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# Vesicular-Arbuscular Mycorrhizae (VAM) Spore Abundance and Soil Characteristics along a Neotropical Premontane Forest Successional Gradient

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## ABSTRACT

Tropical soils are generally nutrient poor, though they support high biodiversity and productivity. Most tropical plants are able to thrive in these soils because they form a symbiosis with Vesicular-Arbuscular Mycorrhizae (VAM). VAM increase the nutrient-absorption capabilities, and therefore the fitness, of the host plant. Previous studies suggest that as a pasture is allowed to regenerate, the soil should become less compact, more nutrient-rich, and the pH should become more neutral. These soil characteristics, in conjunction with spore number, may drive the rate and direction of some successional communities following disturbance. This study examines how VAM spore abundance, macronutrient levels (nitrogen, phosphorus, and potassium), bulk soil density, and pH change between plots at various stages of regeneration from pasture (ages 0-45, and primary forest, N=15) in the Premontane Wet Forest near San Luis, Costa Rica. The data show substantial variation within and between sites in spore number and other soil characteristics. For instance, the three pasture sites sampled varied from 4 to 83 spores ( $\pm 28$ ), with nitrogen levels from 16.81 to 46.7 kg/ha ( $\pm 16.46$ ). Within each individual site there was also variation: the 25 year old forest ranged from 46 to 132 spores ( $\pm 45$ ). Regeneration age did not significantly affect VAM spore number, macronutrient levels, or bulk density, though pH was positively correlated with increasing site age. Spore abundance was not significantly affected by variations in the other soil characteristics. These findings suggest that VAM spores may not be evenly distributed throughout the soil, instead having patchy distributions determined by soil heterogeneity and location of mycotrophic hosts. VAM do not appear to be limiting in the young pastures of San Luis, suggesting that VAM are unlikely to direct forest regeneration.

## RESUMEN

Los suelos tropicales son generalmente pobres en nutrientes, aunque soportan una alta diversidad y productividad. La mayoría de las plantas tropicales son capaces de prosperar en estos suelos debido a relaciones simbióticas con micorrizas. Las micorrizas aumentan la capacidad de absorber nutrientes, y por lo tanto el éxito reproductivo de la planta hospedera. Estudios previos sugieren que conforme se deja regenerar los pastizales, el suelo debería ser menos compacto, más rico en nutrientes y el pH debería ser más neutro. Estas características del suelo, en conjunto con el número de esporas, puede llevar la tasa y dirección de algunas comunidades en sucesión después de disturbios. Este estudio examina como la abundancia de esporas de micorrizas, niveles de macro-nutrientes (N, P, K), la densidad aparente del suelo y el pH cambian entre diferentes parcelas en pastizales con diferente tiempo de regeneración (edades de 0 a 45 años, y bosque primario, N = 15) en el bosque húmedo premontano cercano a San Luis, Costa Rica. Mis datos sugieren una variación sustancial entre y dentro de las parcelas en el número de esporas y otras características del suelo. Los tres sitios varían entre 4 y 83 esporas ( $\pm 28$ ), con niveles de nitrógeno de 16.81 a 46.7 kg/ha ( $\pm 16.46$ ). Dentro de cada sitio individual existe también variación; por ejemplo, la abundancia de esporas en el fragmento de 25 años varía de 46 a 132 ( $\pm 45$ ). La edad de regeneración no afecta el número de esporas de micorrizas, los niveles de macro-nutrientes o la densidad del suelo, aunque el pH está correlacionado positivamente al aumentar la edad de regeneración. La abundancia de esporas no se ve significativamente afectada por las variaciones en las características del suelo. Estos descubrimientos sugieren que las micorrizas pueden no estar distribuidas equitativamente a lo largo del suelo, en cambio tiene una distribución por parches determinado por la heterogeneidad y ubicación de los hospederos. Las micorrizas parecen no verse limitadas en los pastizales más jóvenes en San Luis, sugiriendo que éstas no están directamente relacionadas con la regeneración del bosque.

## INTRODUCTION

Deforestation rates are dramatically high in some parts of the world, such as in South America, which suffered a net forest loss of about 4.3 million hectares per year from 2000 to 2005 (FAO). However, other tropical areas are actually experiencing net forest gains. Such is the case in Costa Rica, which from 2000 to 2005 increased its total forest cover by about 15,000 hectares (FAO). As Costa Rica shifts from an agriculture and cattle-ranching economy to one of industry and tourism, farmland is being abandoned and allowed to regenerate into secondary forest. Though conservation efforts tend to focus on preserving old-growth forests, secondary forests are also an asset to conservation. Many important reserves in Costa Rica, such as Santa Rosa National Park, would not exist today had it not been for the foresight of conservationists (such as ecologist and conservationist Dr. Daniel Janzen) to purchase cheap pasture and let it regenerate naturally (UNEP). Now, only a few decades later, these reserves act as important refuges of biodiversity (UNEP). Thus, from a conservation standpoint, it is important to understand how land naturally regenerates from anthropogenic disturbance. Soil characteristics are one especially influential ecological force affecting the rate and direction of forest succession (Janos 1983, Neale 1997, Smith and Read 1997).

Tropical rainforests are known for their biodiversity and high productivity. However, a rainforest's fertility does not come from the richness of the soil, as most neotropical soils are shallow, nutrient-poor, acidic, and phosphorus-deficient (Janos 1983). Instead, a rainforest's luxurious growth is in part due to a very common mutualism with Vesicular-Arbuscular Mycorrhizae (VAM) (Janos 1983). Though there are many species of mycorrhizae, most tropical plants associate specifically with species of VAM (Family Glomales, Division Zygomycota). These fungi are dependent upon their hosts for the products of photosynthesis (energy-rich carbon compounds like glucose). In return, mycorrhizae provide host plants with increased nutrient and water absorption potential, thus allowing for a higher level of photosynthetic productivity (Janos 1983, Smith and Read 1997).

This mutualism increases the competitive abilities of the host plant, and is therefore influential in determining dynamics of both intact, old growth forests and the trajectory of successional communities (Janos 1980). Mycotrophic hosts (plants that depend on this mutualism), may not be able to grow to maturity (or even germinate successfully, in the case of many tropical canopy trees) without the presence of fungal inocula (Janos 1983, Johnson *et al.* 1991). This is especially true in nutrient-poor soils, like those in the Tropics, where these plants cannot absorb sufficient nutrients or water from the soil without VAM (Janos 1980). In these conditions, such plants may be easily outcompeted (at least temporarily) by non-mycotrophic species, which are frequently pioneer species adapted to rapid growth in low-nutrient conditions (Janos 1983).

Thus, mycorrhizae spore number and other soil characteristics like nutrient composition may work together to determine which plants can or cannot inhabit a regenerating area. As a forest is allowed to regenerate, the soil should become less compact and acquire more leaf litter (Guariguata and Ostertag 2001). This leaf litter acts as a pH buffer (encouraging a more neutral pH), increases humus in the soil, and enriches the soil as the detritus decomposes (Guariguata and Ostertag 2001). Abandoned pastures, for example, are usually nutrient poor (as they are not normally fertilized), and have compacted soil due to frequent trampling by cattle (Neale 1997). One study in the Monteverde area found that the soils of abandoned pastures dramatically increased in their leaf litter and humus content and became less compacted over time (Neale

1997). However, some intensive land-use activities, in some cases rangeland, may degrade the soil to the point where the original habitat type will never be able to regenerate (Guariguata and Ostertag 2001).

Not only do spore levels potentially influence the variety and quantity of plants that can live in a given area, plants may conversely affect spore abundance. Because VAM produce few spores that are fragile and do not last long in the soil, if a dominantly non-mycotrophic plant community inhabits an area for long enough, it may deplete the rhizosphere of spores. This, in turn, could affect the composition of future plant communities (Janos 1980). However, it is not clear how a Neotropical soil's biotic and abiotic nutrient systems change as a plot of land is allowed to regenerate from pasture to secondary forest, or how this impacts the quantity of VAM spores in the soil.

Janos (1980) found that poor soils usually favor either obligatory mycotrophic species-dominant or non-mycotrophic species-dominant communities. He theorizes that if VAM are initially present in poor soils, obligatory mycotrophic species should be the most competitive. This would support a continuous abundance of VAM spores and ensure the long-term dominance of mycotrophic species. However, if the soil is both nutrient-poor and lacking VAM, it should favor non-mycotrophic species, often pioneer species, which can out-compete mycotrophic species when VAM are not present. Without a host, the VAM (as well as the short-lived spores) would soon die, ensuring the continued dominance of non-mycotrophic species in the area (Janos 1980).

In contrast, Rogers (1998) hypothesized that forest succession following disturbance is not determined as much by original soil characteristics as it is by the success of pioneer species. Pioneer species are adapted to poor soils and are usually not dependent on mycorrhizae, but they may also outcompete mycotrophic plants in soil rich in nutrients and VAM spores. Maybe the trajectory of succession depends on the creation of better growing conditions through these pioneer species, which alter the microclimate—they block the wind, decrease the temperature, block direct sunlight, and add detritus to the soil which increases the forest's humidity and water storage potential (Guariguata and Ostertag 2001). This provides a better habitat for understory and later-succession plants, as well for the primary dispersal agents of spores, such as burrowing vertebrates like moles and rabbits (Killham 1994, Kwan 1995, Wolf 1998). Thus, it is possible that mycorrhizal spore abundance in the soil does not affect pioneer communities as much as it does later successional stages.

These initial conditions in nutrient levels and VAM spore abundance may result from the type of land-use or the previous plant community prior to forest regeneration. For example, the farming of an obligately mycotrophic crop like *Lactuca sativa* (common lettuce) could maintain a high VAM abundance in the soil (Janos 1983, Miller and Jackson 1998). Conversely, if the soil contains an excess of nutrients (such as from intensive fertilizer input), this could render VAM obsolete (Janos 1983). In this case, host plants would do better to absorb the soil's abundant nutrients themselves, rather than trade away their photosynthetic products for this function. This means few VAM spores in the soil, because without their host, mycorrhizae inocula soon become inviable. Pastureland is also usually found to have low numbers of VAM spores, because most grasses, though technically mycotrophic, do not frequently form VAM mycorrhizal associations, even in situations of low fertility (Johnson *et al.* 1991).

In light of this literature, and with the premise that pastures should be nutrient poor and have few VAM spores, I expect to find that spore number increases with regeneration as the plot is colonized first by non-mycotrophic pioneer species, which over time will attract mycorrhizal

dispersal agents and be colonized by later-successional mycotrophic species. These changes should also be reflected by other changes in the soil as the area regenerates from pastureland, such as decreasing bulk density, increasing macro-nutrient concentrations, and a more neutral pH. Alternatively, the spore abundance could stay low with regeneration time if the community continues to be outcompeted by non-mycotrophic plants, in which case the soil characteristics would remain relatively degraded and the climax community would differ from plots of “old growth” forest.

## METHODS

I collected soil samples in the Premontane Neotropical Wet Forest near San Luis, Costa Rica on November 1st-15th, 2010, at an elevation of 1055 to 1200 m. I first met with landowners in the San Luis area, as well as with staff members of the University of Georgia Research Station to determine each forest’s age since regeneration and previous land use type. I selected 13 sites of varying regeneration time (0 to 45 years) from the pastures and secondary forests of San Luis and on the trails around the UGA campus. I also selected three additional plots of primary forest to compare with the pastureland and secondary growth. I defined primary forest in this study as old-growth forest for which the age is unknown and which is thought to be historically undisturbed by humans (excluding trails). Though primary forest sites lay “outside of the spectrum” because they have presumably never been disturbed, it seems reasonable to assume that if the soil composition follows some trend with increasing regeneration time, then the plots would eventually converge on the primary forests characteristics.

I removed three soil samples at each site with a soil borer (volume of 365 cm<sup>3</sup>) to a depth of 15 cm, after brushing away surface debris and plant material. This depth is commonly used to capture the best representation of spore numbers, (Johnson *et al.* 1991). All samples were taken from level areas with at least 15 cm of topsoil, and that were 10 m or more from the nearest road, stream, trail, or other habitat type. Each sample was kept separate for individual analysis. Each soil sample was dried for 12 hours in a drying oven set to 63° C (145° F), and was then weighed to obtain the dry weight. This was divided by the soil borer’s volume to determine each soil sample’s bulk density (a measure of soil compaction). I then used a wet-sieving and centrifugation technique (slightly adapted from Wolf 1998) to separate the VAM spores from 7.5 grams of each soil sample. I stirred each soil sample into 500 mL of water to dislodge spores and clumps of soil, and rinsed the solution through a series of nested sieves (250 µm and 125 µm) Therefore, I counted spores with a diameter of roughly 125-250 µm, which captures the midrange of most VAM spores (Wolf 1998). I removed and distributed the sievate with water into 16 2mL plastic “bullet” centrifuge tubes, and centrifuged each soil sample at 3600 rpm for 45 seconds. I removed the supernatant from each centrifuge tube with a pipette, and examined it drop by drop on a Petri dish under a dissecting microscope (Figure 1) to count the number of spores. The dried soil samples were then tested using a LaMotte soil testing kit for pH and for the major soil macronutrients nitrate nitrogen, potassium, and phosphorus.

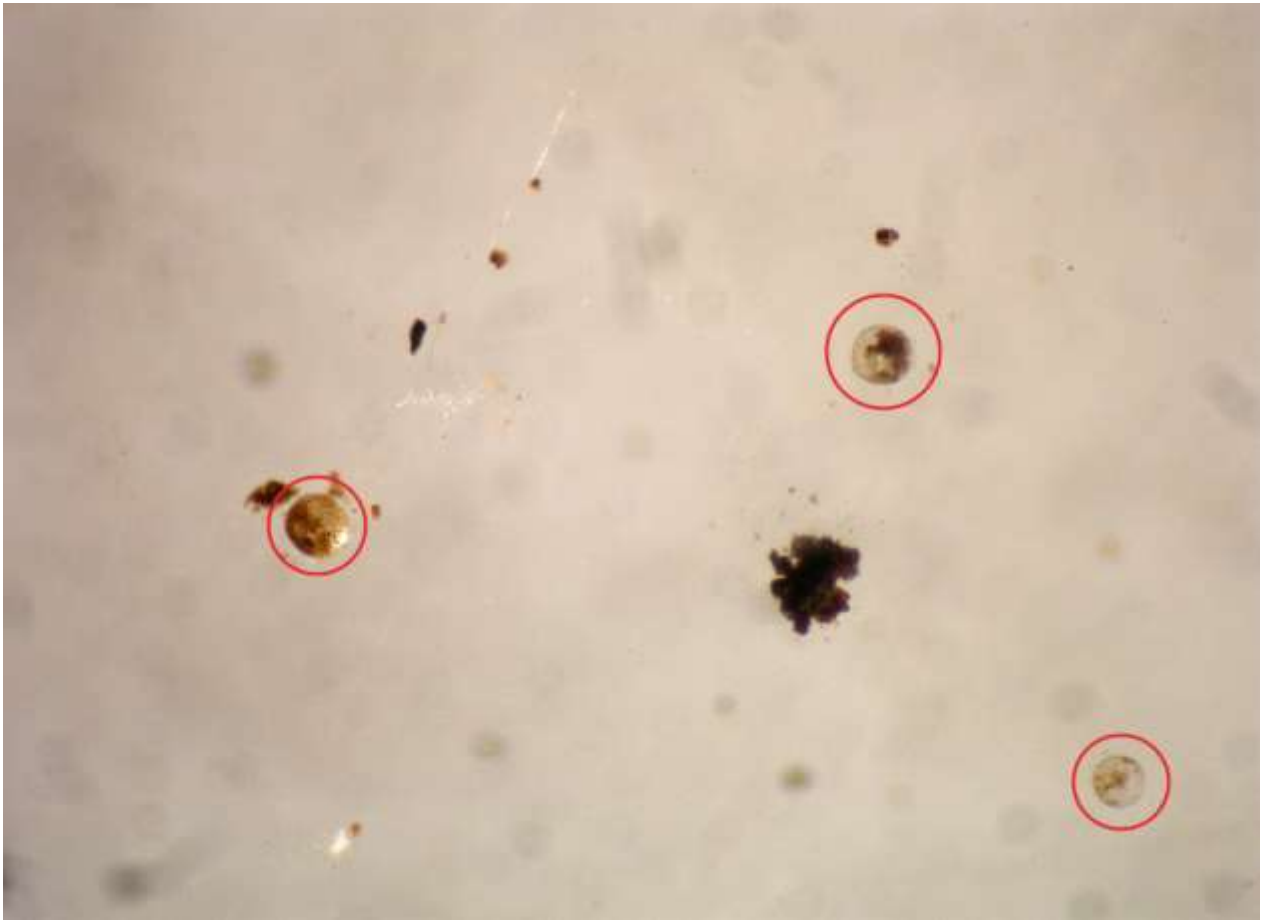


FIGURE 1. Three Vesicular-Arbuscular Mycorrhizae (VAM) spores, circled in red, as seen under a dissecting microscope (magnification 40X). Most neotropical plants depend on their association with VAM to overcome the nutrient-deficiency of tropical soils. These spores were extracted from 7.5 g dry weight of soil from a regenerating pasture near San Luis, Costa Rica using a wet-sieving and centrifugation method.

## RESULTS

### VAM Spore Abundance and Chemical Analysis

The number of spores in each 7.5 g subsample varied substantially within sites (mean spore number  $54 \pm 22$ ,  $N = 15$ ), thus for each site I averaged the VAM spore numbers from each of the three separate soil samples for my calculations (see Table 1). The sites ranged from an average spore count of about 4 to 97 spores, with the lowest from a pastureland site, and the highest found in the soil of 25 year old pastureland re-growth. These averaged spore numbers also varied substantially between the three separate pastureland and primary forest sites, respectively (the average spore count in pastureland ranged from 4 to 83 spores, and in primary forest from 33 to 71 spores). Spore number was not significantly affected by regeneration age (regression,  $p$

= 0.6382,  $R^2 = 0.0174$ ,  $N=13$ ). A correlation table (Table 2) was constructed to determine how the physical and chemical soil characteristics were inter-related. However, none of the factors, except regeneration age with pH and K with bulk density, showed any correlation ( $p$ -values < 0.05). Spore number was not significantly affected by bulk soil density ( $p=0.2513$ ,  $R^2=0.1291$ ), N ( $p=0.8423$ ,  $R^2=0.0042$ ), P ( $p=0.7814$ ,  $R^2=0.0081$ ), K ( $p=0.9785$ ,  $R^2=0.0001$ ), or pH ( $p = .2556$ ,  $R^2 = 0.127$ ; multiple regression,  $N=13$ ).

TABLE 1. Average soil characteristics of the 15 study sites of pasture, regenerating forest, or primary forest near San Luis, Costa Rica.  $N=3$  for each average. Total mean spore abundance =  $54 \pm 22$  per 7.5 g dry weight of soil; total mean bulk density =  $0.46 \pm 0.09$  g/cm<sup>3</sup>; total mean N =  $35.62 \pm 16.46$  g/ha; total mean P =  $108.73 \pm 45.83$  g/ha; total mean K =  $0.56 \pm 0.11$  mL<sup>-1</sup>, total mean pH =  $6.23 \pm 0.3$ .

Site Age/ Type	Avg. Spore #	Avg. Bulk Soil Density (g/cm <sup>3</sup> )	Avg. N (kg/ha)	Avg. P (kg/ha)	Avg. K (mL <sup>-1</sup> )*	Avg. pH
Pasture 1	3.67	0.50	16.81	106.48	0.44	5.43
Pasture 2	83.33	0.38	14.95	78.46	0.50	6.17
Pasture 3	62.00	0.59	46.70	117.69	0.65	5.83
5 years	26.33	0.62	26.15	74.73	0.59	6.27
10 years	81.33	0.48	44.84	37.36	0.54	6.30
15 years	24.33	0.41	18.68	102.75	0.47	6.67
18 years	56.33	0.44	59.78	186.82	0.66	6.30
20 years	42.67	0.40	63.52	46.70	0.47	6.23
25 years	97.00	0.32	13.08	140.11	0.43	6.53
30 years	71.33	0.53	44.84	121.43	0.54	6.30
37 years	40.67	0.45	37.36	95.28	0.59	6.40
45 years	50.67	0.58	39.23	84.07	0.83	6.33
Primary 1	33.00	0.33	18.68	168.13	0.44	6.50
Primary 2	71.67	0.44	44.84	84.07	0.62	6.10
Primary 3	66.33	0.38	44.84	186.82	0.64	6.10

\* The measurement for K (mL<sup>-1</sup>) is an expression of relative abundance based on the soil testing procedure in the LaMotte Soil Testing Kit. This does not directly correlate to lbs/acre of K at each site.

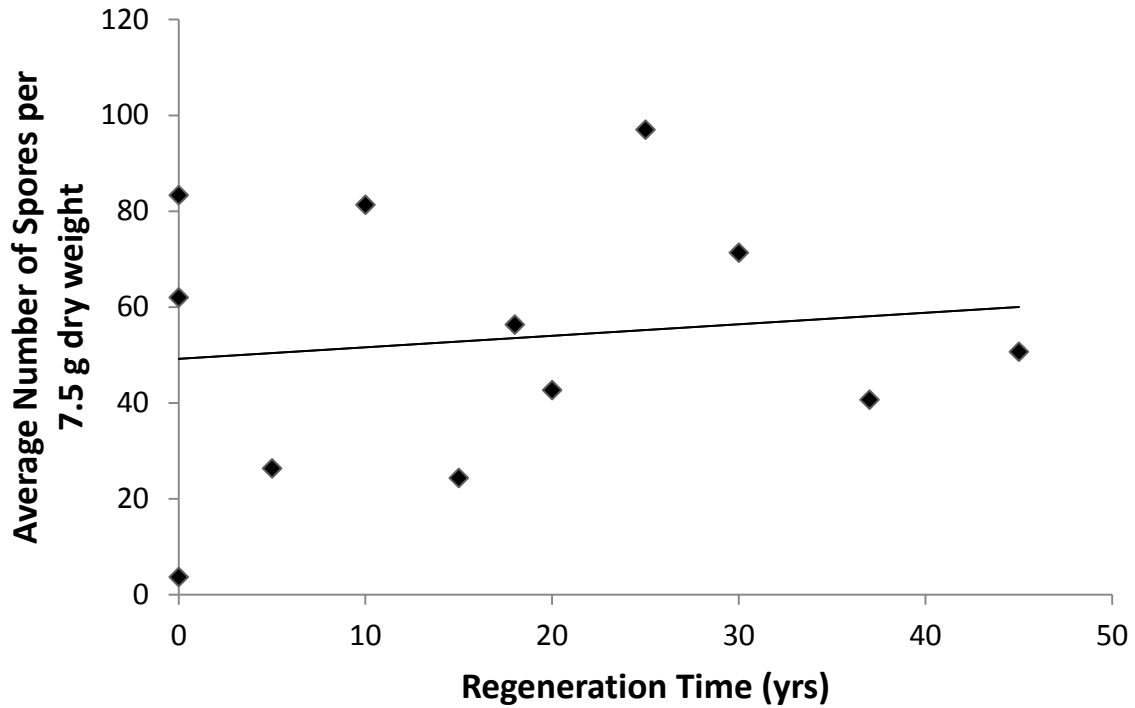


FIGURE 2. Number of average VAM spores per 7.5 gram subsample with increasing natural regeneration time since pastureland. Spore number does not significantly increase with time ( $p = 0.6832$ ,  $R^2 = 0.0174$ ,  $N = 13$ , line of best fit:  $y = 0.2406x + 49.195$ ).

TABLE 2. Correlations between VAM spore number, regeneration age, and the other soil properties of 13 pasture and secondary forest sites. Only site age with pH, and bulk density with K, were significantly correlated ( $p < 0.05$ ). Spore abundance was not significantly correlated with any other soil characteristic.

	Spore #	Age	Bulk Density	N	P	K	pH
Spore #	--	--	--	--	--	--	--
Age	+ 0.13	--	--	--	--	--	--
Bulk Density	- 0.35	- 0.05	--	--	--	--	--
N	+ 0.06	+ 0.26	+ 0.24	--	--	--	--
P	+ 0.09	+ 0.10	- 0.16	- 0.01	--	--	--
K	+ 0.02	+ 0.43	+ 0.62*	+ 0.42	+ 0.11	--	--
pH	+ 0.36	+ 0.55*	- 0.37	+ 0.02	+ 0.01	+ 0.05	--

\* indicates  $p < 0.05$



## Qualitative Observations

I observed a typical pattern of Neotropical community succession (previous personal observations), with grass-dominated pastureland first being colonized by ground-sprawling vines, small shrubs and herbaceous plants, and *Cecropia* and early successional tree seedlings. This seemed to progress to dominance by *Heliconia* and vines as the seedlings increased in height. The understory continued to grow denser with Melastomataceae and aroid species, and some understory palms. Finally, the older sites seemed to be dominated by large trees (and no *Cecropia*), with lianas, Piperaceae, Melastomataceae, ferns and understory palms present.

I also observed that pasture soil samples were extremely muddy and compacted from being trampled by cows, only covered in a thin layer of grass and little humus. Humus and leaf litter seemed to increase with regeneration time, and compaction seemed to decrease, though leveling out at a mid-regeneration time of approximately 25 years. This qualitative observation, therefore, contradicts my measure of compaction (bulk density) for which there was no trend with increasing regeneration age. I did not notice any large differences in macro- or micro-invertebrate soil composition, except that the pasture soils seemed to contain more annelids.

## DISCUSSION

Trends in age were difficult to discern because of high within site and between site variability in spore number. This was obvious by looking at the three pasture sites (average spore numbers: 4-83, average N: 14.91-46.7 kg/ha, etc). Moreover, the sites sometimes varied dramatically (such as the 25 year old secondary forest, which had from 46-132 spores) between the three samples at each site, demonstrating that even one site with the same regenerational history was heterogeneous in the number of spores it contained. Because of this soil heterogeneity, the data collected from each site could have reflected different regenerational trajectories, rather than a point on a single successional path. A longer-term experiment would have been able to judge the interaction of mycorrhizae and nutrient composition along a successional gradient while holding all baseline conditions constant (rather than assuming these starting conditions to be equivalent).

In terms of my predicted results, these observations support neither the hypothesis that VAM spores were consistently uncommon due to the dominance of non-mycotrophic pioneer species, nor the hypothesis that spore numbers increased as mycotrophic species colonized the area. There was no trend between number of spores and regeneration age, and it seems that soil compaction, N, P, K, and pH are not acting as confounding variables. It is possible that these trends do exist in the natural regeneration of pastureland, but that the starting spore or nutrient levels were different in the initial pastureland of each site. However, the macronutrient content and the bulk density of the soil also did not follow a trend with regeneration age. Though I qualitatively observed an increase in leaf litter and humus with regeneration, and with it a decrease in soil compaction, this was not represented in the data. The bulk soil density may be a flawed measure of soil compaction, as a more sandy or heavy soil would have a greater mass per volume of the soil borer, possibly obfuscating the results. PH did increase with regeneration time (becoming more neutral), which may reflect this increase in leaf litter, which sometimes acts as a pH buffer. Ultimately, the soil in most sites seemed to be heterogeneous both in spore abundance and macronutrient levels.

One possible explanation for the observed lack of trend between spore number and regeneration time is that spores may not always linearly affect the infection rate of their host

plants, and therefore spore levels may not increase or decrease predictably with regeneration time. For example, the successful infection of some mycotrophic species (like canopy trees) may require a high density of VAM inocula in the soil, while other species may require a relatively low inocula density (Johnson *et al.* 1991). Therefore, looking directly at mycorrhizal root infection percentage might be more strongly correlated with forest regeneration time (Johnson *et al.* 1991). Additionally, spores are not the only representation of VAM inocula, thus using spores as a proxy for fungal spores, hyphae, and other kinds of inocula may confound results.

Furthermore, the absence of trend in spore number with regeneration time could suggest that spores are mostly distributed randomly or in clumps throughout tropical soils. One study found a patchy distribution of fungal spores in sites due to both proximity to mycotrophic hosts and amount of organic matter (Carvalho *et al.* 2003). Another possible cause for this heterogeneity could be random nutrient deposition into the soil from cattle feces. These patches of greater nutrient availability could offer nearby plants enough nutrients to allow them to forgo their usual mycorrhizal association, creating occasional pockets of lower VAM abundance (Wolf 1998).

If VAM spore abundance is determined by patchy spatial distributions of soil microclimates and mycotrophic hosts, then this heterogeneity could imply the creation of several distinct successional community patches resulting from a single disturbance. However, because of this widespread but patchy distribution, it is unlikely that VAM are a limiting factor in successional communities and it is not clear how they might drive succession. It would be beneficial for further research to explore proximity to mycotrophic host plants and proximity to undisturbed habitat. The effects of VAM spore abundance may be more pronounced for sites that are distant from living mycorrhizae infections, because this distance may limit certain mycotrophic plants from colonizing a regenerating area. Also, mycorrhizae could potentially be important drivers of succession in areas with a more homogeneous soil composition, or they could be critically important to the recovery of sites that have undergone severe disturbance, such as losing topsoil due to years of industrial agriculture practice. Such degraded sites are abundant, but could regenerate to a field of grasses and weedy non-mycotrophs, never to regenerate the canopy these sites carried before transformation.

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