

Glomeromycotina Guild Spore Abundance Correlates with Soil Organic Carbon in Homegardens and Seasonal Forests in Yucatan, Mexico Correlación entre la abundancia de esporas de Glomeromycotina y el carbono orgánico del suelo en sistemas agroforestales y bosques estacionales de Yucatán, México

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SUMMARY

Soil organic carbon (SOC) is a key indicator of soil health. Arbuscular mycorrhizal fungi (AMF) have been shown to increase SOC and respond to SOC content, which in turn, is directly related to land use. We investigated the relationships between land use and Glomeromycotina AMF spore abundance of two AMF functional guilds, rhizophilic (having high root internal AMF hyphal length) and edaphophilic (with high external soil hyphal length) to SOC content in agroforestry systems (AS) and seasonal forest (SF) of Tzucacab, Yucatan, Mexico. Our results indicate greater SOC values in SF than AS of the same age with a trend of increasing SOC with system age. Rhizophilic spore abundance correlated with SOC content, showing differences among land uses and system ages but not between seasons. No relationship was observed between edaphophilic spore abundance to SOC. Thus, we suggest that Glomeromycotina spore abundance (measured at any time of the year) of rhizophilic fungi could be a good indicator of SOC and, a tool to monitor soil health due to land use changes.

Index words: *arbuscular mycorrhizal fungi, edaphophilic fungi, rhizophilic fungi.*

RESUMEN

El carbono orgánico del suelo (COS) es un indicador clave de la salud del suelo. Se ha demostrado que los hongos micorrízicos arbusculares (HMA) pueden aumentar el COS, y también responder al contenido de COS; el cual, a su vez, está directamente relacionado con el uso del suelo. Investigamos las relaciones entre el uso del suelo y la abundancia de esporas de HMA de dos grupos funcionales de HMA, los rizófilos (con alta longitud hifal interna de HMA en las raíces) y los edafófilos (con alta longitud hifal externa del suelo) con el contenido de COS en sistemas agroforestales (SAF) y un bosque estacional (BE) en Tzucacab, Yucatán, México. Nuestros resultados indican valores de COS más altos en BE que en SAF de la misma edad, con una tendencia a aumentar el COS con la edad del sistema. La abundancia de esporas rizofílicas se correlaciona con el contenido de COS, mostrando diferencias entre los usos del suelo y las edades del sistema, pero no entre las estaciones. No se observó relación entre la abundancia de esporas edafófilas y el COS. Por lo tanto, sugerimos que la abundancia de esporas (medida en cualquier momento del año) de hongos rizófilos podría ser un buen indicador del COS y una herramienta para monitorear la salud del suelo debido a cambios en el uso del suelo.

Palabras clave: *hongos micorrízicos arbusculares, hongos edafófilos, hongos rizófilos.*



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INTRODUCTION

Soil organic carbon (SOC) has a pronounced impact on soil quality, functionality, and health. Plant and animal residues as well as active microbial biomass and detritus are the main components of SOC (Lal, 2016). SOC stocks depend on the balance between inputs and outputs (Jobbágy and Jackson, 2000); thus, quantity, quality, and turnover rates of SOC determine soil health (Lal, 2014); and, ultimately, plant and ecosystem health. Plant C stocks in ecosystems vary with stand age, vegetation type, soil characteristics, and disturbances (Dai *et al.*, 2014). Land cover and land use changes regulate SOC stocks as the SOC reaches the maximum sequestration or saturation point (Chen, Arrouays, Angers, Martin, and Walter, 2019). When forests are converted to agricultural lands, carbon stocks are reduced (Ekblad and Bastviken, 2019), affecting microbe community size, composition, and functioning (Tilston, Sismur, Dixon, Otten, and Harris, 2010). Global conversion of forests to agriculture is causing major changes in SOC stocks and associated soil microbiota (Hendrickson, 2003; Murty, Kirschbaum, Mcmurtrie, and Mcgilvray 2002; Wei, Shao, Gale, and Li, 2014).

Arbuscular mycorrhizal fungi (AMF) have an important role in soil carbon cycling by regulating carbon fluxes between the biosphere and the atmosphere which ultimately leads to a long-term stabilization of SOC (Zhu and Miller, 2003; Jansa and Treseder, 2017). Hyphae lifespans range from 5 to 40 days, releasing C to soil (Friese and Allen, 1991; Hernández and Allen, 2013). From these hyphae, there are several compounds and pathways produced by or catalyzed by AMF that contribute to SOC, the most abundant including β -glucan, a C compound comprising up to 50-60% of its dry weight (Fesel and Zuccaro, 2015) and cross-linked with chitin (Gooday, 1994). These form a group of proteins called glomalin that can contribute up to 5% of the total SOC and 25% of the stable organic carbon pool in tropical forests (Rillig, Wright, Nichols, Schmidt, and Torn, 2001; Lovelock, Wright, Clark and Ruess, 2004). In turn, SOC holds nutrients and provides structure, facilitating AMF growth and functionality e.g. (Allen, Allen, Violi, and Gómez, 2003a). Spores can persist in an inactive vegetative state after production (Hays and Watson, 2019), representing a window into accumulated AMF activity. AMF spore densities have been positively correlated with soil organic matter and soil moisture levels (Soka and Ritchie, 2018). Treseder & Allen (2000) found shifts in AMF communities through spore counts on different N availability scenarios. van der Heyde, Ohsowski, Abbott, and Hart (2017) showed that AMF species of Gigasporaceae are less tolerant to disturbances than Glomerales.

One challenge is that a low number of AMF taxa are found with each sampling, and the species undergo shifts in response to plants, soils, and organic matter dynamics. Tracking the functionality of any individual taxon or even family has proven to be difficult. However, the recent taxa arrangement into guilds of AMF provides another means to examine functional relationships between the composition and the impacts of the different AMF. While individual species respond rapidly to small resource changes, the functional guilds may respond to larger ecosystem processes. Weber *et al.*, (2019) organized guilds of AMF to study larger-scale phenomena associated with global change. They focused on guilds within which similar characteristics existed. The two main guilds are especially relevant to our studies, the rhizophilic AMF taxa are characterized by high root internal hyphal length e.g. (Glomeraceae), while edaphophilic AMF taxa have a more extensive external hyphal length e.g. (Gigasporaceae). The rhizophilic and edaphophilic guilds of AMFs as they have relevant characteristics, are especially useful in assessing land use and global change impacts (Phillips *et al.*, 2019; Weber *et al.*, 2019).

We hypothesize that Glomeromycotina AMF spore abundance correlates to SOC variations due to land use changes, system ages, and seasons; thus, allowing the assessment of SOC stock and soil health. In this study, we investigated the relations between land use and Glomeromycotina AMF spore abundance of the two predominant Glomeromycotina AMF functional guilds (rhizophilic and edaphophilic) with SOC contents in agroforestry systems (AS) and seasonal forest (SF) of Yucatan, Mexico.

MATERIALS AND METHODS

Study Site and Sample Collection

The research was undertaken in the municipality of Tzucacab, Yucatan, Mexico (Figure 1). This is a region of seasonal tropical deciduous forest with average temperatures from 24-28 °C and 1000-1200 mm total annual precipitation, most of it falling during the summer wet season; the main soil groups are Luvisols (42.97%), Vertisols (22.01%), Phaeozems (19.66%) and Leptosols (14.43%) (INEGI, 2009). This is a karstic environment where no superficial flowing water exists (Estrada-Medina, Jiménez, Álvarez, and Barrientos (2019), and soils are calcium-rich, promoting P insolubility (Avnimelech, 1980).

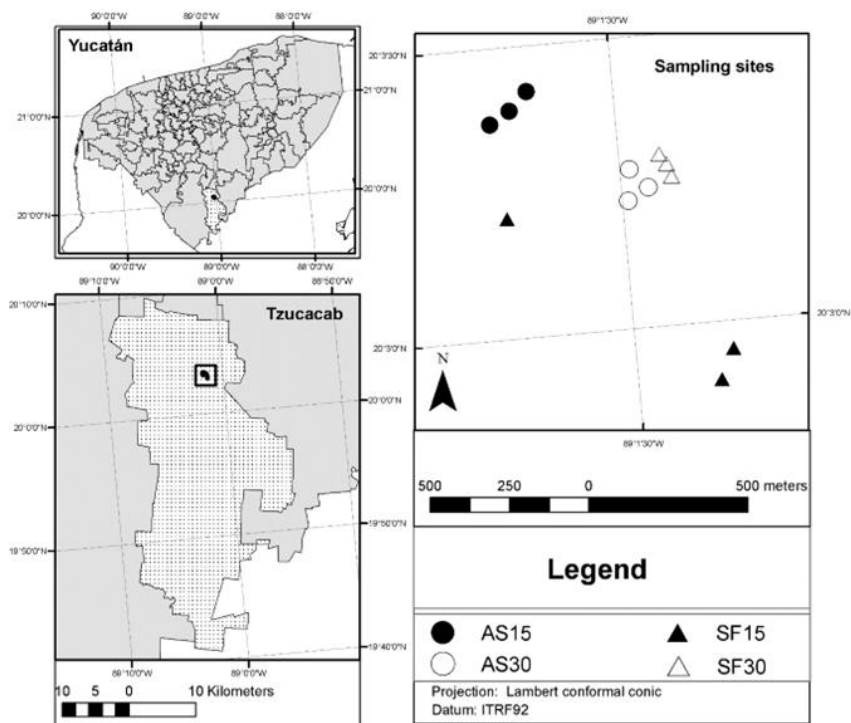


Figure 1. Study sites. AS15 = agroforestry system 15-year-old; AS30 = agroforestry system 30-year-old; SF15 = seasonal forest 15-year-old; SF30 = seasonal forest 30-year-old.

Much of the area includes mixed-species homegardens for fruit and fiber, and shifting agriculture of abandoned and active maize fields or Milpas (a traditional Mayan agricultural system, integrated by a polyculture based on maize, bean, and squash).

For this study, six agroforestry systems (AS) were chosen, three that have been established for 15 years and another three that have reached a 30 year maturation. In addition, six secondary vegetation sites were selected, three with 15 years and three with 30 years of regeneration; these sites were previously utilized for maize cropping in a milpa system for up to three years. These patches of vegetation belong to regeneration sites of the region's original seasonal forests, so we will refer to them as seasonal forests (SF) for the purposes of this study.

Agroforestry systems consist of home gardens dominated by citrus, fruit-bearing, and timber trees. Additionally, species from the Burseraceae, Euphorbiaceae, Fabaceae, Polygonaceae, and Rubiaceae families predominantly inhabit the secondary vegetation areas (Zamora, García, Flores, and Ortiz, 2008).

Bulk soil samples were collected at 0-30cm depth after removing surface litter from the soil and then placed into plastic bags for transport and subsequent laboratory processing. In each of the 12 study sites, plots of 100 × 100m were selected, nine samples were collected in each plot systematically, and the sampling was carried out during both the dry (April) and wet (August) seasons (Figure 1).

A total of 216 samples were collected, encompassing 2 systems (AF and SF) × 2 ages (15 and 30 years) × 3 replicates of the systems × 2 seasons (dry and wet) × 9 samples per plot.

Samples Processing and Laboratory Analysis

Soils were air-dried and sieved (2mm) to separate soil particles and aggregates from fine roots, with coarse organic matter materials predominating. Each soil sample was divided into two subsamples, and the fine root samples were separated for further analysis. Following sieving, the first soil sub-samples were analyzed for organic C by the Walkley-Black method (Nelson and Sommers, 1996).

The second soil subsample was used for analyzing Glomeromicotyna AMF spores, which were rehydrated and isolated by sucrose flotation from 10 g of soil (Allen, Moore, Christense, and Stanton 1979), counted under a dissecting microscope at 10X magnification, and morphologically identified to rhizophilic and edaphophilic taxa by taxonomic keys (Weber *et al.*, 2019).

The rhizophilic AMF guild is comprised of the families Glomeraceae, Claroideoglomeraceae, and Paraglomeraceae, and the edaphophilic guild includes the Gigasporaceae and Diversisporaceae. A third guild, the ancestral AMF, is also recognized by Weber *et al.*, (2019), and consists of Archaeosporaceae, Ambisporaceae, Pacisporaceae, and Acaulosporaceae. However, this guild is based on phylogeny rather than function, and the total spore numbers we observed from this guild were < 1 spore g^{-1} soil. Therefore, we did not include the ancestral guild in the statistical analyses.

Finally, fine roots (< 2 mm) obtained from each soil sample were cleared with KOH (10%) and washed twice with distilled water until white; then, roots were rehydrated, stained with trypan blue, and 100 intersections per slide were microscopically observed for mycorrhizal colonization at 100x magnification; of which, depending on the number of colonized intersections, the percentage of colonization was obtained (Allen, Swenson, Querejeta, Egerton, and Treseder, 2003b).

Statistical Analyses

Glomeromicotyna spore numbers were calculated as spores g^{-1} of dry soil for rhizophilic and edaphophilic species. Differences in number of spores and soil organic carbon (SOC) among land uses (AS and SF) and system ages (15, 30 years) per season (wet, dry) were tested by a two-way ANOVA upon verifying the assumption of homogeneity of variances using the Levene's test. Tukey's post hoc test was employed to examine significant effects.

A principal component analysis on the correlation matrix of the categorical (codified as dummy variables) and numerical variables was used to explore patterns (similarities and differences) within the dataset. Relationships between SOC and number of spores were analyzed by a linear regression for each season and guild. All analyses were run in PAST Ver. 4.01 (Hammer, Harper, and Ryan 2001)

RESULTS AND DISCUSSION

Soil Organic Carbon

Soil Organic Carbon (SOC) varied according to land use and system age, showing lower values in AS than SF and lower values in younger systems (15 years) than older ones (30 years) (Table 1). The interaction (land use age / seasons) of the two-way ANOVA was not significant; thus, only the land use differences are shown.

As expected, SOC contents show different patterns in the studied land uses in the Yucatan Peninsula, which could be related to the management practices in each system. SOC is usually greater in a forest than any other land use, but it varies by region (Ali, Begum, Hayat, and Bohannan, 2017; Mukhtar, Nurliana, Aningtias, and Anugrah, 2021). SOC values in the SF were lower than those found in drier deciduous forests of central and northern Yucatan, with 14.6% in a 12-year-old SF in Hocaba, Yucatan (Shang and Tiessen, 2003) and 27.9% in a 15-year-old SF in Dzibilchaltun, Yucatan (Ceccon, Olmsted, and Campo, 2002), respectively.

This trend was also observed in a study conducted by Álvarez-Rivera *et al.* (2021), in which they investigated homegardens and successional forest sites (> 30 years of fallow) in Tzucacab, reporting an average SOC of 6.24 and 14.61%, respectively.

Table 1. Soil organic carbon content (%) in agroforestry systems and seasonal forest of Tzucacab, Yucatan, Mexico.

Land use and age	Dry Season	Wet Season
AS15	5.98±1.46 c	5.93±1.55 c
AS30	6.86±0.45 c	7.92±0.78 c
SF15	9.81±1.38 b	9.26±0.34 b
SF30	13.61±2.21 a	13.06±1.48 a

AS15 = agroforestry system 15-year-old; AS30 = agroforestry system 30-year-old; SF15 = seasonal forest 15-year-old; SF30 = seasonal forest 30-year-old. Different lowercase letters in columns indicate statistical differences among land uses and age ($P < 0.05$).

On the contrary, management practices applied in AF systems can have diverse impacts on the physicochemical properties of the soil, particularly on SOC. It has been reported that land use changes, can have a negative effect on SOC reservoirs (Matos, Freese, Mendonça, Slazak, and Hüttl, 2011), while specific management practices i.e., (manure amendments, crop rotations) can foster an increase in SOC content (Wolka, Biazin, Martinsen, and Mulder, 2021).

Broadly, the studied AF sites had lower SOC compared to SF, as they are former citrus home gardens turned into mixed species homegardens (Padilla-Vega, Jiménez, and Estrada, 2015) with a history of practices that reduce soil cover and SOC accumulation (weeding control, litter burning, agrochemical application, biomass pruning for forage, etc.). But AF sites also showed increasing SOC with site age, ranging from 6% at 15 years to 7-8% at 30 years. This pattern has also been reported by Lawrence (2005) in tropical dry forests of southern Mexico.

Despite the AF sites having lower SOC than the SF sites, they are showing the same increase with age of maturation or regeneration. The increase in SOC reservoirs can be influenced by various factors (such as climate, microbial activity, vegetation composition, and pH, among others). Nevertheless, it has been reported that as vegetation matures, SOC also tends to increase, until the vegetation reaches climax conditions, and SOC stabilizes or might experience a slight decline (Guan *et al.*, 2019).

Mycorrhizal Colonization and Glomeromycotyna Spore Abundance

For all samples, hyphae accounted for 80% or more of the mycorrhizal colonization, complemented by vesicles and infrequent occurrence of arbuscules. Mycorrhizal colonization was lower in AS during both the dry season (21%) and wet season (32%) than in SF (34 and 38% in both seasons respectively). There were no significant differences in percent colonization due to site age (values are averages of site ages). Therefore, no additional statistical analyses were done, as there was no relationship between the age of the site and mycorrhizal colonization within a land use type.

The most notable differences in colonization were observed among the younger systems (15-years-old), which experience higher disturbance levels. Among these, the AF systems exhibited the lowest mycorrhizal colonization. On the other hand, both age groups of SF systems and the 30-year-old AF systems exhibited higher AMF colonization resilience, as mycorrhizal colonization remains consistent between seasons. Nonetheless, a trend can be observed wherein higher colonization occurs during the rainy season, with this pattern being more pronounced in AF systems. A high mycorrhizal colonization has also been documented in Cacao agroforestry systems in Brazil (de Oliveira and de Oliveira, 2005) and seasonal forest of the Pacific coast of Mexico (Allen, Rincón, Allen, Pérez, and Huante, 1998), associated with an increment in microbial diversity due to the less disturbed conditions of these systems (Matos *et al.*, 2022; Polo-Marcial, Solís, Murillo, Ávila, and Andrade, 2023).

Seasonal variations in mycorrhizal colonization have been explored in previous studies. Several investigations have documented heightened mycorrhizal colonization during the dry season e.g., (Sivakumar, 2013; Chareesri, De Deyn, Sergeeva, Polthanne, and Kuyper, 2020). However, in this study, we observed an opposing trend. This pattern might be attributed to soil moisture and available phosphorus, both of which are pivotal factors influencing AMF responses (Chu *et al.*, 2020; Chudaeng and Teamkao, 2020; Liu, Shen, Li, Xiao, and Song, 2021); due to the region's environmental and karstic conditions, moisture and P are limited during the dry season.

Statistical differences were detected in the number of rhizophilic spores between AS and SF land uses, with differences between AS15 and AS30 only during the wet season; for edaphophilic spores, the only difference identified was among AS15 and the other three systems in both seasons (Table 2). The number of spores was consistently higher in the 30-year-old than 15-year-old systems. Spore abundances of rhizophilic AMF were higher during the wet than the dry season, but not statistically different. The mean rhizophilic Glomeromycotyna AMF spore abundance was 7.17 spores g⁻¹ in the wet season versus 6.67 in the dry season ($P = 0.037$, $n = 108$). The mean of edaphophilic Glomeromycotyna AMF spore abundance was 1.37 spores g⁻¹ in the wet season versus 1.43 in the dry season ($P = 0.037$, $n = 108$). The limited number of spores found in these soils is probably not only related to the high contents of organic carbon but also the pH and P content in these soils, as a low number of fungi spores is often associated with a combination of high organic carbon contents, acid pHs and low available P contents (Alori, Fawole, and Akanji, 2020).

The PCA biplot of the first two principal components indicates a contrary gradient between seasons (dry, wet) and a better relationship of SF to SOC than AF (Figure 2). Within systems, a stronger relationship of 30-year-old systems with SOC compared to 15-year-old systems is shown. AS have a lower importance than SOC content. However, both edaphophilic and rhizophilic AMF spores are more related to SF than to AS. Edaphophilic AMF are more related to SF30 whereas rhizophilic AMF are more related to SF15.

Table 2. Spore abundance (spores g⁻¹) in agroforestry systems and seasonal forest of Tzucacab, Yucatan, Mexico.

Land use/age	Dry season Rhizophilic	Edaphophilic	Wet season Rhizophilic	Edaphophilic
AS15 (n=27)	4.78±1.37 a	0.85±0.66 a	4.48±1.01 a	0.86±0.68 a
AS30 (n=27)	5.19±1.80 a	1.41±1.34 ab	6.30±1.86 b	1.44±0.80 ab
SF15 (n=27)	7.30±1.64 b	1.59±1.50 ab	7.89±1.85 cd	1.61±0.56 ab
SF30 (n=27)	9.41±2.06 c	1.89±0.93 ab	10.00±2.5 cd	1.90±0.93 ab

AS15 = agroforestry system 15-year-old; AS30 = agroforestry system 30-year-old; SF15 = seasonal forest 15-year-old; SF30 = seasonal forest 30-year-old. Different lower-case letters in rows indicate statistical differences ($P < 0.05$) among systems per spore type.

Rhizophilic species spore abundance was sensitive to land use changes and system age but not to seasonality. On the other hand, edaphophilic AMF spore abundance did not show a clear relationship to SOC suggesting that they were more tolerant to the perturbations imposed than rhizophilic AMF. This response was opposite to our expectations. Based on taxonomic classifications, the rhizophilic species fall under the previous Glomeraceae classification, while the edaphophilic species are in the Gigasporaceae (Weber *et al.*, 2019), and can be compared with previous studies reporting taxon names.

Rhizophilic AMF tend to be early successional taxa that have well-developed root internal hyphal development, while edaphophilic are late successional species with extensive external hyphal networks (Allen, Allen, Egerton-Warburton, Corkidi, and Gómez, 2003c, Weber *et al.*, 2019). Rhizophilic species tend to produce a higher density of spores, especially many with small diameters (50-100 μm), while edaphophilic species tend to produce fewer spores with larger diameters (150-200 μm), as we observed here and as well as at another Yucatecan seasonal forest (Allen *et al.*, 2003c).

Based on their late successional status, we expected edaphophilic spores to increase as well as rhizophilic spores with increasing SOC. Instead, we found that spores of rhizophilic AMF species were more sensitive to changes in SOC than those of edaphophilic species, showing more spore abundance as SOC increases. Van der Heyde *et al.* (2017) found that Gigasporaceae species (edaphophilic) are less tolerant to disturbances than species of Glomaceae (rhizophilic). An increase in Gigasporaceae spores was indeed observed during the recovery of a recently burned site in the Yucatan, where both spore density and organic matter recovered to levels observed in mature forests after only three years (Allen *et al.* 2003c).

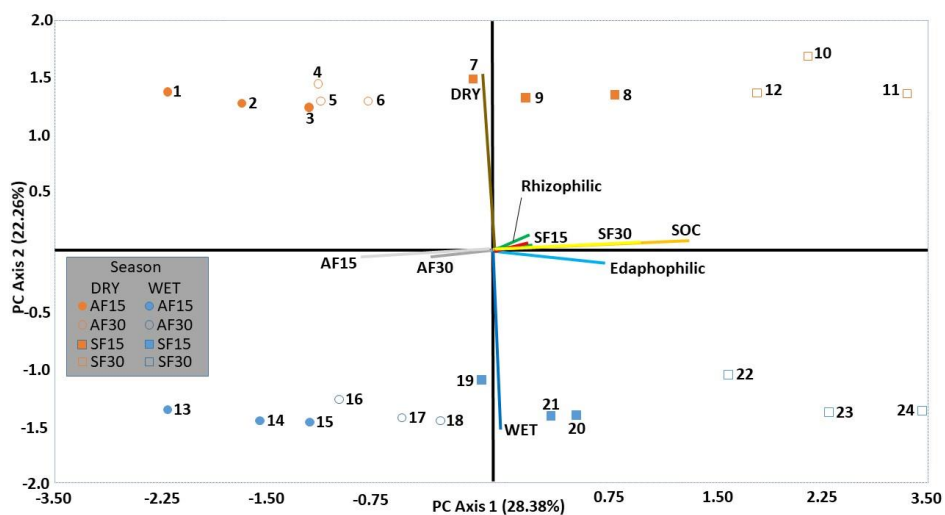


Figure 2. Principal components analysis biplot. Data points represent the average value of the 9 replicates per plot. AS15 = agroforestry system 15-year-old; AS30 = agroforestry system 30-year-old; SF15 = seasonal forest 15-year-old; SF30 = seasonal forest 30-year-old; SOC = soil organic carbon; DRY = dry season; WET = wet season.

A recently established homegarden was not available for this study, and edaphophilic AMF may have already established their hyphal network (and can sporulate) in 15-years-old vegetation. Nevertheless, only the rhizophilic spores continued to increase sporulation with increasing SOC beyond 15 years. The shallower soils and lower precipitation in these sites promote SOC accumulation, conditioning a different relationship between AMF and SOC than van der Heyde *et al.*, (2017) observed. By contrast, the percentage of mycorrhizal colonization was not related to site age or SOC. This is likely because colonization percentage varies with the observation date, while spores persist longer in the soil (Allen *et al.*, 2003c).

SOC Content and Glomeromicotyna Spore Number Relationships

Regression analyses show linear relationships between SOC content and the number of rhizophilic AMF spores for both wet and dry seasons (Figure 3). No relationships were found between SOC and the number of spores of edaphophilic AMF in any season (data not shown).

We postulate that the Glomeromicotyna spore abundance of rhizophilic AMF species could be used as an indicator of soil health at any time of the year. SOC and AMF have intimate feedback, but they must still be better understood. AMF contribute to the stable organic pool. The implications of change rates of rhizophilic and edaphophilic AMF are still unknown. Rhizophilic AMF are presumed to provide pathogen protection but also predominate in arid environments, such as during the dry season in these forests. Edaphophilic AMF are postulated to be especially important in nutrient uptake (Weber *et al.*, 2019). These fungi are needed consistently at all stages of vegetative regrowth. In contrast, pathogen protection and drought stress become increasingly crucial as root mortality and leaf area for transpiration increase with increasing plant density and leaf area of older vegetation.

Importantly, this generalization must be validated for other sites before use because soil conditions and vegetation type can change the relationships found here. For example, Ceccon *et al.*, 2002 found SOC values of 27.9 and 22.0% in 15 and 60-year-old deciduous forest plots in northern Yucatan, suggesting other factors than age affect SOC. In this case, do the rhizophilic AMF spore numbers show the same regression with time, or SOC, or is this a site-specific relationship? Thus, a preliminary examination is mandatory in other sites to check for the relations found here and whether this assessing tool is site-specific. A comprehensive community analysis based on sequencing approaches would also provide important insights into the structure of AMF communities, as this analysis of spores only revealed the composition of AMF in the soil, and not in the roots.

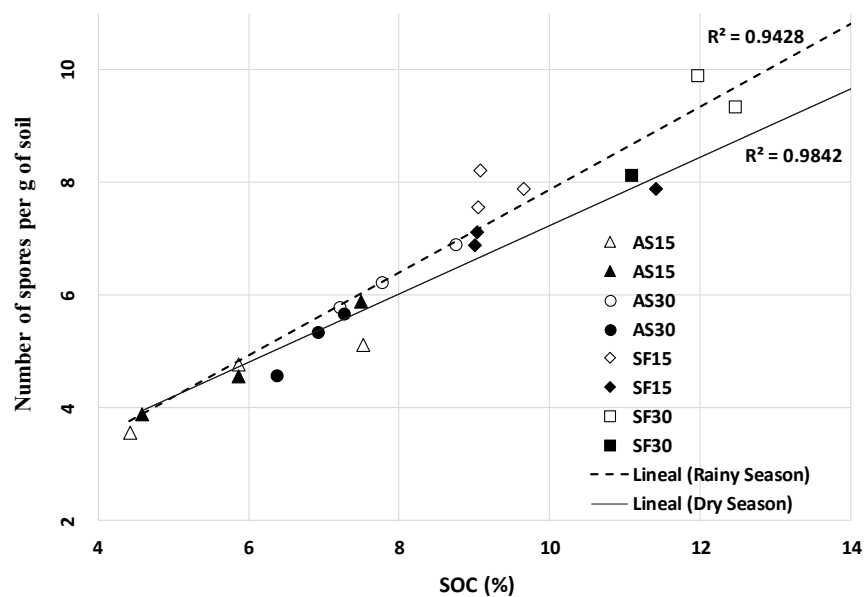


Figure 3. Soil organic carbon (SOC) vs number of rhizophilic AMF spores, linear regression. AS15 = agroforestry system 15-year-old; AS30 = agroforestry system 30-year-old; SF15 = seasonal forest 15-year-old; SF30 = seasonal forest 30-year-old.

CONCLUSIONS

SOC in agroforestry and forest systems varied according to land use and system age; agroforestry systems had lower SOC contents than forest soils. SOC content was related to the number of rhizophilic mycorrhiza spores but not to those of edaphophilic species. The spore abundances of rhizophilic AMF were especially sensitive to land use changes and system age but did not show seasonal differences. Glomeromycotyna spore abundance of rhizophilic species can be used in the studied region as a soil health indicator at any time of the year.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

Study conceptualization: H.E.M., J.J.J.O., M.F.A., and E.B.A. Development of the methodology: H.E.M., J.J.J.O., M.F.A., E.B.A. Experimental validation: S.M.C., H.E.M., J.J.J.O., M.F.A., E.B.A., and O.O.A.R. Results analysis: S.M.C., M.F.A., and R.C.B.M. Data management: S.M.C., M.F.A., and R.C.B.M. Writing and draft preparation: H.E.M., J.J.J.O., M.F.A., E.B.A., O.O.A.R. Redaction, revision and editing: H.E.M., J.J.J.O., M.F.A., E.B.A., R.C.B.M., and O.O.A.R. Project administration: J.J.J.O. Fund acquisition: H.E.M., J.J.J.O., and M.F.A. All authors of this manuscript have read and accepted the published version of the manuscript.

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REFERENCES

- Ali, S., Begum, F., Hayat, R., & Bohannan, B. J. (2017). Variation in soil organic carbon stock in different land uses and altitudes in Bagrot Valley, Northern Karakoram. *Acta Agriculturae Scandinavica*, 67(6), 551-561. <https://doi.org/10.1080/09064710.2017.1317829>
- Allen, M. F., Moore, T. S., Christensen, M., & Stanton, N. (1979). Growth of vesicular-arbuscular-mycorrhizal and nonmycorrhizal *Bouteloua gracilis* in a defined medium. *Mycologia*, 71(3), 666-669. <https://doi.org/10.1080/00275514.1979.12021056>
- Allen, E. B., Rincón, E., Allen, M. F., Pérez-Jimenez, A., & Huante, P. (1998). Disturbance and Seasonal Dynamics of Mycorrhizae in a Tropical Deciduous Forest in Mexico. *Biotropica*, 30(2), 261-274.
- Allen, E. B., Allen, M. F., Violi, H. A. & Gómez-Pompa, A., (2003a). Restoration of tropical seasonal forest in Quintana Roo. In A. Gómez-Pompa, M. F. Allen, S. L. Fedick, & J. J. Jiménez-Osornio (Eds.). *The Lowland Maya Area: Three Millennia at the Human-Wildland Interface* (pp. 587-598). Nueva York, USA: Food Products. ISBN: 1-56022-971-3

- Allen, M. F., Swenson, W., Querejeta, J. I., Egerton-Warburton, L. M., & Treseder, K. K. (2003b). Ecology of mycorrhizae: A conceptual framework for complex interactions among plants and fungi. *Annual Review of Phytopathology*, 41(1), 271-303. <https://doi.org/10.1146/annurev.phyto.41.052002.095518>
- Allen, E. B., Allen, M. F., Egerton-Warburton, L., Corkidi, L., & Gómez-Pompa, A. (2003c). Impacts of early- and late- seral mycorrhizae during restoration in seasonal tropical forest, Mexico. *Ecological Applications*, 13, 1701-1717. <https://doi.org/10.1890/02-5309>
- Alori, E. T., Fawole, O. B., & Akanji, M. O. (2020). Occurrence of arbuscular mycorrhizal fungi spores in soils of some legumes and their response to varying concentrations of phosphorus application. *Open Agriculture*, 5(1), 539-547. <https://doi.org/10.1515/opag-2020-0052>
- Álvarez-Rivera, O. O., Estrada-Medina, H., Jiménez-Osornio, J. J., O'Connor-Sánchez, I. A., Navarro-Alberto, J. A., Ferrer, M. M., ... & Tzuc-Gamboa, M. D. C. (2021). Differences in oxalate-carbonate pathway of *Brosimum alicastrum* in karst homegarden and forest soils. *Soil Science Society of America Journal*, 85(3), 691-702. <https://doi.org/10.1002/saj2.20228>
- Avnimelech, Y. (1980). Calcium-carbonate-phosphate surface complex in calcareous systems. *Nature*, 288, 255-257. <https://doi.org/10.1038/288255a0>
- Chareesri, A., De Deyn, G. B., Sergeeva, Polthanne, A., & Kuyper, T. W. (2020). Increased arbuscular mycorrhizal fungal colonization reduces yield loss of rice (*Oryza sativa* L.) under drought. *Mycorrhiza*, 30(2), 315-328. <https://doi.org/10.1007/s00572-020-00953-z>
- Chen, S., Arrouays, D., Angers, D. A., Martin, M. P., & Walter, C. (2019). Soil carbon stocks under different land uses and the applicability of the soil carbon saturation concept. *Soil Tillage Research*, 188, 53-58. <https://doi.org/10.1016/j.still.2018.11.001>
- Chu, Q., Zhang, L., Zhou, J., Yuan, L., Chen, F., Zhang, F., ... & Rengel, Z. (2020). Soil plant-available phosphorus levels and maize genotypes determine the phosphorus acquisition efficiency and contribution of mycorrhizal pathway. *Plant and Soil*, 449, 357-371. <https://doi.org/10.1007/s11104-020-04494-4>
- Chudaeng, D., & Teamkao, P. (2020). Effects of soil moisture content and arbuscular mycorrhizal fungi on phosphorus fractions in soil. *International Journal of Agricultural Technology*, 16(2), 247-258.
- Ceccon, E., Olmested, I., & Campo-Alves, J. (2002). Vegetación y propiedades del suelo en dos bosques tropicales secos de diferente estado regeneracional en Yucatán. *Agrociencia*, 36(5), 621-631.
- Dai, Z., Birdsey, R. A., Johnson, K. D., Dupuy, J. M., Hernández-Stefanoni, J. L., & Richardson, K. (2014). Modeling carbon stocks in a secondary tropical dry forest in the Yucatan Peninsula, Mexico. *Water, Air, & Soil Pollution*, 225, 1925. <https://doi.org/10.1007/s11270-014-1925-x>
- de Oliveira, A. N., & de Oliveira, L. A. (2005). Seasonal dynamics of arbuscular mycorrhizal fungi in plants of *Theobroma grandiflorum* Schum and *Paullinia cupana* Mart. of an agroforestry system in Central Amazonia, Amazonas state, Brazil. *Brazilian Journal of Microbiology*, 36, 262-270. <https://doi.org/10.1590/S1517-83822005000300011>
- Ekblad, A., & Bastviken, D. (2019). Deforestation releases old carbon. *Nature Geoscience*, 12, 499-500. <https://doi.org/10.1038/s41561-019-0394-7>
- Estrada-Medina, H., Jiménez-Osornio, J. J., Álvarez-Rivera, O., & Barrientos-Medina, R. C. (2019). El karst de Yucatán: su origen, morfología y biología. *Acta universitaria*, 29, 1-18. <https://doi.org/10.15174/au.2019.2292>
- Fesel, P. H., & Zuccaro, A. (2015). Beta-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants. *Fungal Genetics and Biology*, 90, 53-60. <https://doi.org/10.1016/j.fgb.2015.12.004>
- Friese, C. F., & Allen, M. F. (1991). The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. *Mycologia*, 83, 409-418. <https://doi.org/10.1080/00275514.1991.12026030>
- Gooday, G. W. (1994). Physiology of microbial degradation of chitin and chitosan. In C. Ratledge (Ed.). *Biochemistry of microbial degradation* (pp. 279-312). Dordrecht, The Netherlands: Kluwer Academic. https://doi.org/10.1007/978-94-011-1687-9_9
- Guan, J. H., Deng, L., Zhang, J. G., He, Q. Y., Shi, W. Y., Li, G., & Du, S. (2019). Soil organic carbon density and its driving factors in forest ecosystems across a northwestern province in China. *Geoderma*, 352, 1-12. <https://doi.org/10.1016/j.geoderma.2019.05.035>
- Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1-9.
- Hays, Z., & Watson, D. (2019). *Fungal ecology, diversity and metabolites*. Waltham, Abbey Essex, United Kingdom: ED-Tech Press. ISBN: 978-1-83947-188-9
- Hendrickson, O. (2003). Influences of global change on carbon sequestration by agricultural and forest soils. *Environmental Reviews*, 11(3), 161-192. <https://doi.org/10.1139/a04-001>
- Hernández, R. R., & Allen, M. F. (2013). Diurnal patterns of productivity of arbuscular mycorrhizal fungi revealed with the soil ecosystem observatory. *New Phytologist*, 200(2), 547-557. <https://doi.org/10.1111/nph.12393>
- Jansa, J., & Treseder, K. K. (2016). Mycorrhizas and the carbon cycle. In N. C. Johnson, C. Gehring, & J. Jansa (Eds.). *Mycorrhizal mediation of soil* (pp. 343-355). Amsterdam, Netherlands: Elsevier Inc. <https://doi.org/10.1016/B978-0-12-804312-7.00019-X>
- INEGI (Instituto Nacional de Estadística y Geografía). (2009). *Prontuario de información geográfica municipal de los Estados Unidos Mexicanos, Tzucacab, Yucatán. Clave geoestadística 31098*. Consultado el 13 de febrero, 2022, desde http://www3.inegi.org.mx/contenidos/app/mexicocifras/datos_geograficos/31/31098.pdf
- Jobbágy, E. G., & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 10(2), 423-436. [https://doi.org/10.1890/1051-0761\(2000\)010\[0423:TVDOSO\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2)
- Lal, R. (2014). Societal value of soil carbon. *Journal of Soil and Water Conservation*, 69(6), 186A-192A. <https://doi.org/10.2489/jswc.69.6.186A>
- Lal, R. (2016). Soil health and carbon management. *Food Energy Security*, 5(4), 212-222. <https://doi.org/10.1002/fes3.96>
- Lawrence, D. (2005). Regional-scale variation in litter production and seasonality in tropical dry forests. *Biotropica*, 37, 561-70. <https://doi.org/10.1111/j.1744-7429.2005.00073.x>
- Liu, M., Shen, Y., Li, Q., Xiao, W., & Song, X. (2021). Arbuscular mycorrhizal fungal colonization and soil pH induced by nitrogen and phosphorus additions affects leaf C:N:P stoichiometry in Chinese fir (*Cunninghamia lanceolata*) forests. *Plant Soil*, 461, 421-440. <https://doi.org/10.1007/s11104-021-04831-1>
- Matos, E. S., Freese, D., Mendonça, E. S., Slazak, A., & Hüttel, R. F. (2011). Carbon, nitrogen and organic C fractions in topsoil affected by conversion from silvopastoral to different land use systems. *Agroforestry Systems*, 81, 203-211. <https://doi.org/10.1007/s10457-010-9314-y>
- Matos, P. S., da Silva, C. F., Pereira, M. G., da Silva, E. M. R., Tarré, R. M., Franco, A. L. C., & Zonta, E. (2022). Short-term modifications of mycorrhizal fungi, glomalin and soil attributes in a tropical agroforestry. *Acta Oecologica*, 114, 103815. <https://doi.org/10.1016/j.actao.2022.103815>
- Muktamar, Z., Nurliana, S., Aningias, H., & Anugrah, P. M. (2021). Soil organic carbon in forest and other land use types at Bengkulu City, Indonesia. *Jurnal Manajemen Hutan Tropika*, 27(3), 184-184. <https://doi.org/10.7226/jtfm.27.3.184>
- Murty, D., Kirschbaum, M., Mcmurtrie, R., & MCGilvray, H. (2002). Does conversion of forest to agricultural land change soil carbon and nitrogen? A review of the literature. *Global Change Biology*, 8(2), 105-123. <https://doi.org/10.1046/j.1354-1013.2001.00459.x>

- Lovelock, C. E., Wright S. F., Clark D. A., & Ruess, R. W. (2004). Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape. *Journal of Ecology*, *92*, 278-287.
- Nelson, D. W., & Sommers, L. E. (1996). Organic matter. In D. L. Sparks, A. L. Page, P.A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, & M. E. Sumner (Eds.). *Methods of Soil Analysis: Part 3. Chemical Methods. Agronomy Monograph* (pp. 961-1010). Madison, WI, USA: American Society of Agronomy-Soil Science Society of America.
- Padilla-Vega, J., Jiménez Osornio, J. J., & Estrada-Medina, H., (2015). Análisis de la estructura vegetal de huertas frutícolas del sur de Yucatán, México. *Revista Mexicana de Ciencias Agrícolas*, *6*(7), 1443-1454.
- Phillips, M. L., Weber, S. E., Andrews, L. V., Aronson, E. L., Allen, M. F., & Allen, E. B. (2019). Fungal community assembly in soils and roots under plant invasion and nitrogen deposition. *Fungal Ecology*, *40*, 107-117. <https://doi.org/10.1016/j.funeco.2019.01.002>
- Polo-Marcial, M. H., Solís-Ramos, L. Y., Murillo-Cruz, R., Ávila-Arias, C., & Andrade-Torres, A. (2023). Mycorrhizal and endophytic richness and colonization in *Cedrela odorata* L., in agroforestry systems and secondary forest from southeastern Costa Rica. *Agroforestry Systems*, *97*(4), 647-658.
- Rillig, M. C., Wright, S. F., Nichols, K. A., Schmidt, W. F., & Torn, M. S. (2001). Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant Soil*, *233*, 167-177. <https://doi.org/10.1023/A:1010364221169>
- Shang, C., & Tiessen, H. (2003). Soil organic C sequestration and stabilization in karstic soils of Yucatan. *Biogeochemistry* *62*, 177-196. <https://doi.org/10.1023/A:1021123728639>
- Sivakumar, N. (2013). Effect of edaphic factors and seasonal variation on spore density and root colonization of arbuscular mycorrhizal fungi in sugarcane fields. *Annals of Microbiology*, *63*, 151-160. <https://doi.org/10.1007/s13213-012-0455-2>
- Soka, G. E., & Ritchie, M. E. (2018). Arbuscular mycorrhizal spore composition and diversity associated with different land uses in a tropical savanna landscape, Tanzania. *Applied Soil Ecology*, *125*, 222-232. <https://doi.org/10.1016/j.apsoil.2018.01.013>
- Treseder, K. K., & Allen, M. F. (2002). Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist*, *155*(3), 507-515. <https://doi.org/10.1046/j.1469-8137.2002.00470.x>
- Tilston, E. L., Sizmur, T., Dixon, G. R., Otten, W., & Harris, J. A. (2010). The Impact of Land-Use Practices on Soil Microbes. In G. R. Dixon, & E. L. Tilson (Eds.). *Soil microbiology and sustainable crop production* (pp. 273-295). The Netherlands: Springer. https://doi.org/10.1007/978-90-481-9479-7_7
- van der Heyde, M., Ohsowski, B., Abbott, L. K., & Hart, M. (2017). Arbuscular mycorrhizal fungus responses to disturbance are context-dependent. *Mycorrhiza*, *27*(5), 431-440. <https://doi.org/10.1007/s00572-016-0759-3>
- Weber, S. E., Diez, J. M., Andrews, L. V., Goulden, M. L., Aronson, E. L., & Allen, M. F. (2019). Responses of arbuscular mycorrhizal fungi to multiple coinciding global change drivers. *Fungal Ecology*, *40*, 62-71. <https://doi.org/10.1016/j.funeco.2018.11.008>
- Wei, X., Shao, M., Gale, W., & Li, L. (2014). Global pattern of soil carbon losses due to the conversion of forests to agricultural land. *Scientific Reports*, *4*, 4062. <https://doi.org/10.1038/srep04062>
- Wolka, K., Biazin, B., Martinsen, V., & Mulder, J. (2021). Soil organic carbon and associated soil properties in Enset (*Ensete ventricosum* Welw. Cheesman)-based homegardens in Ethiopia. *Soil and Tillage Research*, *205*, 104791. <https://doi.org/10.1016/j.still.2020.104791>
- Zamora-Crescencio, P., García-Gil, G., Flores-Guido, J. S., Ortiz, J. J. (2008). Estructura y composición florística de la selva mediana subcaducifolia en el sur del estado de Yucatán, México. *Polibotánica*, *26*, 33-66.
- Zhu, Y. G., & Miller, R. M. (2003). Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends in Plant Science*, *8*, 407-409. [https://doi.org/10.1016/S1360-1385\(03\)00184-5](https://doi.org/10.1016/S1360-1385(03)00184-5)