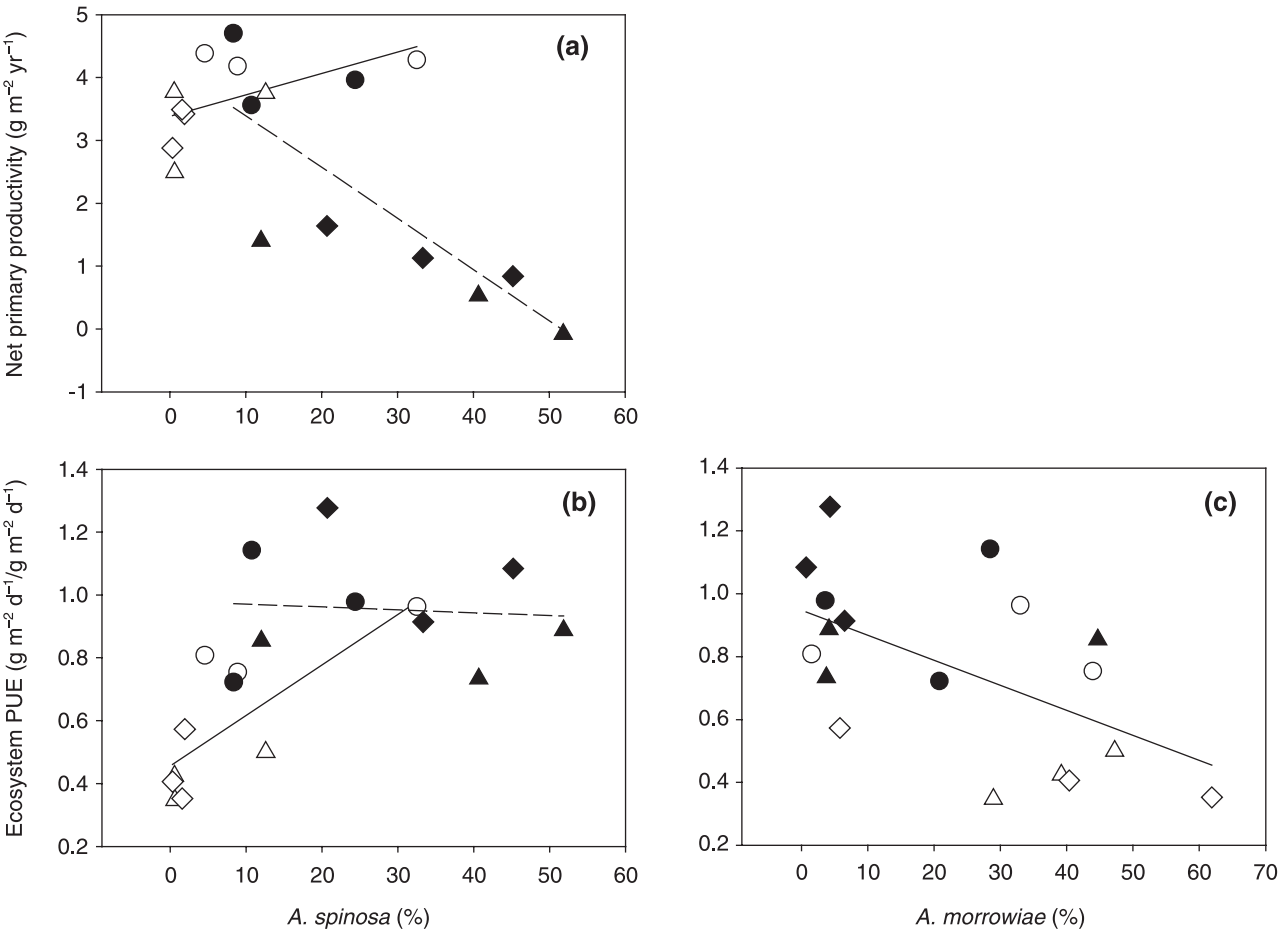

Fig. 4 Relationship between (a) net primary production (r^2 = 0.43) and (b) ecosystem phosphorus-use efficiency (PUE) (r^2 = 0.31) and relative abundance of the arbuscular mycorrhizal fungal species *Acaulospora spinosa.* Lines of best fit for monoculture (solid line) and polyculture (dashed line) plots. (c) Relationship between ecosystem PUE and relative abundance of the AM fungal species *Acaulospora morrowiae* (r^2 = 0.28). Plots planted with tropical tree species *Cedrela odorata* (triangles), *Cordia alliodora* (diamonds) and *Hyeronima alchorneoides* (circles).

Host tree species had a significant effect on both the number of AM species and their community structure, similar to observations in other studies (Johnson *et al.*, 1992; Musoko *et al.*, 1994; Bever *et al.*, 1996; Merryweather & Fitter, 1998b; Eom *et al.*, 2000; Burrows & Pfleger, 2002; Husband *et al.*, 2002a; Lovelock *et al.*, 2003). Also, similar to earlier studies in the forest at La Selva (Lovelock *et al.*, 2003) and in other ecosystems (Frank *et al.*, 2003), differences in host–AM fungal community associations were caused not by differences in the presence or absence of spores of AM fungal species, but by differences in the relative abundance of spores (but see Landis *et al.*, 2004), suggesting that AM fungal species that are sporulating are widely distributed. This is in contrast to results of studies using molecular techniques (Husband *et al.*, 2002a, 2002b), and may be a further indication that methods using identification of AM fungal species based on spores can result in underestimation of the diversity of the AM fungal community, and that tests of significant host or environmental effects on communities of AM fungal spores are likely to be conservative, with a lower likelihood of detecting subtle changes in AM fungal communities.

In the current study, host-species differences in the associated AM fungal community were largely caused by changes in the relative abundance of the spores of the most common AM fungal species (Table 2). Tree species have different growth rates, phenologies, architecture and secondary chemistry, and can also differentially alter fertility and other physical and chemical characteristics of soils (van Breeman, 1998 and references therein). *Hyeronima*, the host species with the highest AM fungal diversity, is evergreen, compared with the semideciduous habit of *Cedrela* and *Cordia*, and develops a denser canopy and higher root densities (Haggar & Ewel, 1997), and sustains higher rates of net primary productivity, than do the other two tree species in the experimental plots (Hiremath & Ewel, 2001). Landis *et al.* (2004) report that AM fungal species diversity increases with soil fertility, which is probably correlated with plant community productivity, and Burrow & Pfleger (2002) observed increases in AM species numbers

with plant cover and soil nitrate during some sampling intervals. Enhanced soil fertility has also been observed to support higher AM hyphal productivity (Lovelock *et al.*, 2004). When viewed together, a possible emerging pattern, similar to that observed in many plant communities (Tilman & Pacala, 1993), could be that the diversity of the AM fungal community increases with increasing productivity of the plant and/or fungal community, at least over some range of productivity.

Within this framework, we assessed the relationship between AM fungal community and NPP and PUE. We expected that enhanced diversity of AM fungal communities would be associated with enhanced utilization efficiency by the plant community, because a diverse fungal community should be able to occupy a broader range of biotic and abiotic niches, accessing more of the soil P, which should ultimately result in higher plant community NPP (Pringle & Bever, 2002). Over all plots, diversity of AM fungal spore communities was not correlated with an increase in ecosystem PUE, but community evenness was correlated with PUE. If relative abundance of spores does reflect the structure of the AM fungal community, then the correlations between AM community evenness could suggest that an equitable distribution of AM fungal species leads to enhanced P capture, a hypothesis similar to that advanced for the function of evenness in plant communities (Wilsey & Potvin, 2000). This remains to be rigorously tested.

As predicted, NPP of plots increased with increasing AM fungal diversity. The increase in NPP with AM fungal diversity was steeper in polycultures compared with monocultures, the monocultures maintaining higher NPP with less diverse AM fungal communities. One hypothesis that could be proposed to explain this pattern is that a diverse AM fungal community reduces competition, leading to enhanced productivity of subordinate plant species (Grime *et al.*, 1987; van der Heijden *et al.*, 1998a, 1998b), which in this case are the understory herb and palm. However, these results must be viewed cautiously because one of the tree species, *Hyeronima*, strongly influences the AM fungal diversity–NPP relationship, particularly in the polyculture plots (Fig. 2). Assessing AM fungal diversity over a broader range of randomly selected plants species, similar to the design of Burrows & Pfleger (2002), would provide a step toward understanding links between plant and fungal diversity in tropical forests.

Because little is known of the biology and ecology of individual AM fungal species, we also assessed the links between plant ecosystem processes and the relative abundance of spores of the dominant fungal species. No significant relationship between the abundance of spores of *A. scrobiculata* and ecosystem PUE and NPP were found, but relative abundance of *A. spinosa* and *A. morrowiae* were correlated with ecosystem PUE and, in the case of *A. spinosa*, with NPP. The relative abundance of spores of *A. spinosa* increased with increasing PUE, but decreased with increasing NPP. These patterns may implicate *A. spinosa* as an AM fungal species that sporulates under conditions where P is limiting plant growth, where P supply is low relative to the demand imposed by plant growth rates. The relative abundance of *A. morrowiae* was not significantly related to NPP, but decreased with enhanced PUE, in an opposing trend to *A. spinosa*. Thus sporulation of *A. morrowiae* could be favoured where supply of soil P is relatively high compared with the demand for P imposed by growth of the plant community. In both cases we propose that the niche of the AM fungal species is a function of both nutrient supply and plant growth rate, which is affected by other environmental factors, including light.

The relative abundance of spores of these two species in the less fertile Oxisols of the undisturbed forests at La Selva, where *A. morrowiae* is dominant and *A. spinosa* is rare (Lovelock *et al.*, 2003), are consistent with their suggested niches in the current study. In the undisturbed forest, the low frequency of canopy gap formation results in light-limited, slow growth rates for most individuals that may not result in a high demand for soil nutrients, although this has not been specifically tested at La Selva. The observation of Howeler *et al.* (1987) and Sieverding (1989), that *A. morrowiae* was a poor symbiotic partner for fast-growing agricultural species, is also consistent with our observations. The opposing trends in relative abundance of spores of *A. morrowiae* and *A. spinosa* over the gradient in PUE could reflect replacement through competition by these fungal species for host carbon resources needed to sporulate; but could also be caused by host selection, or some other unidentified process or gradient. Overall, the patterns in relative abundance of spores of individual AM species are difficult to interpret because we have insufficient data on the ecology and physiology of these species, but they do suggest that individual species are responding to similar host and environmental gradients in different ways, which suggests that different AM fungal species have different ecological niches.

The results of our study provide further evidence that the symbiotic AM fungi in tropical soils are influenced by the identity of the host tree species and the composition of the plant community, as originally proposed by Janos (1980b). Although our data do not provide evidence of a causal relationship, we present the first evidence from a field study that the evenness of the AM fungal community could be influenced by, or is influencing, the PUE of a tropical forest plant community, and that the diversity of the AM fungal community is influenced by, or is influencing, tropical forest ecosystem NPP.

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