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SHORT NOTE

Arbuscular mycorrhizal propagules in soils from a tropical forest and an abandoned cornfield in Quintana Roo, Mexico: visual comparison of most-probable-number estimates

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Abstract The present study was aimed at comparing the number of arbuscular mycorrhizal fungi (AMF) propagules found in soil from a mature tropical forest and that found in an abandoned cornfield in Noh-Bec Quintana Roo, Mexico, during three seasons. Agricultural practices can dramatically reduce the availability and viability of AMF propagules, and in this way delay the regeneration of tropical forests in abandoned agricultural areas. In addition, rainfall seasonality, which characterizes deciduous tropical forests, may strongly influence AMF propagules density. To compare AMF propagule numbers between sites and seasons (summer rainy, winter rainy and dry season), a "most probable number" (MPN) bioassay was conducted under greenhouse conditions employing *Sorgum vulgare* L. as host plant. Results showed an average value of 3.5±0.41 propagules in 50 ml of soil for the mature forest while the abandoned cornfield had 15.4±5.03 propagules in 50 ml of soil. Likelihood analysis showed no statistical differences in MPN of propagules between seasons within each site, or between sites, except for the summer rainy season for which soil from the abandoned cornfield had eight times as many propagules compared to soil from the mature forest site for this season. Propagules of arbuscular mycorrhizal fungi remained viable throughout the sampling seasons at both sites. Abandoned areas resulting from traditional slash and burn agriculture practices involving maize did not show a lower number of AMF propagules, which should allow the establishment of mycotrophic plants thus maintaining the AMF inoculum potential in these soils.

Keywords Arbuscular mycorrhizae · Inoculum potential · Likelihood analysis · Seasonal variation · Tropical forest · Regeneration

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Introduction

Arbuscular mycorrhizal fungi (AMF) are an important biological component of most soils, and several studies have shown their role in soil conservation (Miller and Jastrow 1992). Arbuscular mycorrhizae are formed when a suitable root is available to fungi propagules in the form of either hyphae, spores and/or vesicules within roots (Douds and Millner 1999). In tropical areas, most AMF propagules have shown seasonal fluctuations in abundance either as spores (Guadarrama and Alvarez-Sánchez 1999) or in colonized roots (Corkidi and Rincón 1997a). Importantly, previous work has also shown that AMF propagules viability (Jasper et al. 1989; Kabir 2005), abundance (Lovelock et al. 2003; Tchabi et al. 2008), and species richness (Tchabi et al. 2008) are negatively influenced by soil disturbance such as that resulting from agricultural activities. Nonetheless, other studies indicate that the number of propagules can be higher in abandoned agricultural areas than in adjacent forest (Fischer et al. 1994). In Mexico, slash and burn agriculture is common and it has been observed that this agriculture practice has a negative short-term effect on AMF propagules (Aguilar-Fernández et al. 2009); however, this practice does not result in long-term impacts on the AMF community, one of the reasons for this being that not all propagule sources are affected (Muthukumar et al. 2003; Guadarrama et al. 2008; Gavito et al. 2008).

Attempts to evaluate AMF propagule density in soil to diagnose soil quality have included direct counts of spores extracted from soil (Allen et al. 2003; Carvalho et al. 2003), assessment of percentage of colonized roots (Corkidi and Rincón 1997a), and estimation of length and biomass of hyphae in the soil (Pearson and Jakobsen 1993; Simard and Durall 2004). On the other hand, although there are some techniques to assess propagule viability (Brundrett et al. 1994), the role of such condition for the formation of new mycorrhizae remains unclear. One of these techniques, the most probable number (MPN) method, was used by Porter (1979) to assess propagule viability. This microbiological technique provides a realistic estimation of the overall condition of the AMF community (An et al. 1990; Cabello 1997) and even allows the detection of AMF species which do not produce spores (Troeh and Loynachan 2003). Nonetheless, several authors have criticized the MPN method because certain experimental variables (e.g., host plant, temperature, soil humidity, pot size, soil conditions) may influence estimations (Wilson and Trinick 1982; Adelman and Morton 1986), the disturbance associated to soil dilution may also produce an underestimation of propagules (Dalpé and Hanel 2008), in addition to it being labor-intensive (An et al. 1990; Woomer 1994). Despite this, propagules estimations obtained from the maximum likelihood estimates (MLE) of the MPN method (see the Methods section) using similar conditions (host plant, growth conditions, pot size) may allow for the relative comparison of samples from different sites and seasons.

Given that agricultural management practices in tropical forests may lead to a decrease in the number and viability of AMF propagules in soils, it is of central importance to evaluate propagule densities of abandoned agricultural areas because AMF abundance will increase the chance of plant establishment success and forest regeneration. Thus, previously managed agricultural sites which maintain high levels of viable AMF propagules probably will not suffer from halted regeneration. The main goal of this study was to compare the number of AMF propagules in soil samples from a mature tropical forest and from a nearby abandoned cornfield for three different seasons. In order to achieve this, we used a visual comparison method based on propagule estimations obtained from the MPN technique. Our hypotheses are that (1) the availability and viability of AMF propagules will change across seasons within a year, but that (2) such AMF community parameters will not differ between the mature tropical forest and the abandoned cornfield, the rationale behind this hypothesis is that traditional slash and burn agricultural practices are of low ecological impact, and allow the full recovery of the AMF community, at least in terms of abundance, after a given period of abandonment. Such recovery of the AMF community will likely contribute to tropical forest regeneration.

Materials and methods

Soil samples were taken at the “ejido” (i.e., communal land ownership) of “Noh-Bec” (19°02′30″–19°12′30″ N, 88°13′30″–88°27′30″W), in the state of Quintana Roo, southeast Mexico. The mature forest site is a semi-evergreen tropical forest. The abandoned cornfield site was originally composed of semi-evergreen forest and subjected to traditional Mayan slash and burn agriculture (low input agriculture with no fertilization added). Corn was cultivated for 2 years, period after which the site was abandoned for approximately 8–10 years prior to sampling (Ramos-Zapata et al. 2006a, b) (Table 1).

At each site, five soil samples (2 kg each) were randomly collected at depths ranging from 2 to 20 cm during the summer rainy season (September, 2001), winter rainy season (January, 2002) and dry season (March, 2002). Soil samples were then transported to the laboratory, where arbuscular mycorrhizal fungi (AMF) propagules were estimated immediately after samples collection. Fifty milliliters sub-samples of soil were used as recommended for the MPN test (Porter 1979), using a fourfold soil dilution series (one non-diluted and six dilutions sub-samples with four replicates each), *Sorghum vulgare* L. was used as the trap plant.

Steam-sterilized soil (for 1 h three consecutive days) was used to dilute the soil samples. Pots were filled with 300 ml of steam-sterilized soil and 50 ml of the diluted soil. To prevent contamination, 50 ml of steam-sterilized soil was added to the surface of the pots. One-week-old seedlings of *S. vulgare* were transplanted to the pots filled with the diluted samples and were randomly placed on tables in the greenhouse where ambient temperature and humidity were similar during all bioassays (25–37°C and 80–100% RH). After 6 weeks, roots of *S. vulgare* from the diluted soil layer were collected, stained (Phillips and Hayman 1970), and examined microscopically for the presence or absence of AM colonization.

For the estimation of MPN (here denoted as λ), we applied the procedure based on maximum likelihood estimation (MLE, λ_{MLE}) proposed originally by Fisher (1922). As an alternative to MLE in an age in which computers did not exist, Fisher gave also an approximate “moment estimation method” for the MPN (Fisher and Yates 1970), the same method adapted by Porter (1979) for

Table 1 Vegetation, pH values, phosphorous (P), and organic matter (OM) content of soil samples from a mature forest site and an abandoned cornfield in the locality Noh-Bec, in Quintana Roo, Mexico (modified from Ramos-Zapata et al. 2006a, b)

Characteristics	Mature forest site	Abandoned cornfield site
Vegetation type	Semi-evergreen mature forest	Early secondary vegetation
Canopy (m)	20–30	10–15
Dominant tree species	*Alseis yucatanenses* Standl. (RUBIACEAE), *Metopium brownei* (Jacq.) Urban (ANACARDIACEAE), *Pouteria unilocularis* (Engel) Eyma (SAPOTACEAE)	*Bursera simaruba* (L.) Sarg. (BURSERACEAE), *Cecropia peltata* L. (MORACEAE), *Guazuma ulmifolia* Lam.(STERCULIACEAE), *Guettarda combsii* Urb. (RUBIACEAE)
Understory	*Cryosophila stauracantha* (Heynh.) R. Evans (ARECACEAE), *Sabal mauritiiformis* (H. Wendl. Ex-H. Karst.) Griseb. and H. Wendl. (ARECACEAE)	–
pH	7.3	7.1
Extractable P (mg kg^{-1} soil)	18.8	13.5
OM (%)	5.2	3.3

the estimation of propagule number in mycorrhizal studies. Although Fisher's moment estimator of MPN is a good approximation of λ_{MLE}, Hurley and Roscoe (1983) pointed out that computation of the former is now unnecessary, as computers performs the MLE quite easily. MLE methods not only offer a point estimate of the MPN but any degree of replication at each dilution and any number of dilutions. It also allows graphical and analytical comparisons of estimated values of the MPN under different conditions, using relative likelihoods and the likelihood ratio test (Pawitan 2001).

Given the observed data, x, the MLE method relies on the likelihood function $L(\lambda;x)$ that ranks the plausibility of possible values of λ based on how probable they make the observed data (Pawitan 2001). For convenience, $L(\lambda;x)$ is standardized with respect to its maximum value $L(\lambda_{\mathrm{MLE}};x)$, resulting in the relative likelihood function (RL), $R(\lambda;x) = L(\lambda;x)/L(\lambda_{\mathrm{MLE}};x)$, with values ranking from 0 to 1 (Sprott 2000). A graph of $R(\lambda;x)$ shows what λ values are plausible, although the most practical use of the RL is in the construction of likelihood intervals. A level c likelihood interval for λ is given by $R(\lambda;x) \geq c$, $0 \leq c \leq 1$. The RL for the MPN is right-skewed and unimodal; thus, the likelihood is a single interval, whose statistical center is λ_{MLE}, which does not necessarily lies on the midpoint of the interval. Usual values for the level c are 0.05, 0.15, and 0.25. Some studies (Kalbfleisch 1985; Sprott 2000) have suggested that likelihood intervals $R(\lambda;x) \geq 0.15$ (or equivalently, the 15% relative likelihood intervals) are interpretable as approximate 95% confidence intervals in the classic statistical setting. Relative likelihoods of the MPNs were calculated for each season within each site, as well as for each site by pooling all three seasons within a given site. Plots were generated in order to evaluate visually whether the RL curves intersected below the 0.15 level; if this was the case, then the plausibility of differences between MPNs was accepted. The relative likelihood analysis allowed us to compare the values of MPN propagules between abandoned cornfield and forest sites for different seasons.

Results

Results showed an average value of 3.5±0.41 MPN propagules in 50 ml of soil for the mature forest, while the abandoned cornfield had 15.4±5.03 MPN of propagules in 50 ml of soil. The greatest MPN of propagules was observed for the summer rainy season sample of the abandoned cornfield, while the lowest number was observed for the dry season sample of the mature forest (Fig. 1). Although the MPN of propagules was always higher in the abandoned cornfield than in the mature forest, according to relative likelihood analysis the sites differed significantly only in the summer rainy season (Fig. 1), when the abandoned cornfield had an estimated value close to eight times that of the mature forest site. As far as we know, this is the first study which has used likelihood methods to predict AMF propagule numbers. Monitoring mycorrhizae-forming units is relevant in order to understand spatial and temporal variation in the AMF community properties.

Discussion

The presence of viable propagules able to form mycorrhizae during dry season is an important condition for a seasonal tropical forest, because at the onset of the growing season colonization of new roots may occur rapidly (Jasper et al. 1989) and thus increase nutrient acquisition during the growing season (Corkidi and Rincón 1997b). We found that

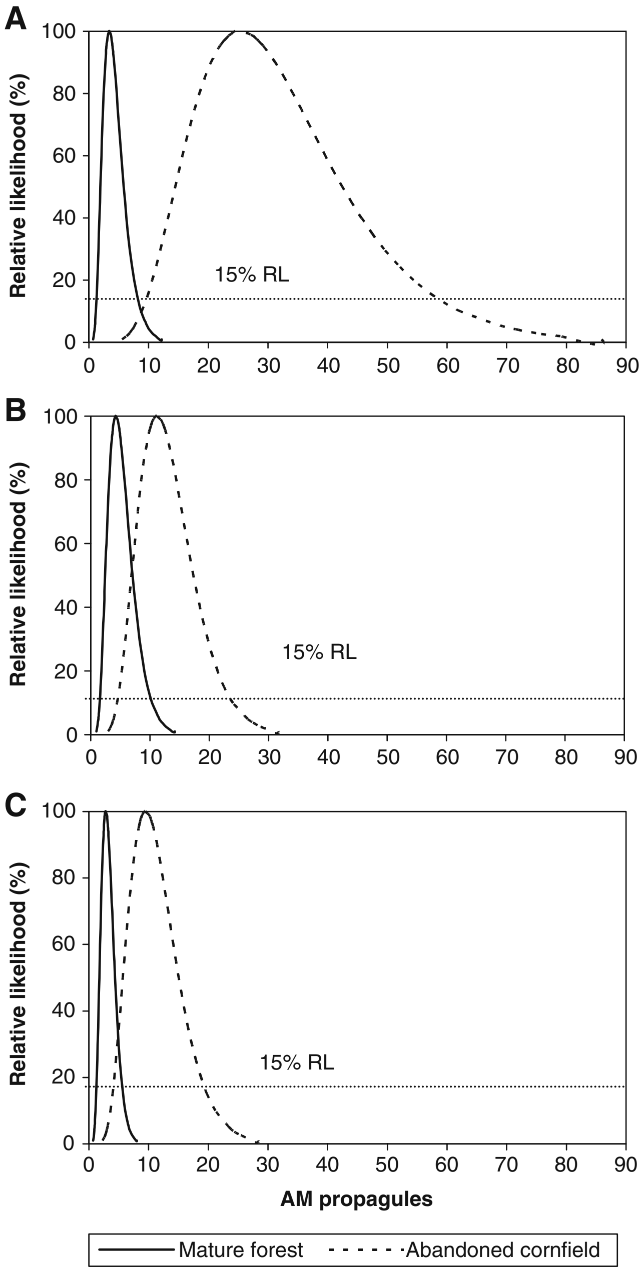

Fig. 1 Relative likelihoods of AMF propagule numbers estimated by the MPN method using soil from mature forest and an abandoned cornfield collected during three different seasons at the locality Noh-Bec, in Quintana Roo, Mexico. **a** Summer rainy season. **b** Winter rainy season and **c** dry season. Intervals ranging from the intersections of the horizontal lines and the relative likelihood functions give the 15% likelihood intervals. For each graph, non-overlapping likelihood intervals are interpreted as MPN values plausibly different

the inoculum potential of the soil is maintained within a given range throughout the year in both sites of study as has been reported for other environments (Brundrett and Abbott 1994; Aguilar-Fernández et al. 2009).

Results indicated a greater number of AM propagules for the abandoned cornfield site rather than the mature forest site (at least during the rainy season), which agrees with previous results from tropical systems (Fischer et al. 1994; Zangaro et al. 2000; Guadarrama et al. 2008), semi-arid grasslands (Richter et al. 2002), and semi-arid temperate steppe (in terms of root colonization for some plant species) (Tian et al. 2009). Our results are also consistent with previous seedling establishment experiments which have shown that AMF propagules number in abandoned agricultural site were four times higher than in forest site (Ramos-Zapata et al. 2006a).

Although the number of infective propagules of AMF apparently is low, similar values (4.4 from forest, 1.6 from pasture) have been reported in tropical dry forest from Jalisco, Mexico (Aguilar-Fernández et al. 2009) and from Oaxaca, Mexico (four from secondary forest, 14 from recent abandoned cornfield) (Guadarrama et al. 2008), and in a tropical forest (0.6 from forest, 56 from pasture) from Costa Rica (Fischer et al. 1994). Anyway, we believe that it is only valid to compare the MPN values from the sites and within seasons from our study because of the particular conditions of the bioassay (growth conditions, pot size, and host plant).

Some reasons why propagules of arbuscular mycorrhizae fungi may remain viable in the soil of abandoned agricultural sites are that: (a) slash and burn agriculture is of low impact and allows the maintenance of spores and colonized roots (Guadarrama et al. 2008; Lekberg et al. 2008; Aguilar-Fernández et al. 2009), (b) the establishment of weeds recently after abandonment (some of which are potentially mycotrophic) may favor propagules multiplication (Jordan et al. 2000), and (c) the immediate colonization of AMF propagules which may be dispersed by wind (Warner et al. 1987) from the surrounding forest, since the traditional slash and burn agriculture exploits small areas conserving the forest around them.

Results from this study indicate that AMF propagules were at least as abundant at the abandoned agricultural site than at the mature tropical forest, and this condition is likely to buffer forest regeneration at sites which have been previously subjected to slash and burn agriculture. Nonetheless, the identity of the AMF species present must be determined given that they differ in terms of their resistance to disturbances (Hart and Reader 2004) and their nutrient acquisition efficiency (Smith and Read 2008; Johnson et al. 1997). Despite this, a recent study showed that the conversion of tropical forests to traditional cornfields does not cause a change in AMF species composition (Violi et al. 2008). To conclude, findings from this study indicated that AMF propagules remain viable after a traditional slash and burn agriculture management. We believe that knowledge on plant-AMF community dynamics will help to understand plant succession, and contribute to successful tropical forest restoration activities. Future work on AMF community dynamics in the study area should consider AMF species identities, the role of soil fauna as dispersal and predator agents, and the effect of abiotic soil parameters.

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